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Bottlenecks in plant phenotyping: getting to the root of the problem

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As genotyping technology has improved, phenotyping has become a bottleneck in crop improvement, particularly regarding root traits.

Issues to overcome:

- Difficult data collection
- Methods often slow
- Technology expensive and inaccessible
- Image analysis challenges
- Lack of interest from breeders





Root Phenotyping techniques



**2D seedling
phenotyping**



Anatomics



Shovelomics



X-ray CT



2D Seedling Root Phenotyping

Growth room

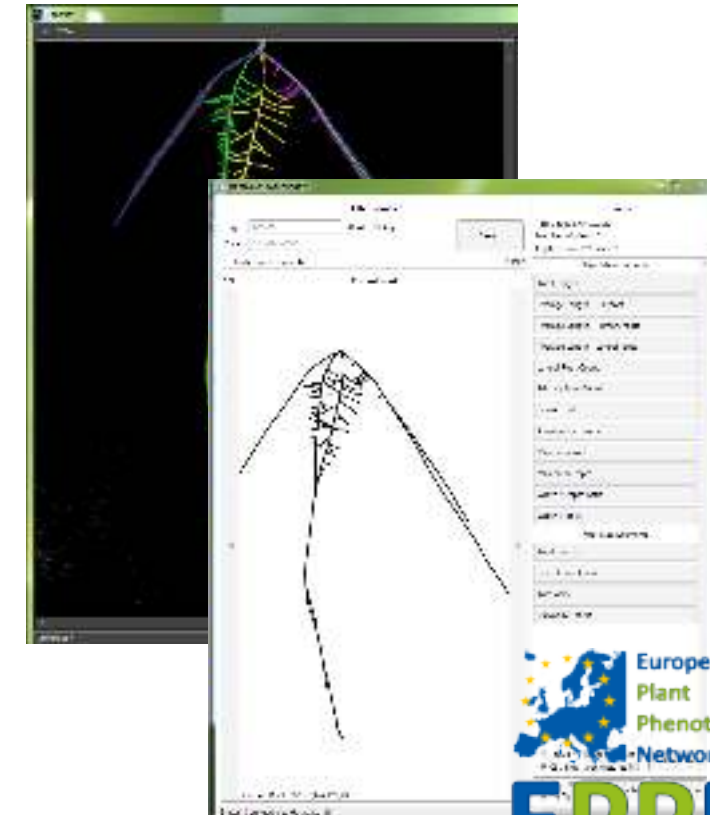
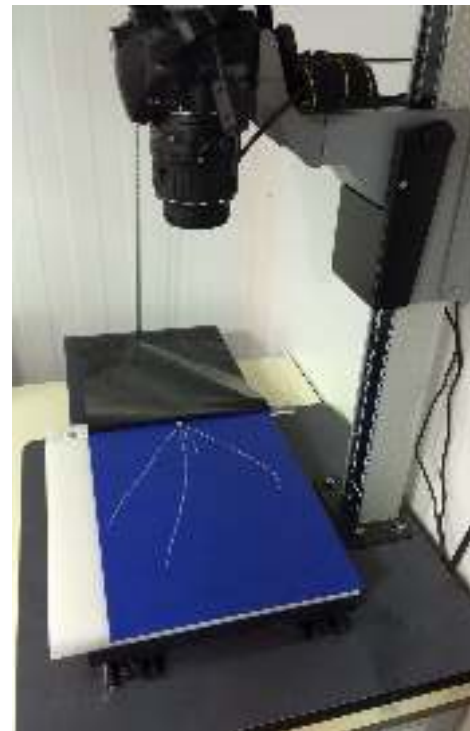
- 600 plants in growth pouches
- 2 weeks growth
- Gravity fed automatic watering

Imaging

- Computer controlled SLR
- Modified copy stand
- QR code system for plant identification

Software

RootNav (Pound et al., 2013)
'semi automatic' image analysis





2D Seedling Root Phenotyping

Savannah

- Group 4
- Feed wheat
- Very High Yield

X

Rialto

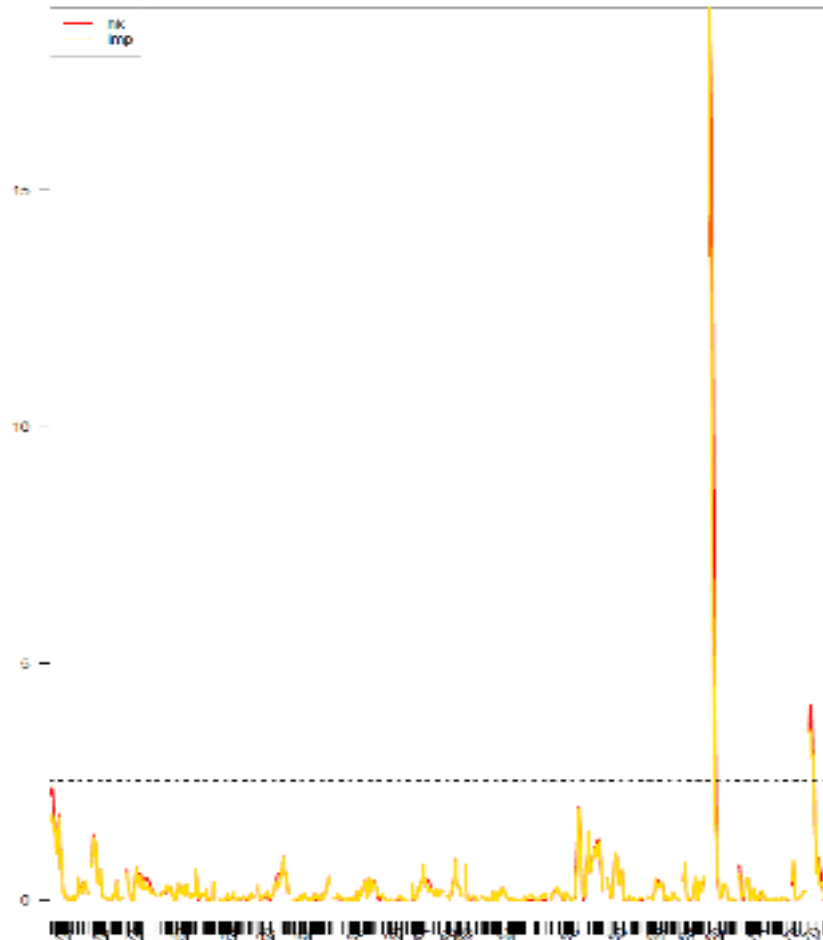
- Group 1
- Bread wheat
- High quality

- 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 the
2D pipeline**

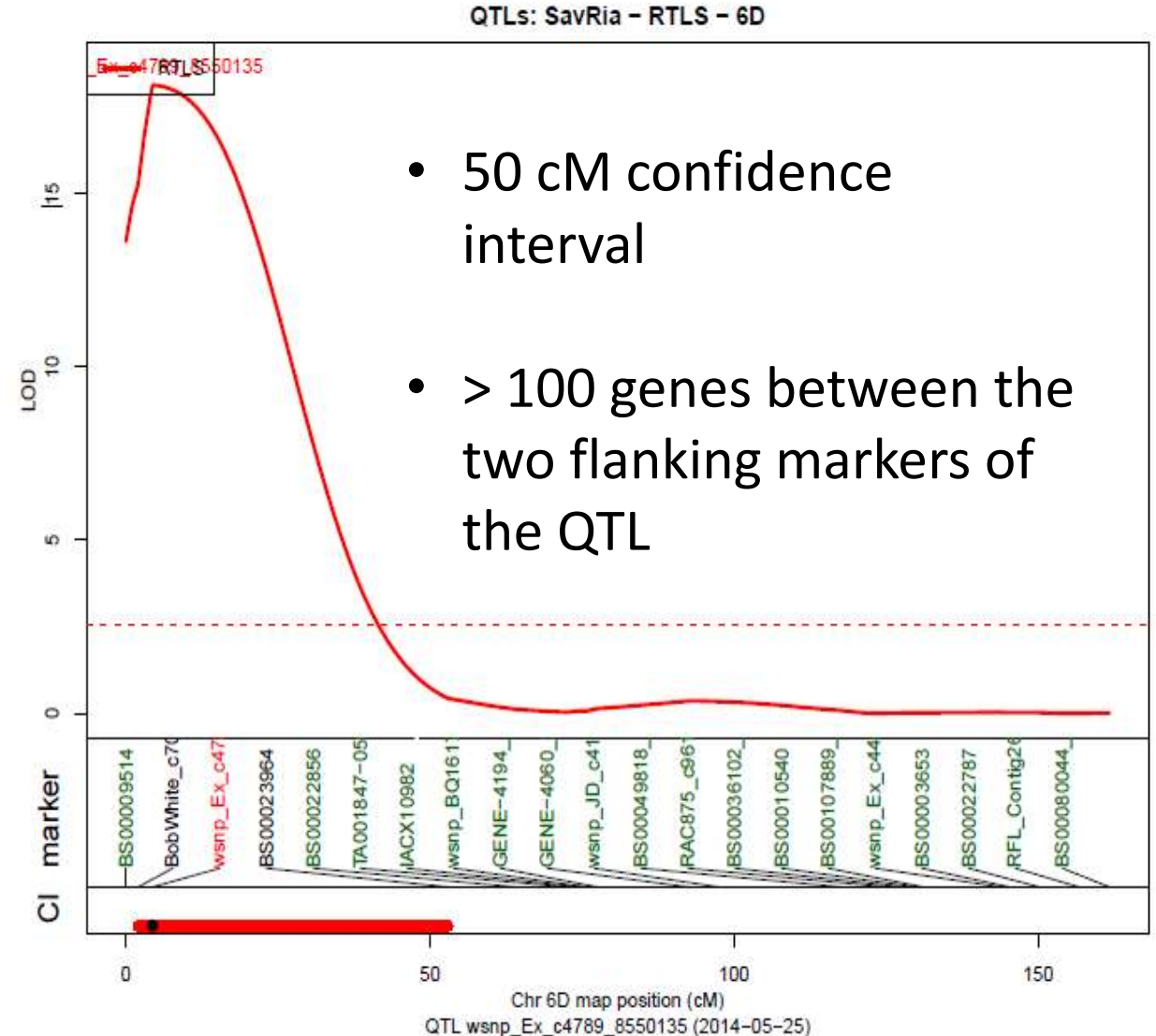


2D Seedling Root Phenotyping

Total Length of seminal roots (LOD > 20)

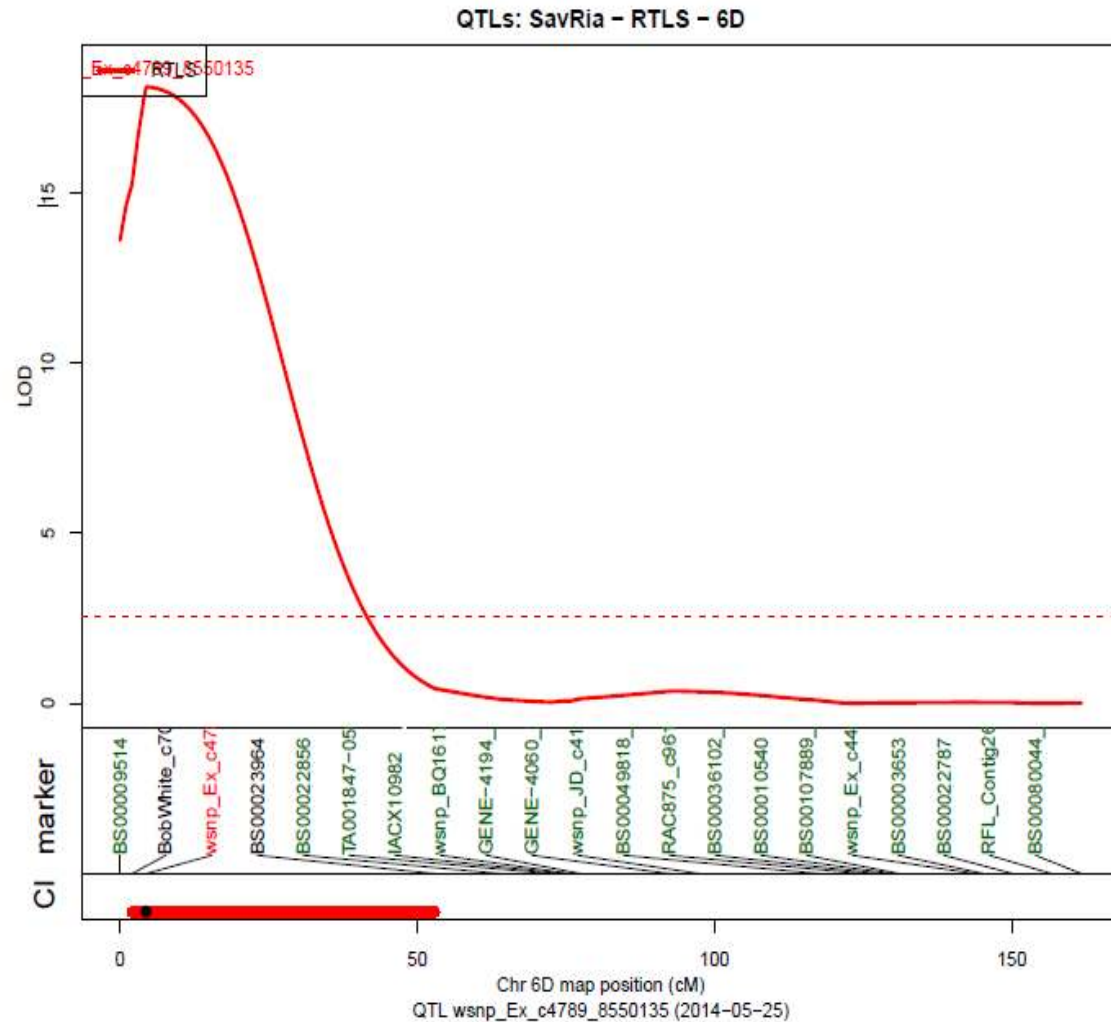


Atkinson et al., 2015





2D Seedling Root Phenotyping



RNAseq

Collaboration with
Laura Gardiner
(Earlham)

18 candidate
genes

NILs

Collaboration with
Limagrain

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



How do you speed up the analysis of 1000's of images?

Machine learning :

1. 'Traditional' machine learning

- PRIMAL - Random Forest

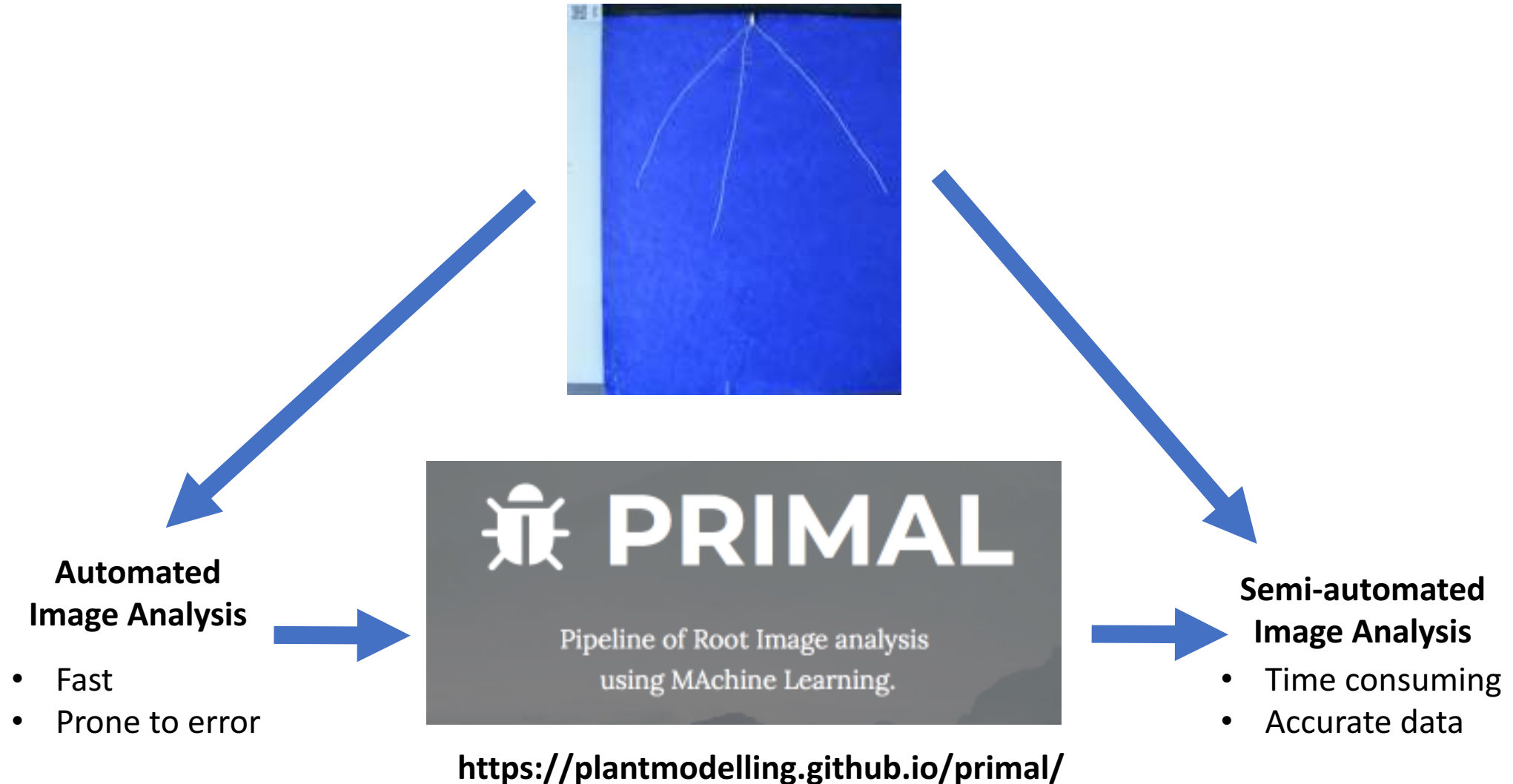
2. Deep learning

- Convolutional neural networks (CNNs)





How do you speed up analyzing 1000's of images?





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 ~ 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



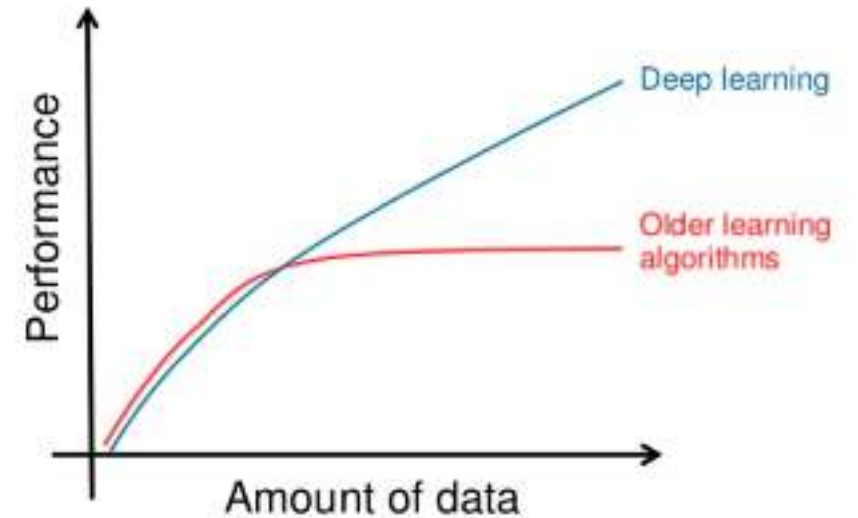
Deep Machine Learning

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 we've tested
- LeMuR: Plant Root Phenotyping via Learned Multi-resolution Image Segmentation



Pound et al., 2017 (Gigascience)

Pound et al., 2017 (ICCV)

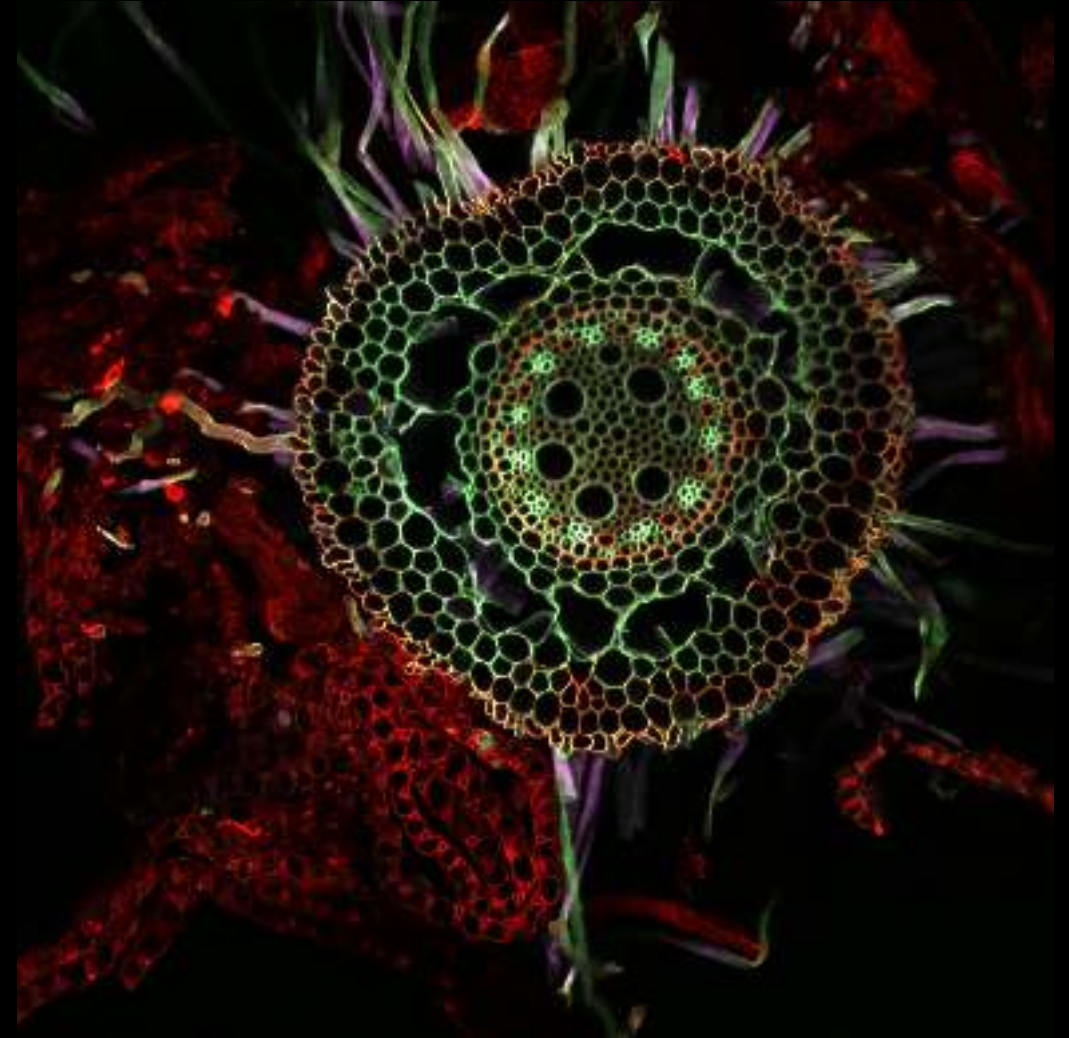


Phenotyping anatomical features

- **Xylem and phloem number**
- **Number of cortical cell files**
- **Presence of root cortical aerenchyma**

Sectioning can be slow and time consuming

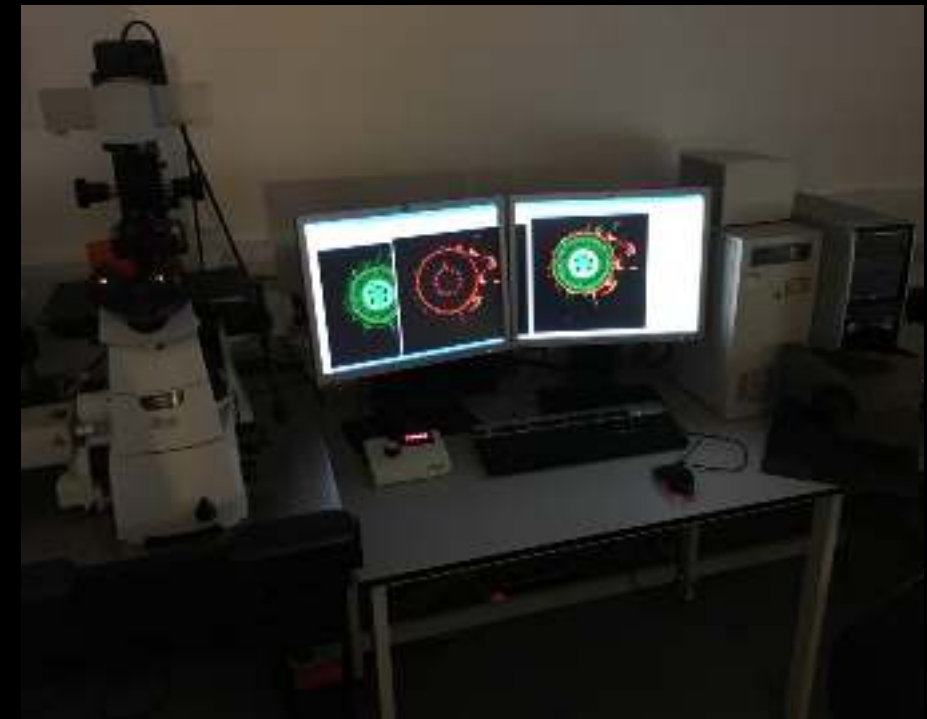
High throughput equipment such as LAT is expensive





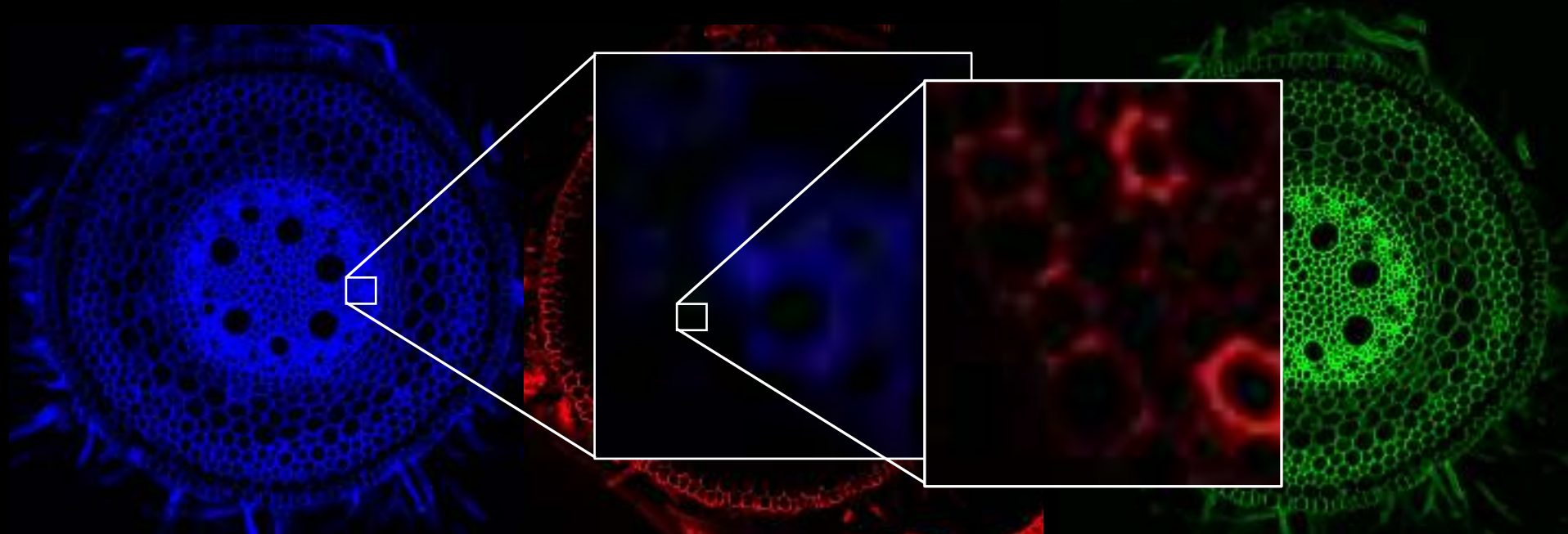
Anatomical sectioning

- Simple protocol using inexpensive 3D printed moulds
- Agarose embedding
- Fast 'thick sectioning' either using a vibratome or by hand
- Rapid staining (30 seconds) using very concentrated Calcofluor
- Imaging on a confocal microscope
- ~200 samples per day (including sampling from the field)





Anatomical sectioning



Channel 1. 408 laser
450/35 (blue) detector
Low gain

Channel 2. 488 laser
605/75 (red) detector.

Channel 3. 408 laser
515/30 (green) detector

Exploiting differential staining properties of calcofluor and autofluorescence to detect xylem and phloem cells

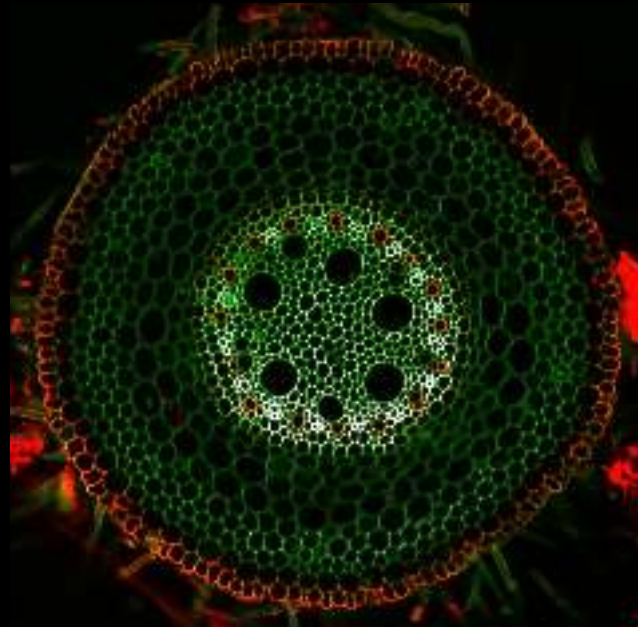
3 separate images are taken using different lasers, detectors and settings captured automatically



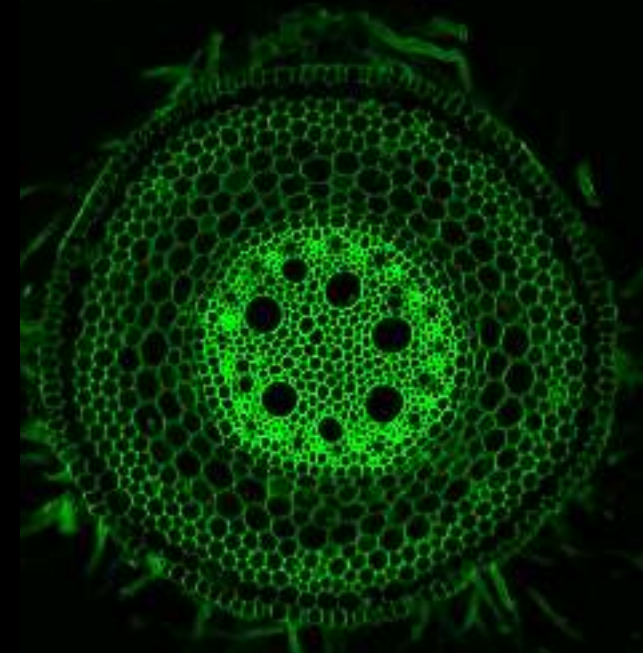
Anatomical sectioning



Channel 1. 408 laser
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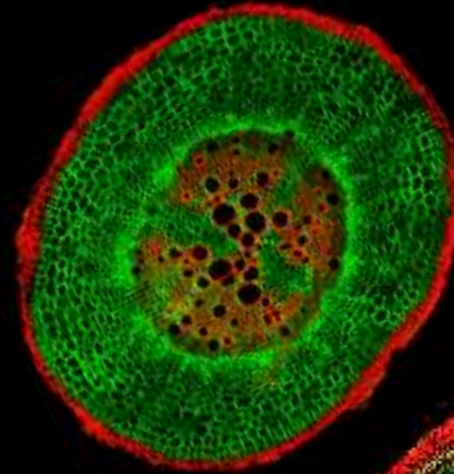
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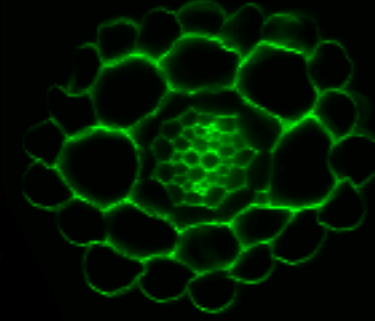
Anatomical sectioning



Wheat (cv. Savannah)



Sugar Beet



Arabidopsis



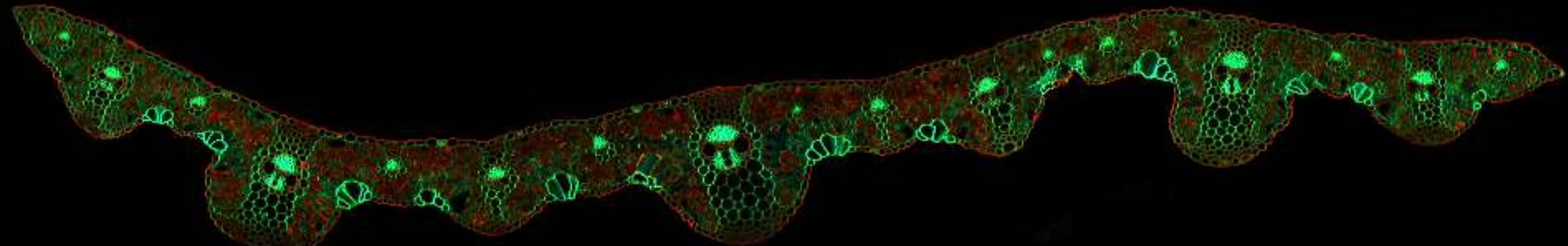
Rice



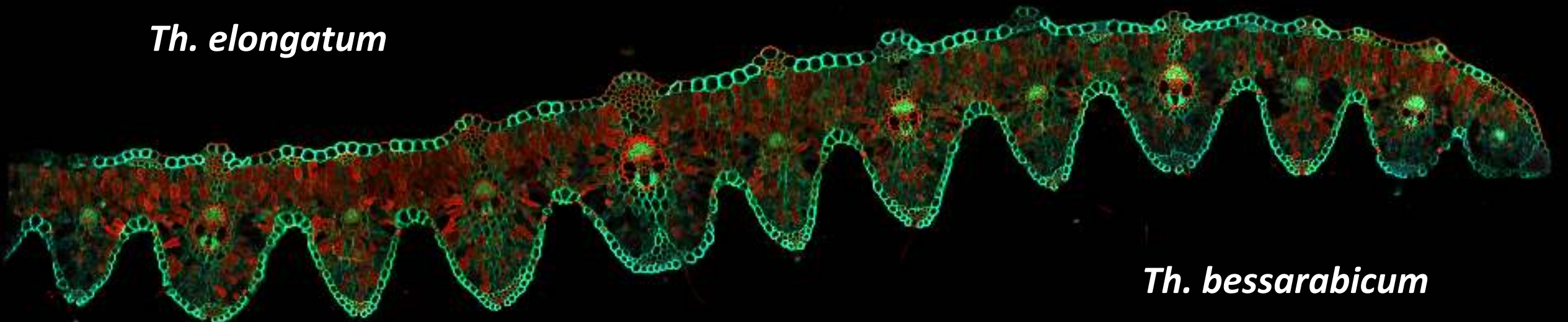
Date Palm



Pavon 76



Th. elongatum

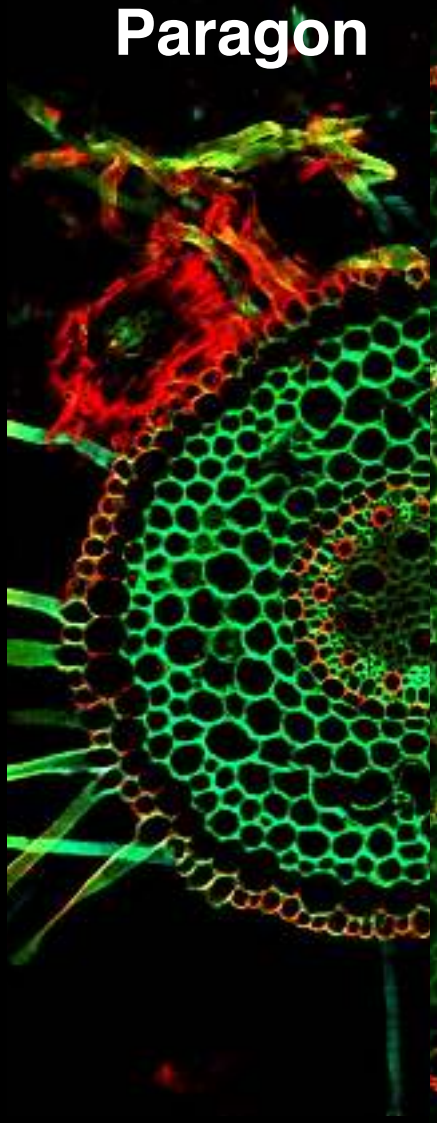


Th. bessarabicum

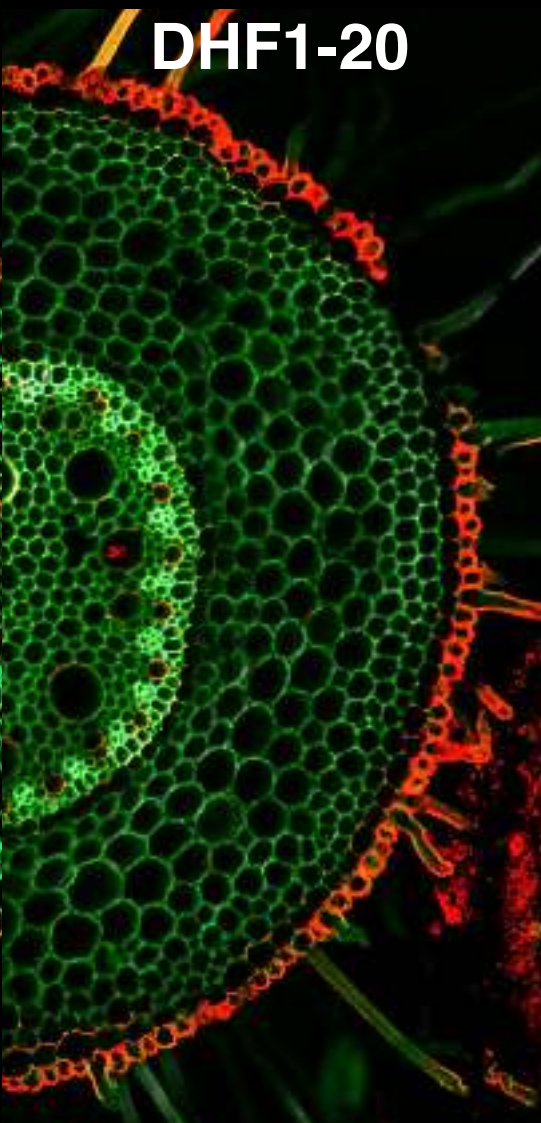


Anatomical sectioning

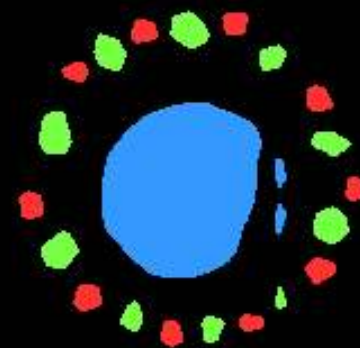
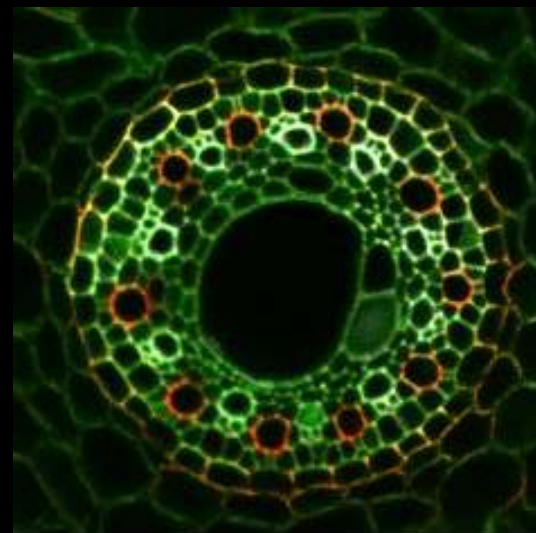
Paragon



DHF1-20

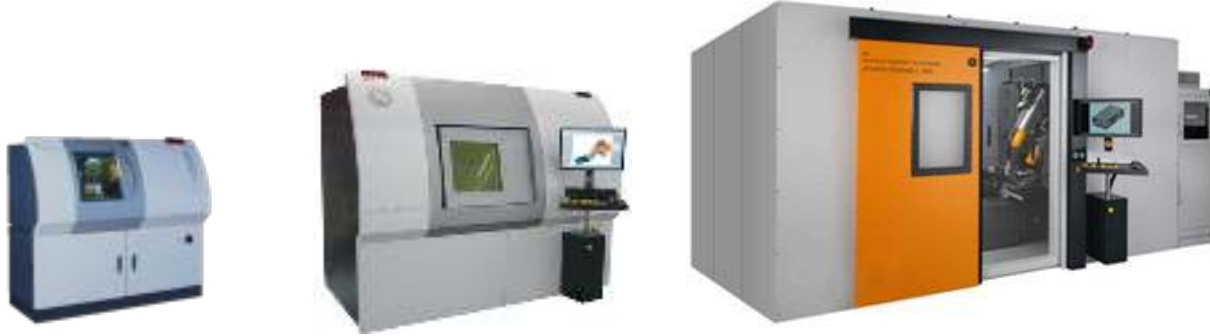


- Screening germplasm from Ian and Julie King
- Changes in root diameter, phloem/xylem pole count, aerenchyma, etc
- The bottleneck again is Image analysis and data quantification
- Another application for Deep Machine Learning





X-ray Computed Tomography



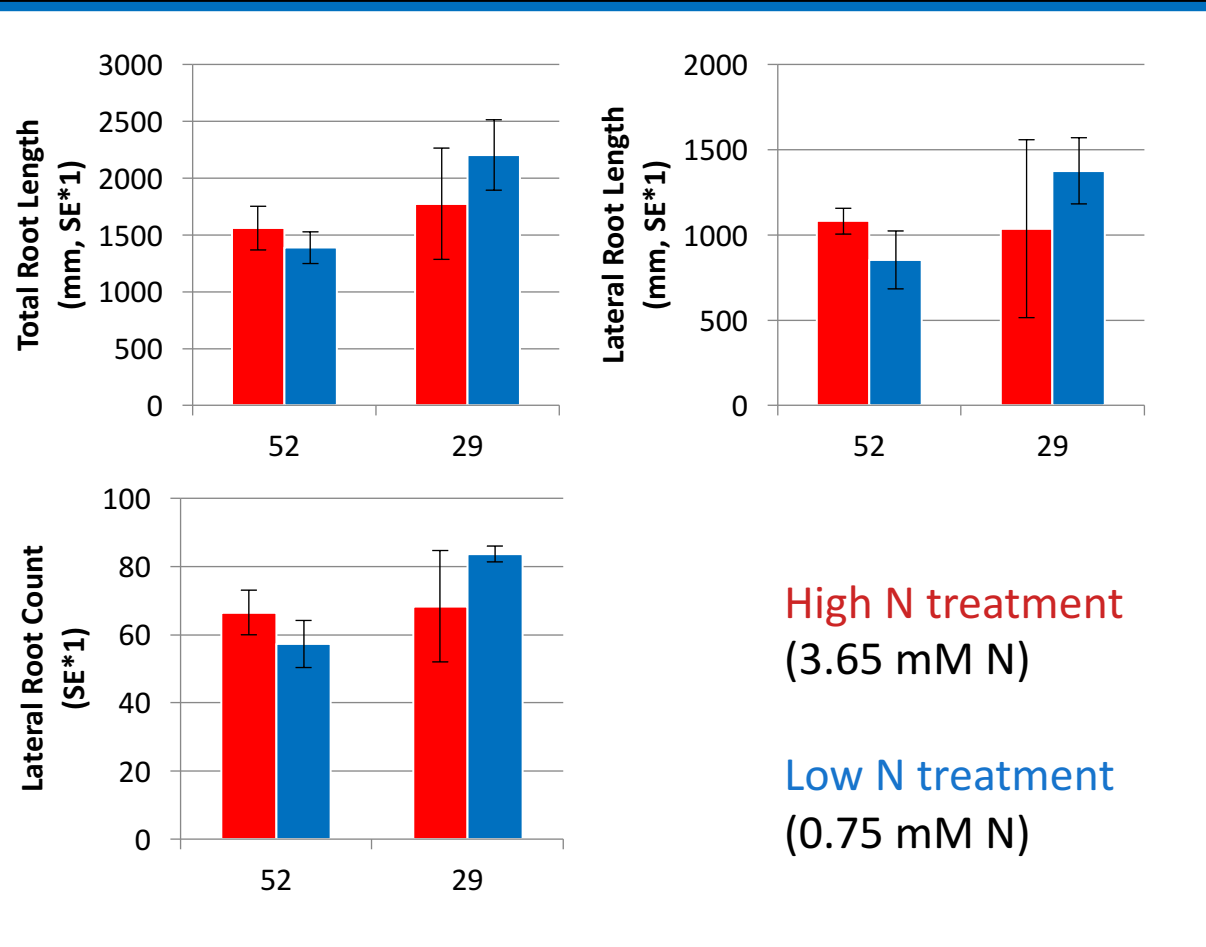
- 3 X-ray CT scanners covering ca. 1 μm to 150 μm resolution
- Samples sizes up to 25 cm diameter & 1 m length
- *Automated sample delivery*





X-ray Computed Tomography

Nitrogen Uptake Efficiency in Wheat



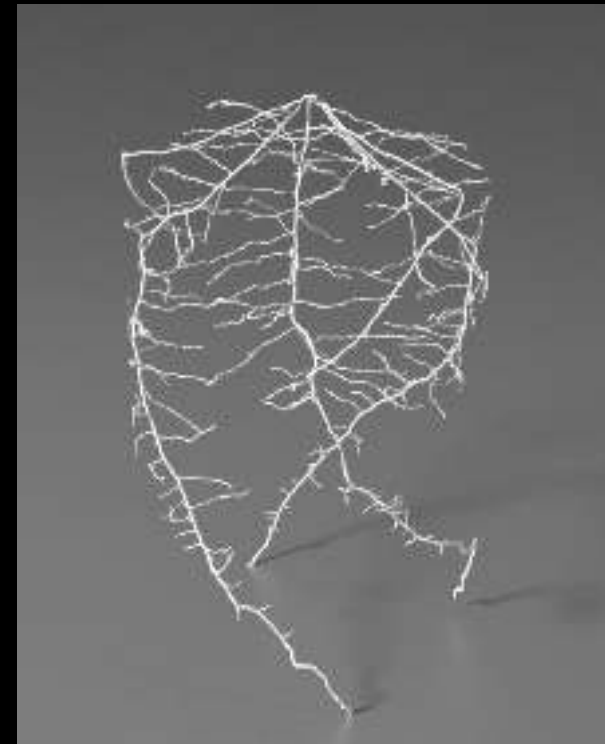
Line 52

Low NUpE in both high & low N field trials



Line 29

High NUpE particularly in Low N field trials





X-ray Computed Tomography

Effect of fungal pathogens on roots

Tomato (control)



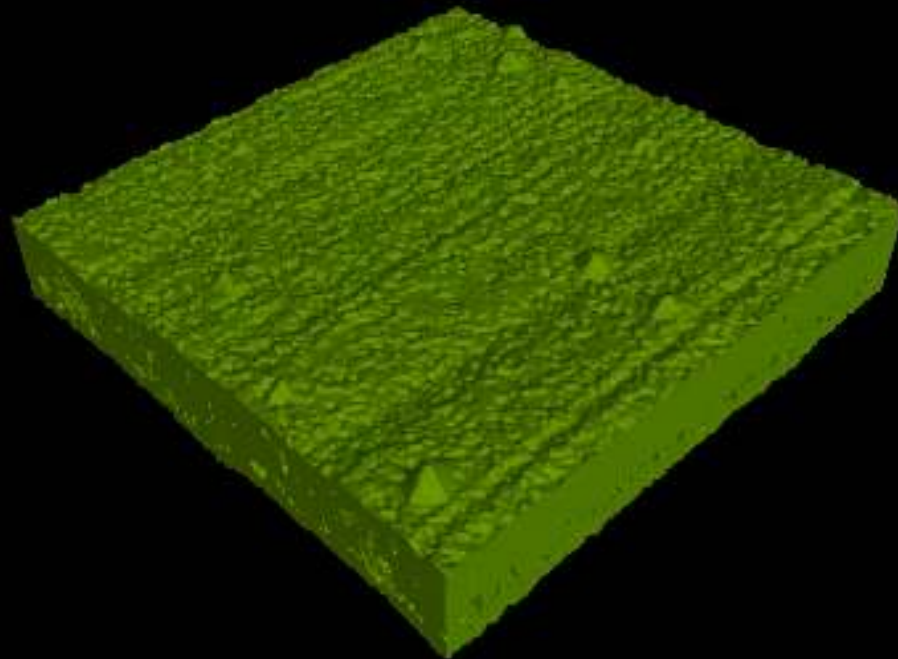
Tomato (+ *Rhizoctonia*)



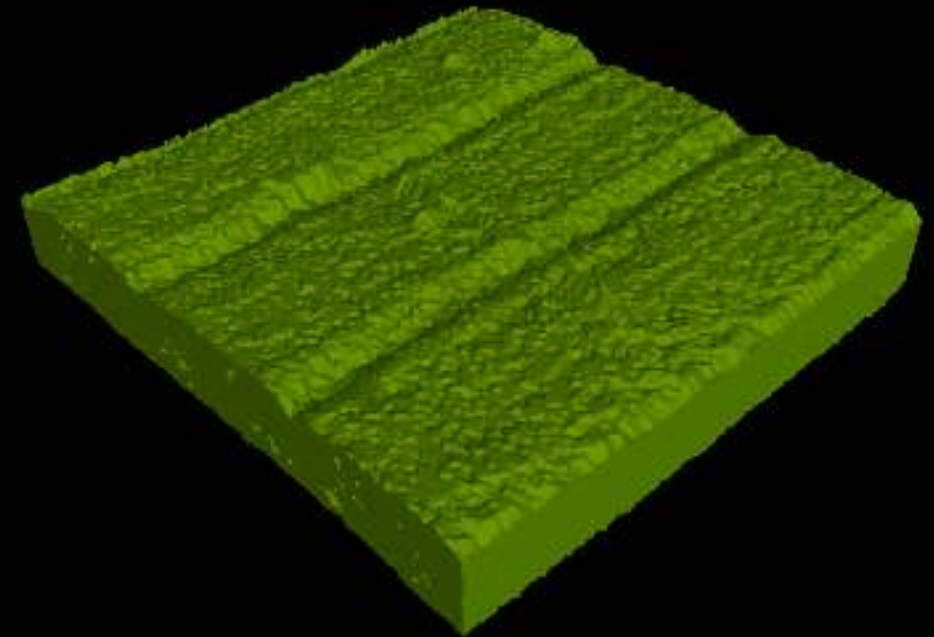


X-ray Computed Tomography

Oryza latifolia



Oryza minuta



14.0
0.00848
0.0181
59.6

Porosity (%)
Mean Thickness (mm)
Max. Thickness (mm)
Surface Area:Vol

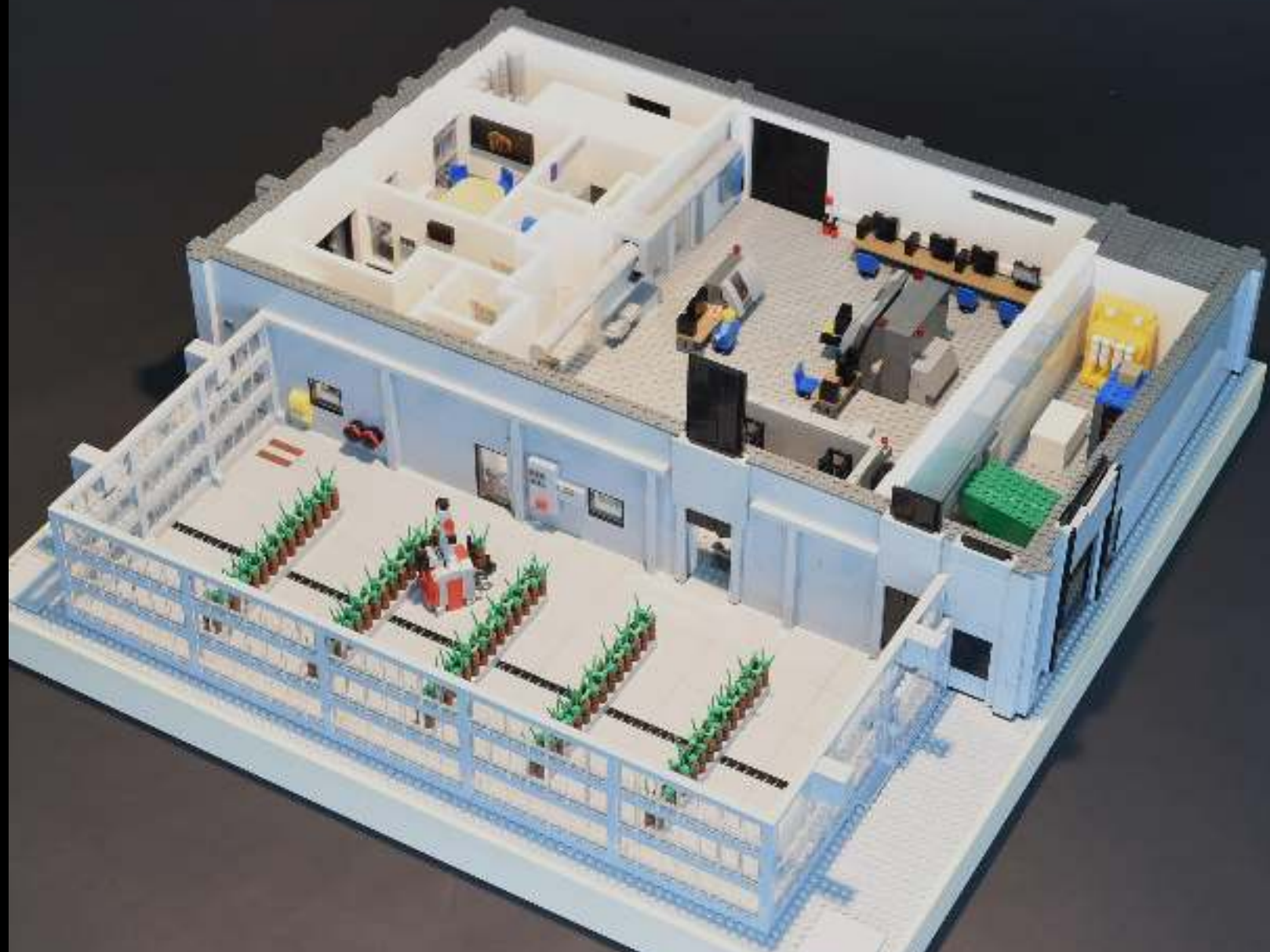
5.57
0.00795
0.0152
27.0



X-ray Computed Tomography

LCT – Large x-ray CT scanner

- Custom built by GE
- Automated sample handling
- 1 m x 25 cm diameter soil columns (~60 kg with wet soil)
- 100-150 μm resolution
- ~ 3-4 hours per scan







X-ray Computed Tomography

Temporal development of wheat
over 6 months , from seedling to
maturity

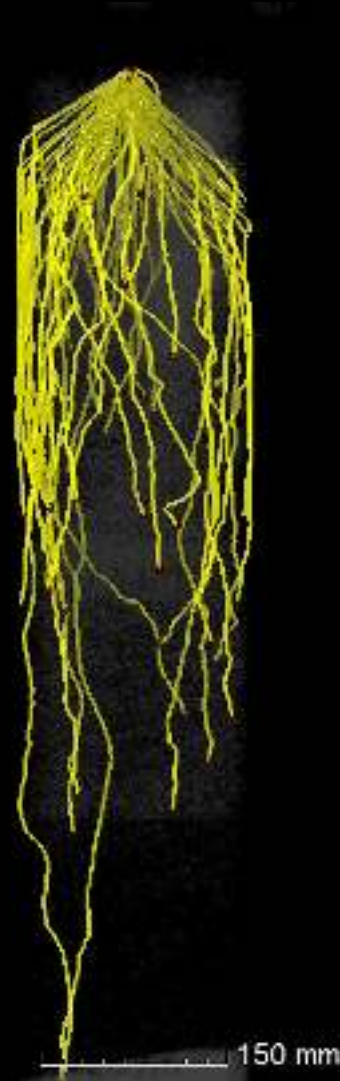




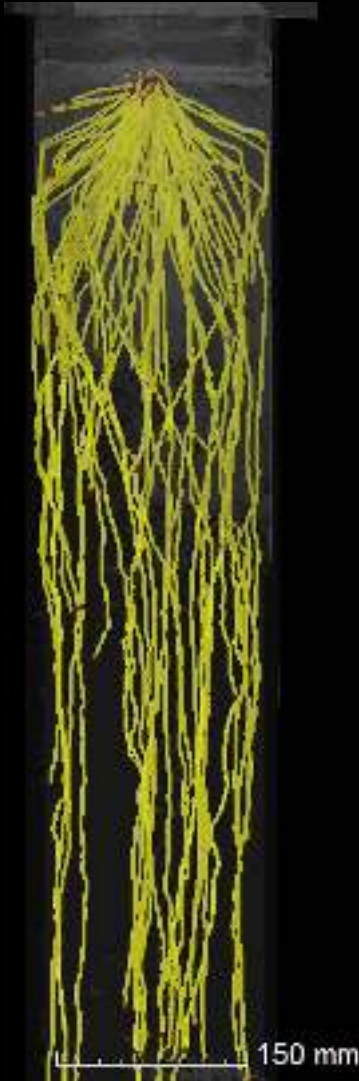
X-ray Computed Tomography

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



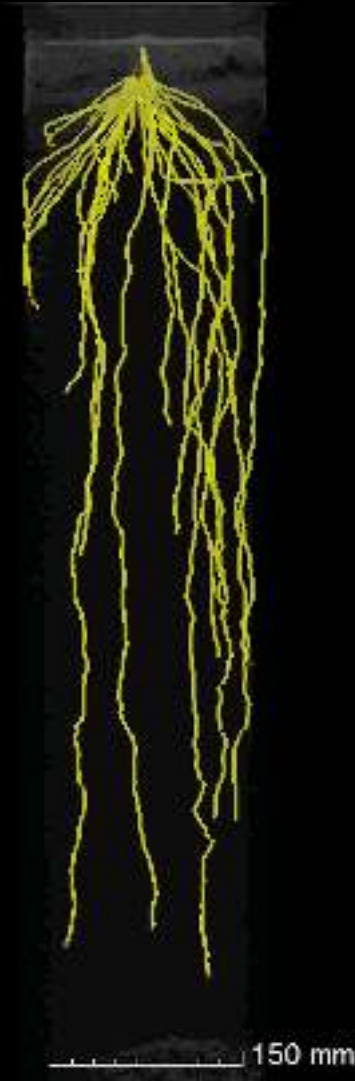
T. urartu



Ae. speltoides



T. dicoccoides



Ae. tauschii

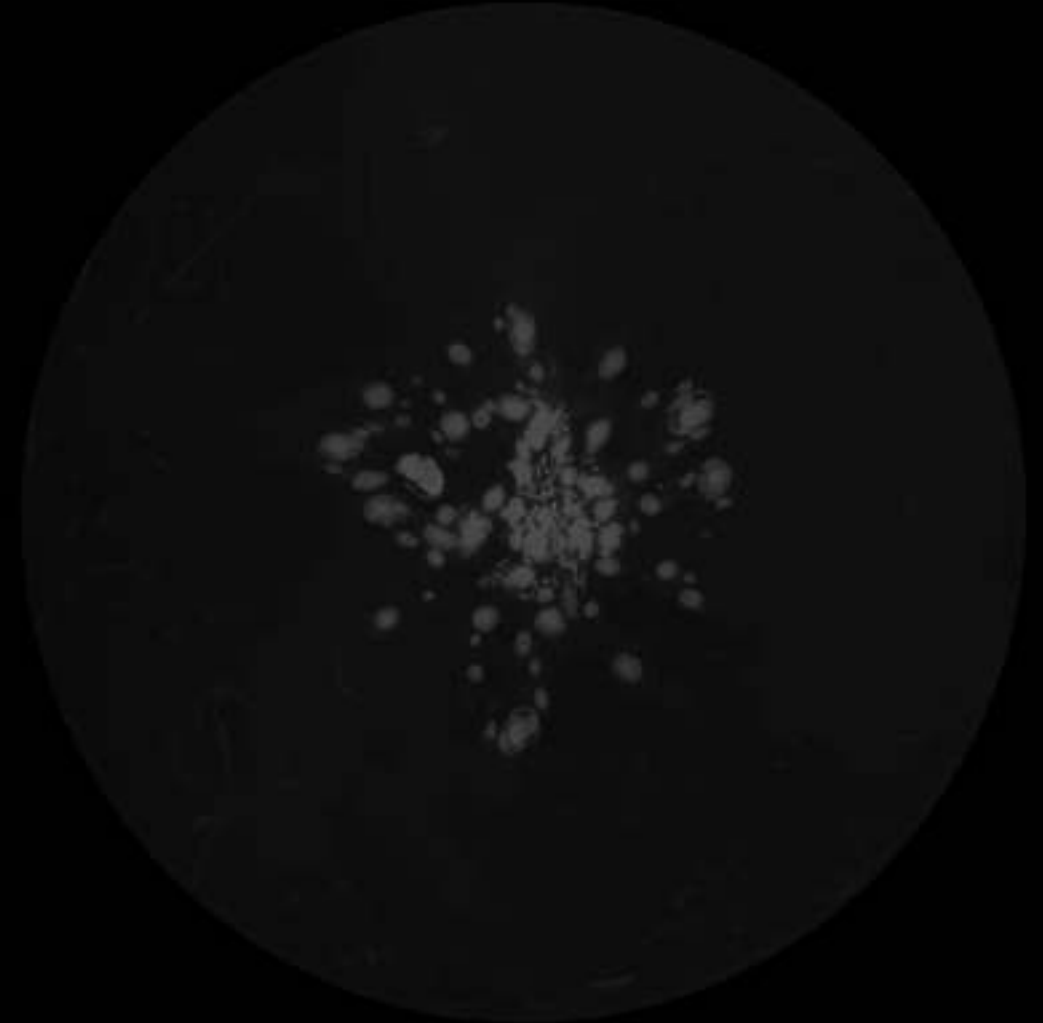


T. aestivum



X-ray Computed Tomography

- Data collection is entirely automated
 - The rate limiting step is analysis
- We are now looking to apply deep machine learning to X-ray CT.
- Due to the volume of data, having enough processing power is the next major hurdle.





Summary

Throughput



- High throughput – 2D seedling screen
- Medium throughput - Anatomics
- Low throughput high detail - X-ray CT

**All of these methods can be improved by better image analysis,
particularly deep learning**



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UoN Biosciences

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Craig Sturrock

Brian Atkinson

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Tony Pridmore

Andrew French

Wheat Research Centre

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(Forschungszentrum
Jülich & Université
catholique de Louvain)

Simon Berry

(Limagrain)

Luzie Wingen

(JIC)

Marcus Griffiths

(Noble, formally UoN)





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Any Questions?



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