

Graduate School Course on Post-genomics  
and bio-informatics

**A practical approach to  
phenomics**

Darren M Wells

*darren.wells@nottingham.ac.uk*

# Overview

- Definitions
- Technologies
- Examples
- Case study & tour – the Hounsfield Facility

# Phenomics – some definitions

Phenotype: observable characteristics of an organism  
– result of genotype x environment interactions

Phenome: “the full set of phenotypes of an individual” (Houle *et al.*, 2010)

Phenomics:

“...an emerging transdiscipline dedicated to the systematic study of phenotypes on a genome-wide scale” (Bilder, 2009)

# Phenomics – characteristics

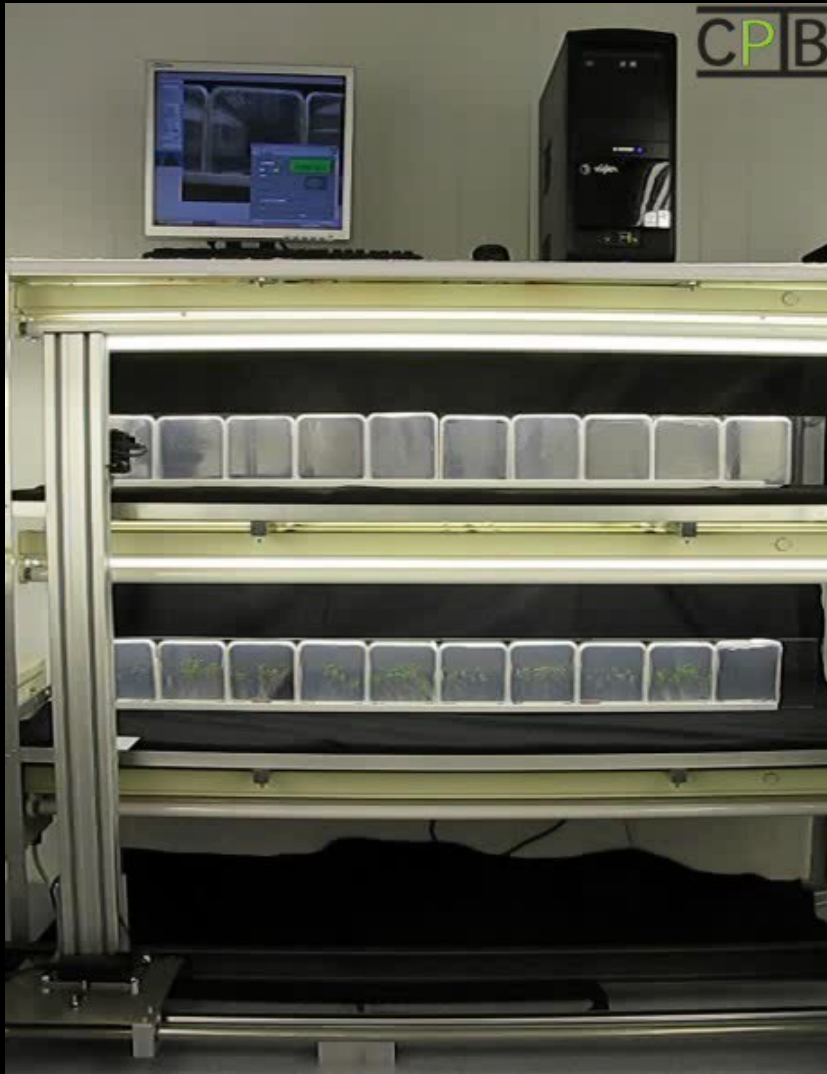
Phenomics is (usually):

- Multi-disciplinary
- Multi-scale (cellular, organ, organism, population)
- High-throughput phenotyping

# Phenotyping technologies

- Many and varied
- Selected according to availability and amenability to automation
- Often based on imaging techniques:
  - Visible/NIR/hyperspectral
  - Tomography (OPT, MRI, CT, PET etc)
  - Microscopy (light, EM)

# Automated image acquisition and analysis



RootTrace I

*Video 8x speed*

*French et al. (2009); Wells et al. (2012)*



RootTrace II

# Examples

- **Cell-scale:** hormone dynamics in *Arabidopsis*
- **Organ-scale:** zebrafish larvae
- **Organism level:** wheat seedling root systems
- **Population level:** field crops
- **Case study:** developing a phenotyping facility at UoN



# Cell-scale phenotyping - example

Development of fluorescence-based hormone sensors in *Arabidopsis* allows cellular-level modelling of distribution and response

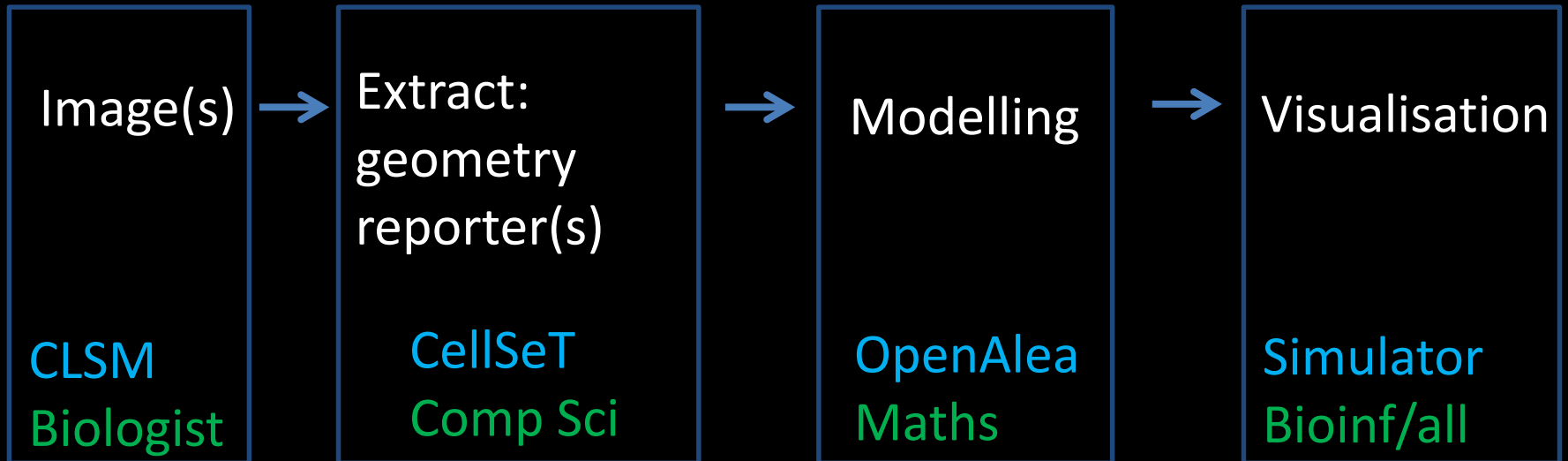
Required input from biologists, biophysicists, bioinformaticians, computer scientists (image analysts), mathematical modellers

# Cellular resolution modelling

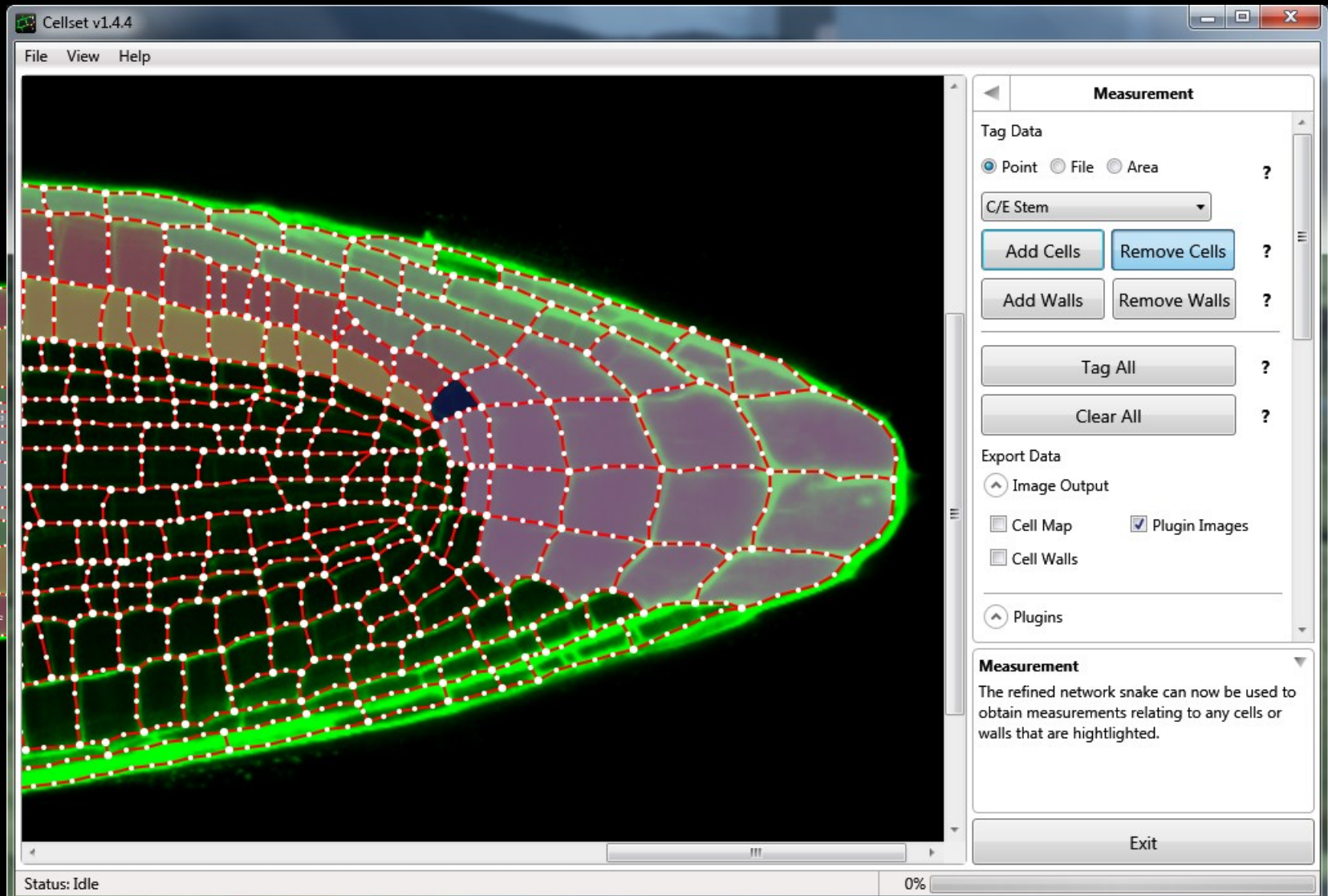
## Requirements:

- extraction of cell-level fluorescence data:
  - to map root geometry
  - to quantify sensor fluorescence
- Biological parameter estimation to populate model with carriers etc.

# Phenotyping pipeline



# CellSeT - Tagging and measurement

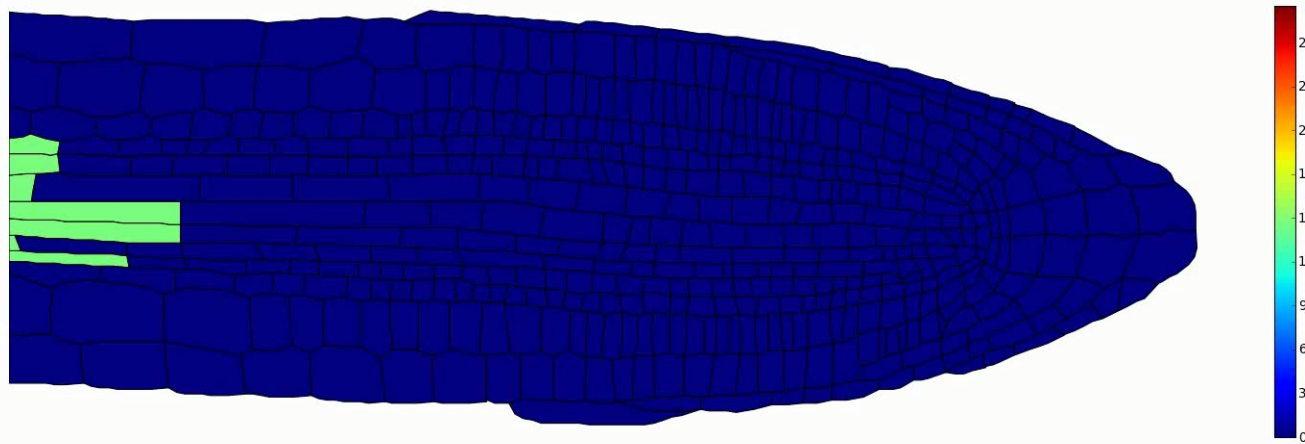


# Modelling

- Extracted geometries read into a vertex-based model, based on the OpenAlea modelling framework.
- carrier distributions prescribed
- ODEs for:
  - diffusion of protonated auxin across cell membranes
  - carrier-mediated auxin transport
  - passive diffusion of auxin within the cell wall
  - degradation of biosensor fluorescence via a parameterised network model



*In silico* simulation of the hormone fluxes through the root tissue, using segmented cell geometries:



Visualisation/interaction via simulator website

## Visualisation – Simulator

The screenshot displays the VRoot Simulator software interface. The window title is "VRoot Simulator". The interface is divided into several panels:

- File Help**: A menu bar at the top left.
- Geometry Parameters Carrier**: A tabbed interface with the "Parameters" tab selected. It contains a list of parameters and their values:

permeability to auxin	2,016.0
permeability due to AUX1	0.0
permeability due to PINs	1,008.0
apoplastic pH	5.3
cytoplasmic pH	7.2
membrane potential	-0.12
auxin production rate	0.0
auxin degradation rate	0.0
cell-wall thickness	0.2

Below the table is a "Reset to default" button.
- Run New**: A section with a "Run" button and a "New" button.
- Snapshots**: A section with a progress bar, "Snapshots: 10", and "Interval: 0.2".
- Information of the selected cell**: A large empty text area.
- Visualisation**: The main area containing two simulation views:
  - Auxin**: A 3D visualization of a root tip showing auxin concentration. The concentration is represented by a color scale from blue (0.0) to red (4.0). The highest concentration is at the root tip.
  - AUX1**: A 3D visualization of the same root tip showing AUX1 concentration. The concentration is represented by a green color scale. The highest concentration is also at the root tip.

At the bottom of the window, there are status messages: "Snapshot 1 ready.", "Snapshot 2 ready.", and "Snapshot 3 ready."

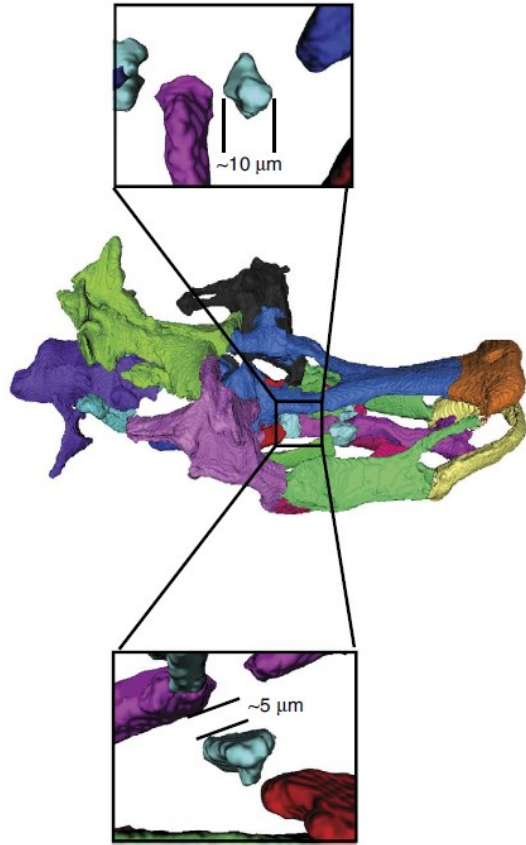
# Organ-scale phenotyping

- Zebrafish anatomy
  - High throughput (18 seconds/larva)
  - Optical projection tomography (320 images)
  - 3D reconstruction of entire larvae
  - Craniofacial cartilage (dyed)
    - 200 independent morphological measurements
    - Used in studies of teratogen action

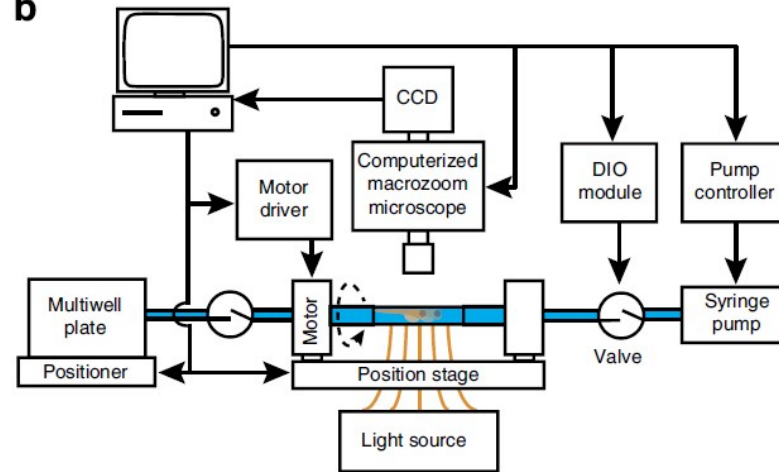


# Organ-scale phenotyping

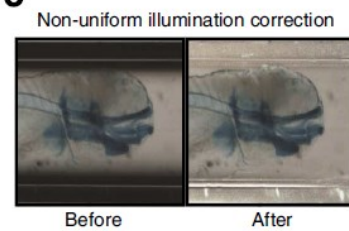
a



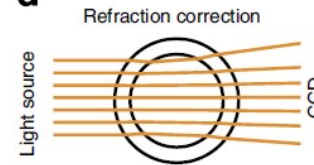
b



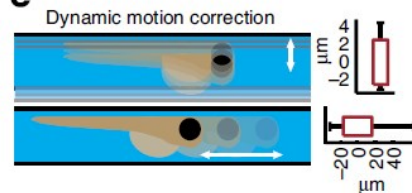
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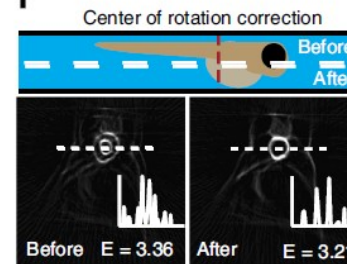
d



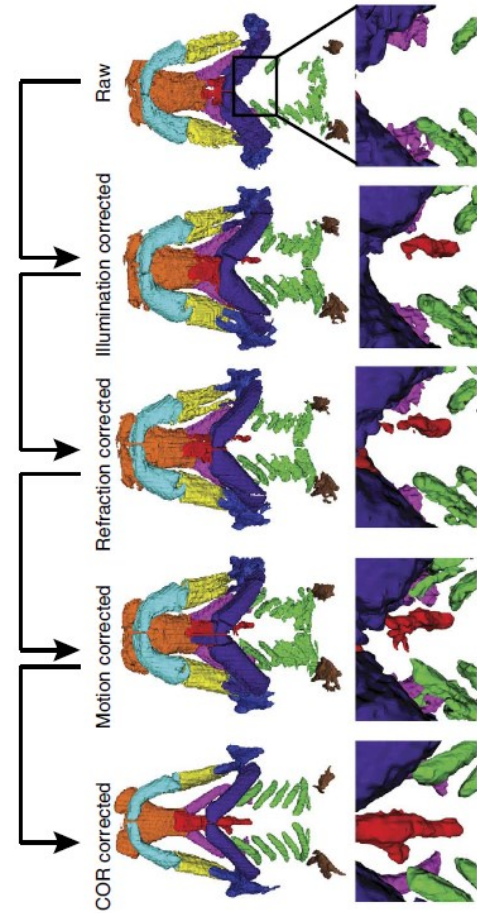
e



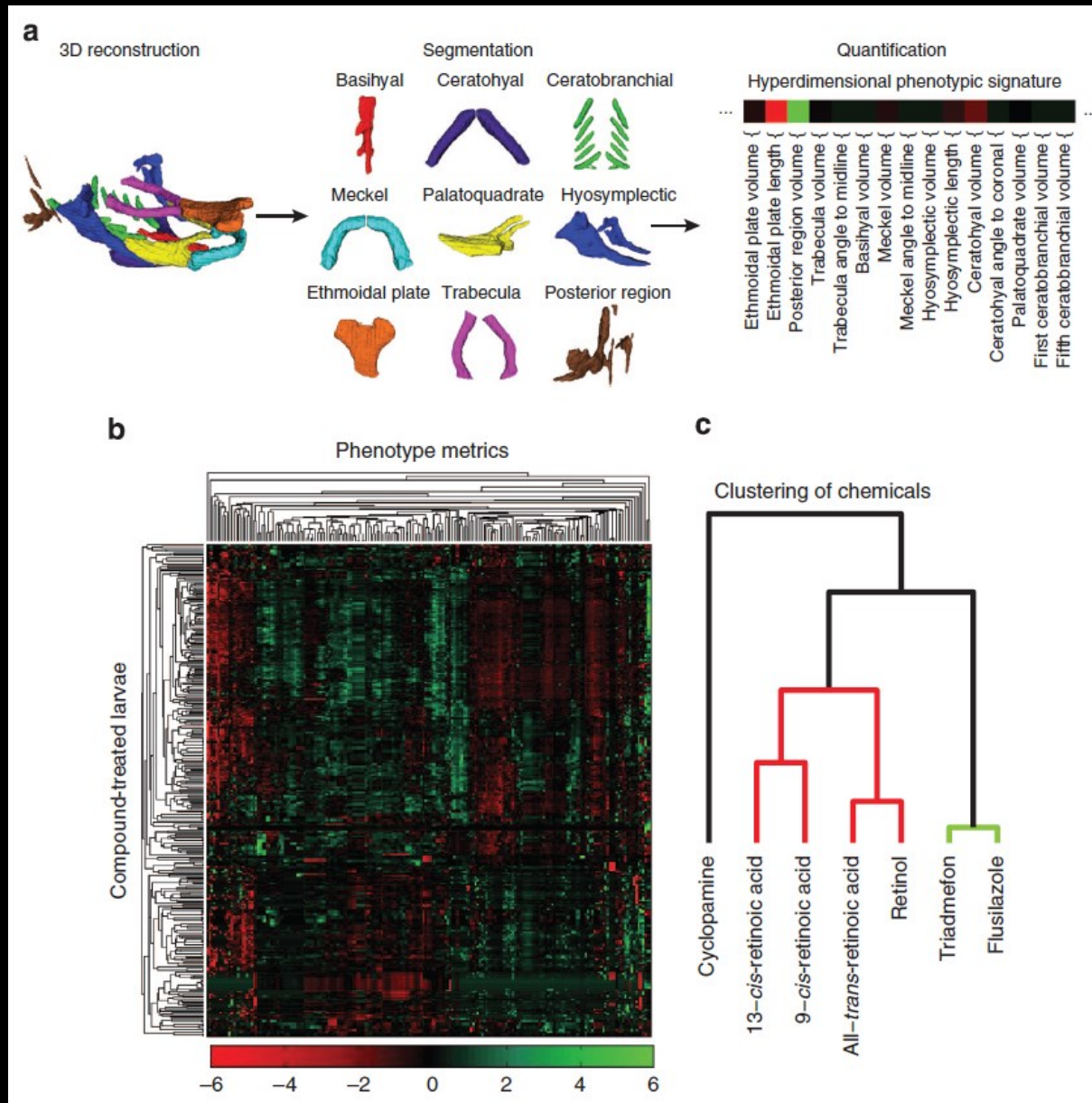
f



g



# Results – teratogen studies



# Organism-level phenotyping

- Root systems architecture may represent untapped genetic resource to improve modern crops
- Screen mapping populations for desirable root traits (e.g.: deep for water and nitrate, shallow for phosphorus)

# Wheat root phenotyping – mapping populations

## Savannah

- Group 4
- Feed wheat
- Very High Yield

**X**

## Rialto

- Group 1
- Bread wheat
- High quality

- 132 doubled haploid lines
- All genotyped using iSelect 80k SNP array –  
publically available maps for 44k of those SNPs
- **96 lines (20 replicates) phenotyped using a 2D-  
imaging pipeline**

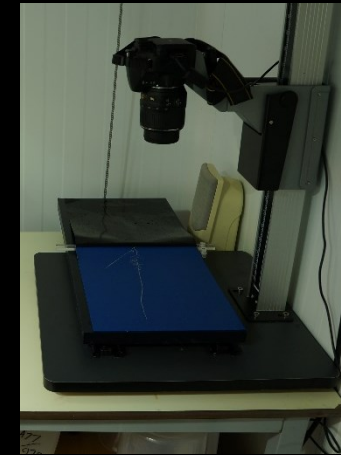
# 2D Phenotyping pipeline

4 components of the 2D root phenotyping pipeline...

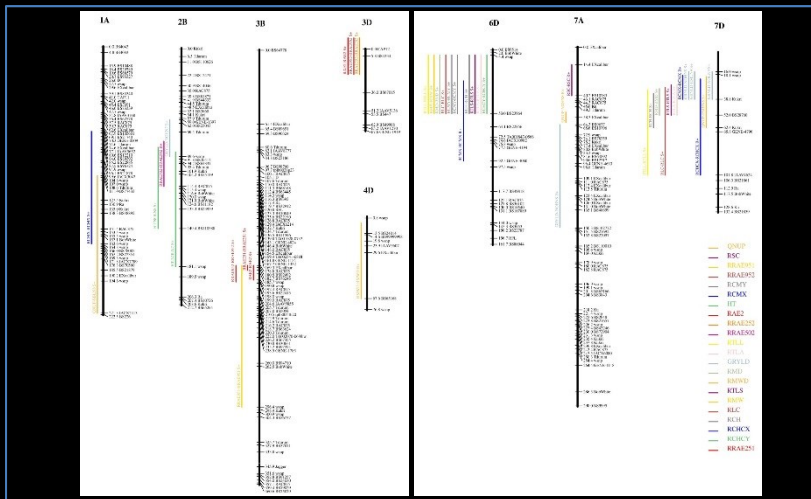
1. Plant growth system



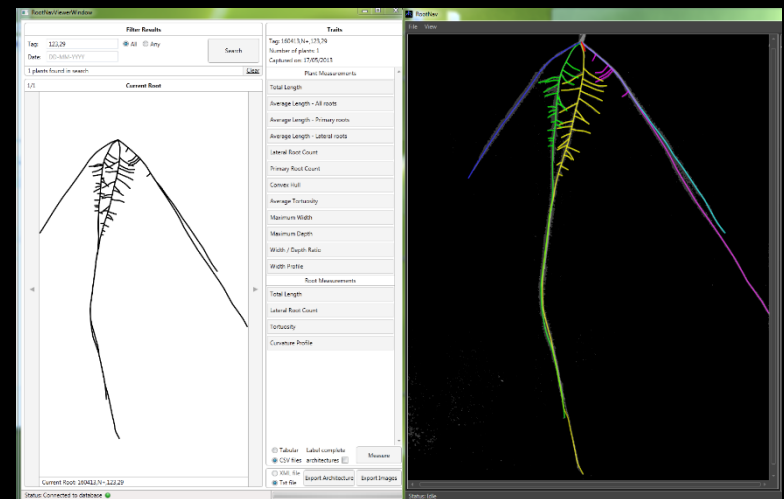
2. Image capture



4. QTL analysis



3. Image analysis & trait quantification



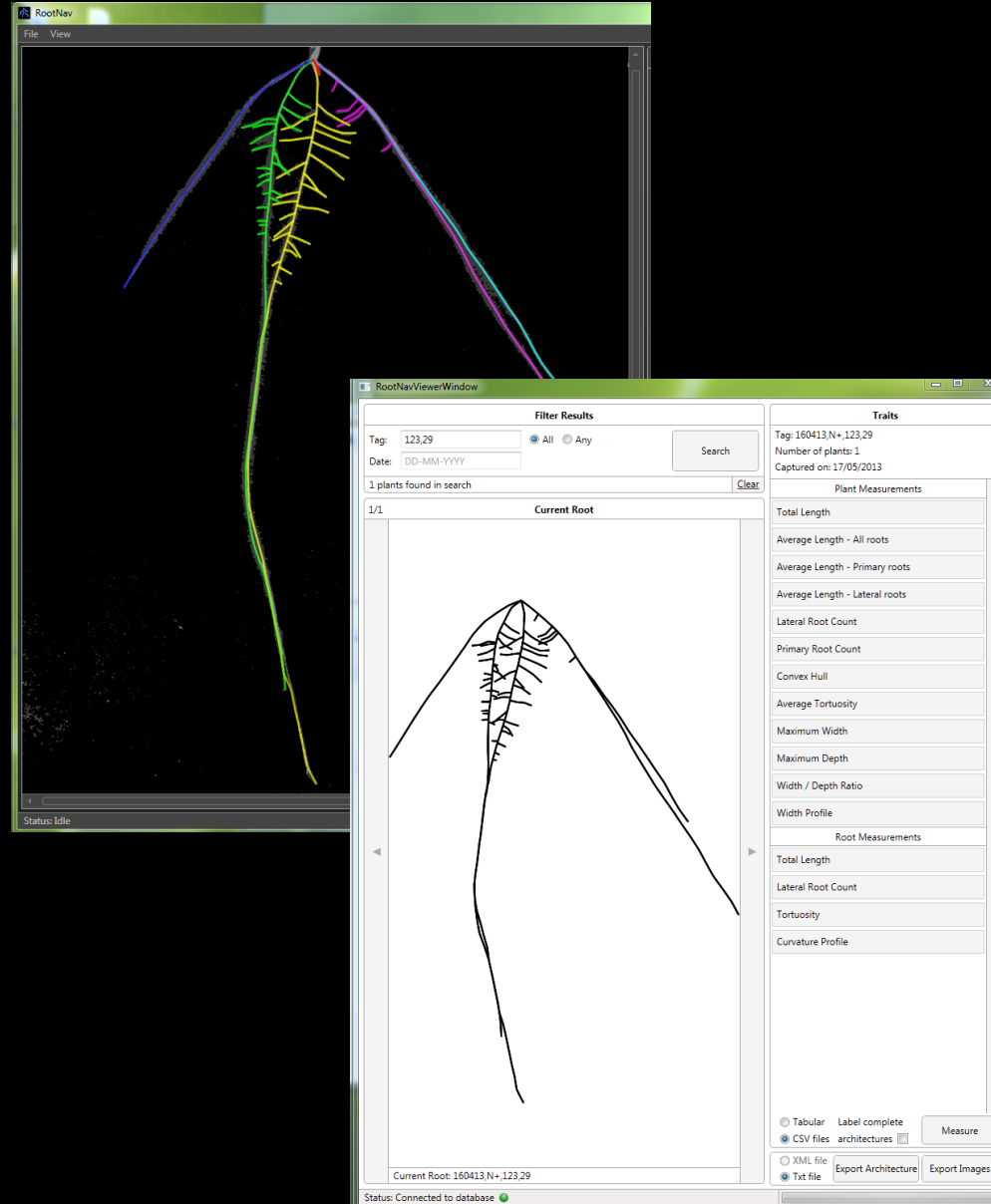


# Plant Growth System

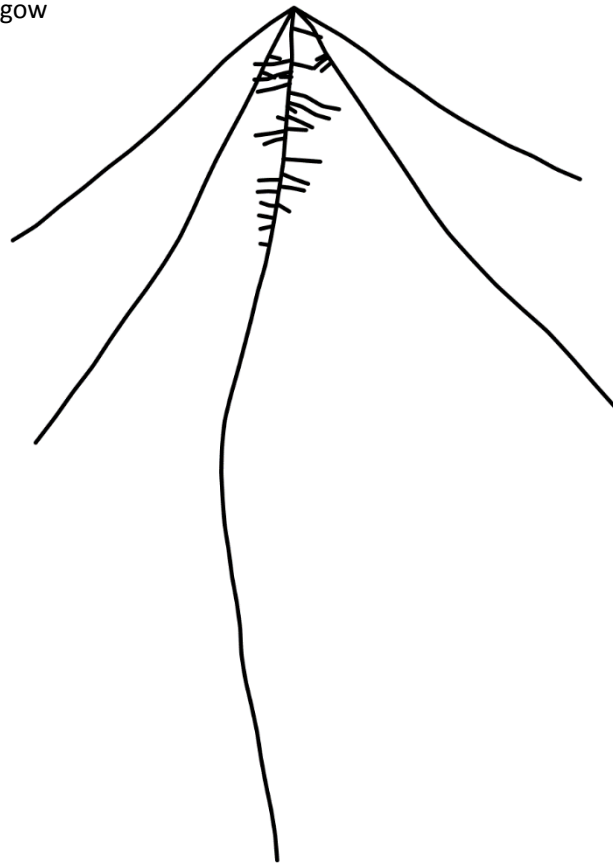


# Image analysis - RootNav

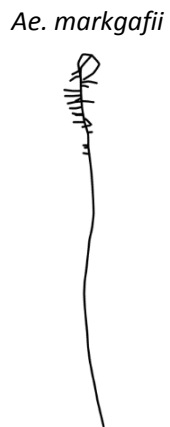
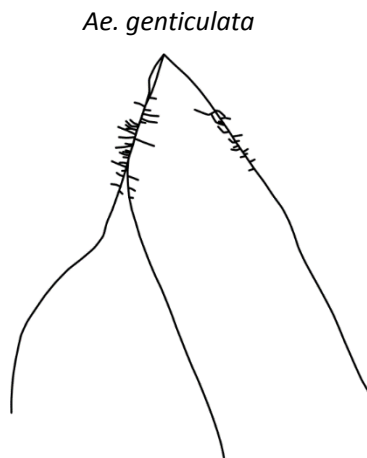
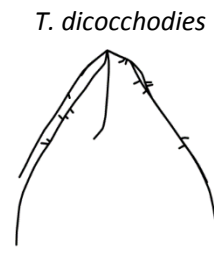
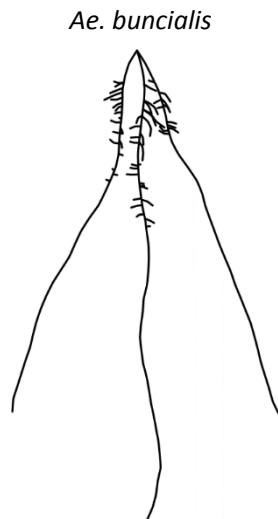
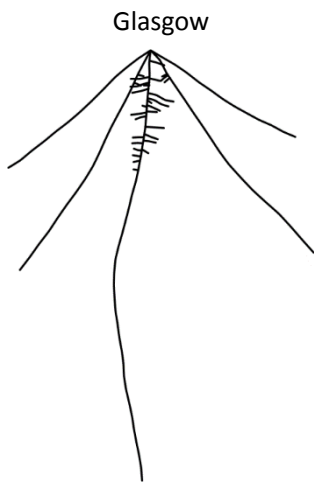
- Semi-automatic analysis and quantification of RSA
- 30s – 2 minutes per image
- Spline data stored on RootNav server
- Root data can be queried using the viewer tool.
- Traits quantified and exported via RSML



Winter Wheat var:  
Glasgow

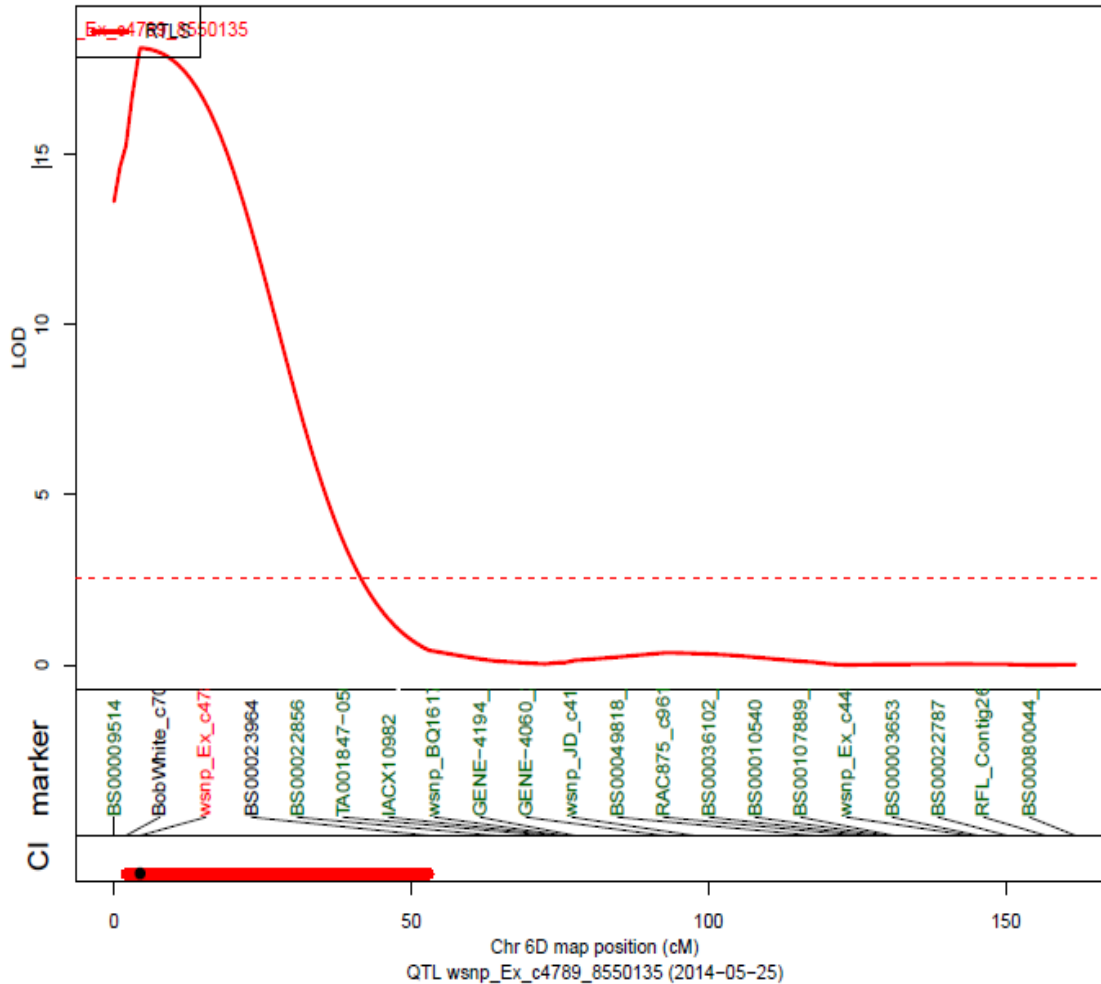






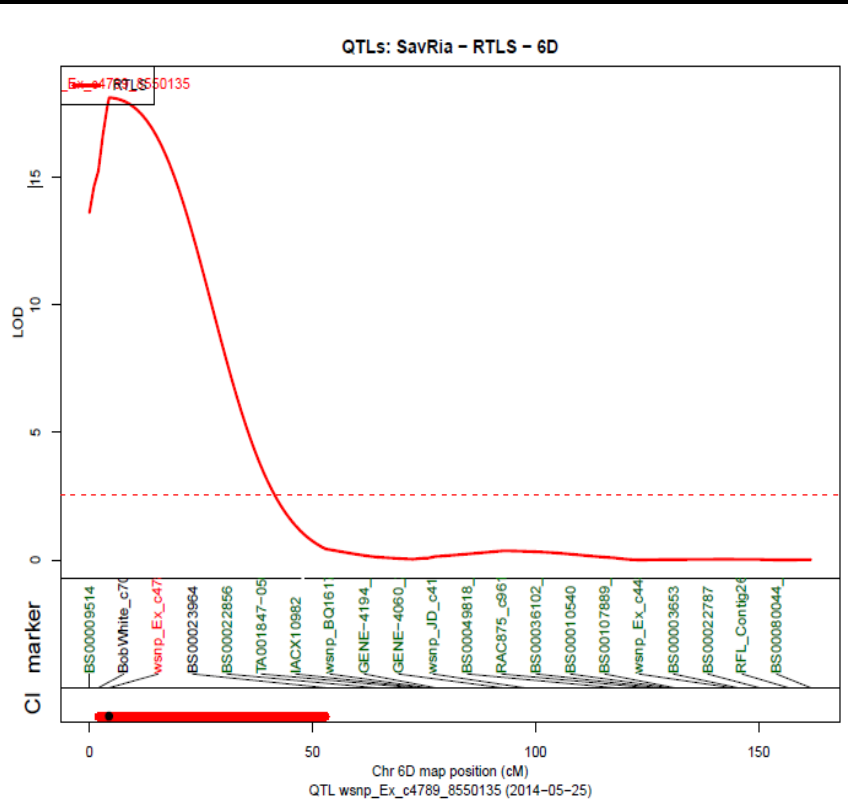
# QTL Analysis Results

QTLs: SavRia - RTLS - 6D



Indicates the presence of a major effect gene regulating seedling root architecture/vigour

# 2D Seedling Root Phenotyping



## RNAseq

Collaboration with  
Laura Gardiner  
(Earlham)



18 candidate  
genes



## NILs

Collaboration with  
Limagrain

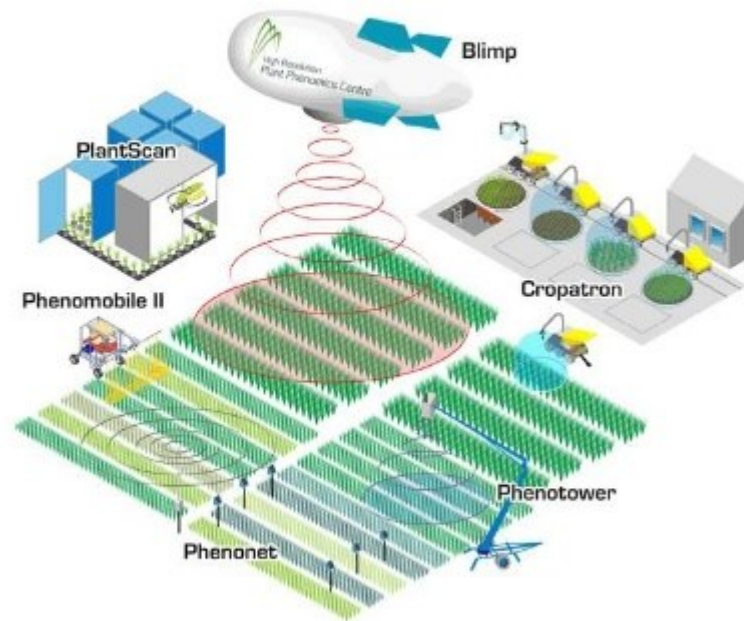


- Currently phenotyping
- Being evaluated in the field
- 18 cM introgression which confers the phenotype

Jonathan Atkinson (*unpublished*)

# Population scale: field phenotyping

## The High Resolution Plant Phenomics Centre



  
Australian  
Plant Phenomics Facility  
The High Resolution Plant Phenomics Centre

Director: [Robert.Furbank@csiro.au](mailto:Robert.Furbank@csiro.au)



# Population scale: field phenotyping

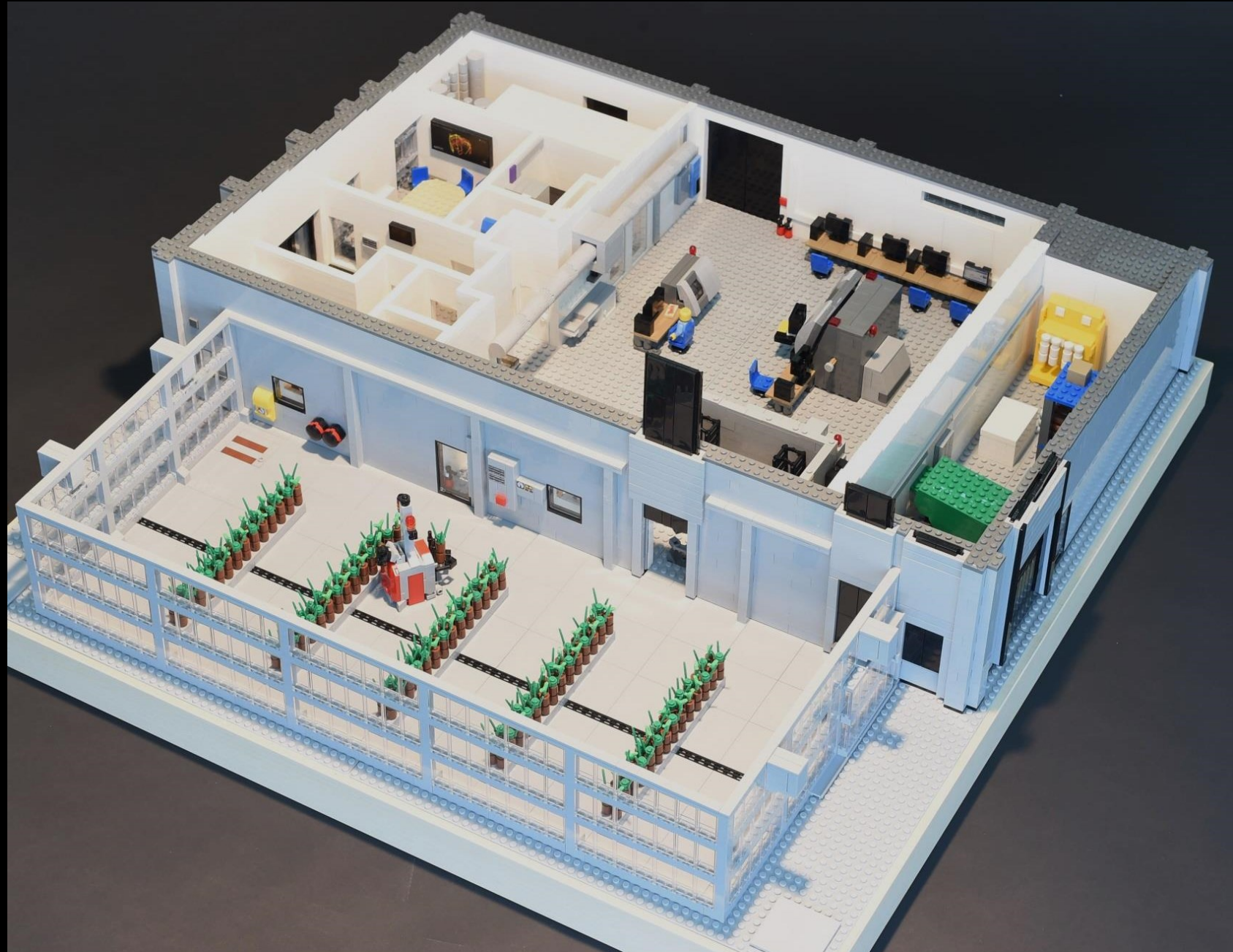
[https://www.youtube.com/watch?v=Wj-U0QH5J\\_M](https://www.youtube.com/watch?v=Wj-U0QH5J_M)



# Case study: developing a phenotyping facility at UoN

- Rationale – micro-computed X-ray tomographic ( $\mu$ CT) scanning allows imaging of root systems in soil.
- Use of mesocosms of realistic dimensions at reasonable throughput presents many technical challenges





# Hounsfeld Facility





*Mesocosm preparation: standardising soils*



# Sample handling

- Glasshouse capacity 140 columns
- Column dimensions 1 m x 25-30 cm OD
- Weight ~60-90 kg
- Minimal disturbance (reduce growth effects, settling of soil etc.)
- 24/7 automated operation
  
- Laser guided vehicle (LGV)
  - autonomous robot







# Scanning

Modified large scanner

high power 320kV minifocus  
X-ray tube

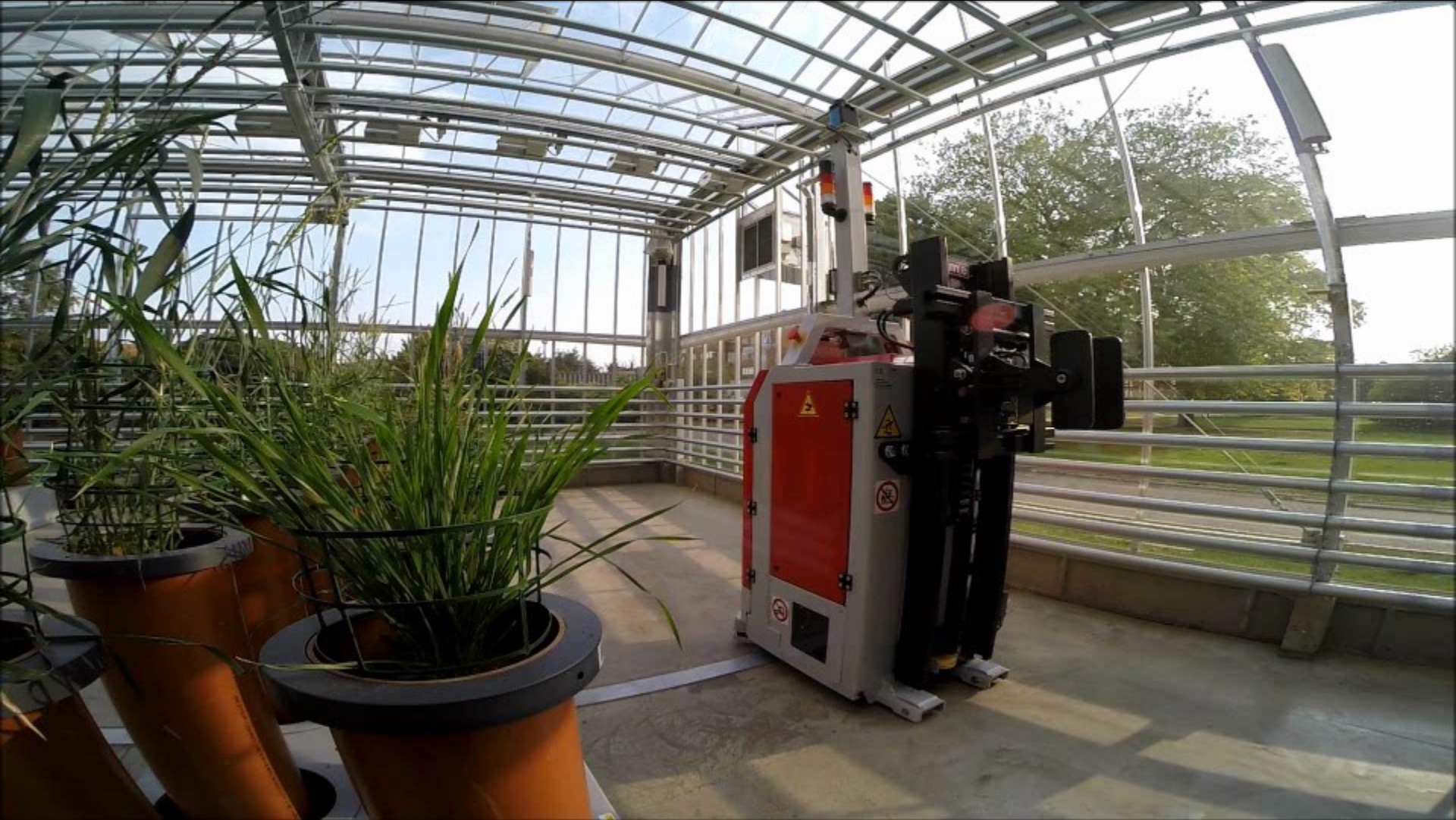
heavy duty manipulator  
stage

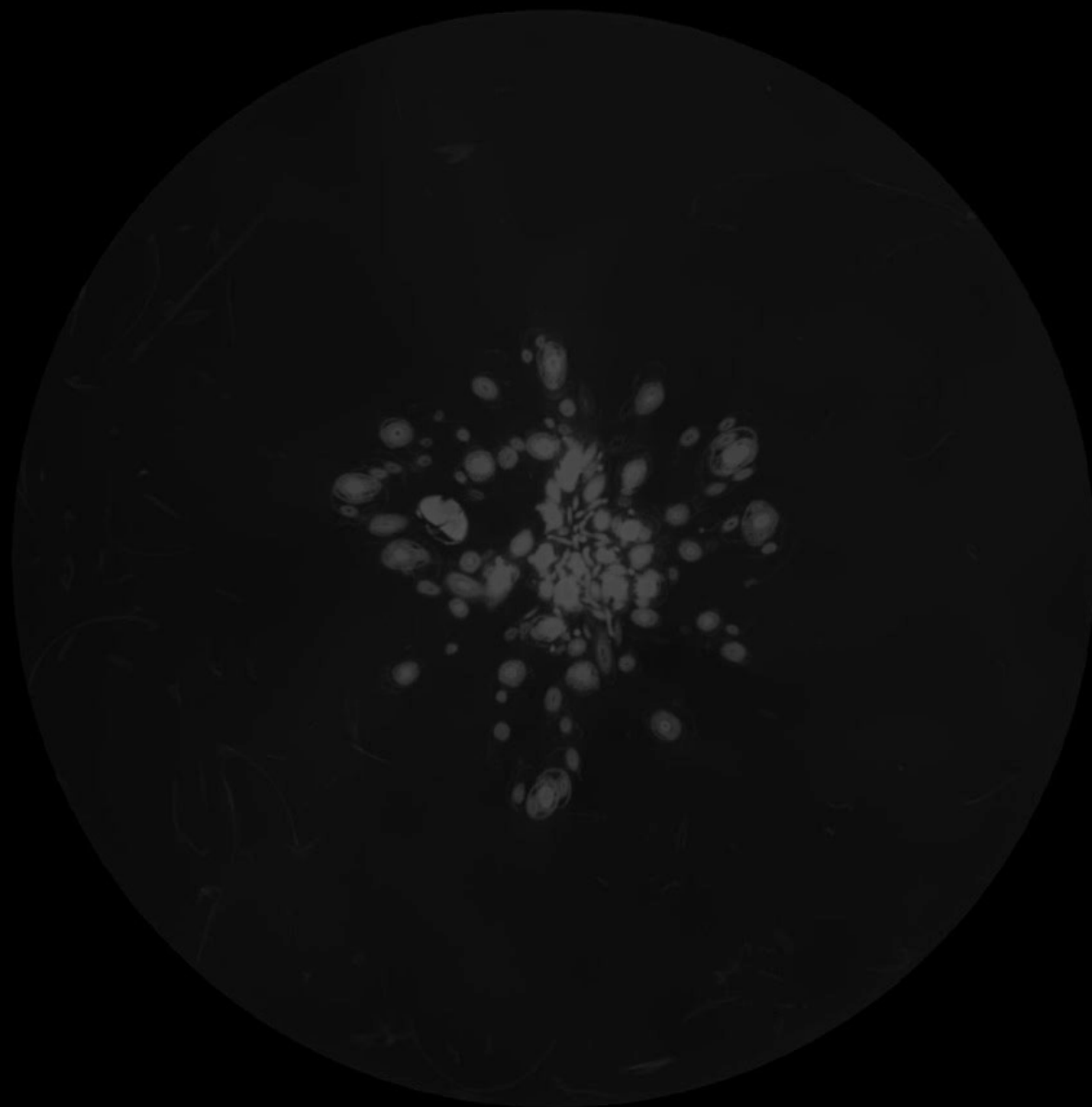
high contrast digital  
detector

Volumes automatically  
stitched and reconstructed  
on acquisition

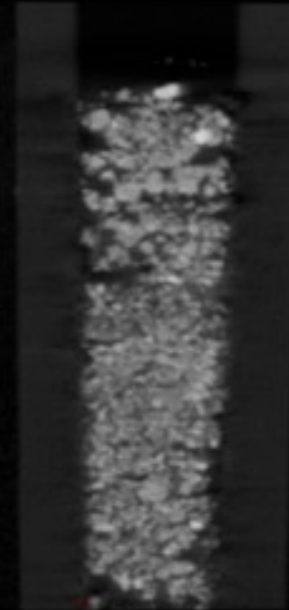
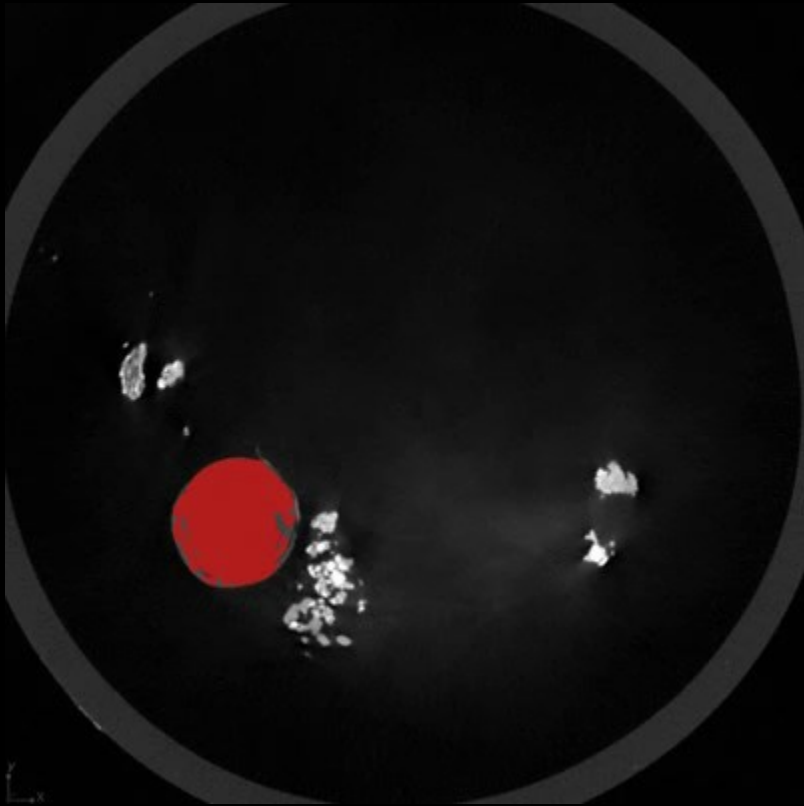








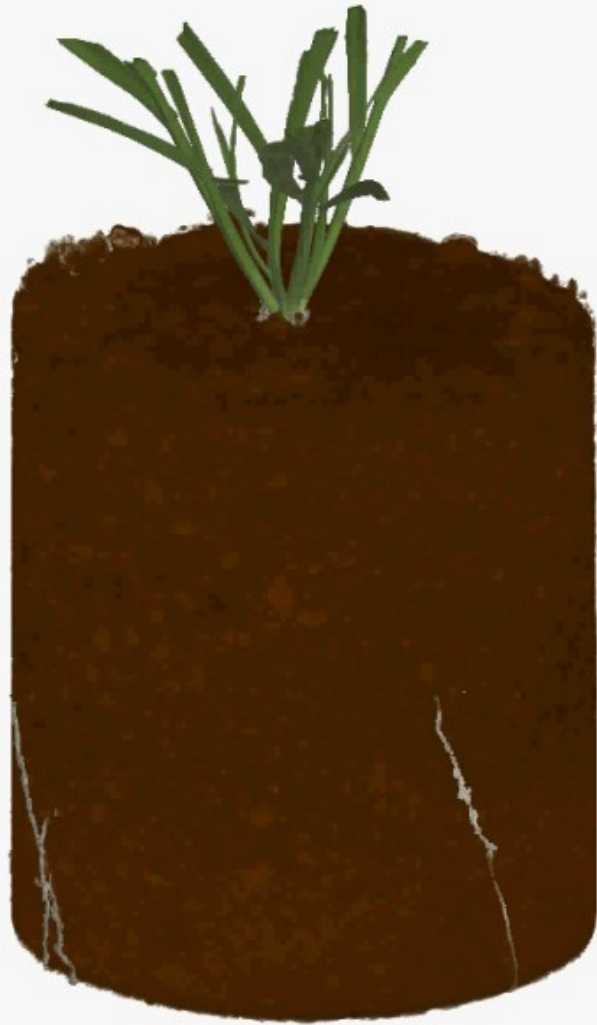
# Image analysis- RooTrak



Maize in sandy loam, resolution 30 $\mu$ m

*Mairhofer et al. (2012); Mairhofer et al. (2013)*

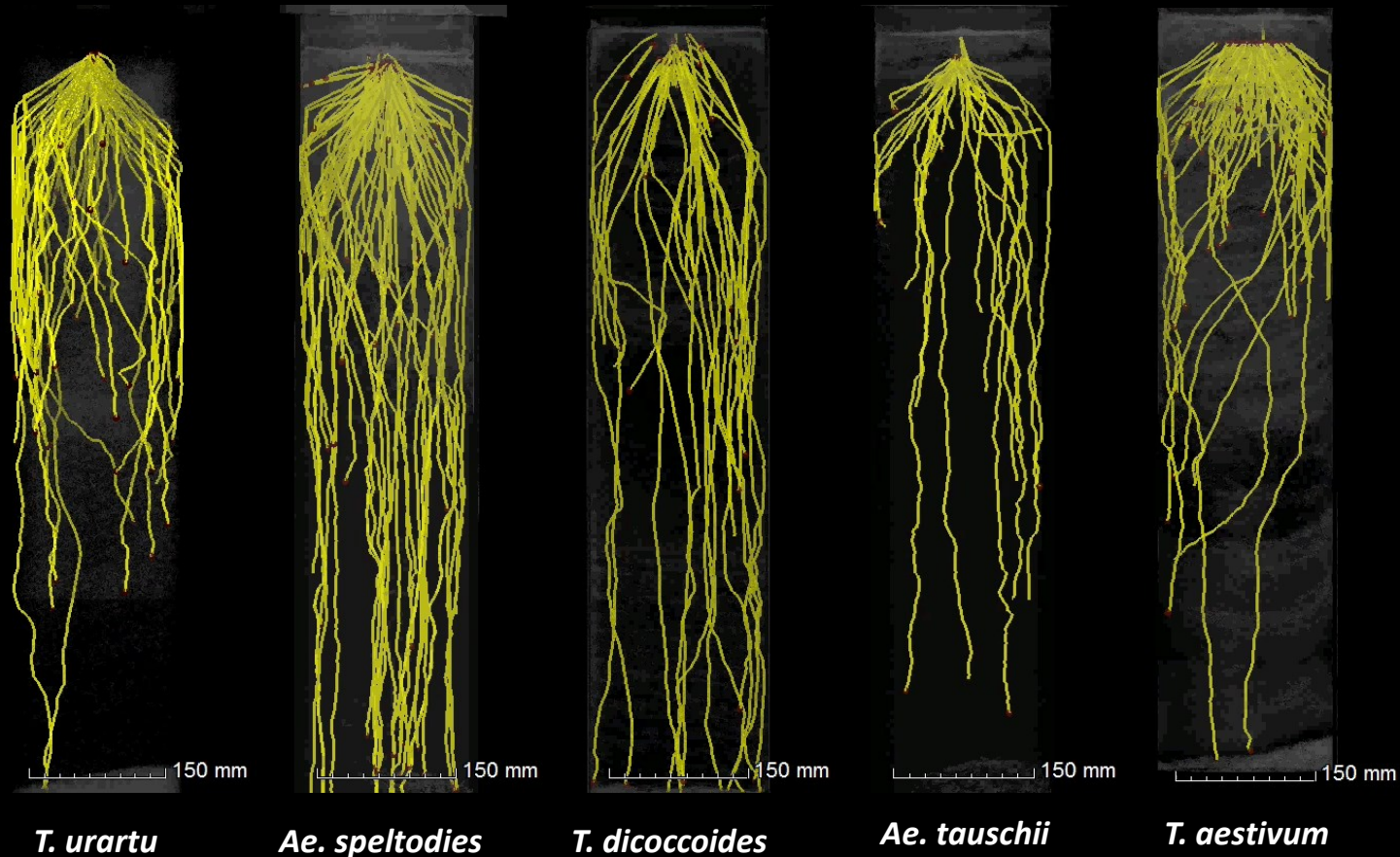




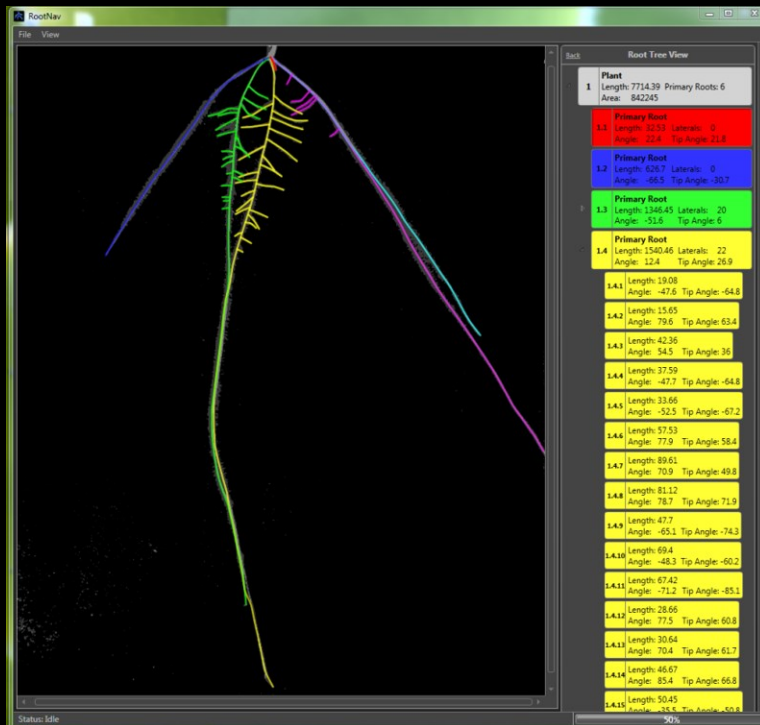
# X-ray Computed Tomography

## Ancient relatives of wheat

- Variation for useful agricultural traits such as more roots at depth
- Select WISP/DFW introgression panels from the Ian and Julie King which may have beneficial root architecture traits



# Unblocking the image analysis bottleneck



In *Arabidopsis*, image analysis unblocked the phenotyping bottleneck.

In larger, more complex plants – image analysis is again a limiting step.

Can new techniques help?

# Machine learning

## Unblocking the image analysis bottleneck

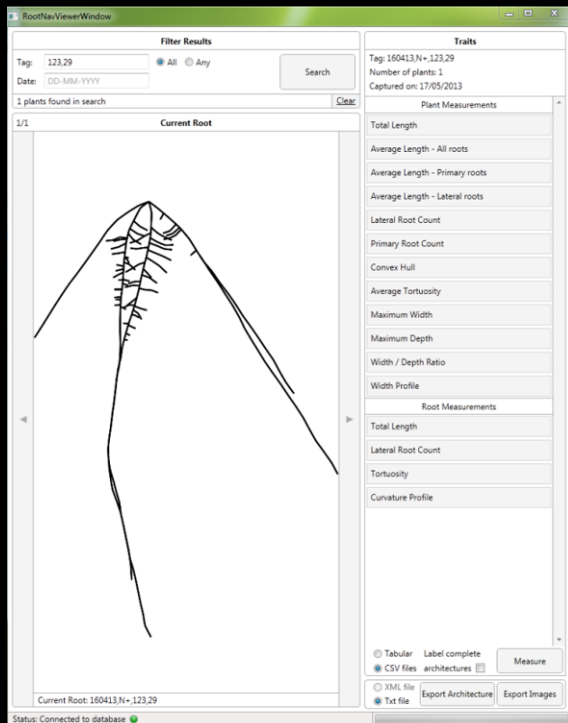
### Machine learning for image analysis

#### 1. 'Traditional' machine learning

- PRIMAL - Random Forest

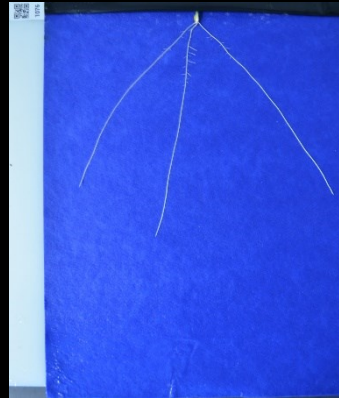
#### 2. Deep learning

- Convolutional neural networks (CNNs)



# Machine learning

## PRIMAL



### Automated Image Analysis

- Fast
- Prone to error



## PRIMAL

Pipeline of Root Image analysis  
using Machine Learning.

<https://plantmodelling.github.io/primal/>

### Semi- automated Image Analysis

- Time consuming
- Accurate data

# Machine learning

Chr	Trait	Manual (RootNav)	Automatic (RiaJ)	Primal (600 images)
4D	W/D ratio	2.7	2.71	2.5
6D	Seminal count			3.3
	Total root length	24	17	16.0
	Mean seminal length	22.2		14.0
	Lateral count	9.1		17.0
	Total lateral length	6.4		12.6
	Total seminal length	25.6		15.2
	Width	6.4	13.5	13.1
	Depth	22.7	13.6	15.0
	W/D ratio			2.2
7A	Seminal number	2.1		
7D	Lateral number	2.4		5.0
	Seminal number			3.4
	Total lateral length	2		4.2
	Total root length	9	4.1	3.1
	Total seminal length	9.7		2.8

- Requires around 600 training images to be analyzed to achieve an  $R^2$  of  $\sim 0.9$
- 12/13 QTL discovered using PRIMAL vs RootNav
- Does sometimes create false positives with low LOD scores, but these often co-localise with other 'real' QTL

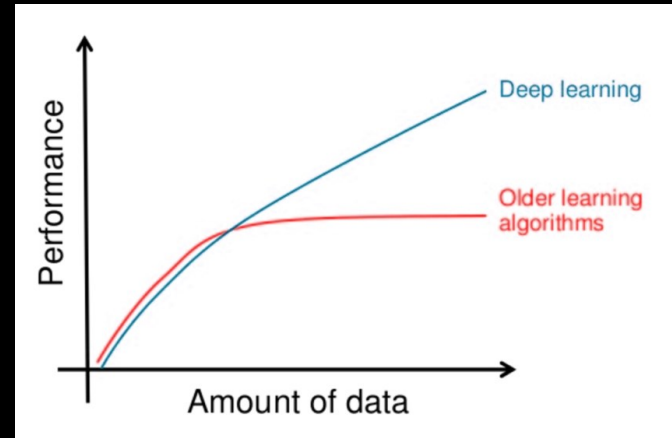
# Deep machine learning

Relies on training a network using a large number of annotated images

- The more training data you use, the better it becomes

Does not use pre-computed features

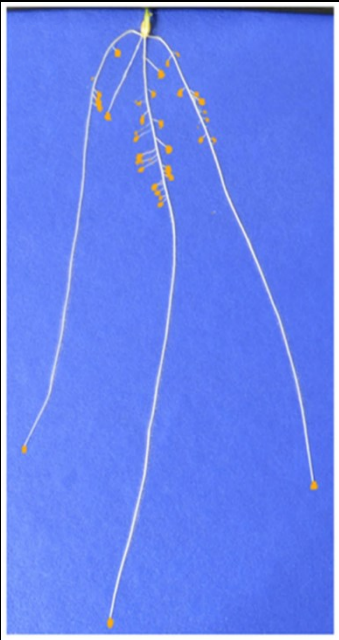
Once trained, the network can annotate new images





# Deep machine learning

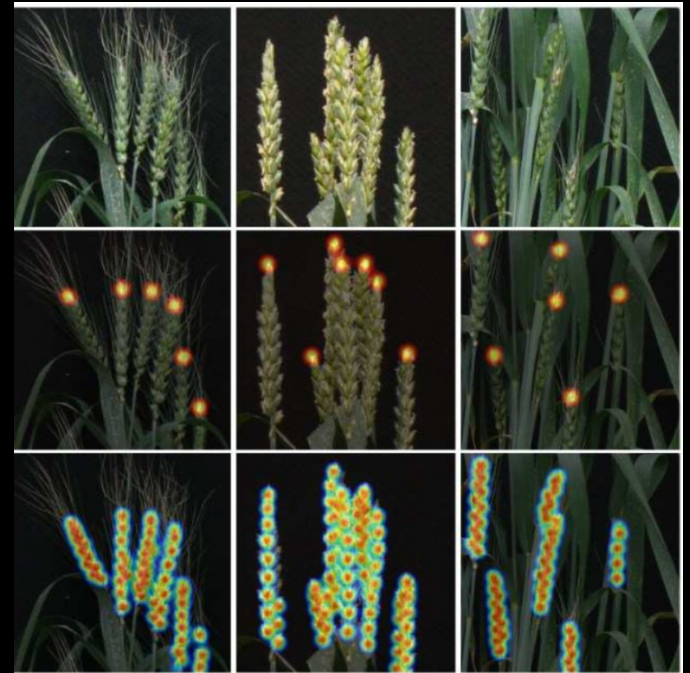
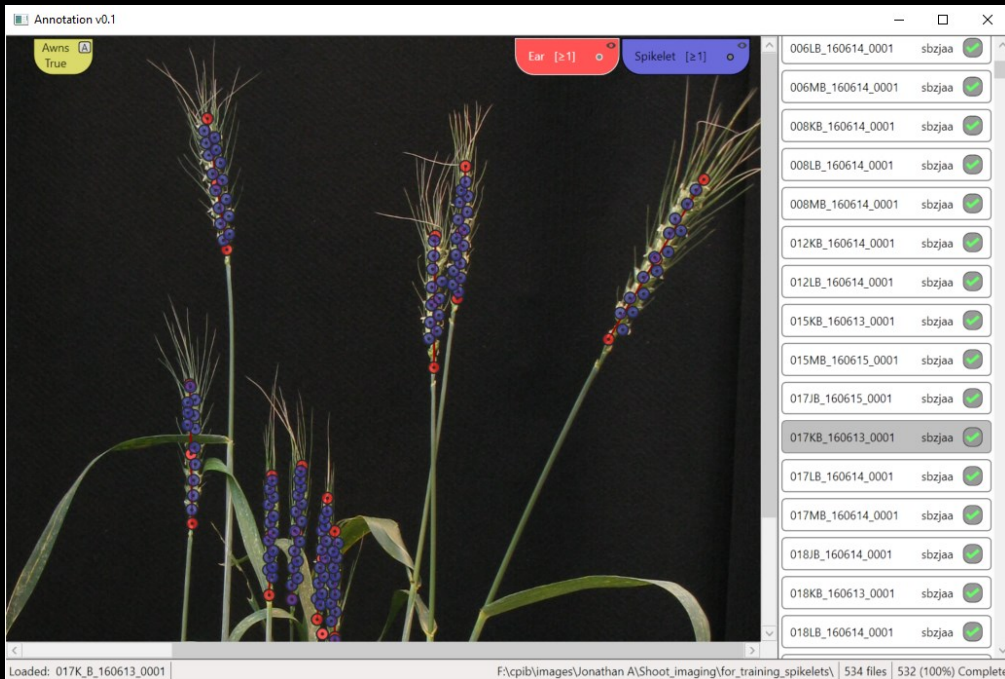
- >97% accuracy in most of the example uses tested to date
- LeMuR: Plant Root Phenotyping via Learned Multi-resolution Image Segmentation (AutoRootNav)



Pound *et al.* (2017)  
Pound *et al.* (2017, ICCV)

# Deep machine learning

## Annotation tool



# Summary

- Phenomics aims to bridge the “genotype-phenotype gap”
- Phenomics involves high throughput acquisition and analysis of multi-dimensional data
- Phenomic pipelines utilise multiple disciplines and technologies

# References/further reading

Houle D., Govindaraju D.R., Omholt S. (2010) Phenomics: the next challenge. *Nature Reviews Genetics* 11 (12): 855–66

Furbank RT, Tester M. (2011) Phenomics-technologies to relieve the phenotyping bottleneck. *Trends Plant Sci.* 16(12):635-44

Tardieu F, Cabrera-Bosquet L, Pridmore T, Bennett M. (2017) Plant Phenomics, From Sensors to Knowledge. *Curr Biol.* 27:R770–R783

Databases: <http://www.phenomicdb.de/>

Resources: <http://www.plant-phenomics.ac.uk/en/resources/>