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Root phenotyping

Image analysis approaches

Dr Andrew French
University of Nottingham
UK

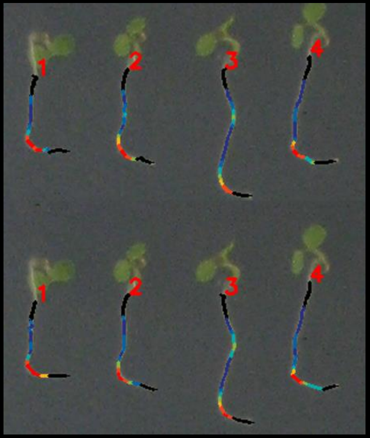
BBSRC bioscience for the future

erc European Research Council Established by the European Commission

This talk

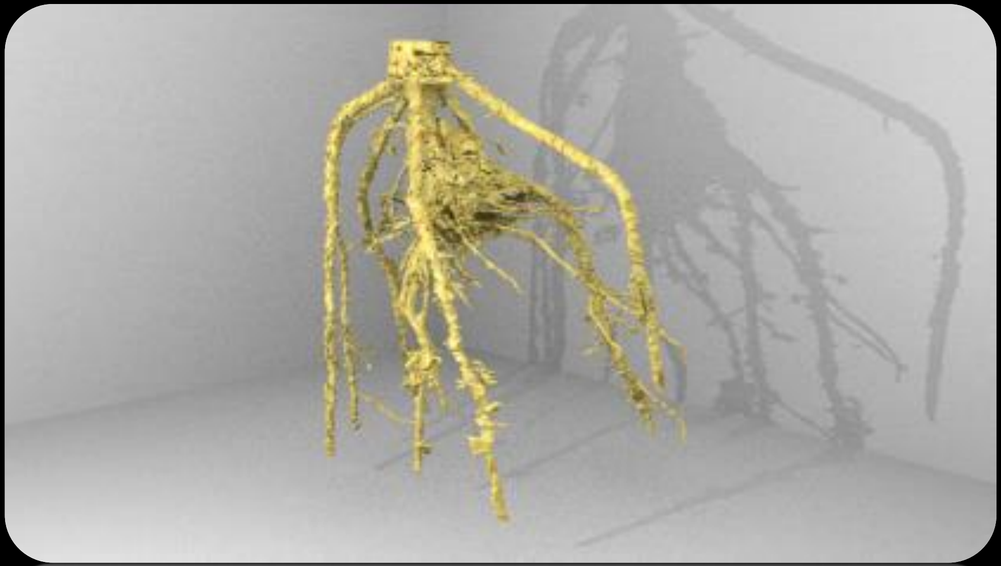
1. Introduction
2. Capturing root images
3. Phenotyping software
   - Across the scales, from sub-cellular to field
   - Some existing software tools
   - A little bit of ‘behind the scenes’ technical detail

Similarities with the previous talk



- **Segmentation** is still a key problem
  - Working out what is a root in the image
  - Then measuring or counting some parameters about the roots

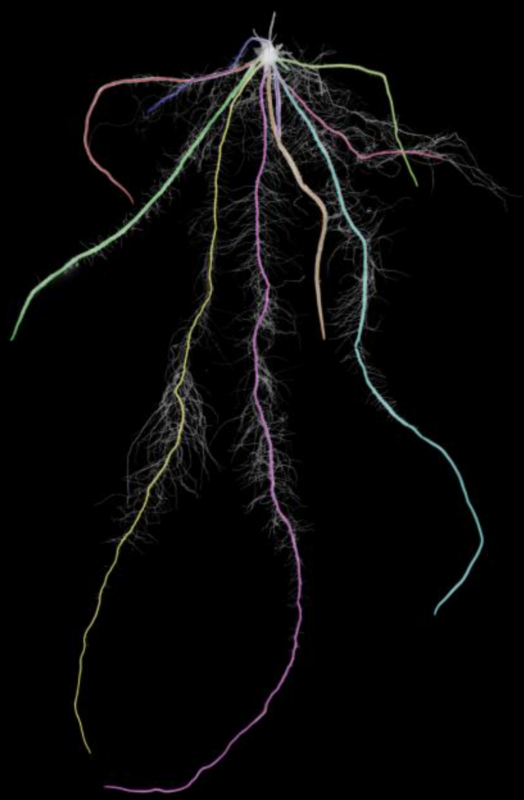
- Image capture is different.
  - Roots are **hidden** by nature in soil
  - So the first problem is, how to image them?



CAPTURING IMAGES OF ROOTS

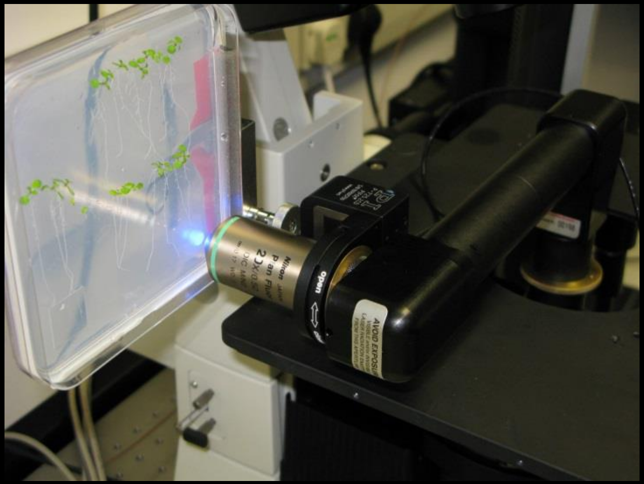
Capturing images

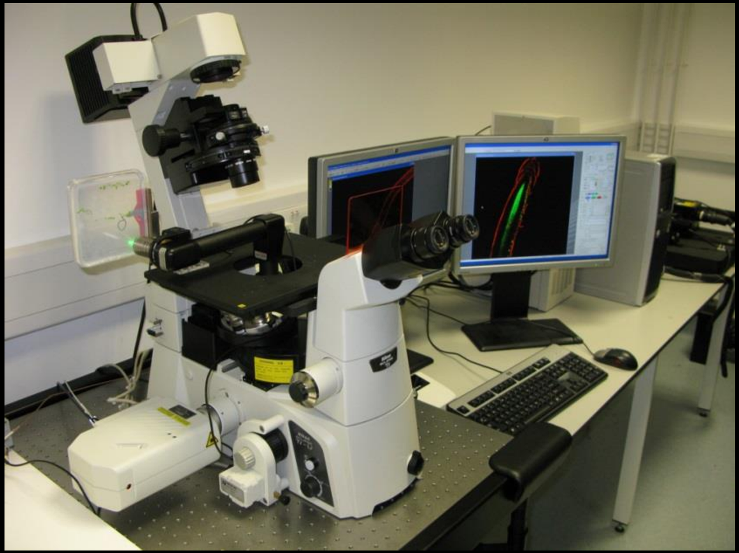
Flatbed scanner



Throughput

Microscope





Vertical plate, as roots grow down!

Digital photos

Hardware: automated image acquisition

- Min. interval ~3 min
- Capacity: 20 standard (125x125mm) plates
  - ~400 plants/run
- LabView for motor control/PSRemote for cameras
- 2 Compact cameras – continuous visible light
- Uses existing lighting

Video 8x speed

French A, Wells D, Everitt N and Pridmore T (2012) Measuring Roots: an updated approach

Hardware: automated image acquisition II

- Commercial components
- Accuracy: ±187 µm
- Repeatability: <2 µm
- 1.5m length
- Max speed: 60 mm/s
  - Min interval: 5 mins
- Higher load (10kg) – more flexibility wrt payload
- Machine vision cameras (industrial spec)
- Near infra-red imaging
- Capacity: 26 125x125mm plates (~500 seedlings/expt)

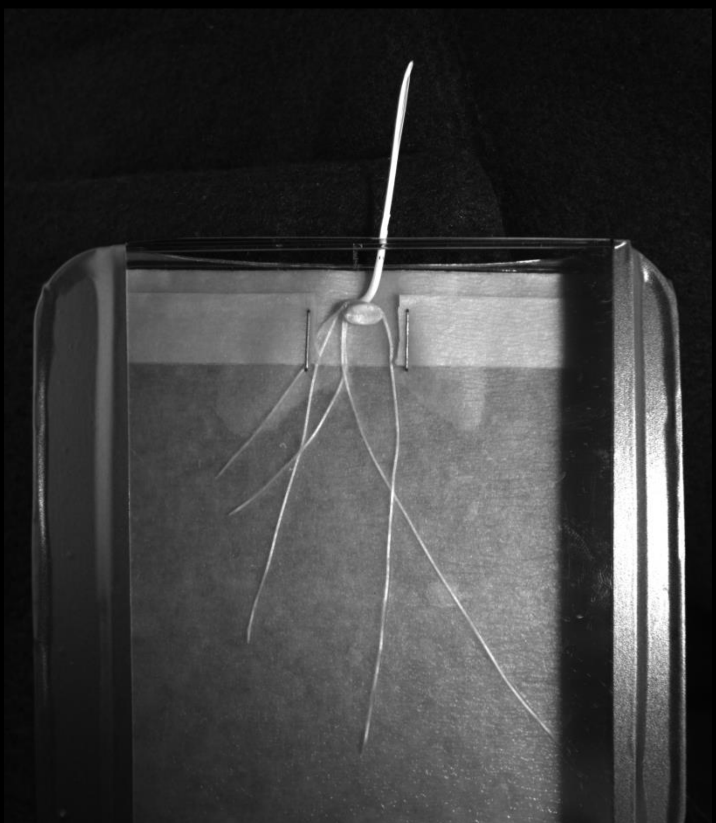
Near infra-red imaging

IR pass acrylic sheet: 0% transmittance below 740 nm, 90% >850 nm

Arabidopsis

pass filter off
IR LEDS off

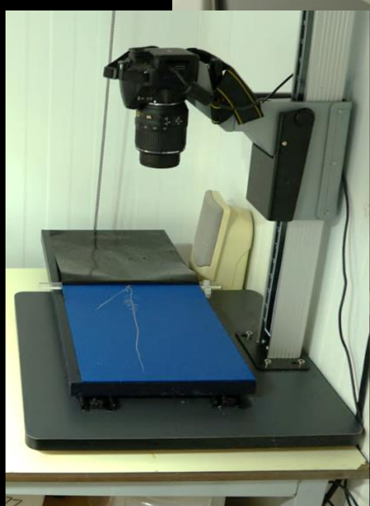
winter wheat (cv. Rialto)



pass filter off
IR LEDS off

Wells D, French A, Naeem A, Ishaq O, Traini R, Hijazi H, Bennett M & Pridmore T (2012) Phil. Trans. B

2D Root Phenotyping



- Capacity: 400 plants (100 plants per unit; 4 units per standard growth room)
- Roots and shoots imaged at regular intervals
- RSA traits quantified via RootNav software
  - exported to user database for further analysis

Field scale

Root Tunnel at East Malling Research, UK

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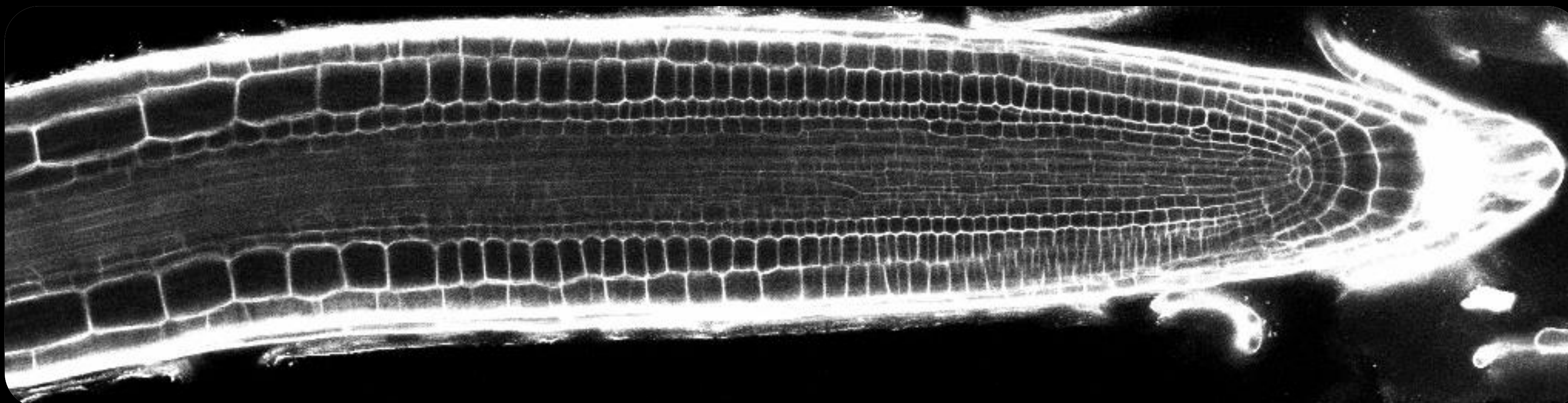

PHENOTYPING ROOTS ACROSS SCALES: SOFTWARE

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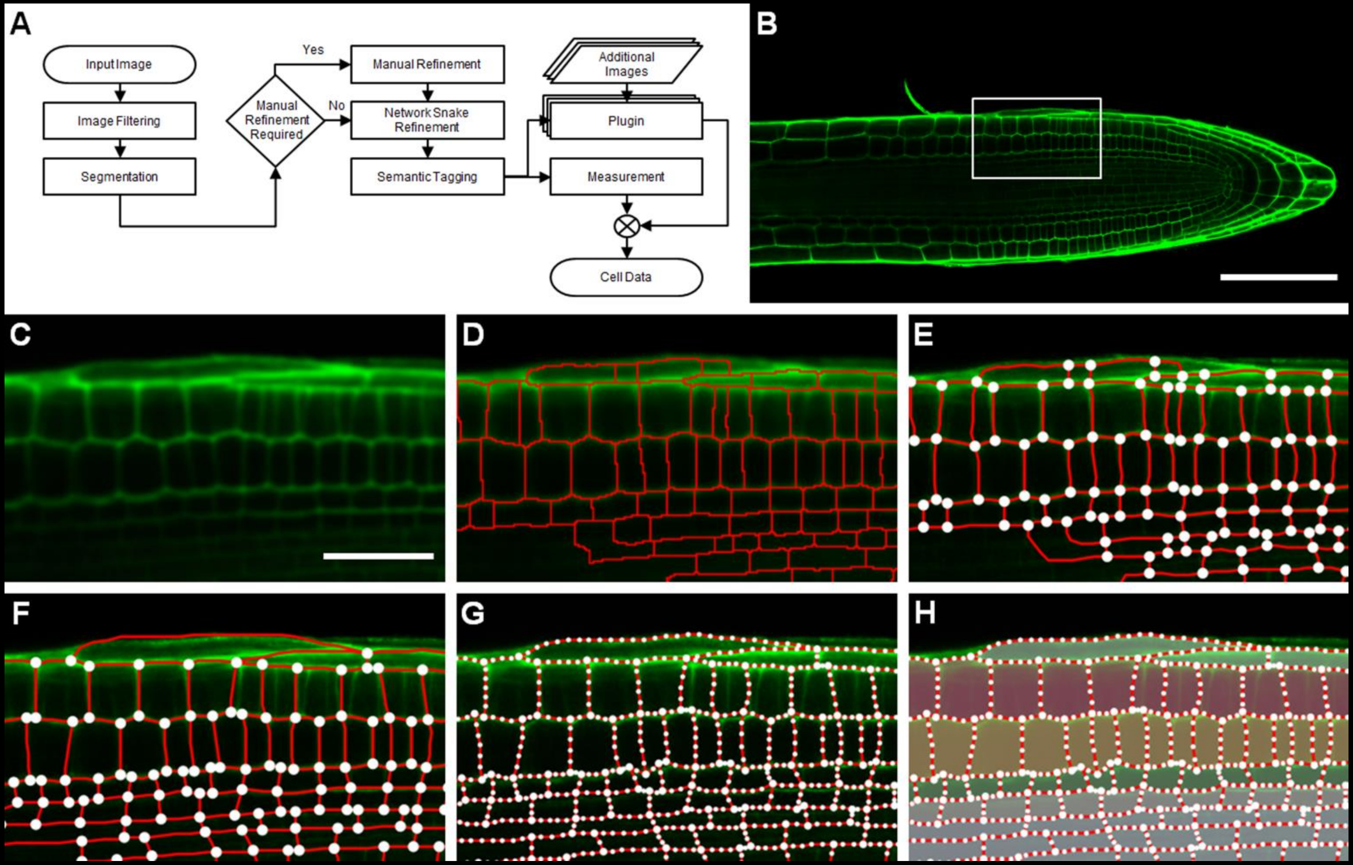
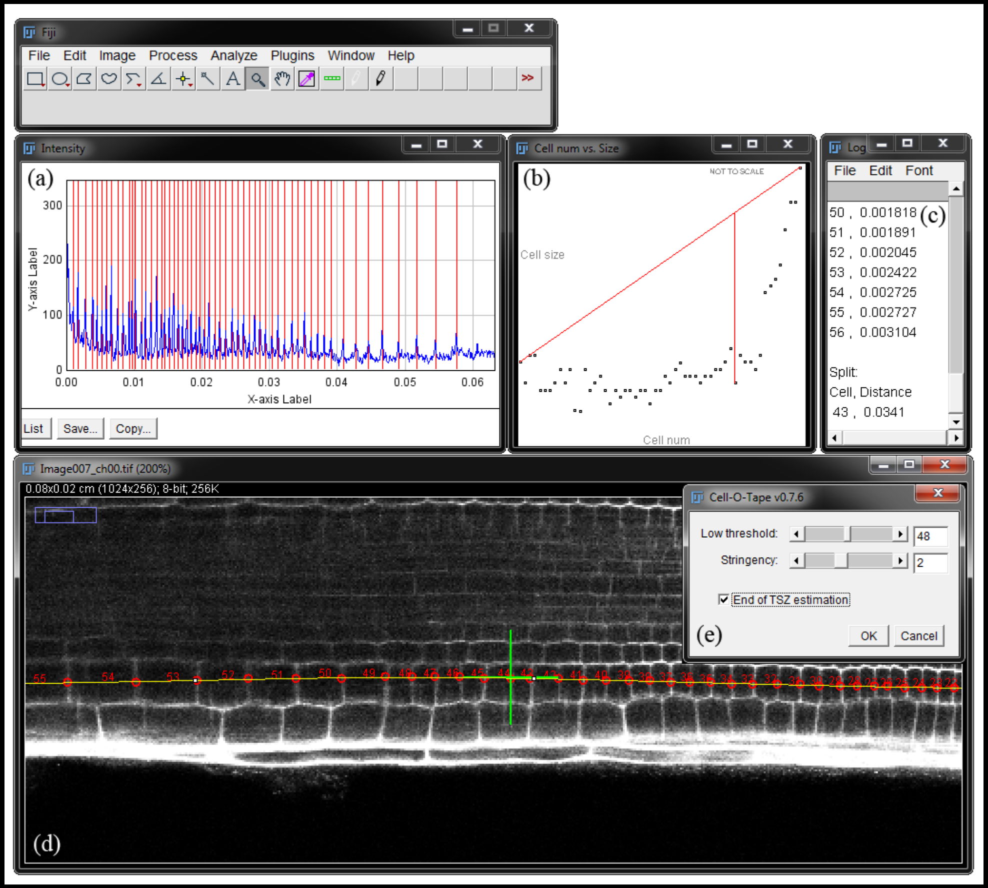
Cell/tissue scale: CellSet



CellSet: M. Pound, A. French et al. , Plant Cell, 2012

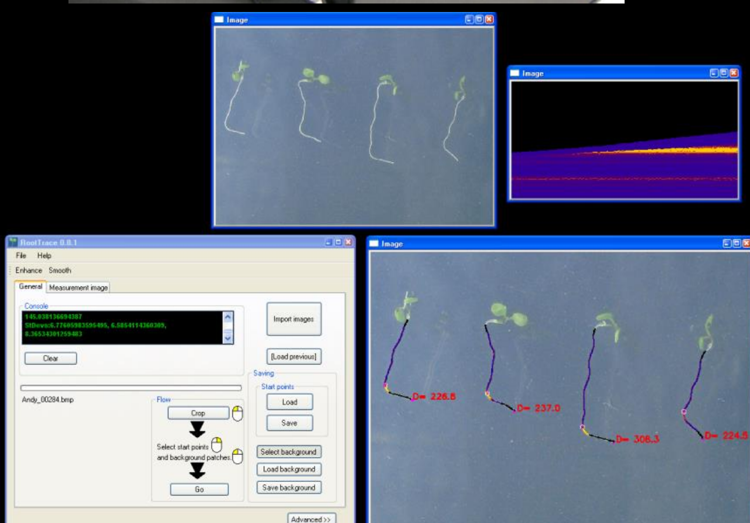
Cell-o-tape



FIJI
'Fiji Is Just **ImageJ**'
http://fiji.sc/Fiji

French et al. (2012) Plant Methods 8:7

Phenotyping software: RootTrace v1

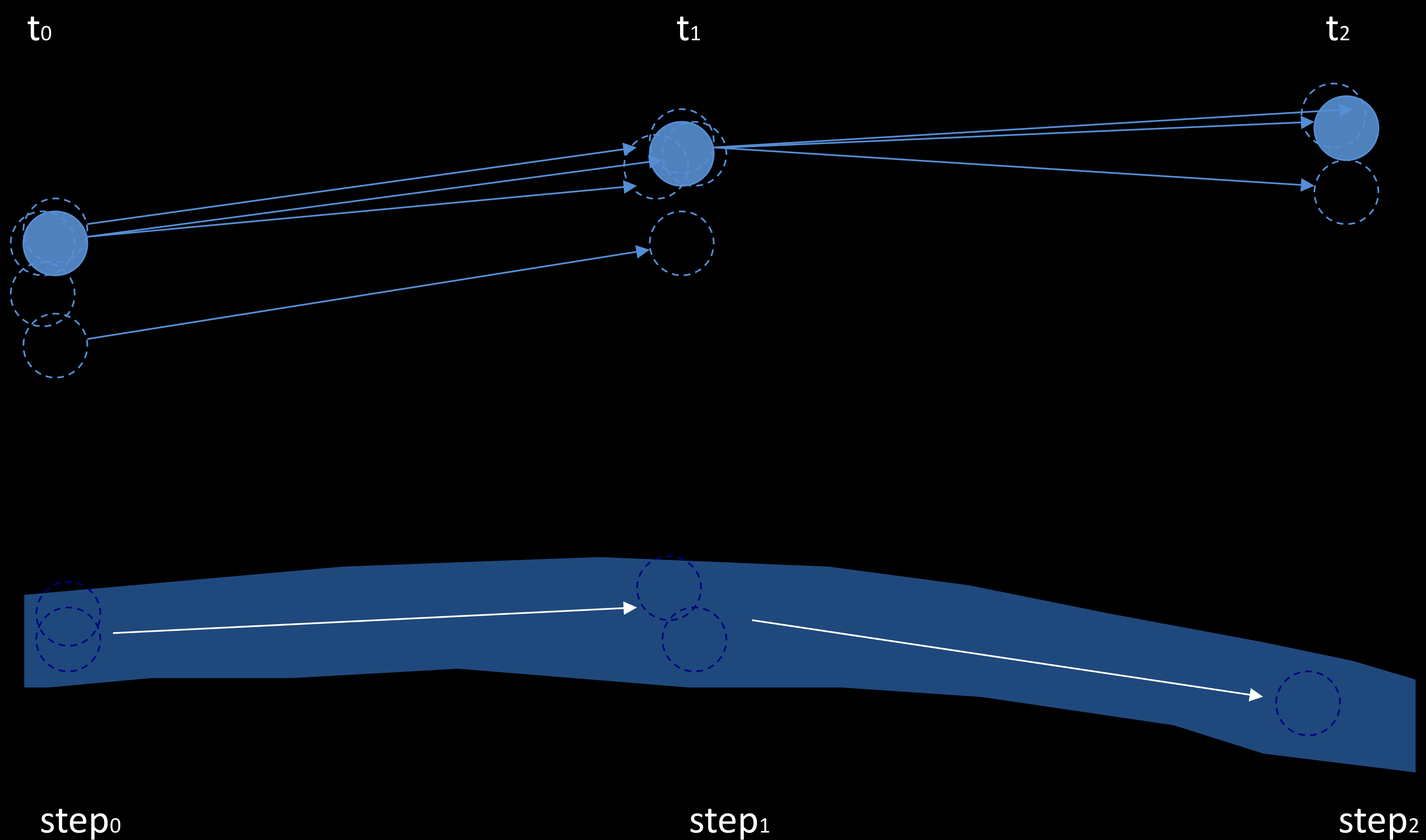


- Fixed cameras
- max two plates
- ~ 40 plants/plate
- Semi-automatic
- Root growth
- Curvature
- Gravitropic response

French AP, Úbeda-Tomás S, Holman TJ, Bennett MJ, Pridmore TP (2009) Plant Physiol. 150, 1784-1795

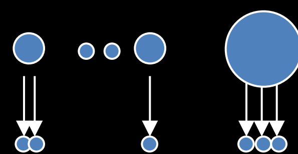
A bit of technical detail: Tracking along the root

Condensation Algorithm – ‘tracking’

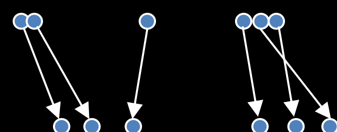


Condensation Algorithm

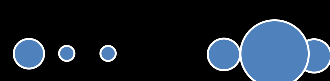
1. Select a sample from the set with a probability proportional to the sample weights



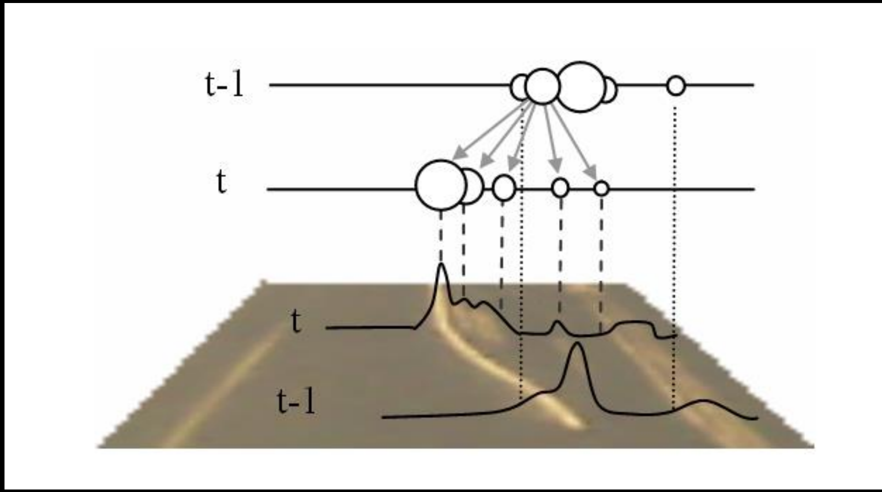
2. Apply dynamic model to particles in new set



3. Weight the new particles based on an appearance model



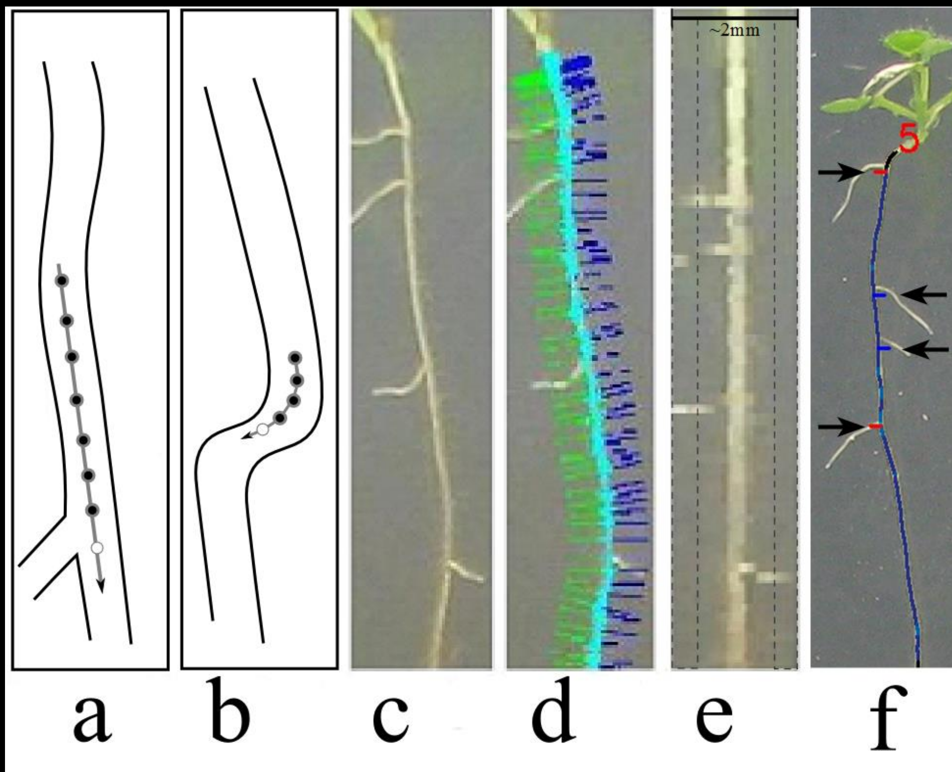
Particle set propagation

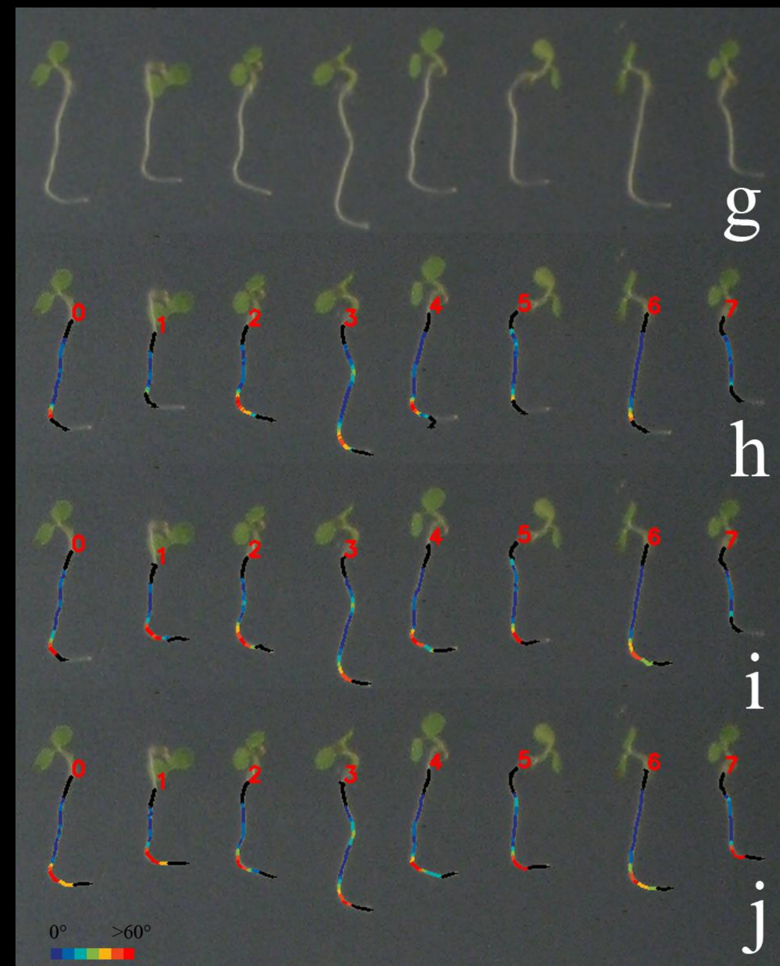


"Motion" model:

- Particles are "driven" in the general direction of root growth
- Additional diffuse component, perpendicular to growth direction
- "Velocity" (directional) component, which uses root smoothness assumption

Software: RootTrace v2





- Improved root detection wrt curving
- Counts laterals
- Tracks angle of tip

Naeem A, French AP, Wells, DM & Pridmore TP (2011) Bioinformatics 27:1337-1338

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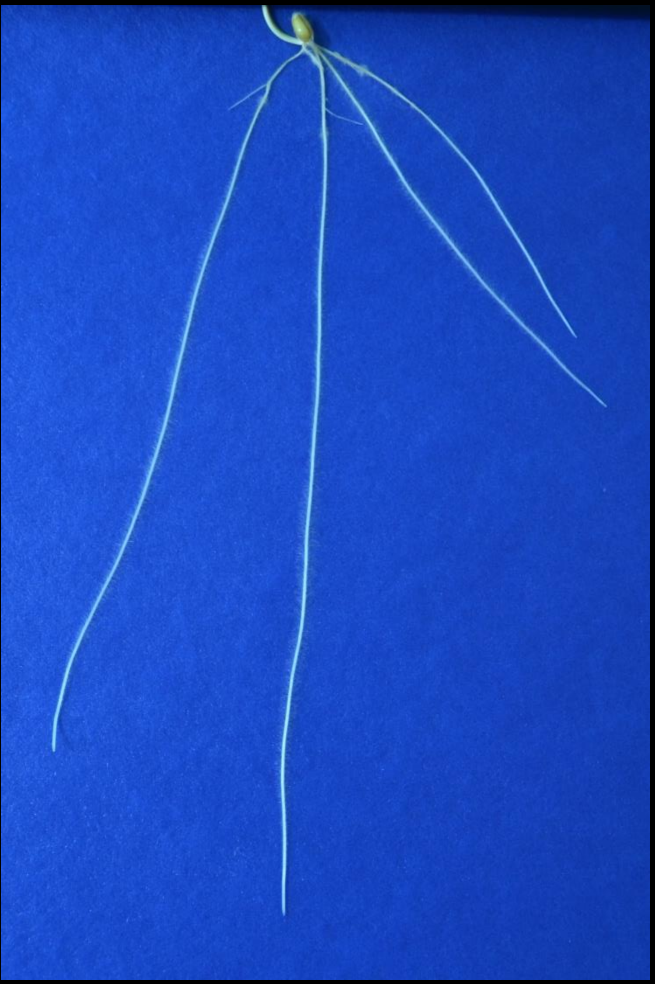
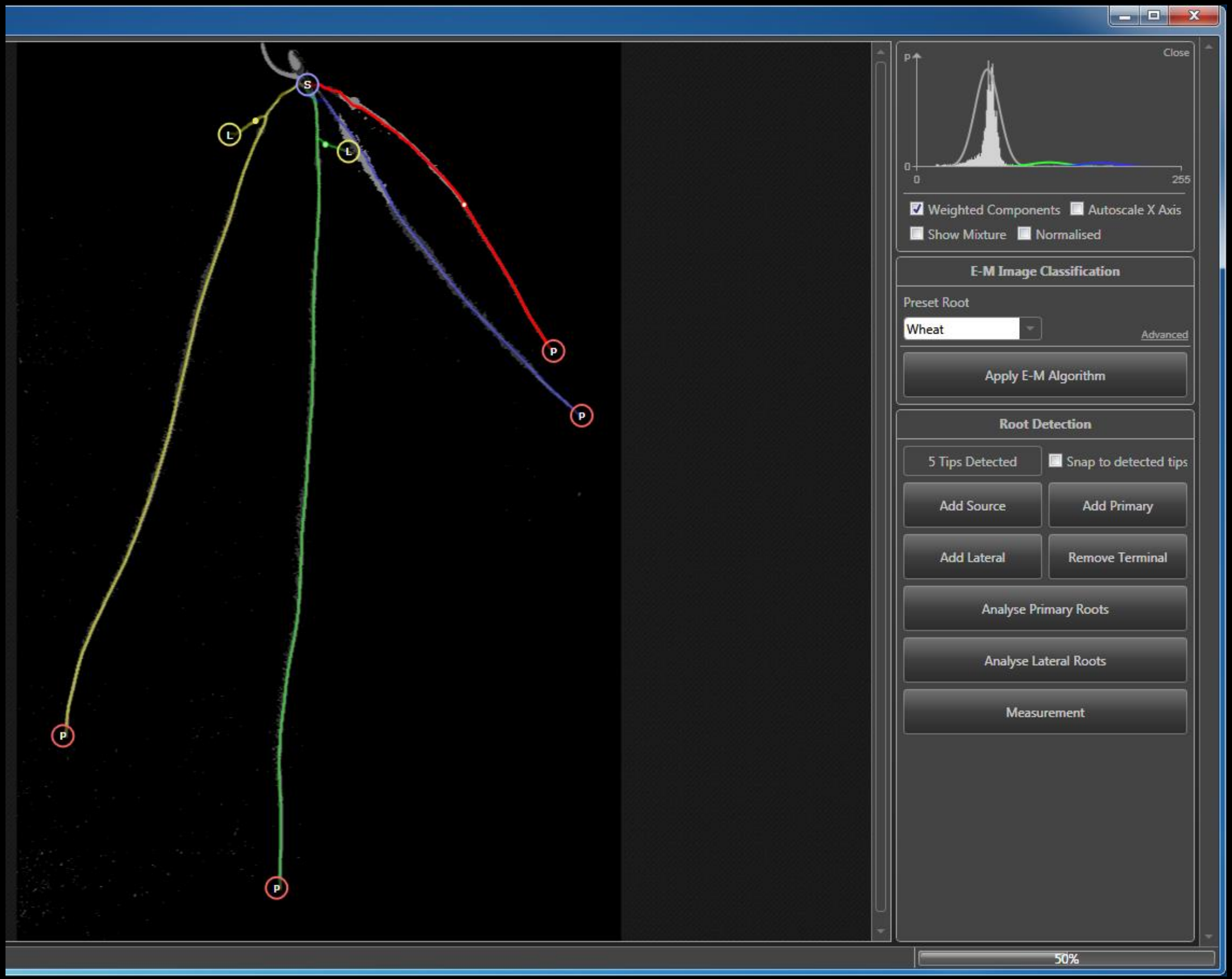
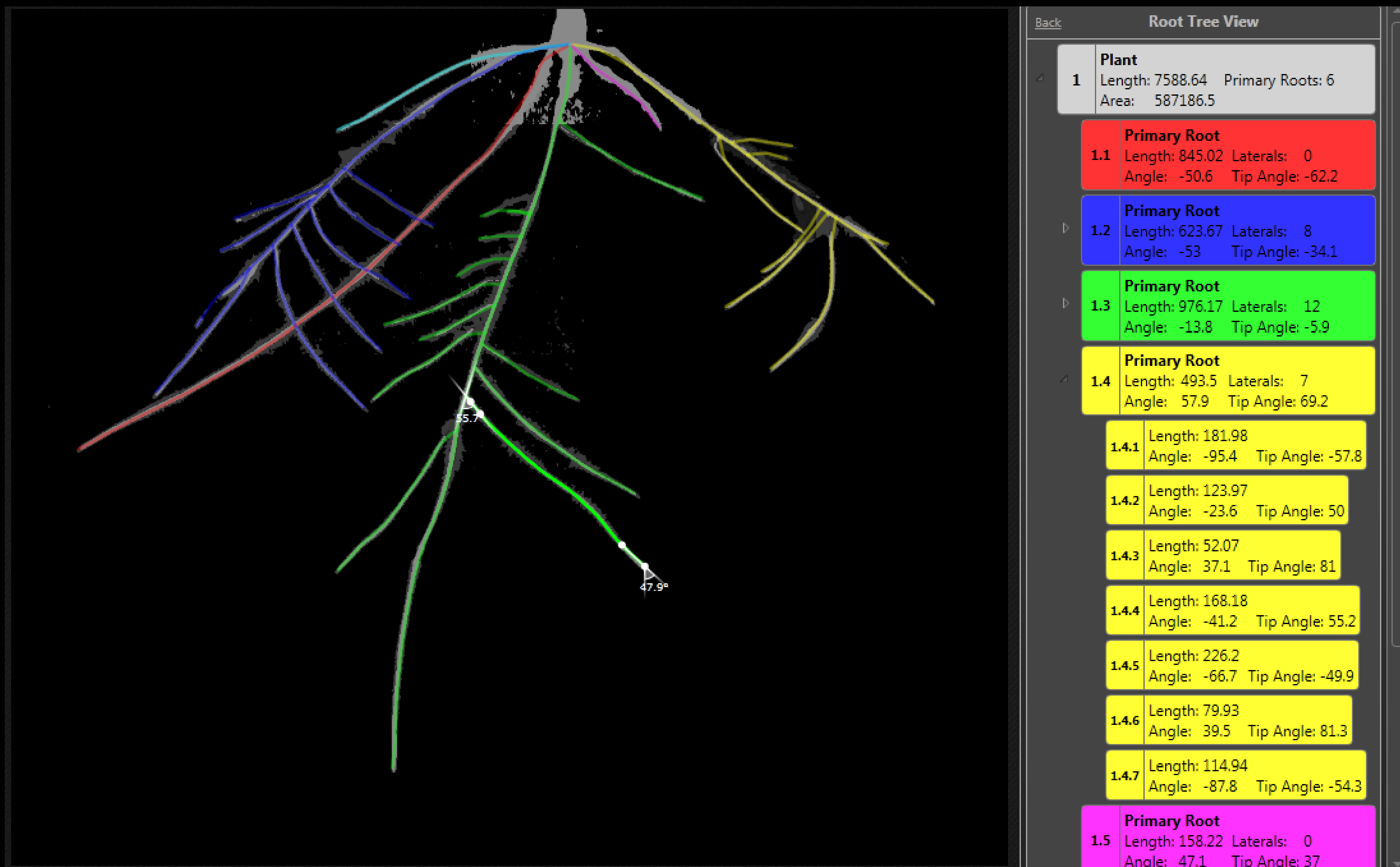
2D Root Phenotyping – RootNav





2D Root Phenotyping – measurement and data storage



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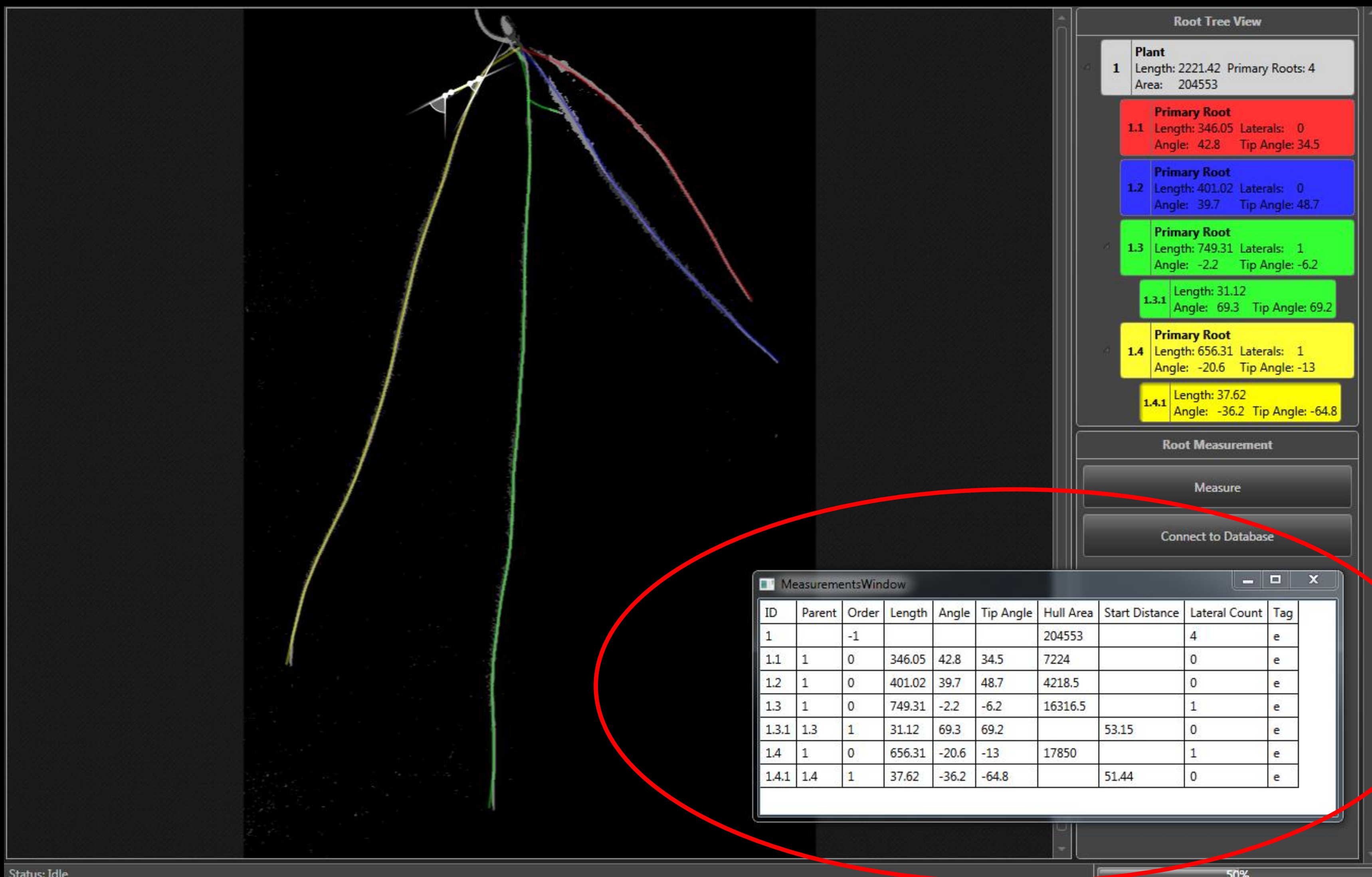
2D Root Phenotyping – measurement and data storage

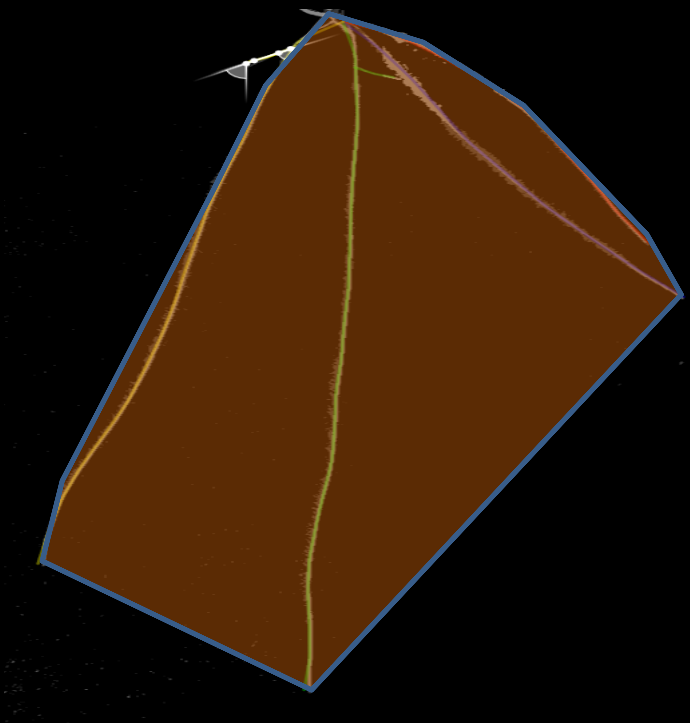


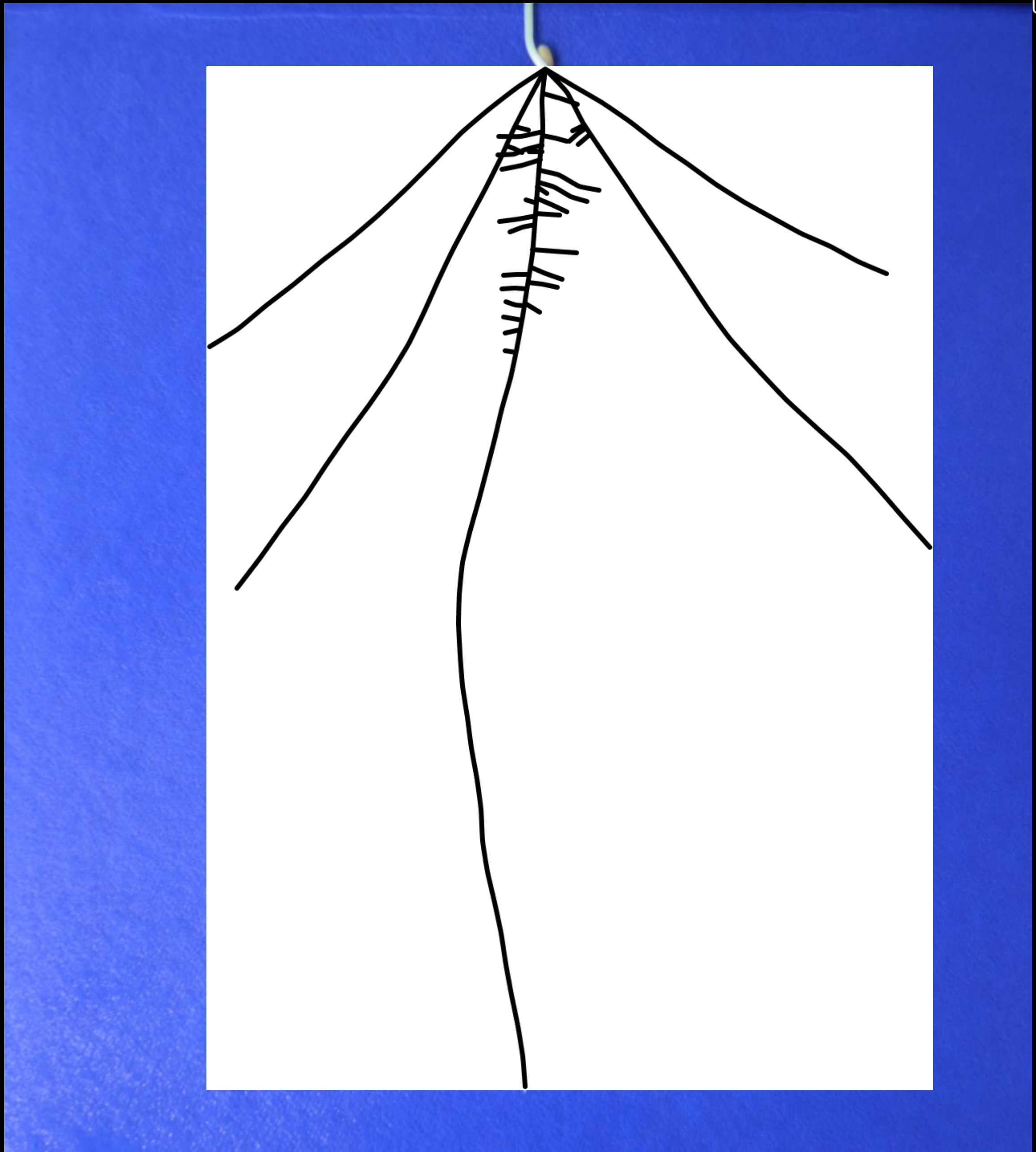
RootNav – example use

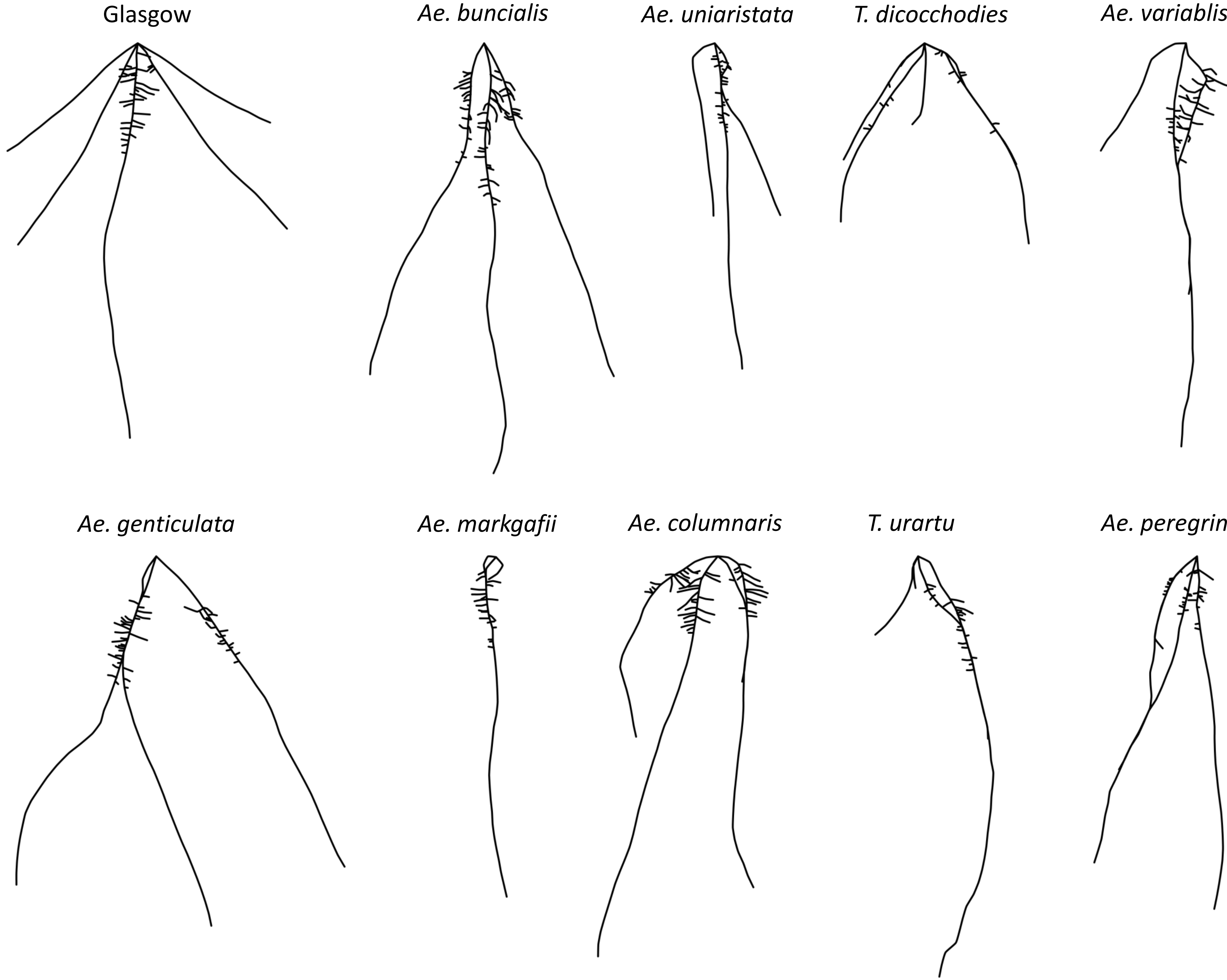
Pound et al. *Plant Physiology*, in press (2013). doi:10.1104/pp.113.221531

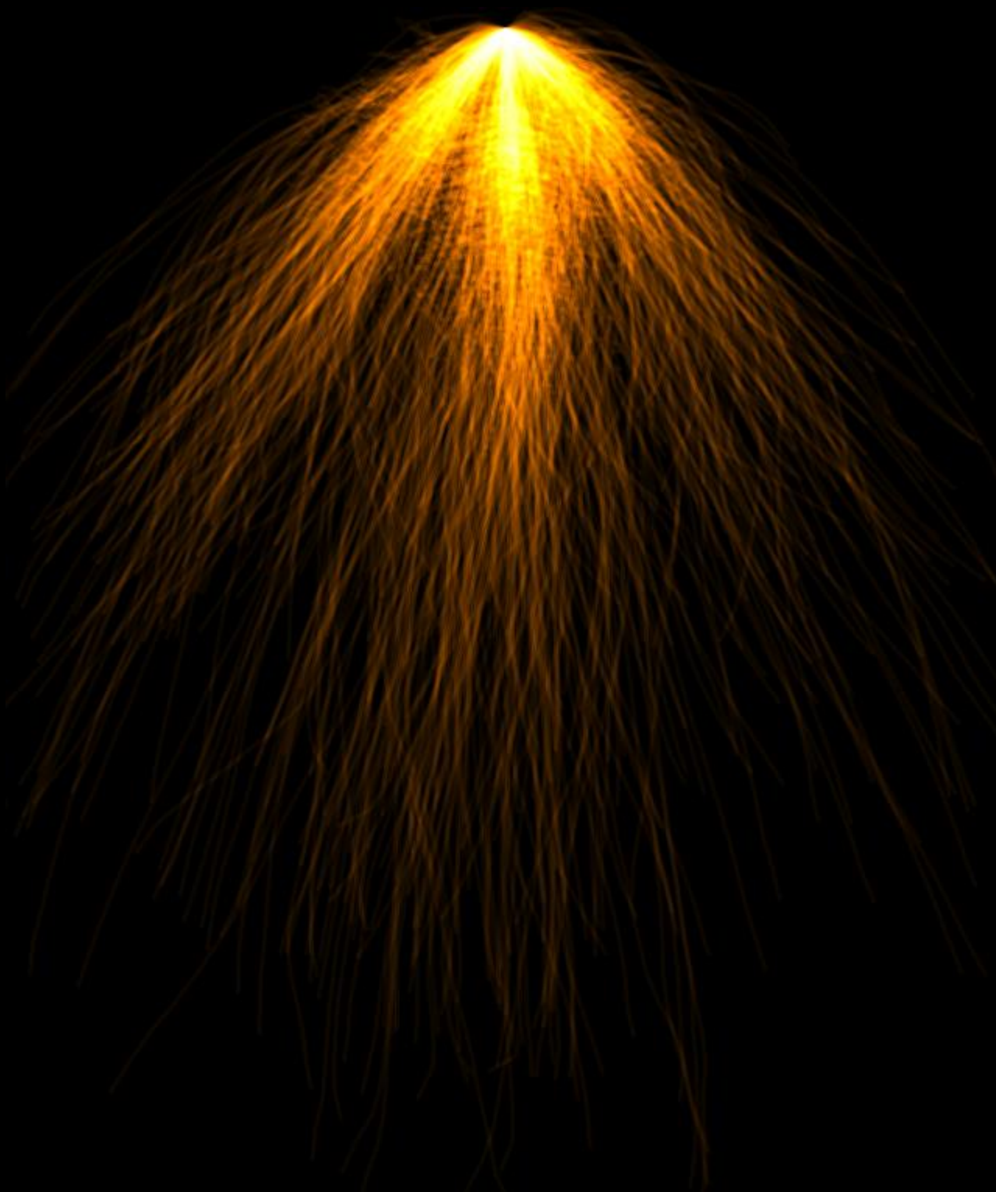
What do we do with the data?

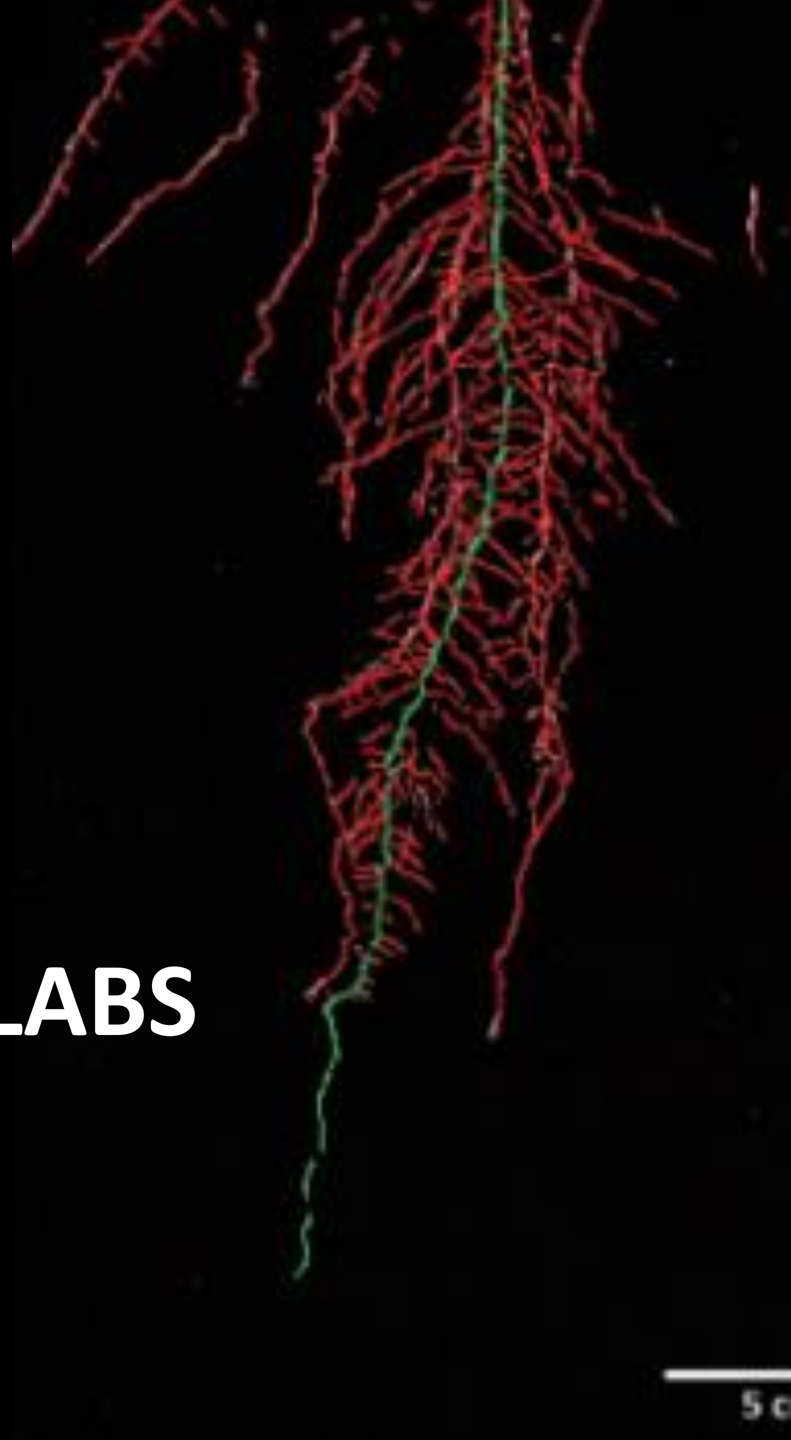
- Calculate some metrics automatically e.g.
  - Convex hull area
  - Count of root types
  - Lengths of roots
  - Angles
  - Relationships






Glasgow
Ae. buncialis
Ae. uniaristata
T. dicocchodies
Ae. variablis
Ae. genticulata
Ae. markgafii
Ae. columnaris
T. urartu
Ae. peregrina

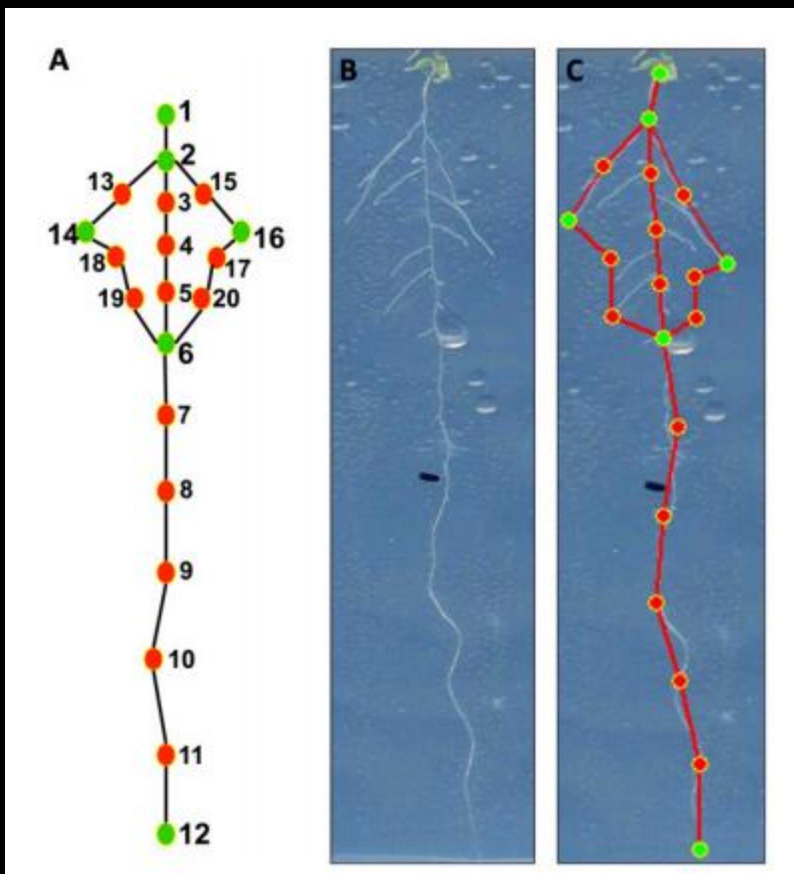


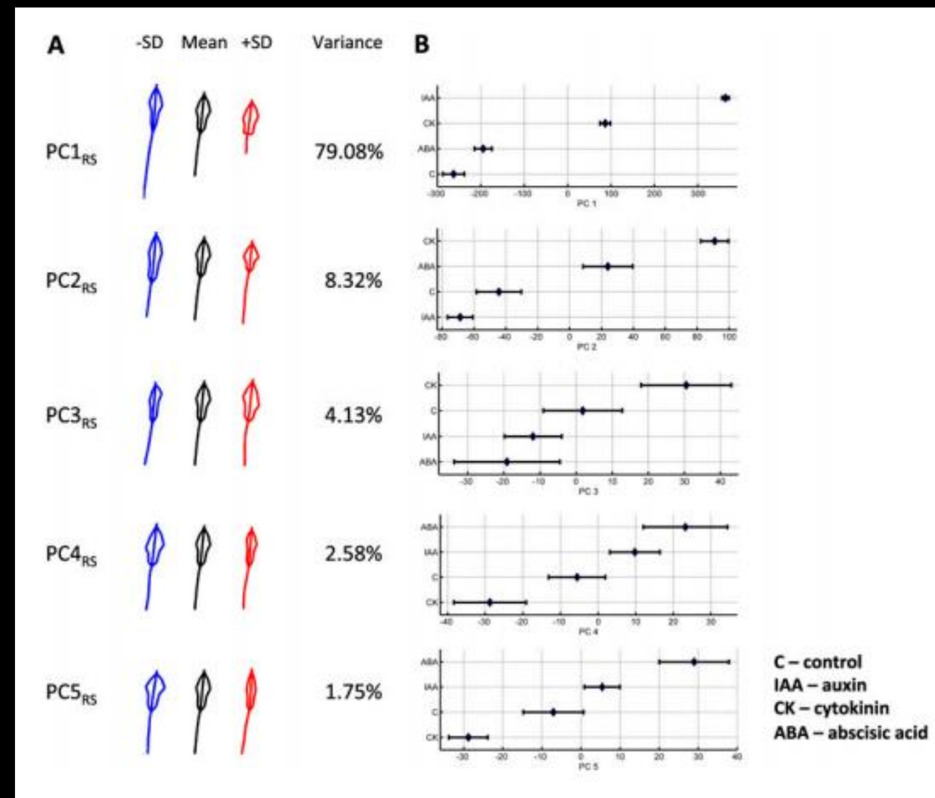


TOOLS FROM OTHER LABS

RootScape

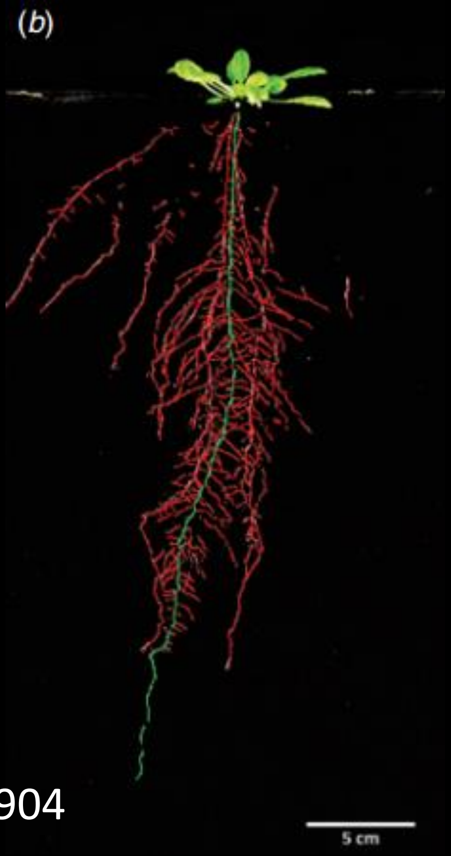
-statistical analysis of overall shape





D. Ristova et al.,
2013 , Plant physiology , 161(3), 1086-96

GROWSCREEN-Rhizo



Nagel et al., Functional Plant Biology, 2012, 36, 891-904

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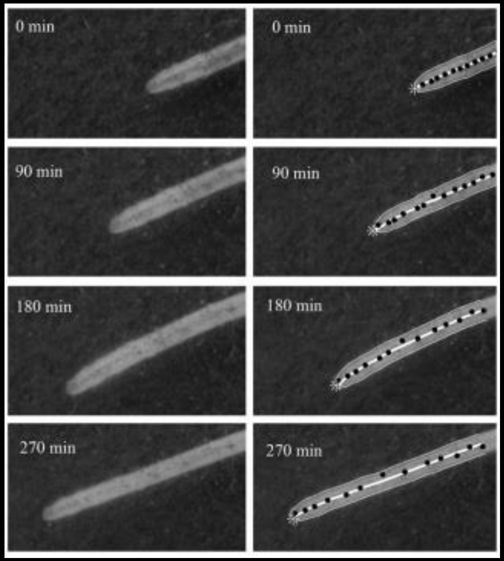
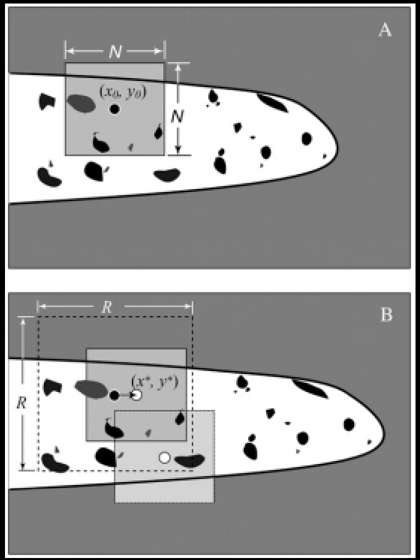
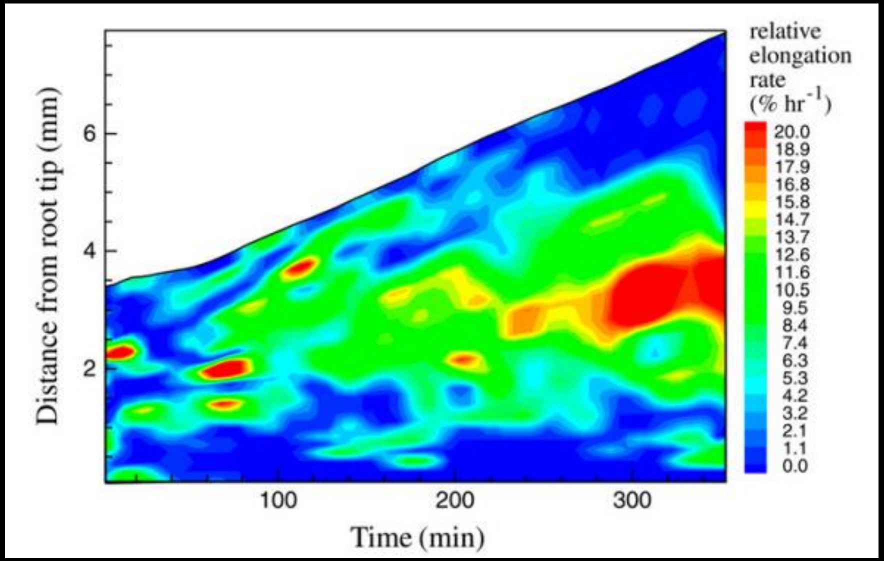
KineRoot





Pattern matching

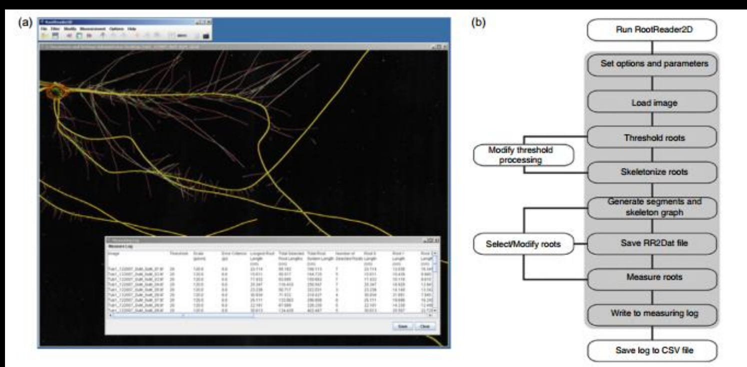


Relative elongation rate

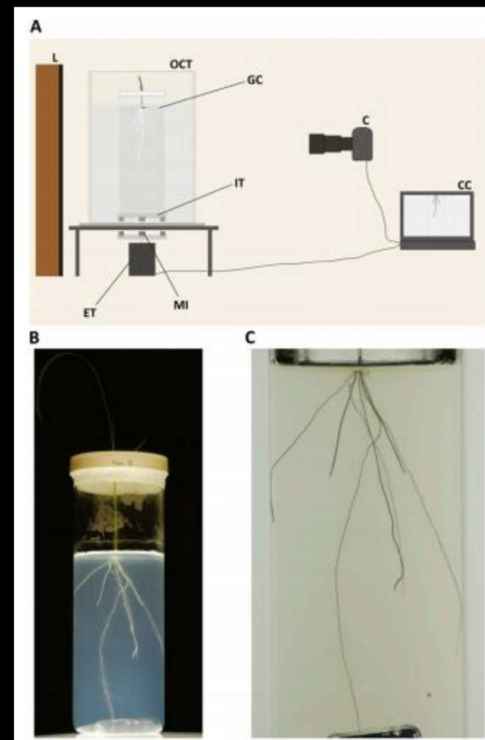
Basu et al, Plant Physiology, 145, p305

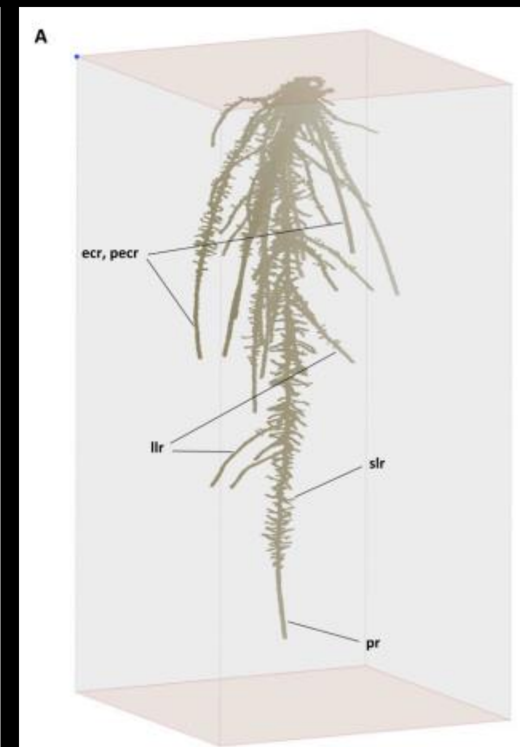
RootReader 2D/3D

2d:



3d:





2d: Clark R et al. 2013, Plant Cell and Environment 36 (2), 454–466
3d: Clark R et al.2011, Plant Physiology, 156, 455-465.

IMAGING ROOTS IN SOIL
AT THE UNIVERSITY OF NOTTINGHAM

Micro X-ray computed tomography

nanotom®

ultra-high resolution

nanoCT system

nanofocus < 800 nm spot size

180 kV / 15 W

Very high resolution

(voxel size 0.5 to 300 µm)

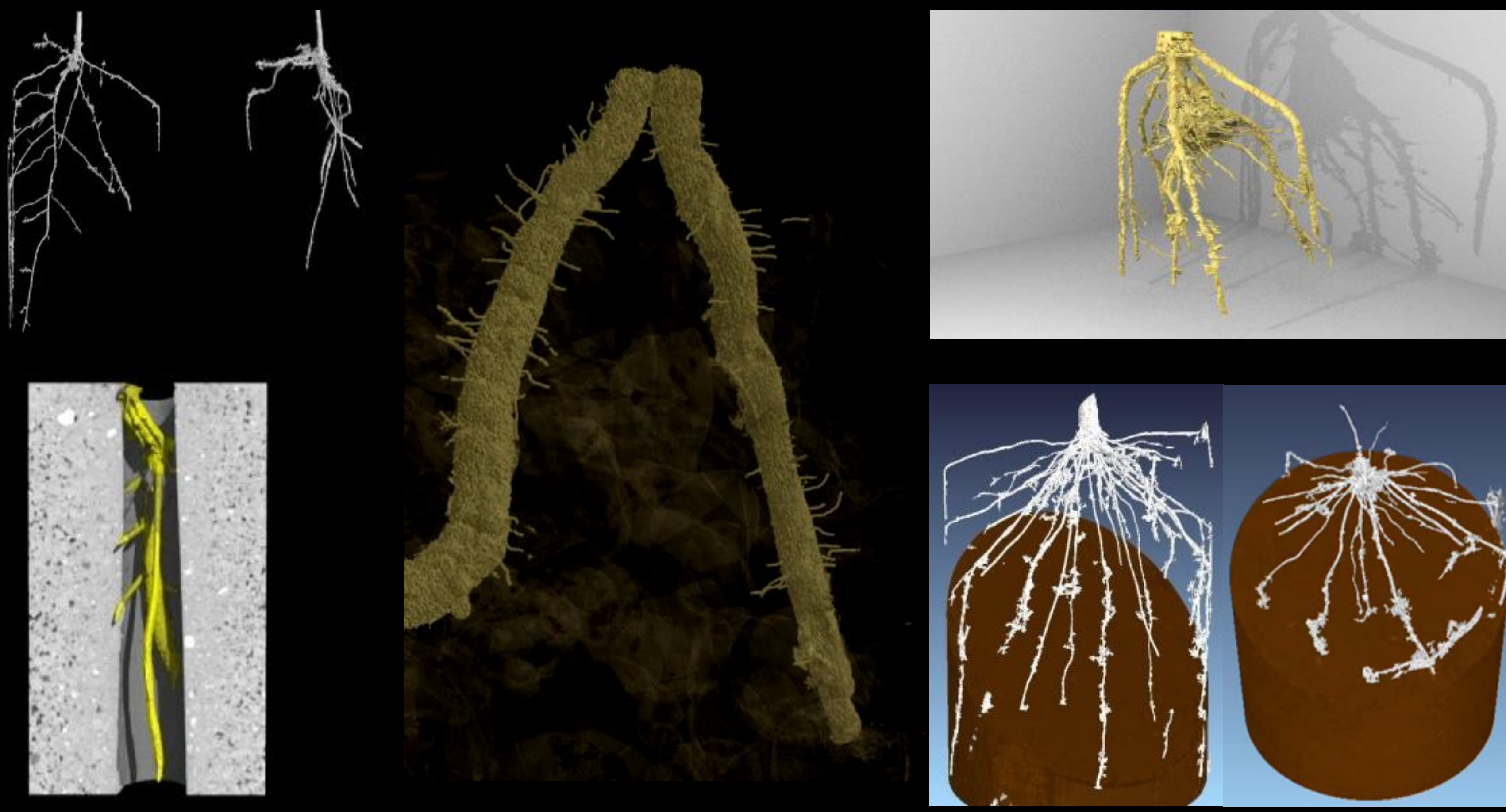
Fast scanning and reconstruction

(scan between 5-40 mins &

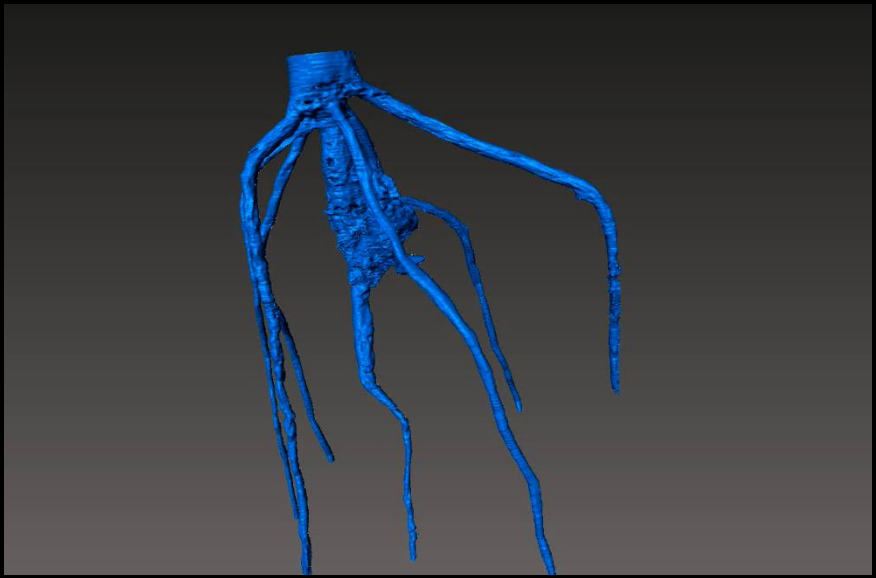
recon in < 5 min)

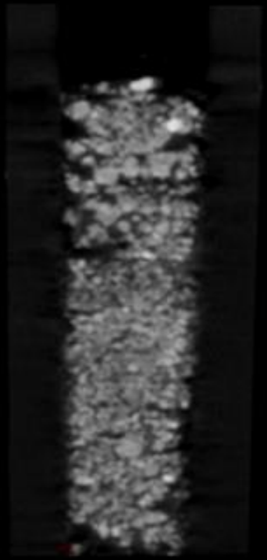
Sample c. 10 cm cube

Micro X-ray computed tomography



Software: thresholding vs *Rootrak* output





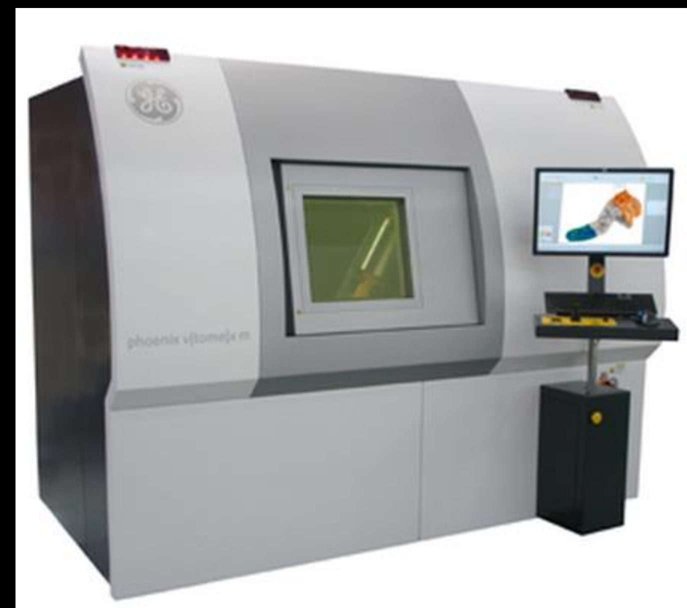
Maize at 22 µm voxel size

21 days old grown in sandy loam

Tracy et al. (2010) Journal of Exp Botany, 61(2), pp. 311-313.

Mairhofer et al. (2012) Plant Physiology, 158, 561-569
Mairhofer et al. (2013) Plant Methods, 9:8

Micro X-ray computed tomography -larger sample capability



- 300 kV
- 500 W
- 600 mm x 500 mm
- 50 kg
- 1 um

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Upscaling from core to column: 1 m deep field sample

Rhizosphere Facility

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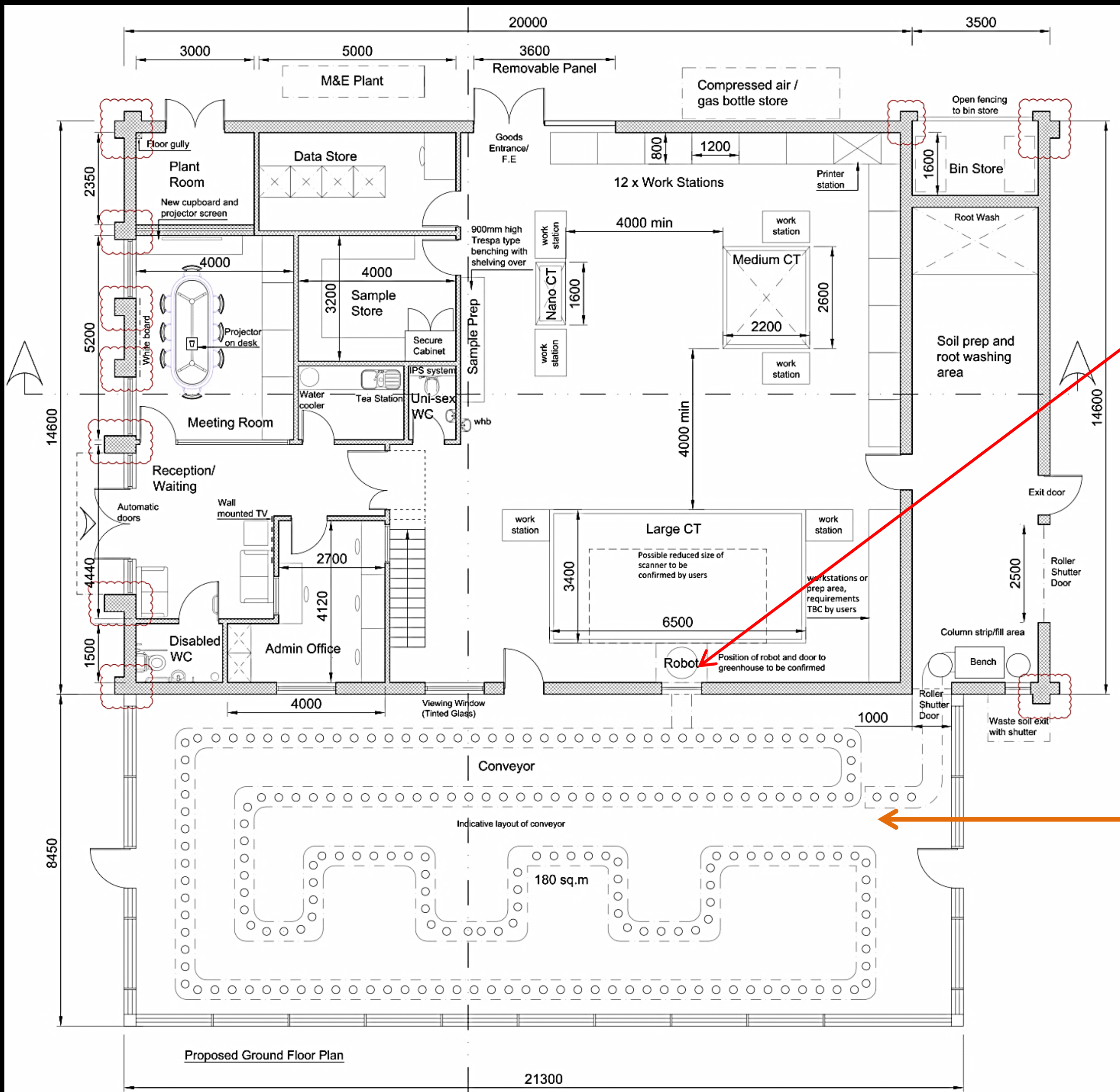

Thanks and references

- Michael Pound
- Stefan Mairhofer
- Darren Wells
- Malcolm Bennett
- Sacha Mooney
- Tony Pridmore

- All at CPIB

Useful Links:

- Guillaume Lobet's list of plant image analysis software tools
  - http://www.plant-image-analysis.org
- CPIB's tools and resources page
  - http://www.cpib.ac.uk/tools-resources

Practical:

On Wednesday, using Fiji to segment roots, seeds etc.

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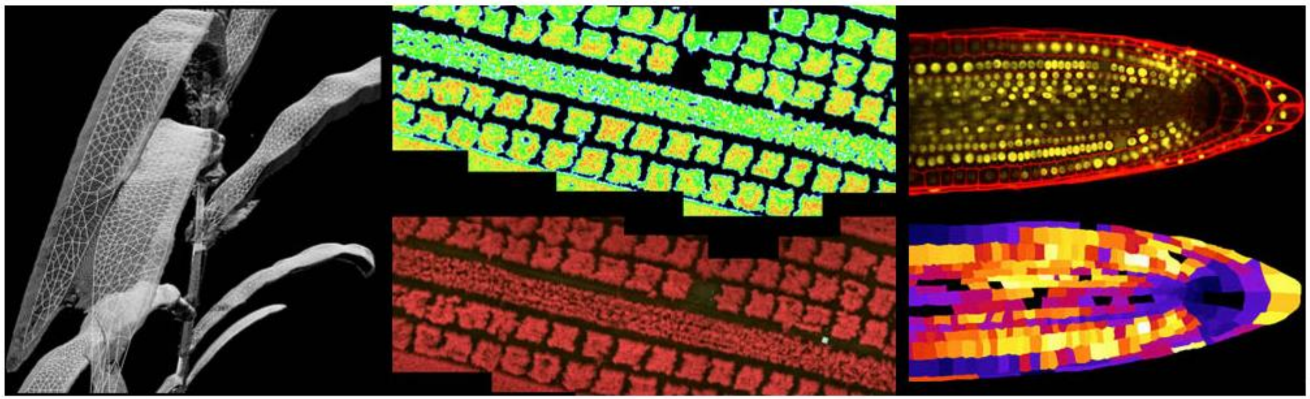
International Workshop on Image Analysis Methods for the Plant Sciences



2nd-3rd September 2013, University of Nottingham, Jubilee campus, UK

For *developers* of algorithms and tools for plant sciences

http://www.cpib.ac.uk/outreach/image-analysis-workshop-2013

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