

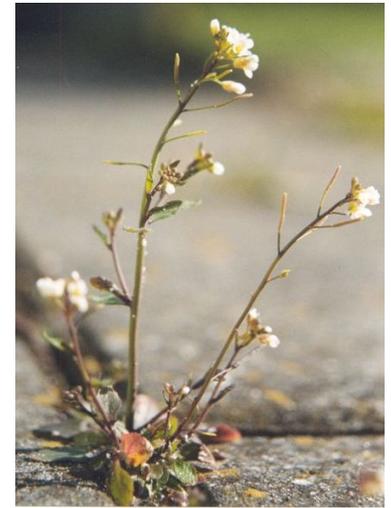
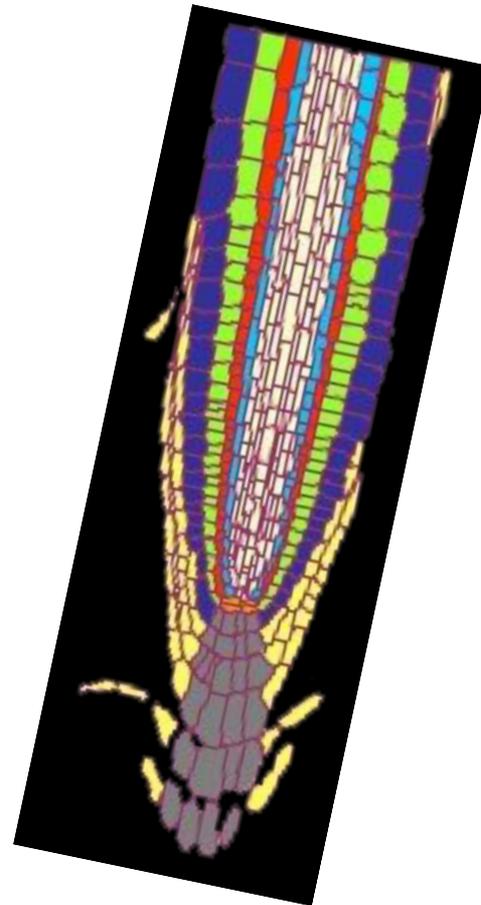
A Systems Solution to Root Phenotyping

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CPIB: *A centre of excellence in Plant Integrative Biology*

- £9.2M BBSRC/EPSRC project, 5 years, 22 PDRAs, 20 PhDs
- Integrative Systems Biology approach to creating a *virtual Arabidopsis root*
- Co-located biologists, soil scientists, mathematicians, computer scientists, engineers

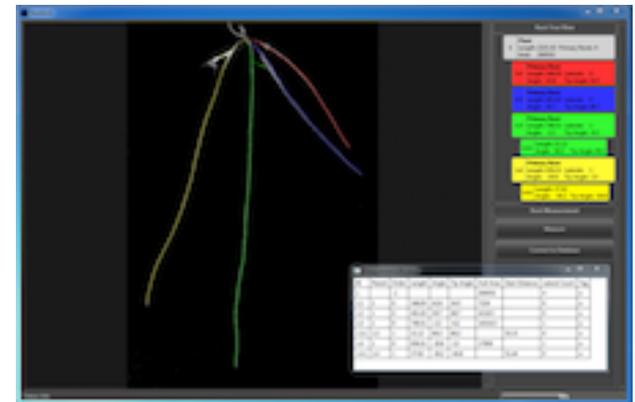
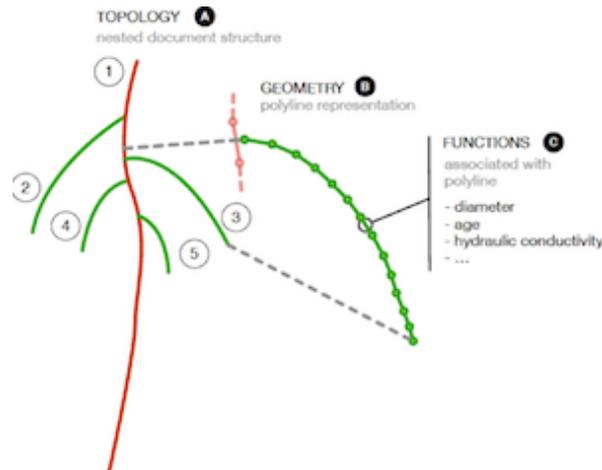
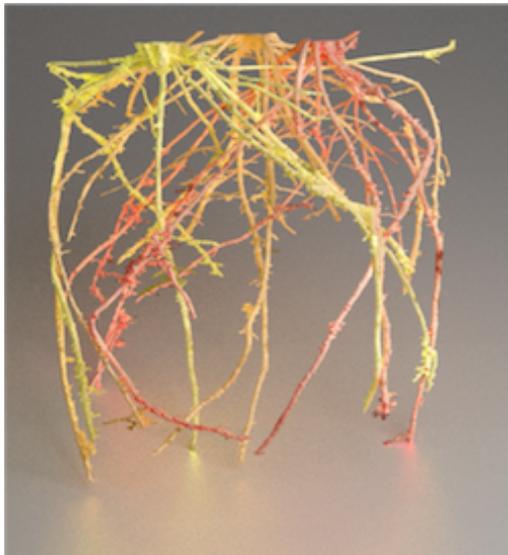
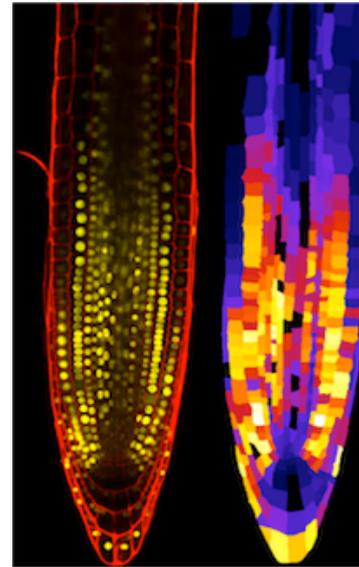


Integrated Phenomics

- Common theme:
 - in-house acquisition hardware and control software
 - in-house analysis software
- Design principles:
 - minimal disruption to sample
 - flexible hardware and image acquisition platforms
 - minimal user interaction analysis software
 - only possible with multi-disciplinary team with input at every stage of development

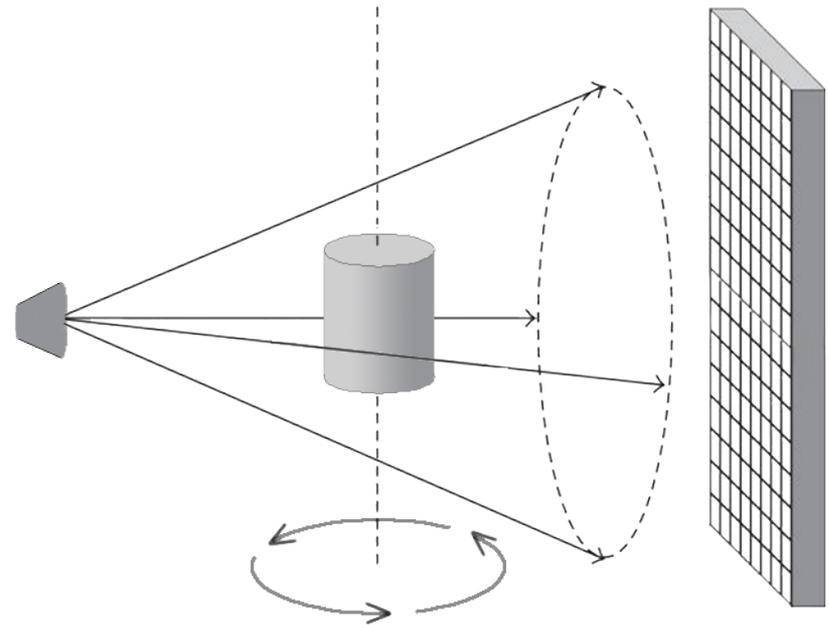
Image-based phenotyping: Roots

- Tissue scale (Cellset)
- Organ Scale (Roottrace)
- 2D root architectures (RootNav)
- 3D root architectures in soil (Roottrak/Rooth)
- Root System Markup Language (RSML)
- Deployment via iPlantUK



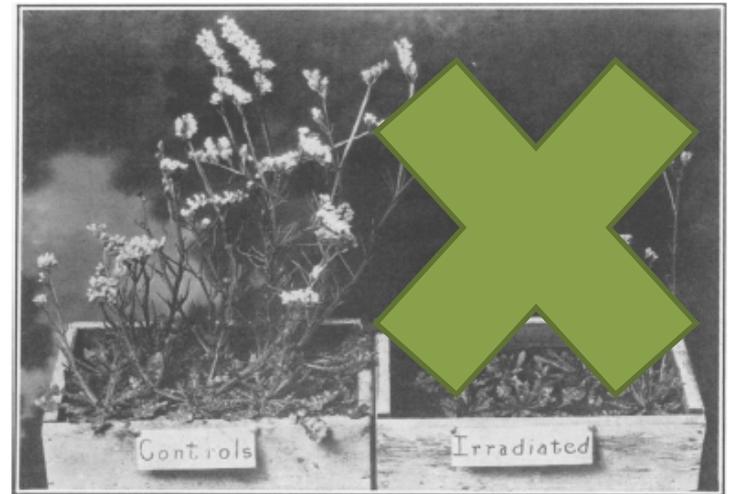
X-Ray μ -Computed Tomography

- General purpose sensor capable of providing rich descriptions of complex objects/situations
- Images values reflect target density
- Non-destructive
- Captures root systems in three-dimensional space
- Can visualise roots and soil simultaneously



Imaging Issues: Sample Exposure

- Typical dose for modern scans of plant and soil samples is 0.1 – 20 Gy
- Most evaluative studies on seeds
- 10Gy at germination reduces shoot elongation and flowering (Johnson 1936)
- BUT Plants show much higher tolerance after germination
- Repeated exposure over 10 days at 7+ Gy produced no effect



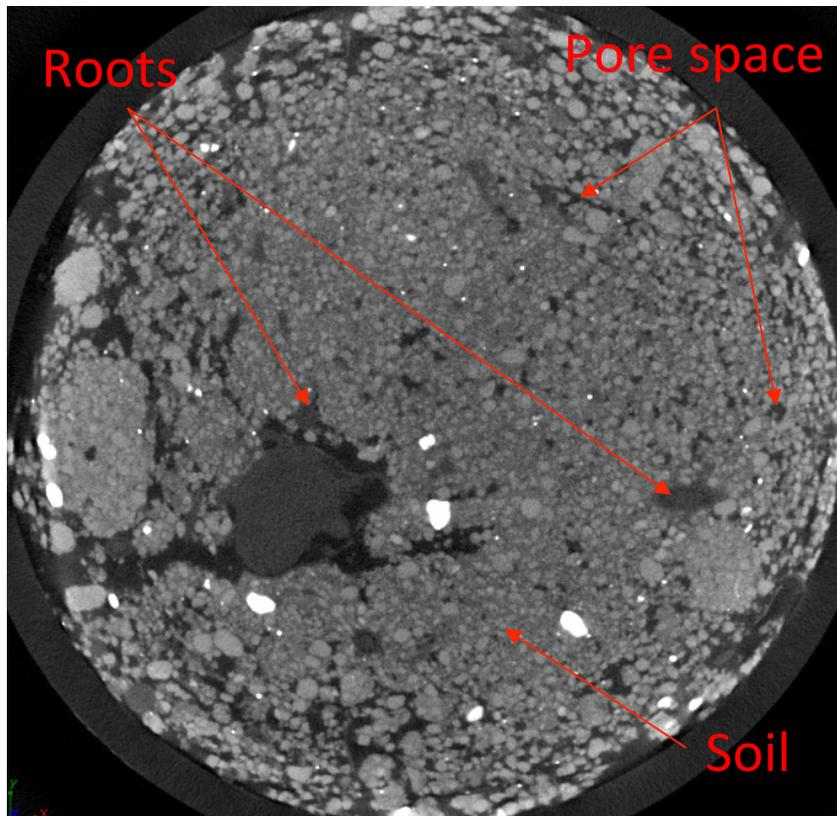
Zappala et al, PLOS-ONE, 2013

Imaging Issues: Water Content

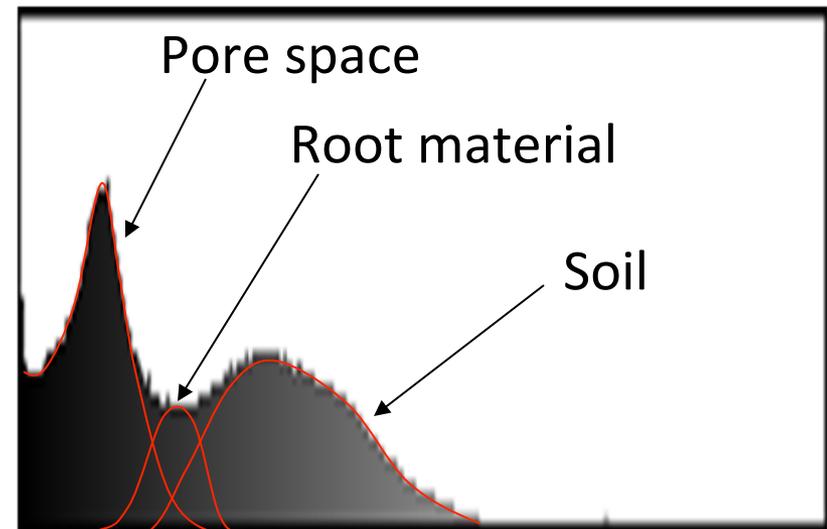
- Increased water content makes root and soil density similar, what is the ideal water content for root/soil segmentation?
- Samples were scanned over 9 days while drying from saturation
- (Manually) segmented volumes compared with root washing
 - At saturation root and soil become (near-) indistinguishable
 - In dry soil, air pockets add image noise
 - Root volume was most accurately recovered after 3 days drainage: at approximately field capacity

Image Analysis Challenges

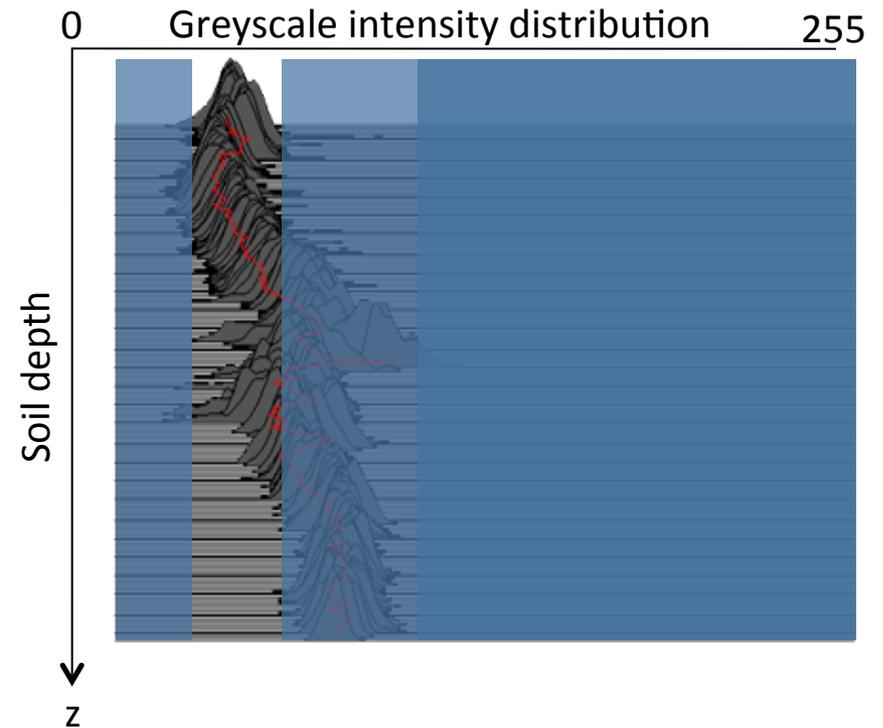
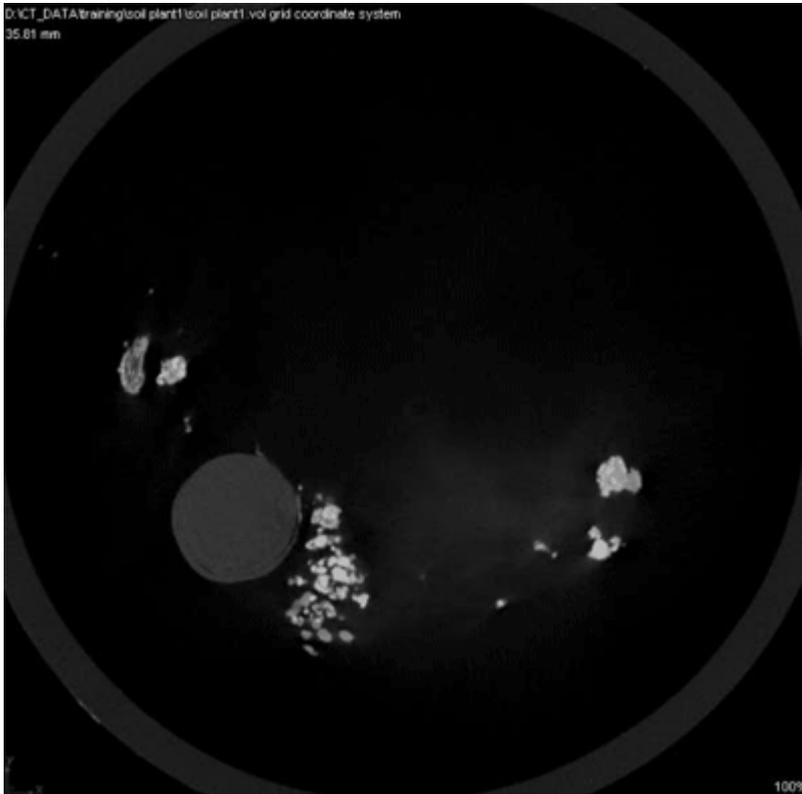
- Similar density of roots, water and pore spaces



Maize in loamy sand, resolution 44.23 μ m

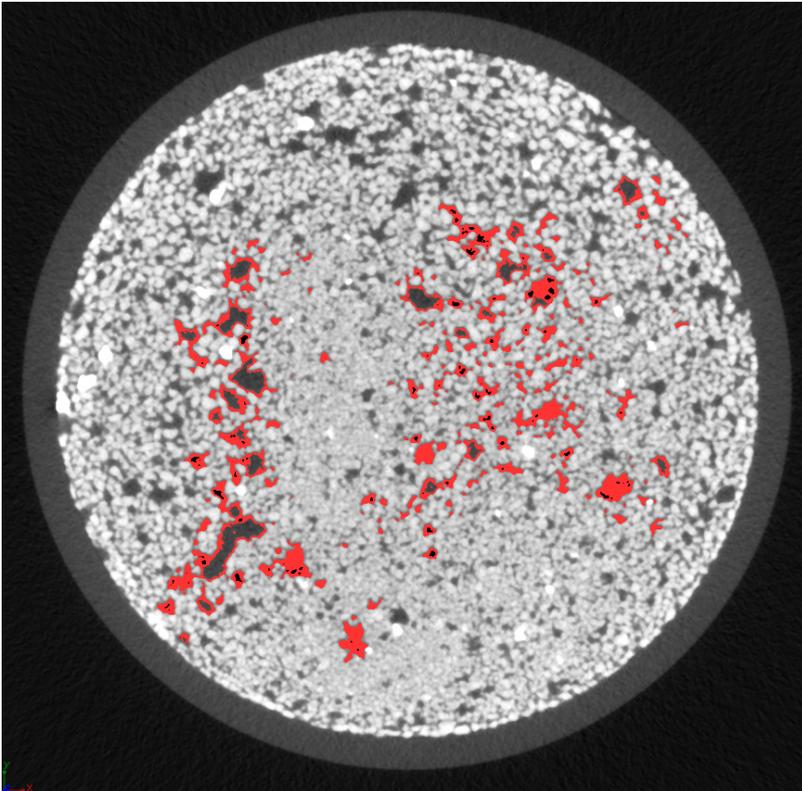


Intensity Variation of Roots



Maize in loamy sand, resolution 44.23 μ m

Threshold-based Segmentation



Wheat in sand, resolution 28.75 μ m



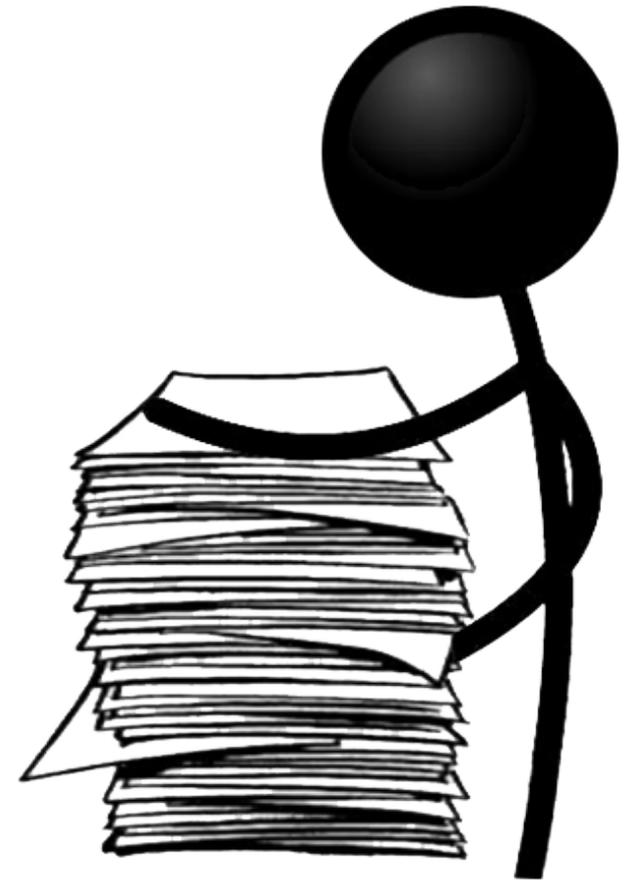
Simple, static tests cannot separate roots and soil

Intensity is Not Enough

- Plants don't grow in isolation
 - Negative: competing for resources
 - Positive: one plant's influence on bacterial flora can improve another plant's nutrient supply
- Interaction is of particular interest when designing intercropping schemes
- Need to separate multiple plants in CT data means **density is not enough**: roots are the same material, and will have the same density characteristics

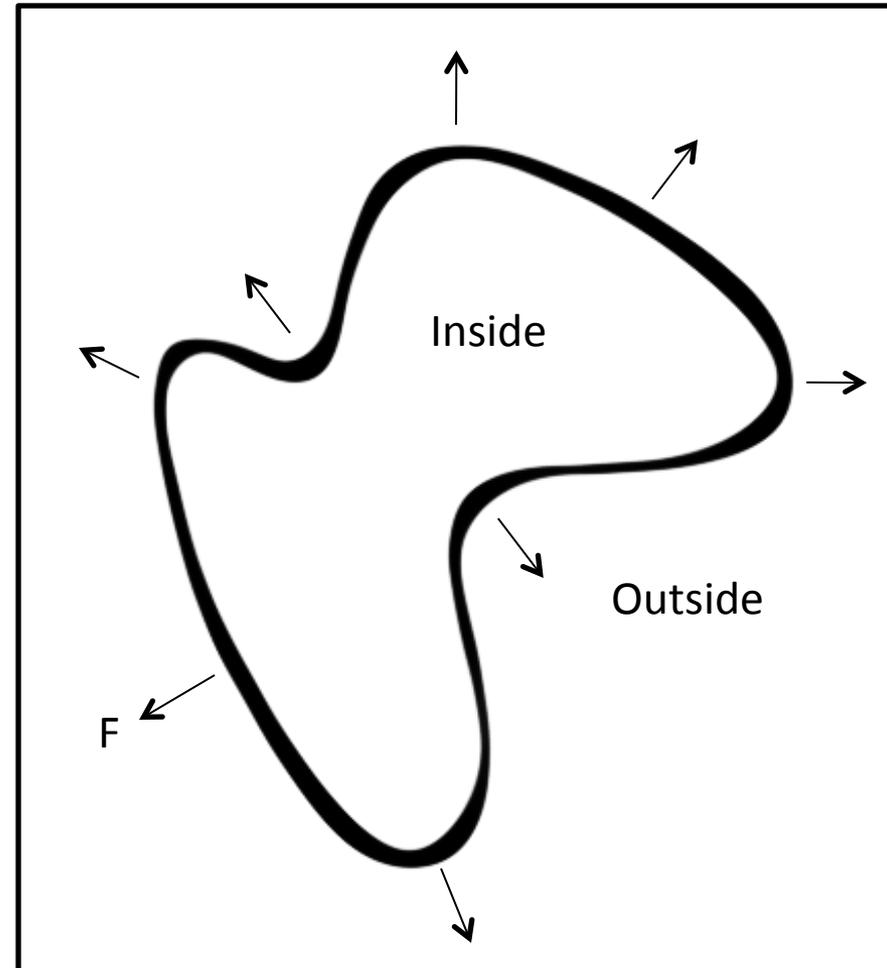
A Tracking Approach

- Volumetric dataset is stored in a three-dimensional grid of voxels
- Can be viewed as a stack of images that are traversed from top to bottom
- Allows analysis to be
 - focussed on roots, avoiding other objects with similar density
 - adaptive, varying its model of root density as tracking proceeds
 - flexible, using exploiting other information, like shape

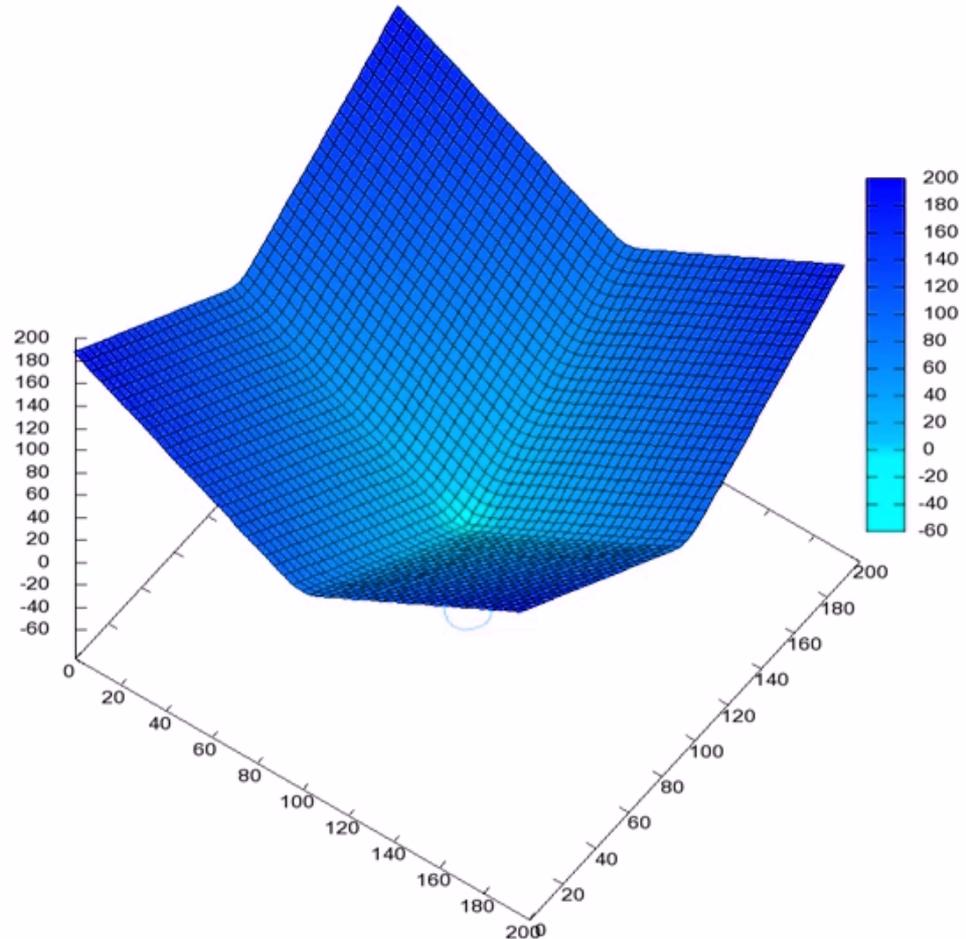
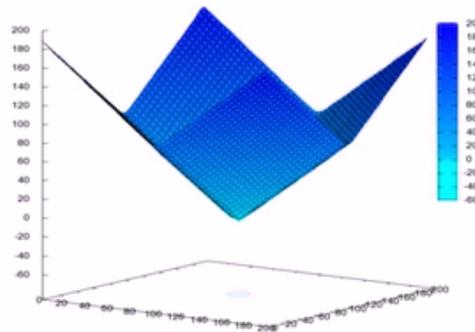
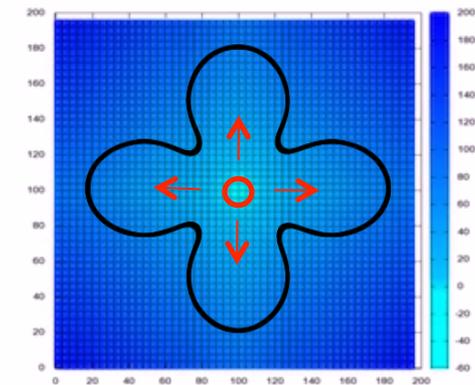


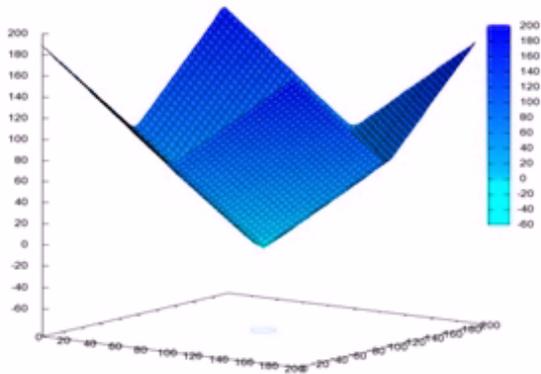
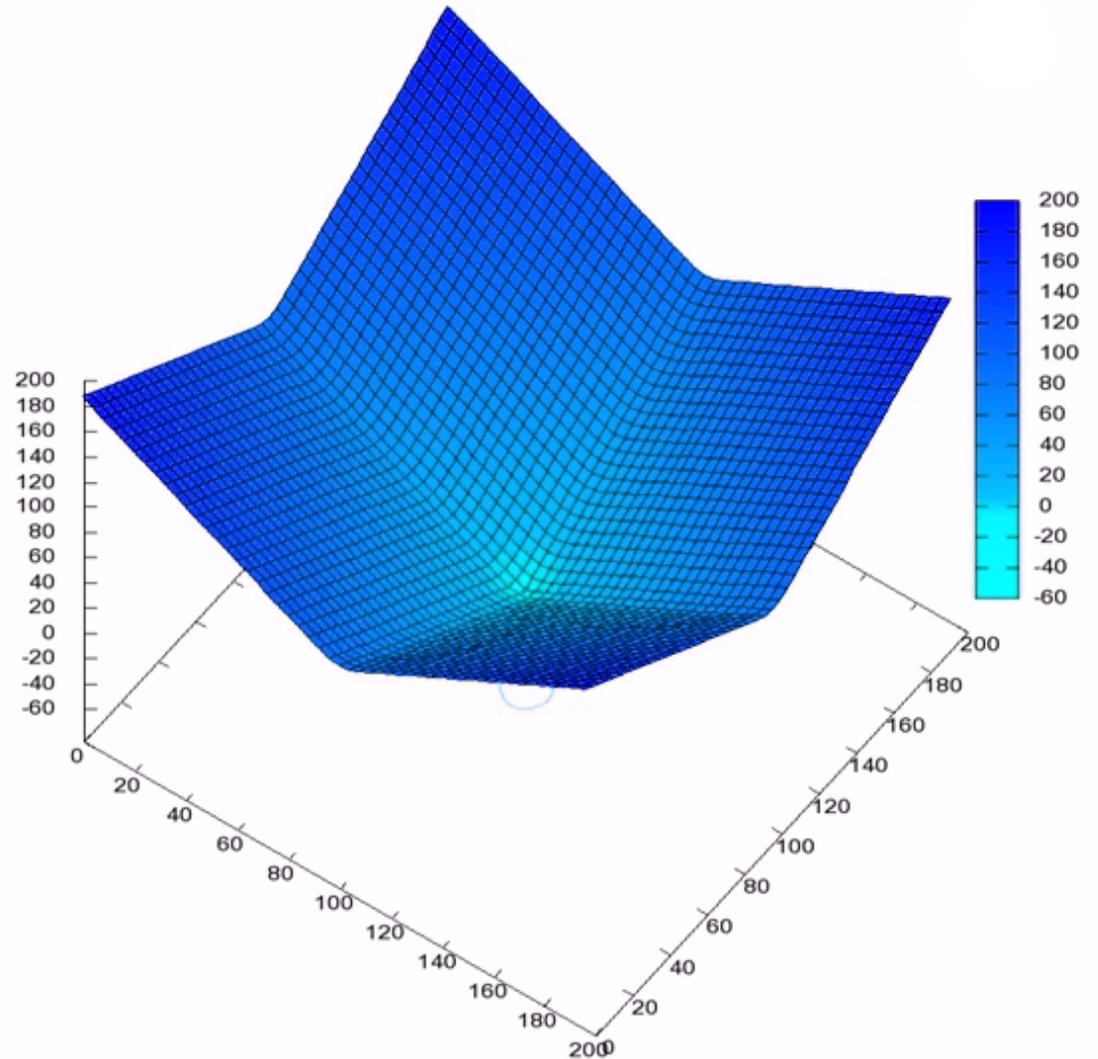
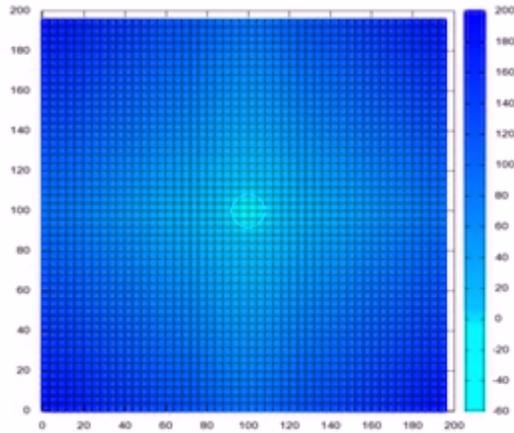
Tracking Root Sections

- Level set method: implicit representation of an object's shape
- Root objects are defined by the intersection of a deforming 3D surface and the image plane
- Interface motion is controlled by a properly defined velocity function
- We assume root objects overlap in adjacent images



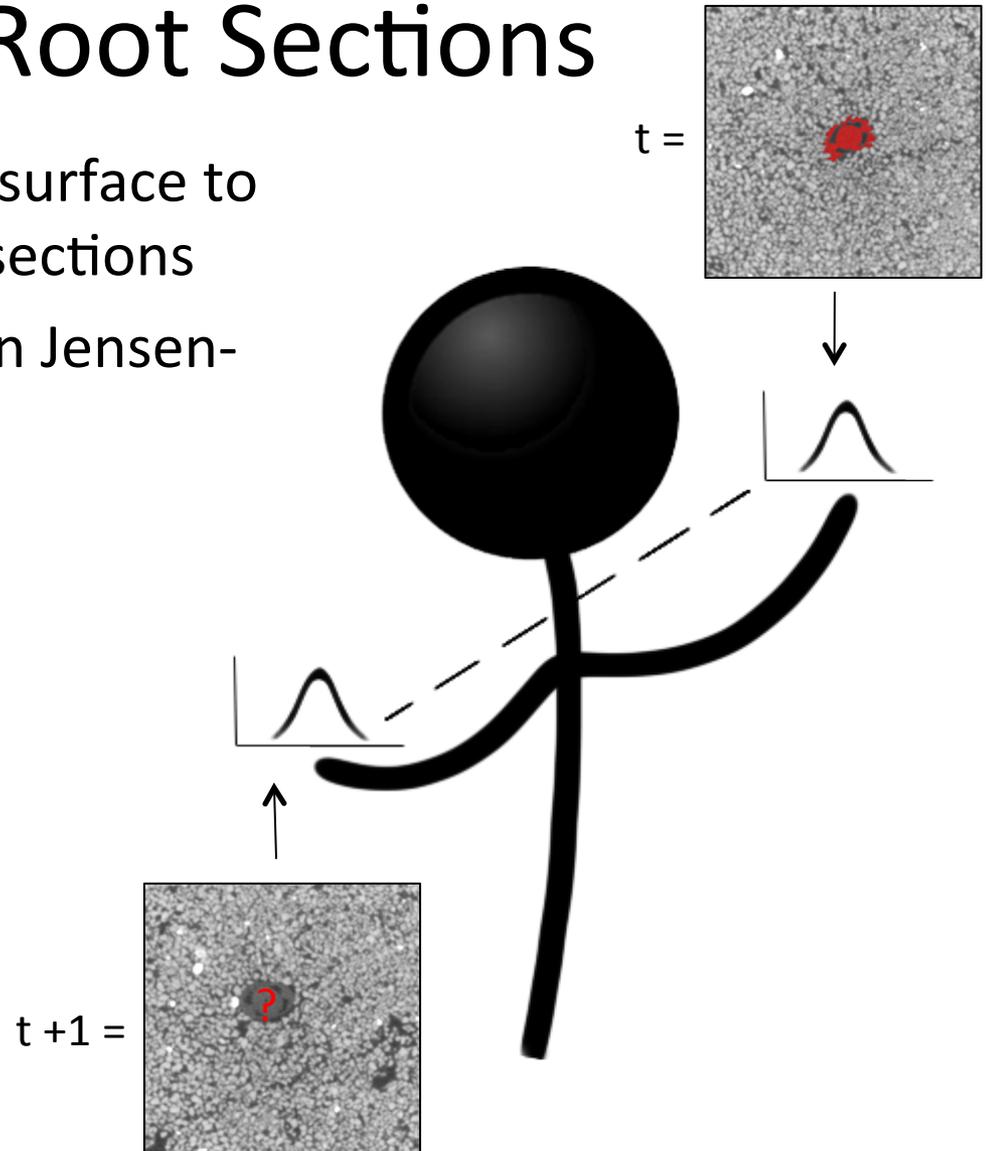
Example of Moving Interface





Tracking Root Sections

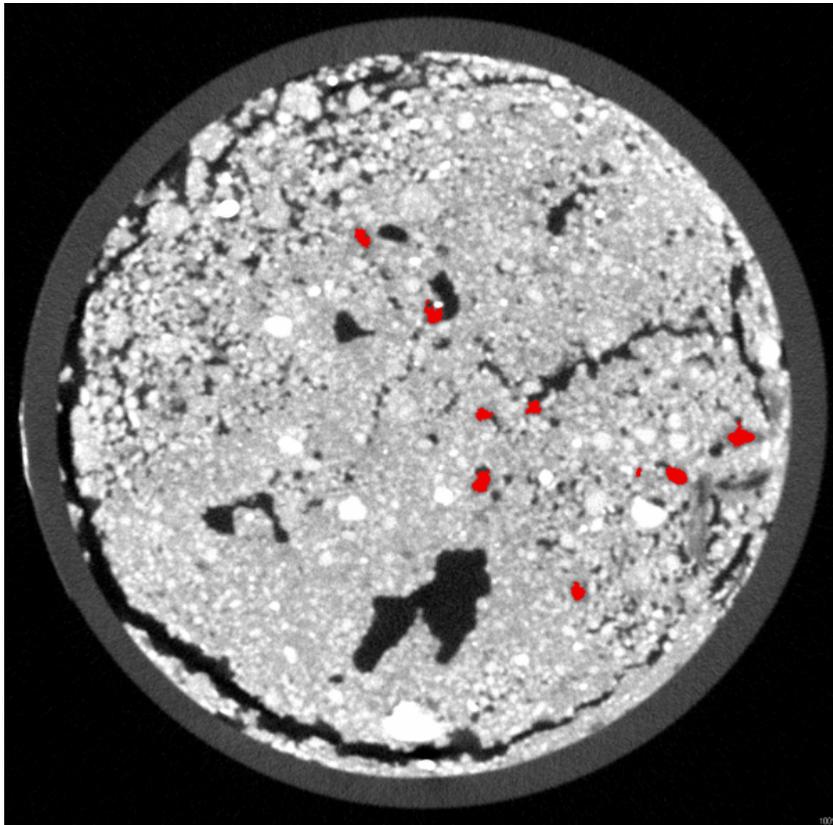
- Need a way to control the surface to find the boundary of root sections
- Statistical analysis based on Jensen-Shannon divergence
- Compute the distance between two probability density functions
 - Root model
 - Actual data in the current image



Adapting to Density Changes

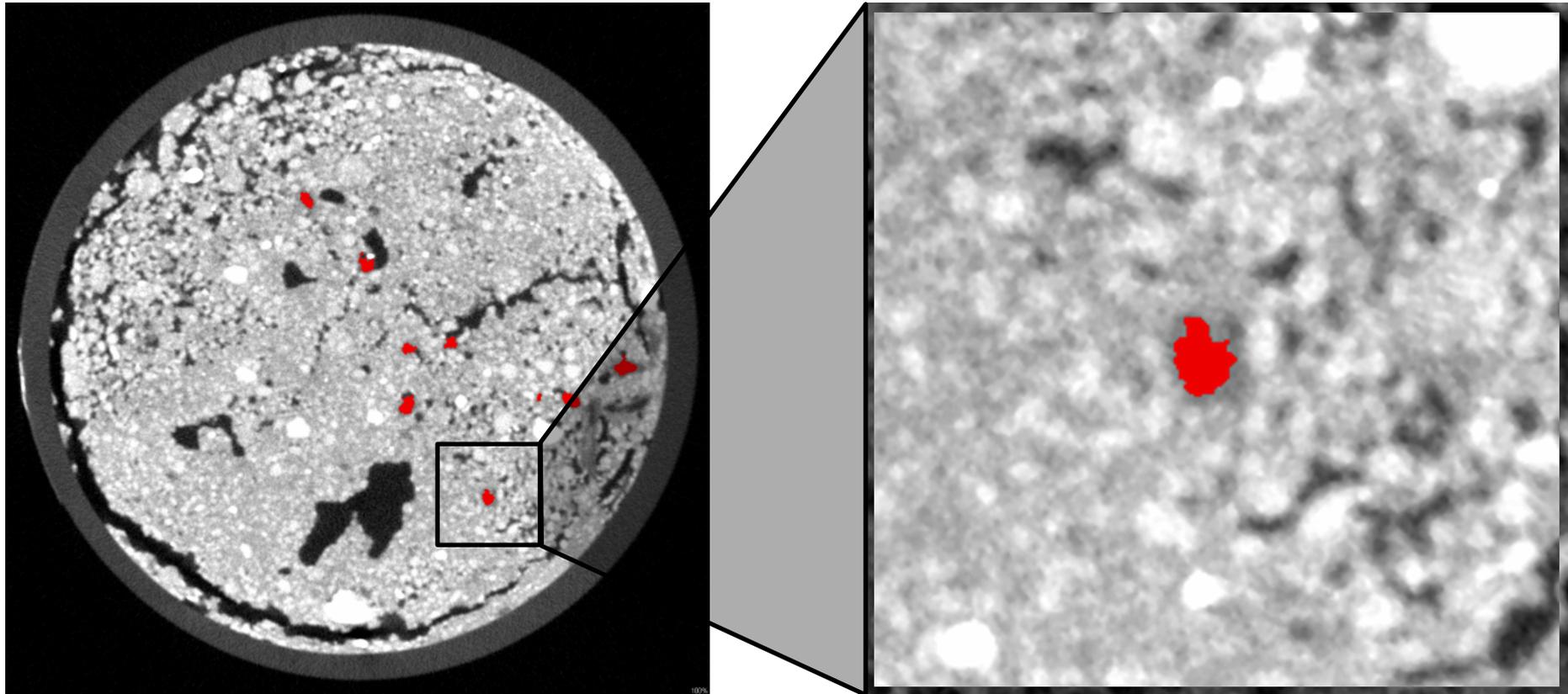
- Root grey level varies; we need to update the intensity model used as tracking proceeds
- The intensity distribution of the object found in image n becomes the model used to search image $n+1$ unless the object's shape is changing
 - Compute Fourier shape descriptor of root object boundaries in frame n and $n+1$
 - Update (ie model at $n+2 = \text{model at } n+1$) unless they are very different
- Avoids using a model that may be bleeding out

Tracking Root Sections

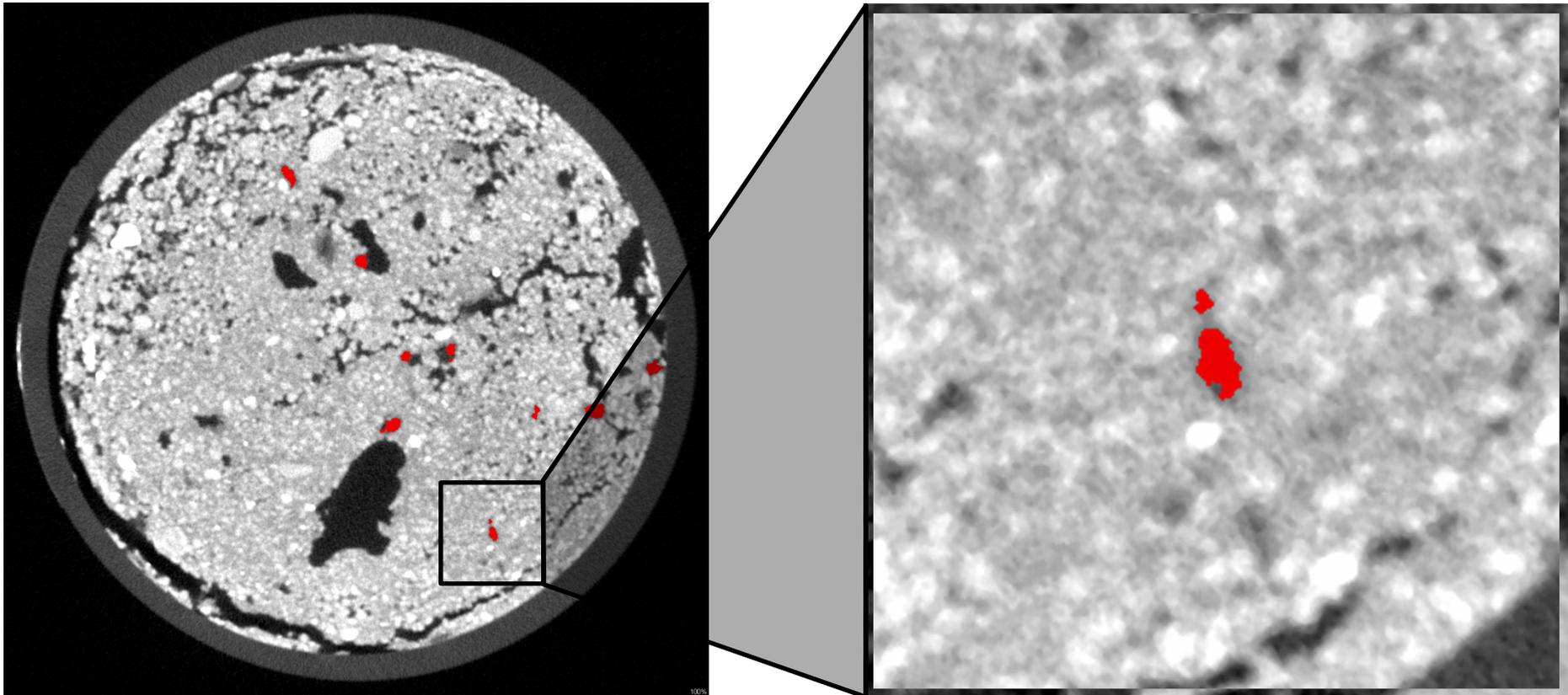


- Assume partial overlap between root cross-section
- Maintain root appearance model for each target
- Update root appearance model to adapt to changing intensity values
- Evolve level set function

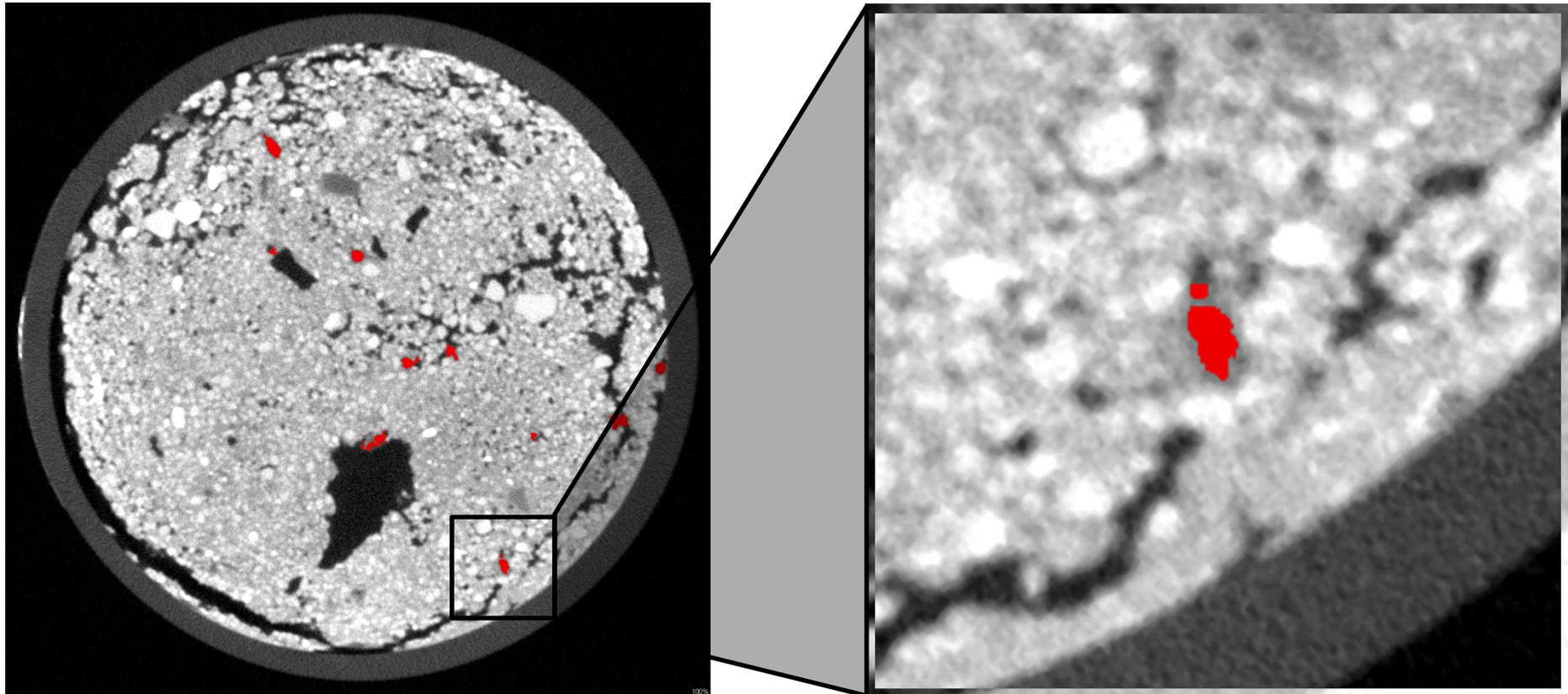
Tracking Root Objects



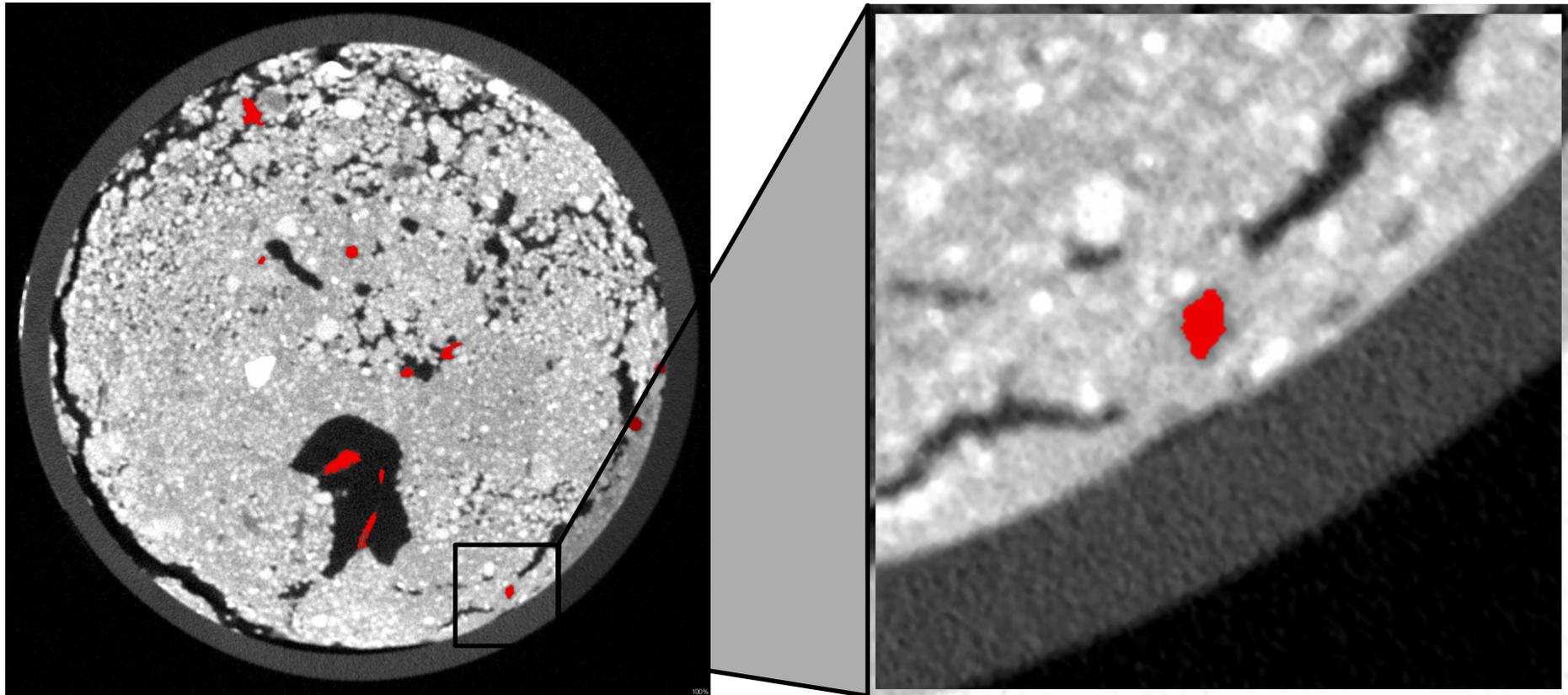
Tracking Root Objects



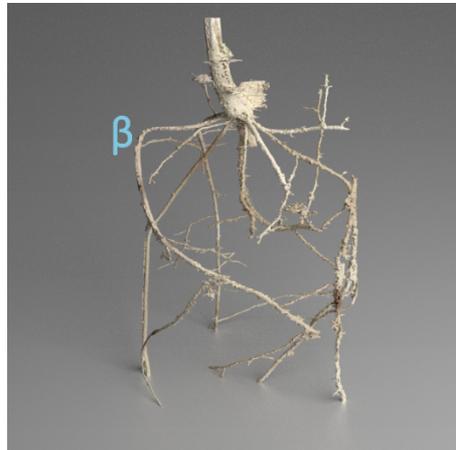
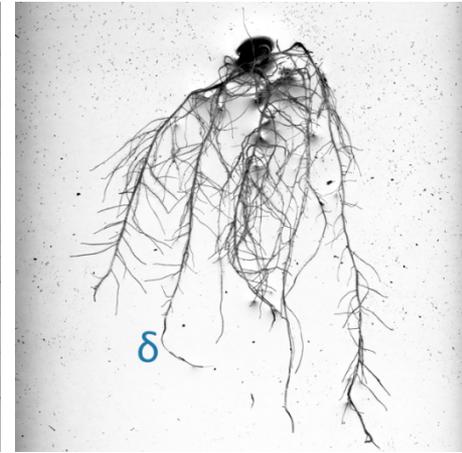
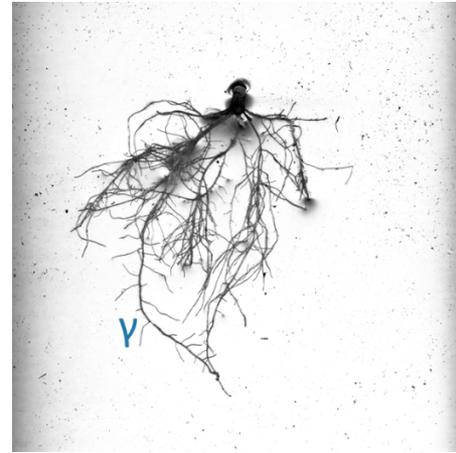
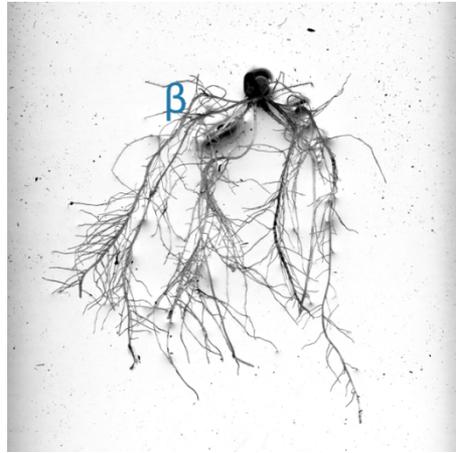
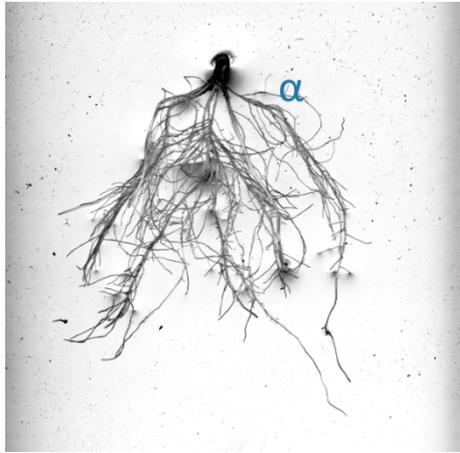
Tracking Root Objects



Tracking Root Objects



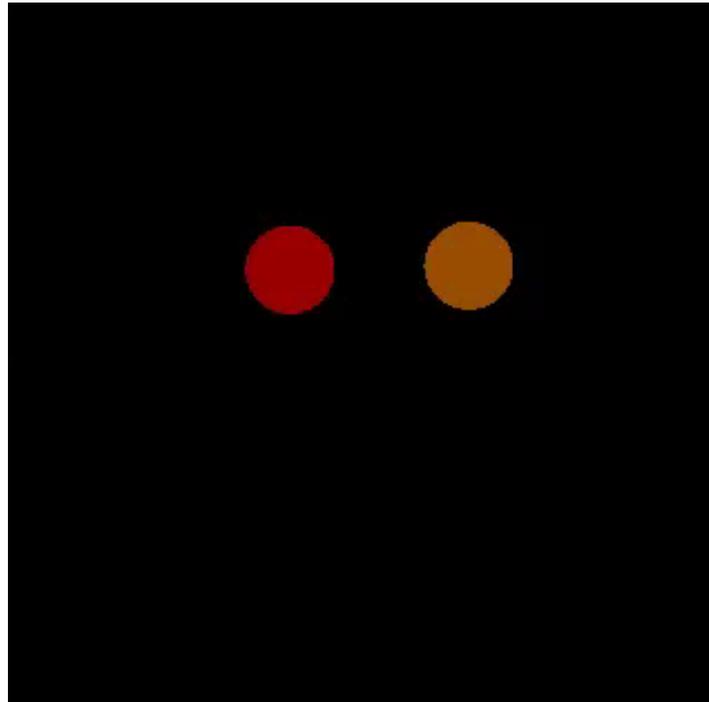
Root Systems of Wheat



Wheat in loamy sand/clay loam scanned at a resolution of 25.00 μ m

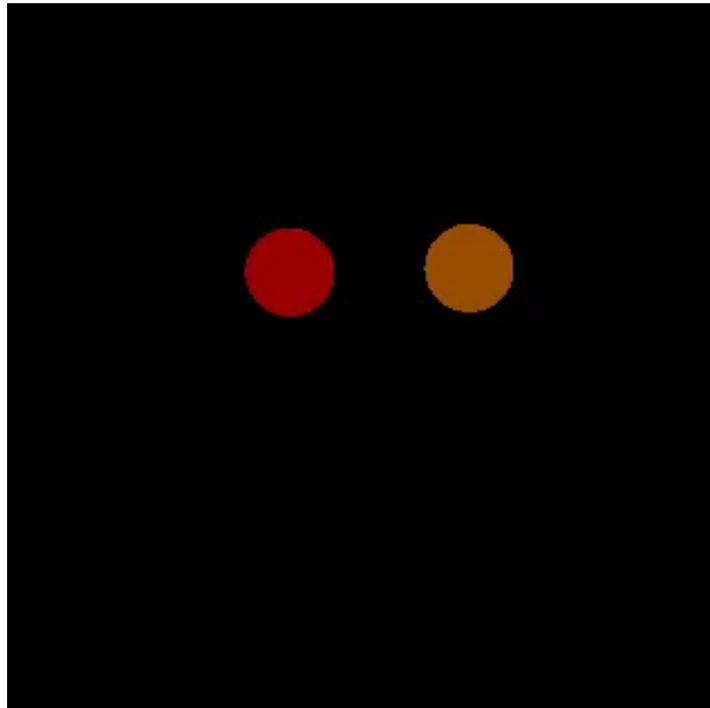
Multiple Roots: Multiple Trackers

- If roots do not touch, no problem
- If they do touch, each tracker will lay claim to pixels with intensity values matching their model, a race either could win



Adding Shape

- The evolving interface (root object) in image $n+1$ is matched to a set of points on the object found in image n
- A modified speed function allows, but penalises, growth away from the shape of the previous root section



Interacting Roots

