Climate Change Science Institute
AT OAK RIDGE NATIONAL LABORATORY

Neutron Imaging of Soil, Rhizosphere & Root Water Dynamics

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SNS
SPALLATION NEUTRON SOURCE

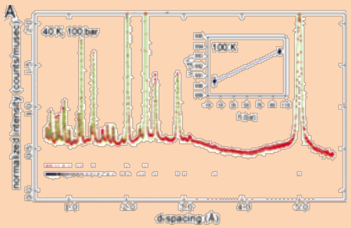
HFIR

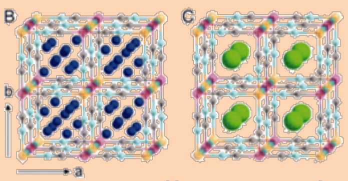
Outline

- Why Neutron Imaging?
- Neutron Sources at ORNL
- Examples of NI of Plants and Soils
  - Structure, Dynamics
  - Water, Water flux
  - Analysis and Modeling
- Advanced Imaging Techniques
- Future Directions

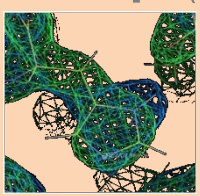
Neutrons Measure Structure (& Dynamics!)

Neutron Diffraction



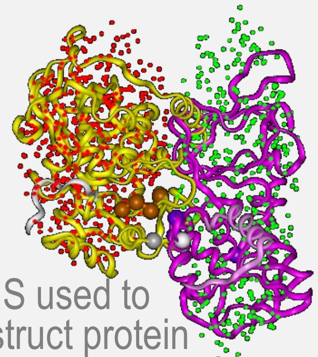


Neutron diffraction of D_2 sorption in $Cu3[Co(CN)_6]_2$

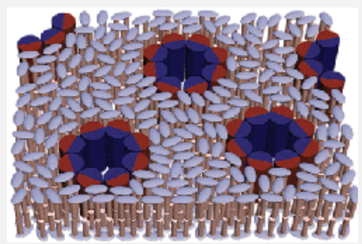


Nuclear and electronic density in enzymes

Neutron Scattering

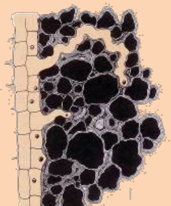


SANS used to construct protein kinase A (PKA)

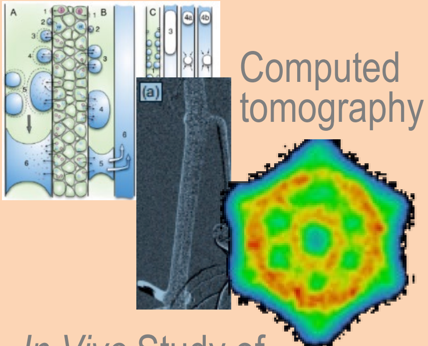


Characterization of biological membranes, colloids, porosity, etc.

Neutron Microscopy



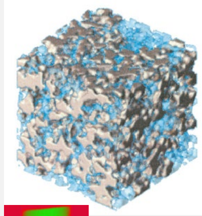
Soil-root interface (rhizosphere)



Computed tomography

In Vivo Study of Embolism Formation

Neutron Imaging



Hydraulic dynamics in plant-soil-earth systems

Ice/water segregation in permafrost structures

Inferred structure (indirect)

Direct structure

10^{-11} 10^{-9} 10^{-7} 10^{-5} 10^{-3}

Dimension (meters)

Kenneth W. Tobin, Director, Reactor & Nuclear Systems Division

Plant & Soil Neutron Imaging at ORNL

- Strong need to further understand complex processes ***in situ***
- Investigate soil and plant <u>responses</u> to external stimuli
- Temporal & spatial <u>dynamics</u> of water within soil and plant
- Understand soil-microbe-root <u>rhizosphere</u> dynamics
- Improve mechanistic <u>models</u> of roots, water, compounds and carbon fluxes
- Carbon sequestration, transformation, mineral <u>interactions</u>

Leaves Easy

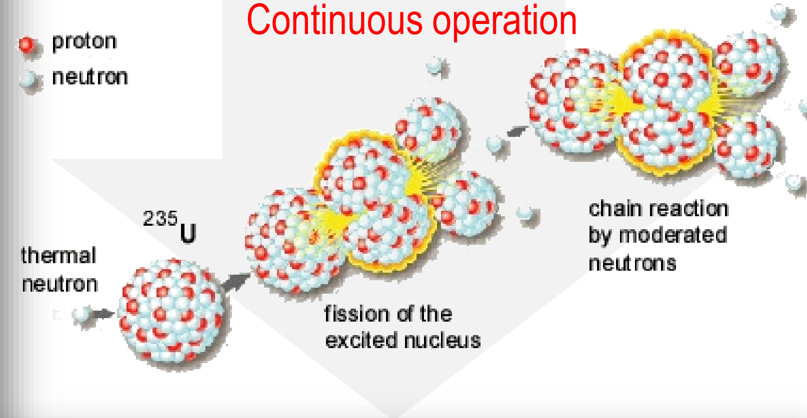
Soil & Roots Hard

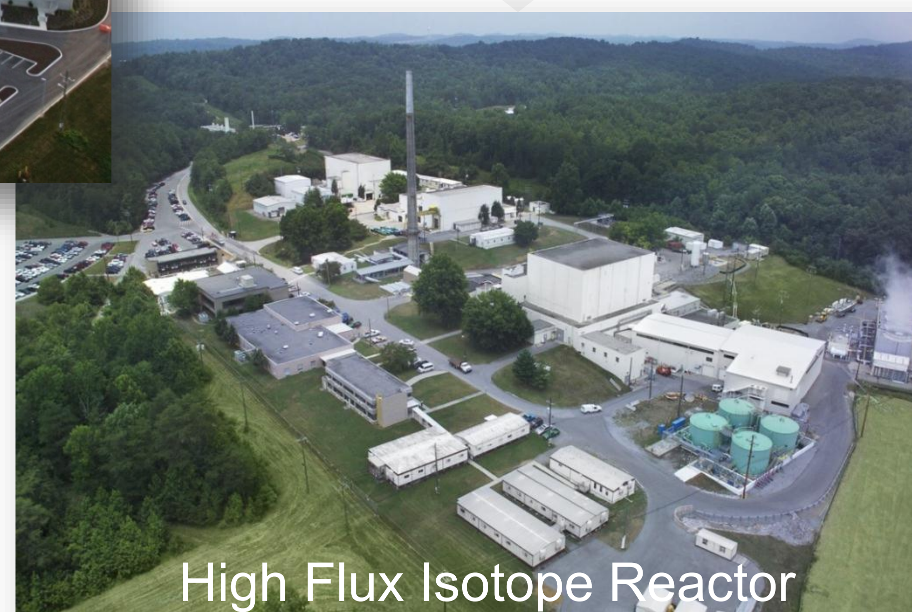
Anna Jensen
in Panama

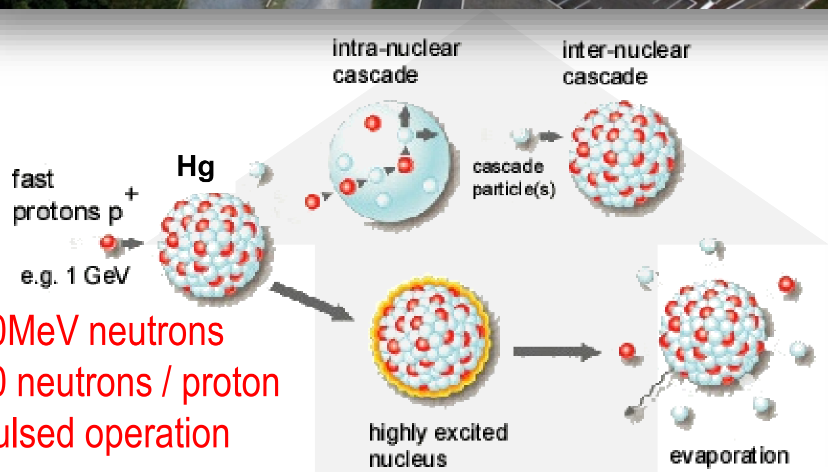
Rob Coulombe
in Dirt

Oak Ridge National Laboratory's SNS and HFIR are World Class Neutron Facilities

10MeV neutrons
1 neutron / fission
Continuous operation







30MeV neutrons
40 neutrons / proton
Pulsed operation

Why Neutrons?

- **Non-invasive**
- **Minimal damage to sample**
- **Penetrate deeply**
- **Heavy elements transparent**
- **Interact with light elements**

WATER!

Material at 1.54Å	Attenuation coefficient
H_2O	**5.39**
Al	0.10
SiO_2	0.29

- **Caveats - not field portable**
  - limited to small plants

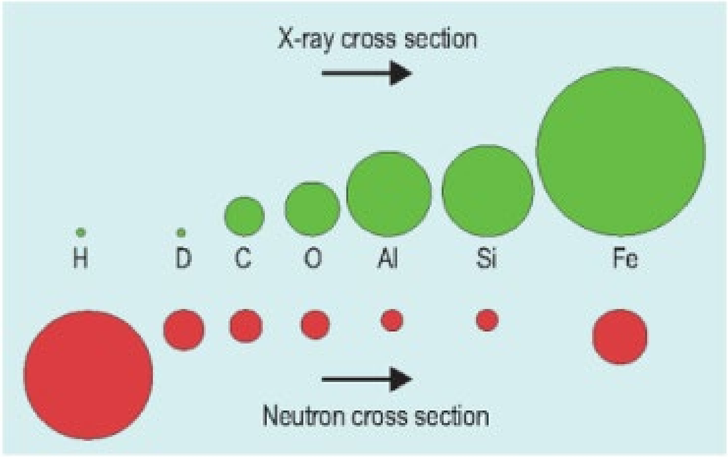
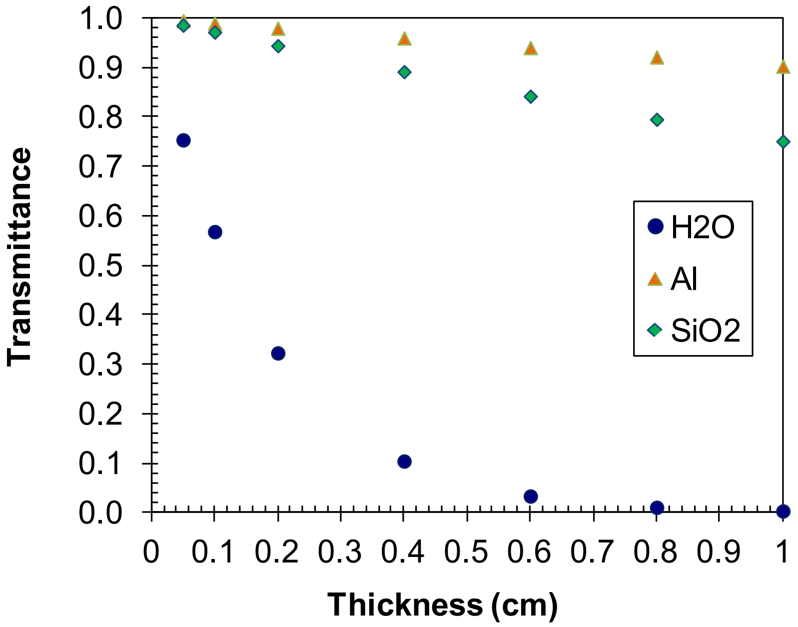
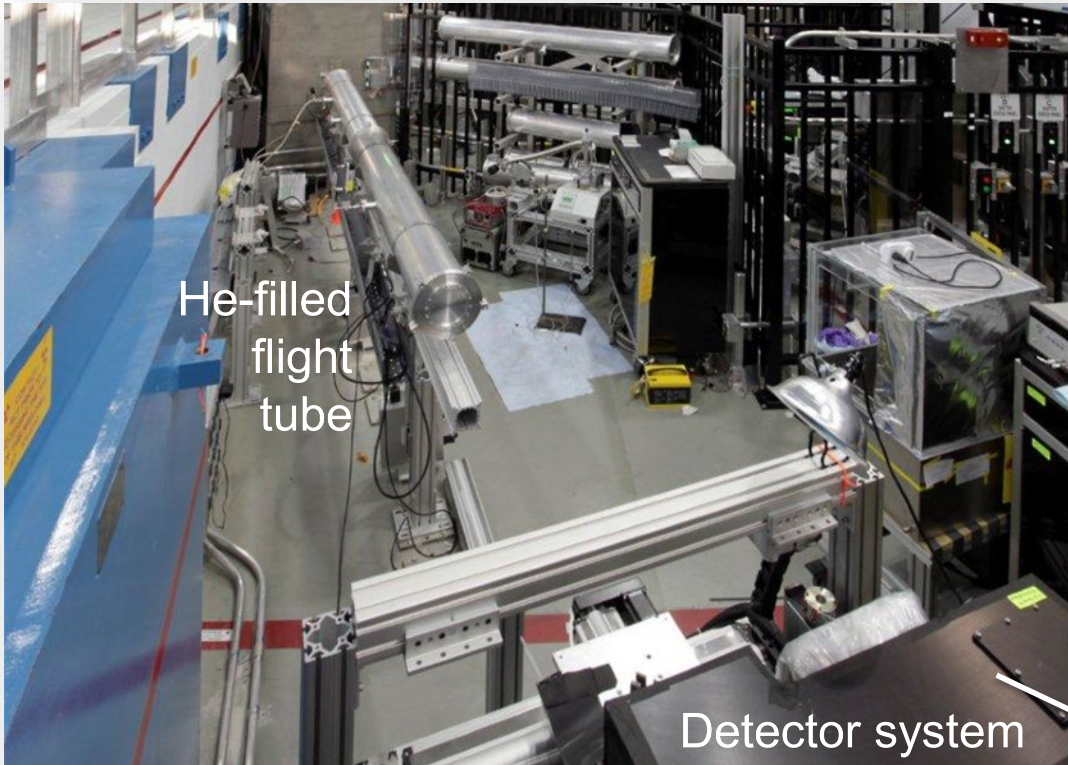


Fig. 2. Neutron and x-ray scattering cross-sections compared. Note that neutrons penetrate through Al much better than x rays do, yet are strongly scattered by hydrogen.

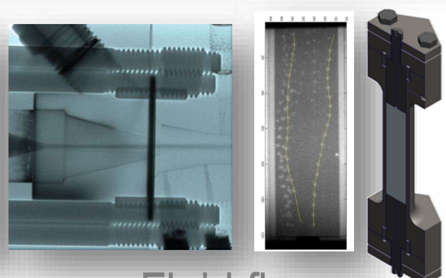


Imaging at ORNL HFIR CG-1D Beamline

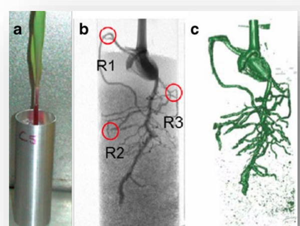


CG-1D Cold Neutron Imaging	
Beam Spectrum	Cold (1.8 Å < λ < 6 Å)
Spatial resolution	40 μm MCP, 50-200 μm LiF/ZnS scint.
L/D ratio	400-800
Flux on sample ($n/cm^2/s$)	1 x 10^7
Field of View	2 cm x 2 cm up to 8 cm x 8 cm in transmission

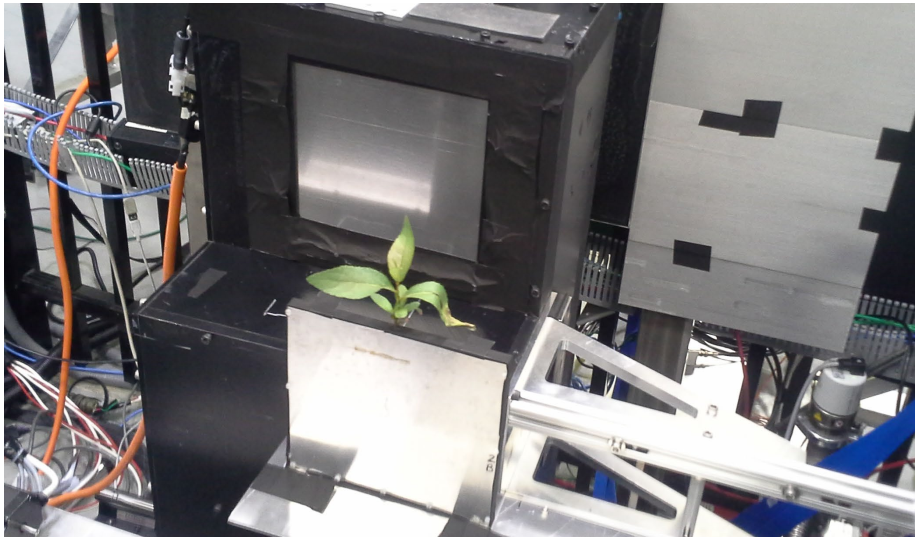
Structure



Fluid flow

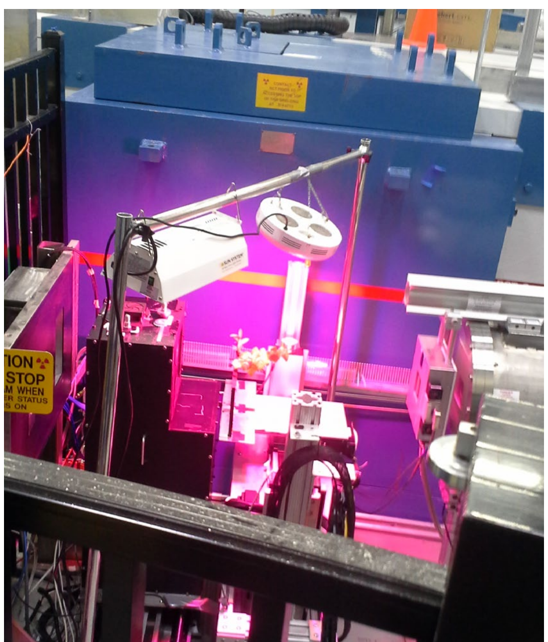


Materials

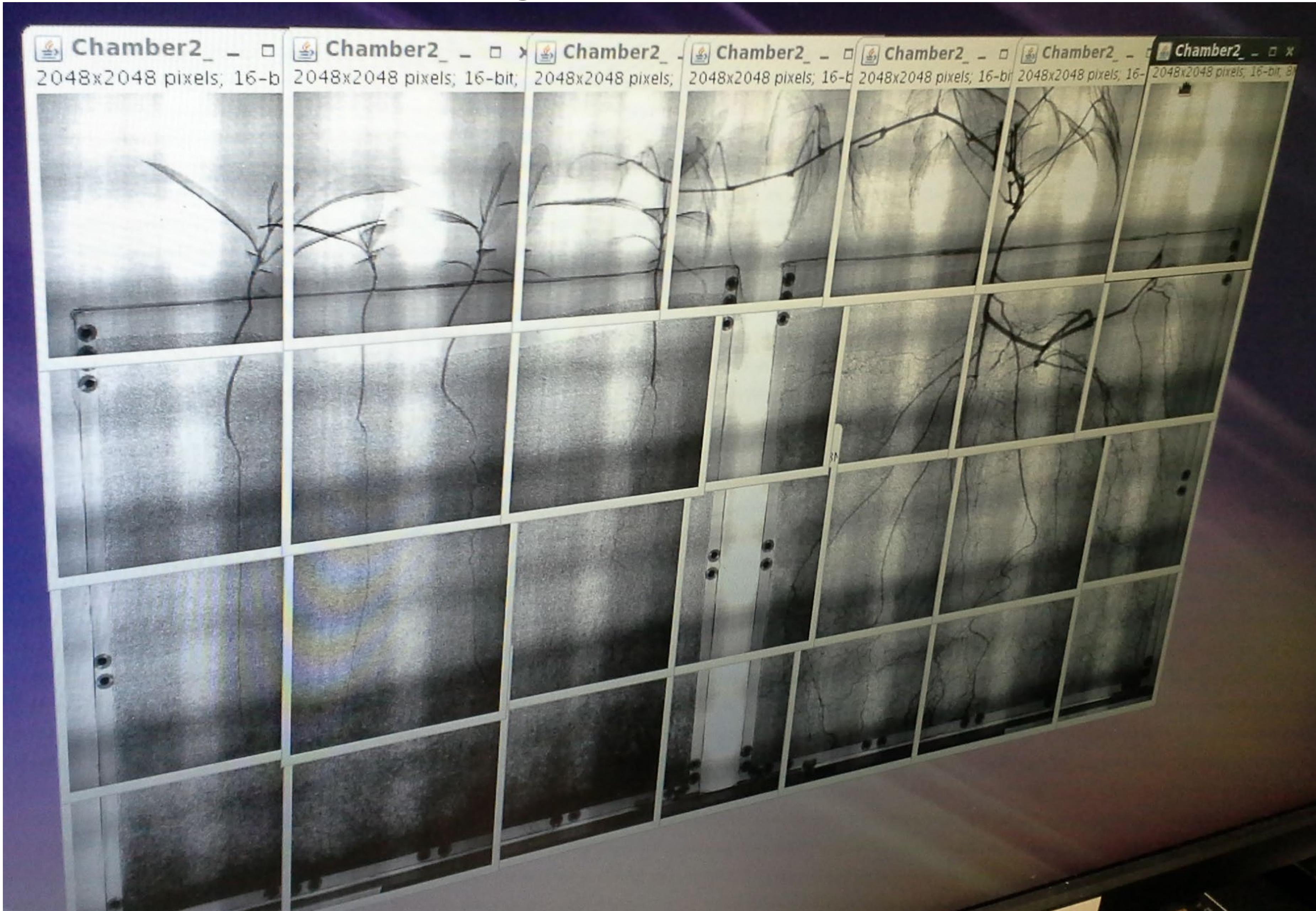


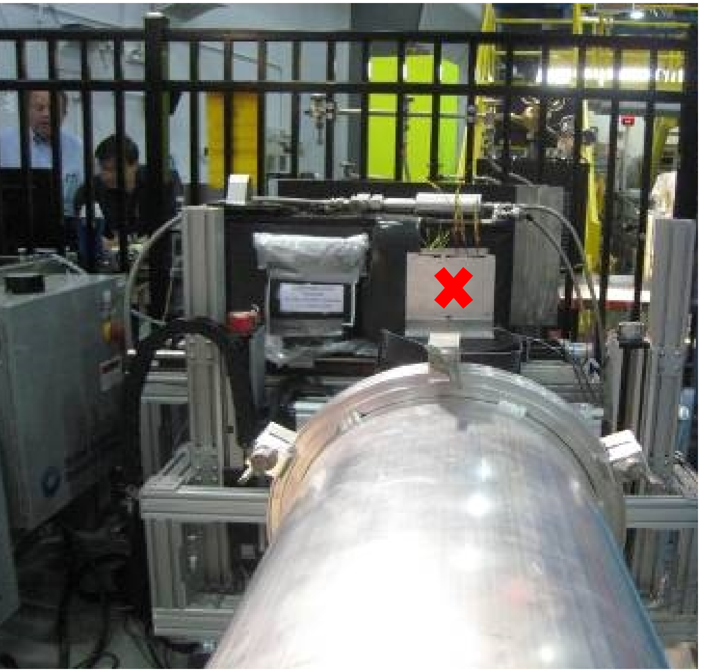
Plants in Beam Line

Populus and *Vitis*



Populus and *Vitis* radiographs - screenshot





Looking down neutron path to target area

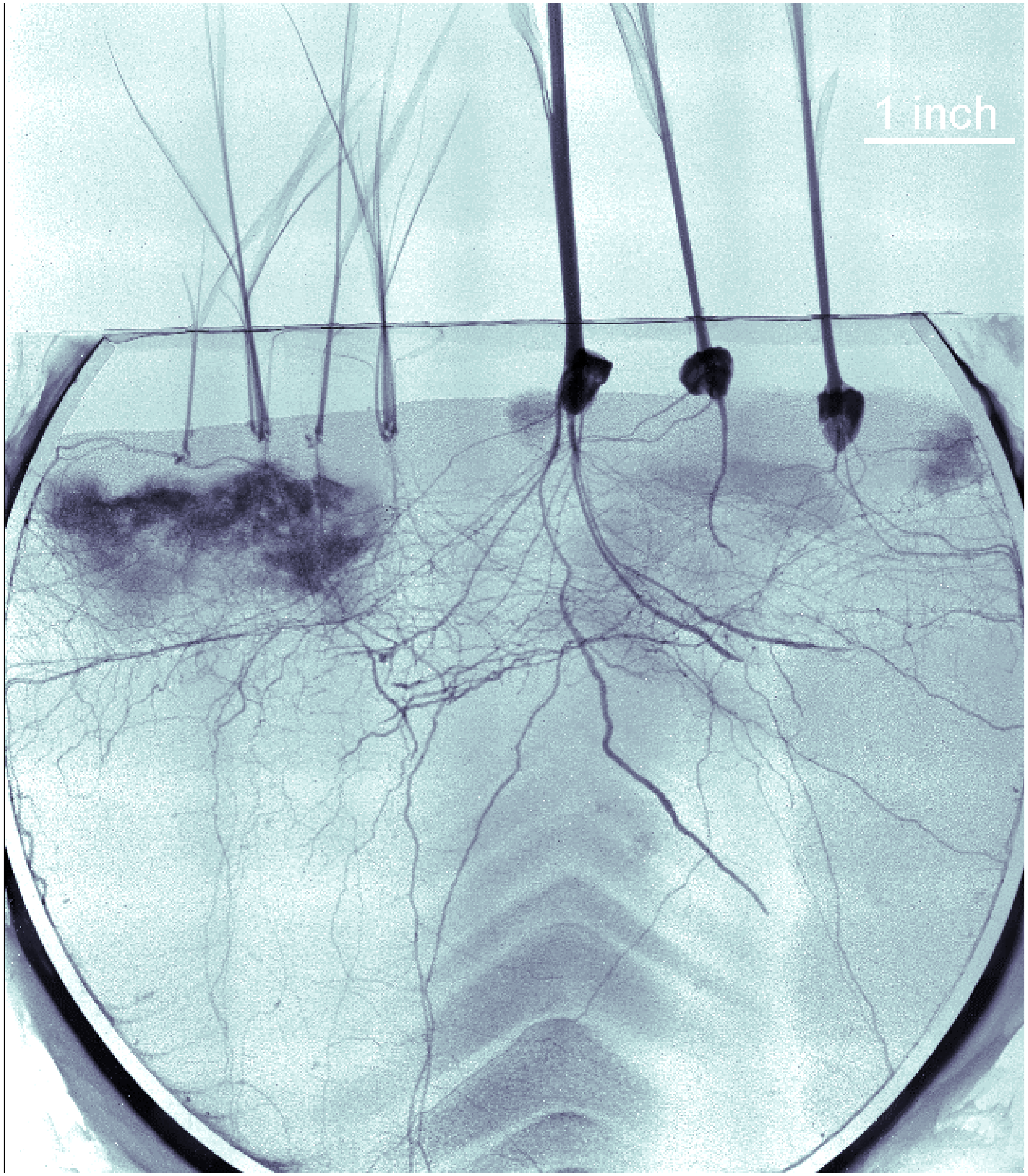
Root distribution, competition, symbiosis

<u>Composite Radiographs</u>

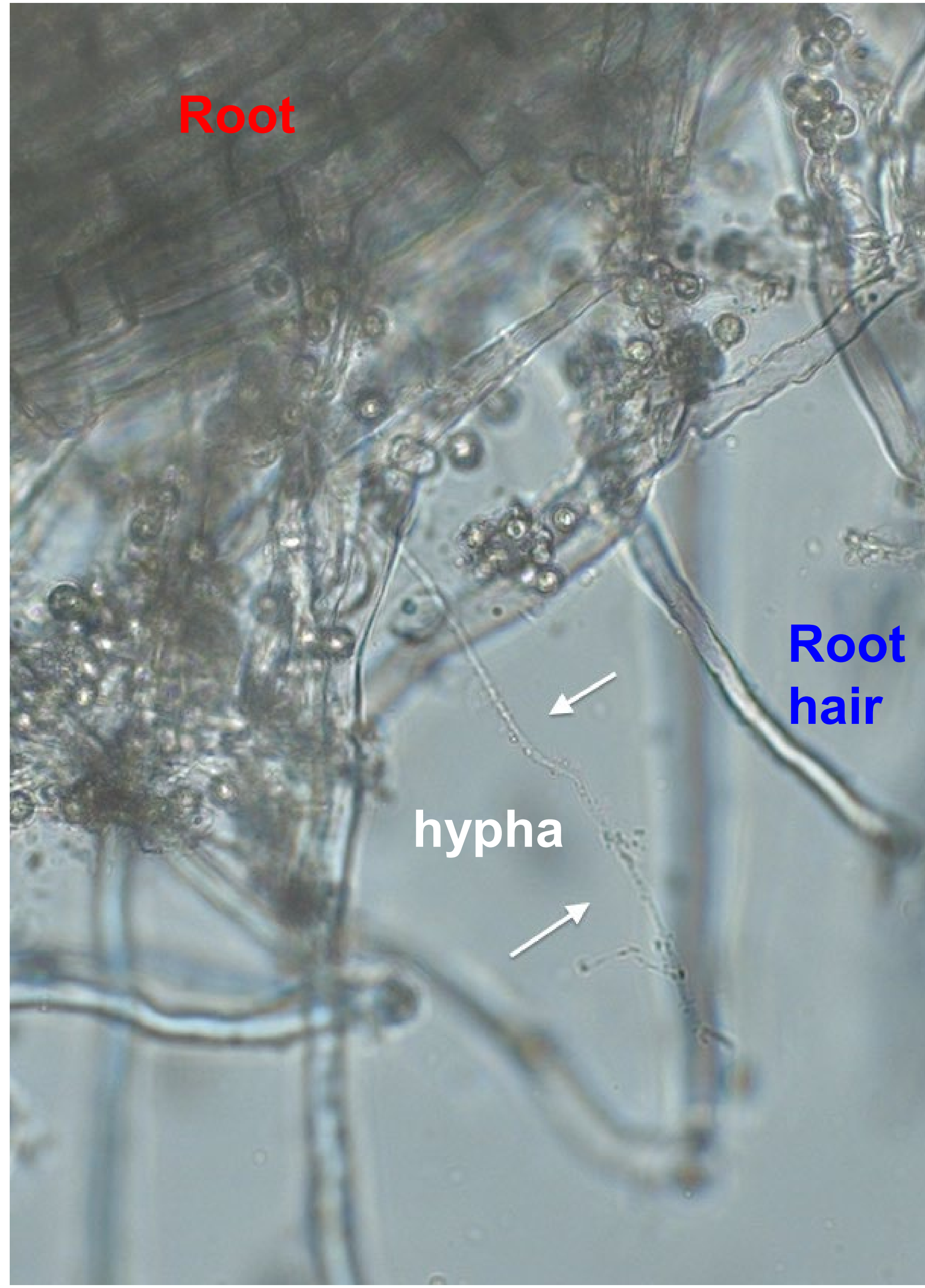
Coarse and fine root morphology and distribution readily visible

Fungal hyphal mass visible near roots of switchgrass, revealing substantial hydration of the rhizosphere

Triangular pattern in soil indicates varying water content & porosity due to separation of particle sizes as chamber was filled with sand



Switchgrass root, root hairs, symbiotic fungi



Importance of Scale!

Organ	Diameter
Root –	200 µm
Root hair –	12 µm
Fungal hypha-	3.5 µm

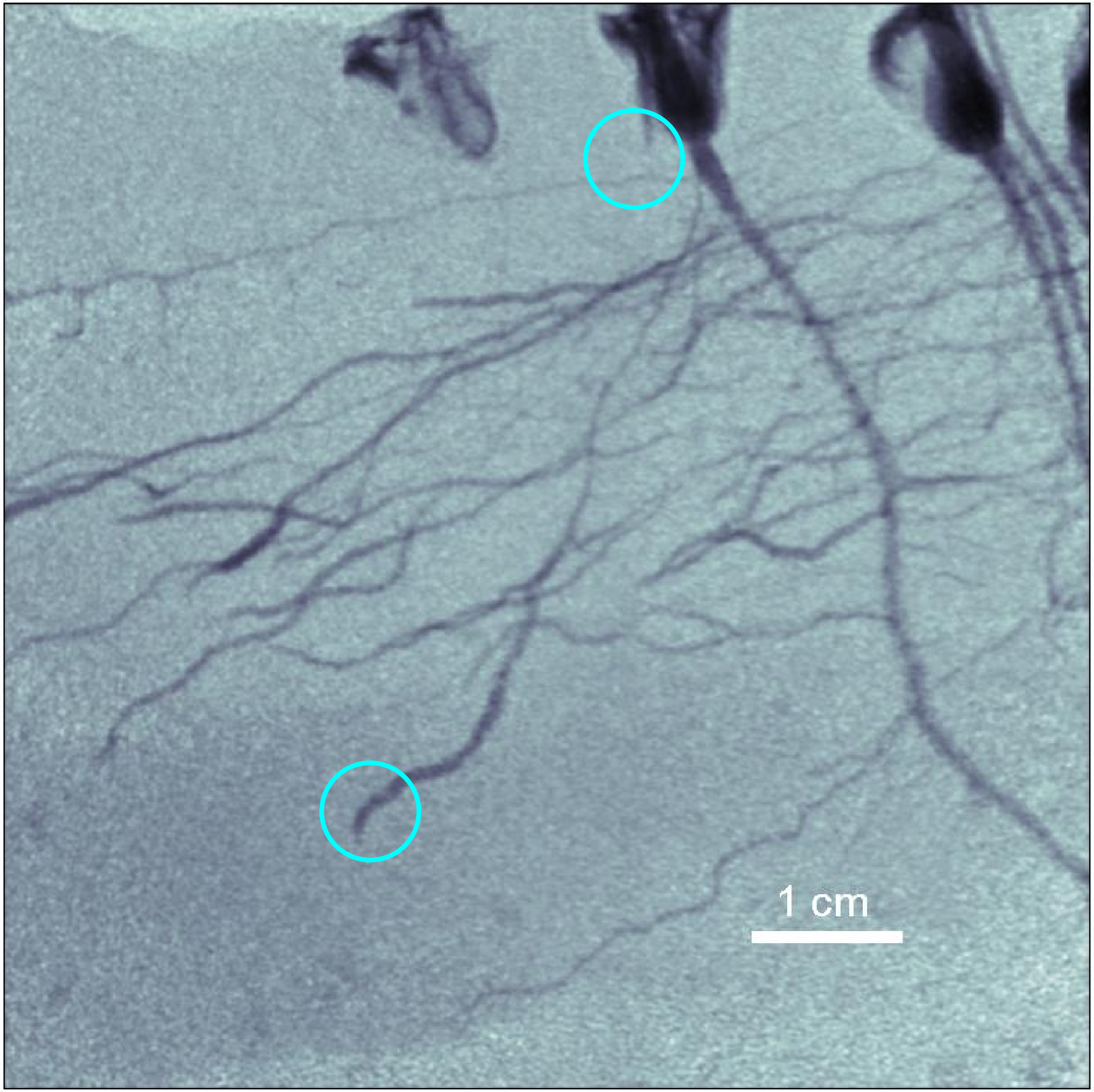
Differential interactions with soil surfaces, soil pores

Development of preferential pathways

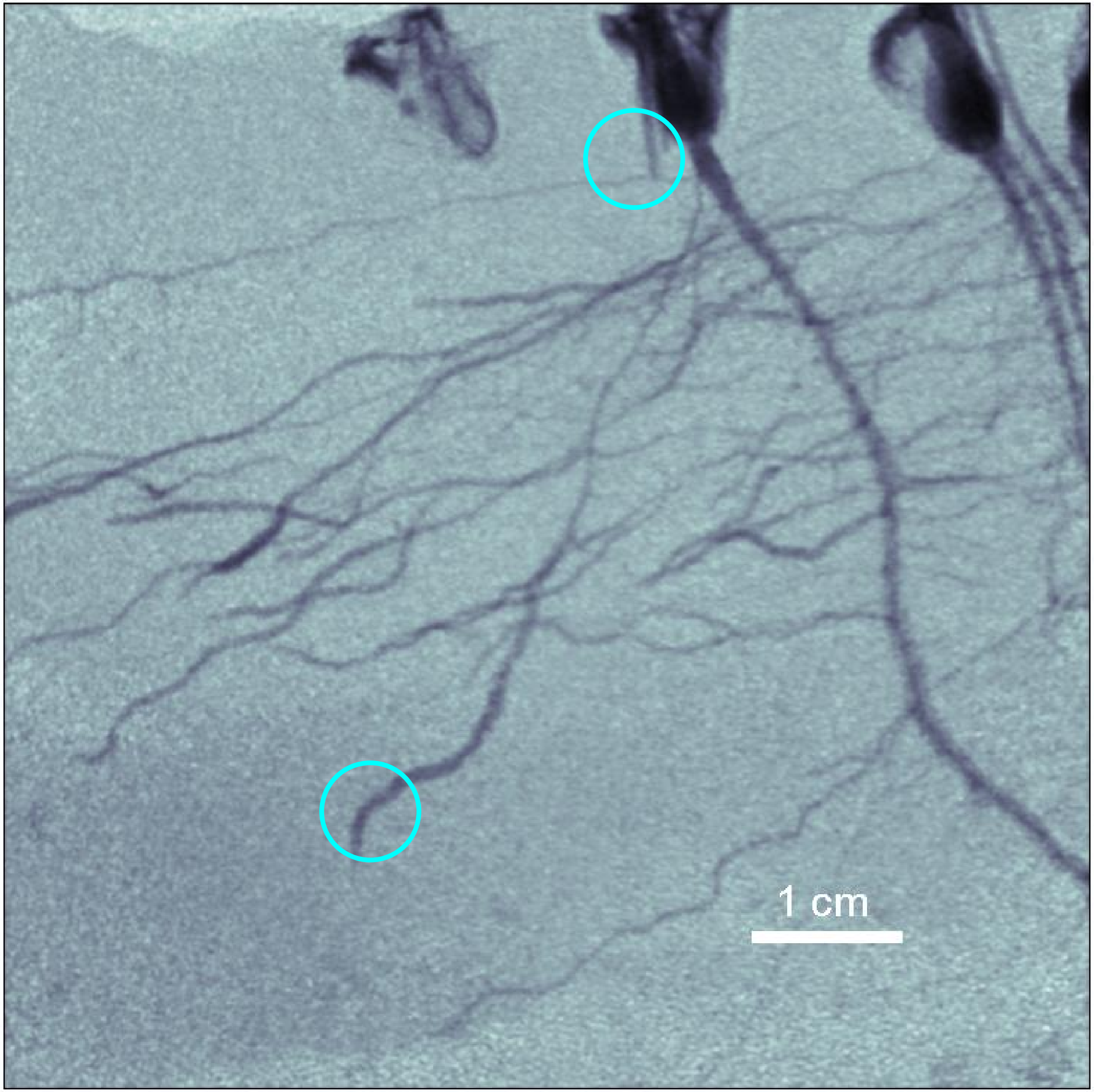
Importance during soil drying as gaps develop

How can we use neutrons to measure function?

Root Growth

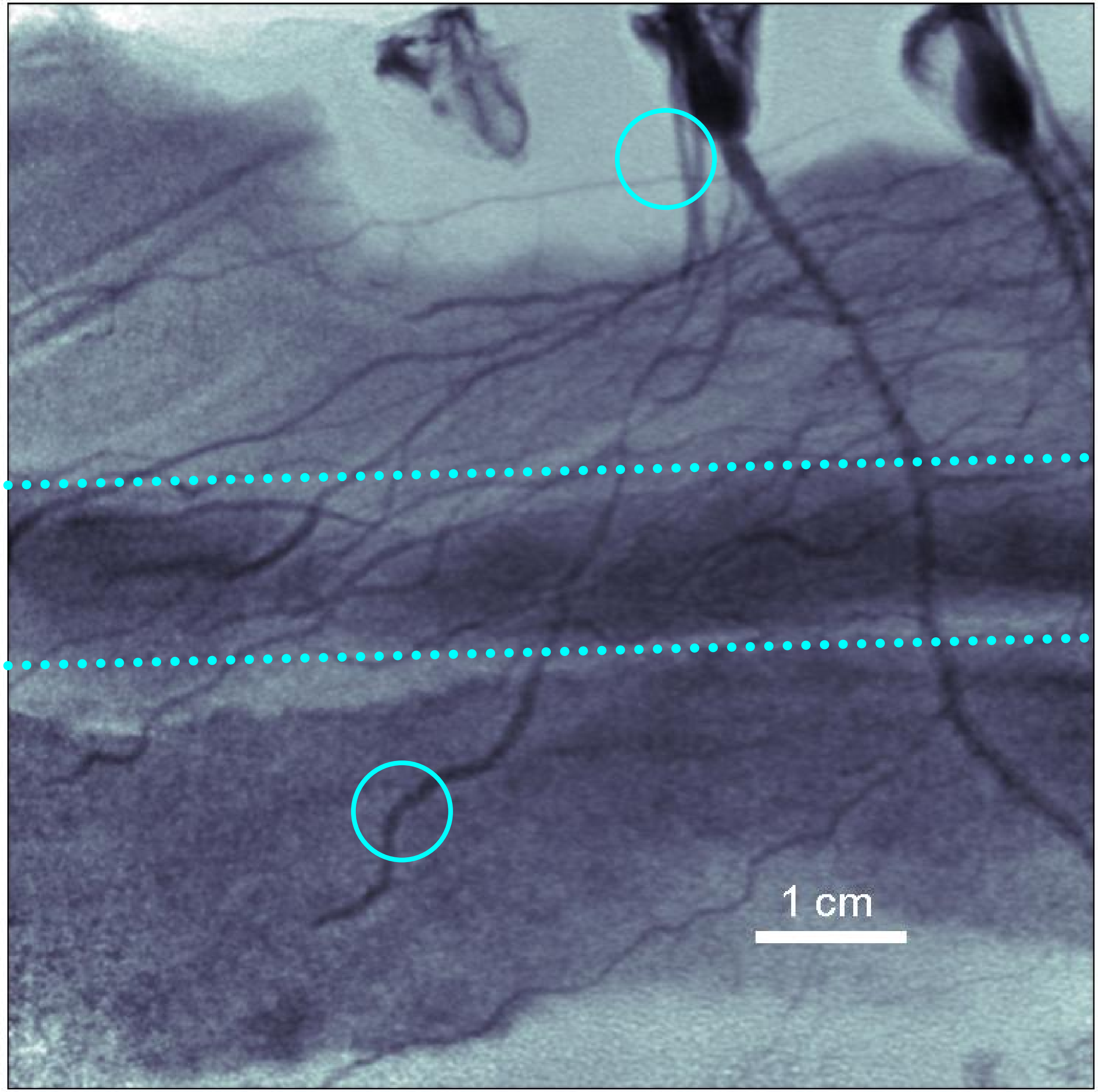


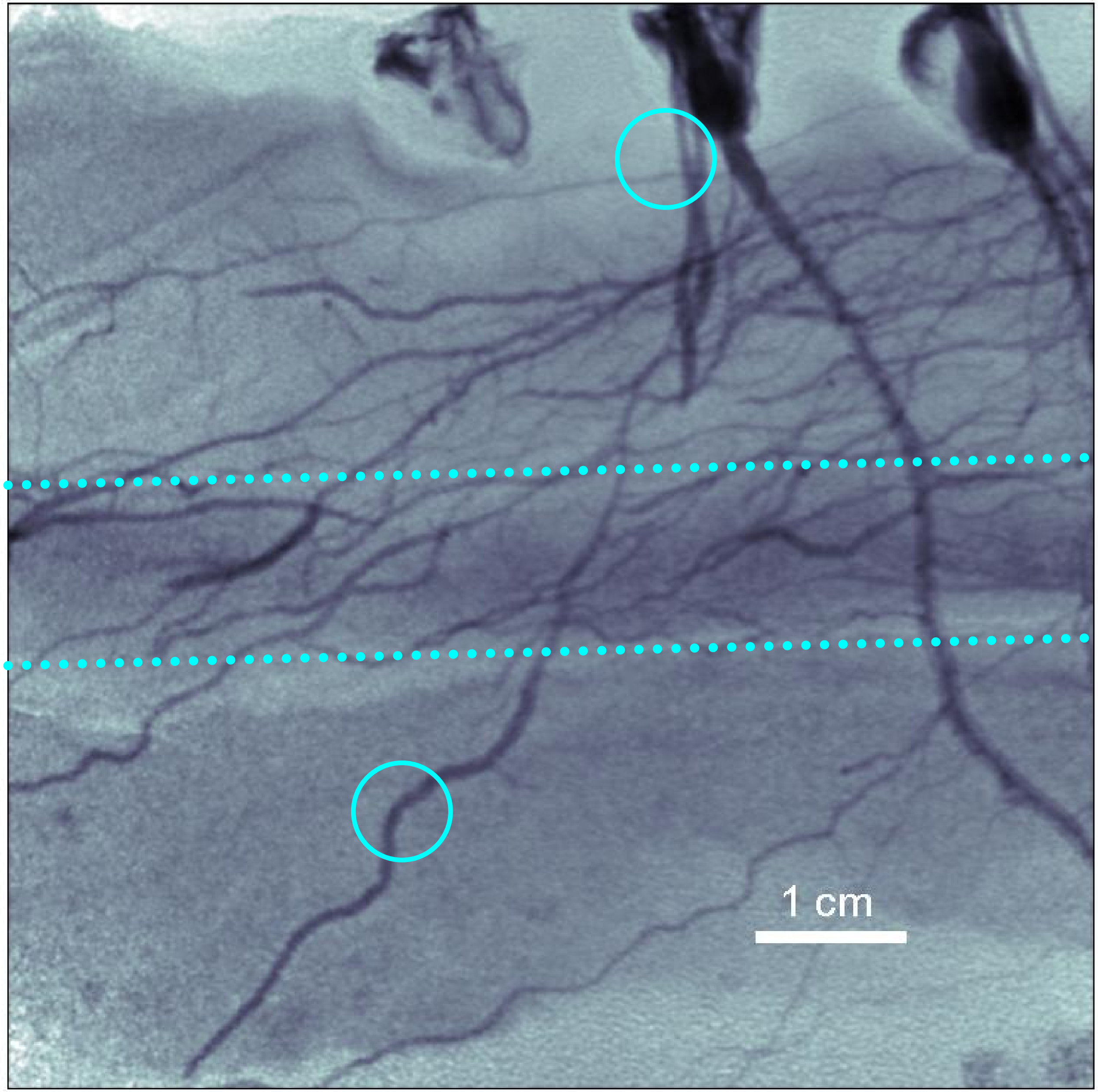
Root Growth



Pulse of water added

Fine sand
more roots
Different SWRC
Interface




1 cm

Dynamics - Root water uptake, root dehydration

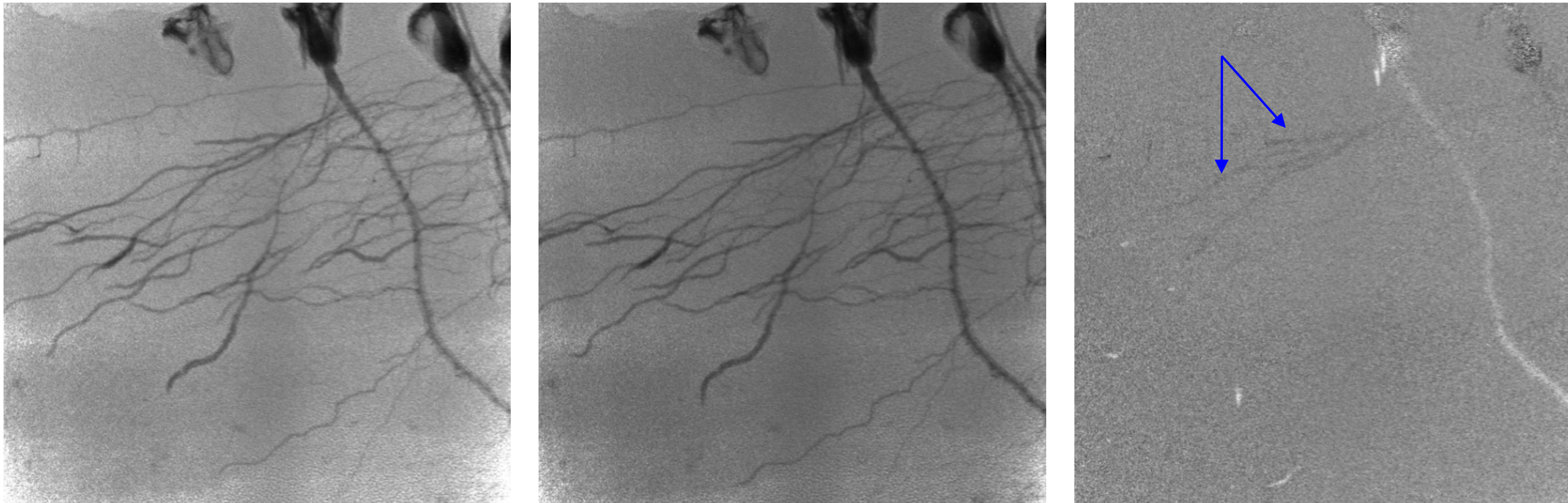


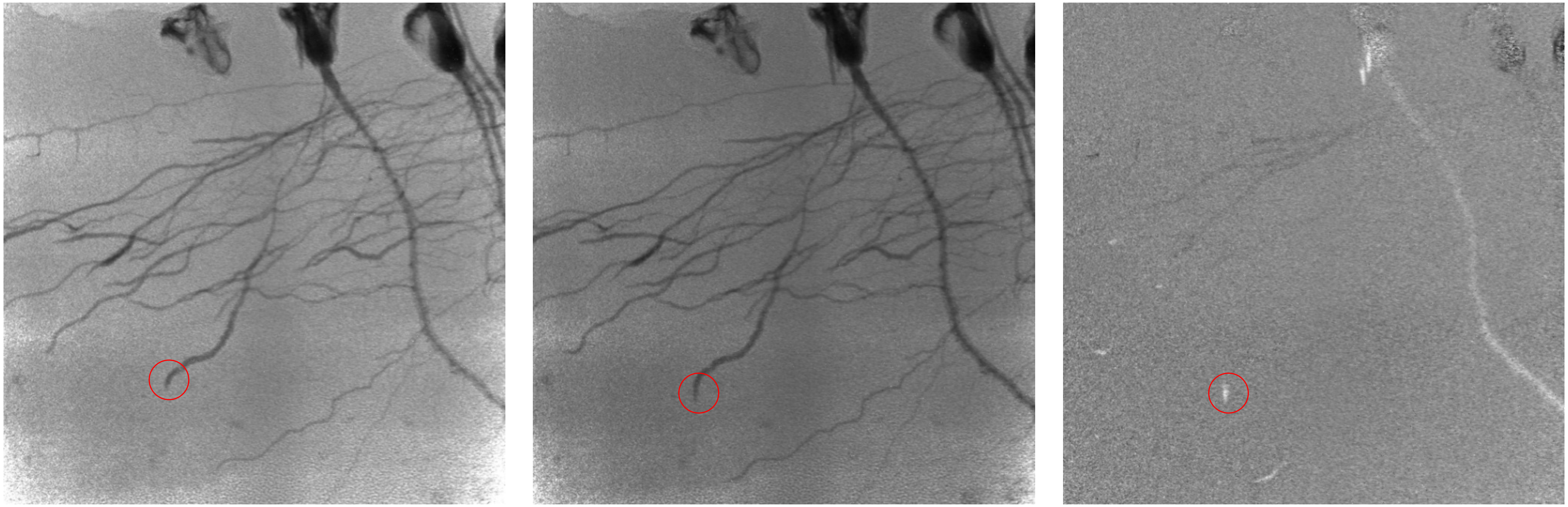
Image 1 → H_2O applied → Image 2 Image 1 – Image 2

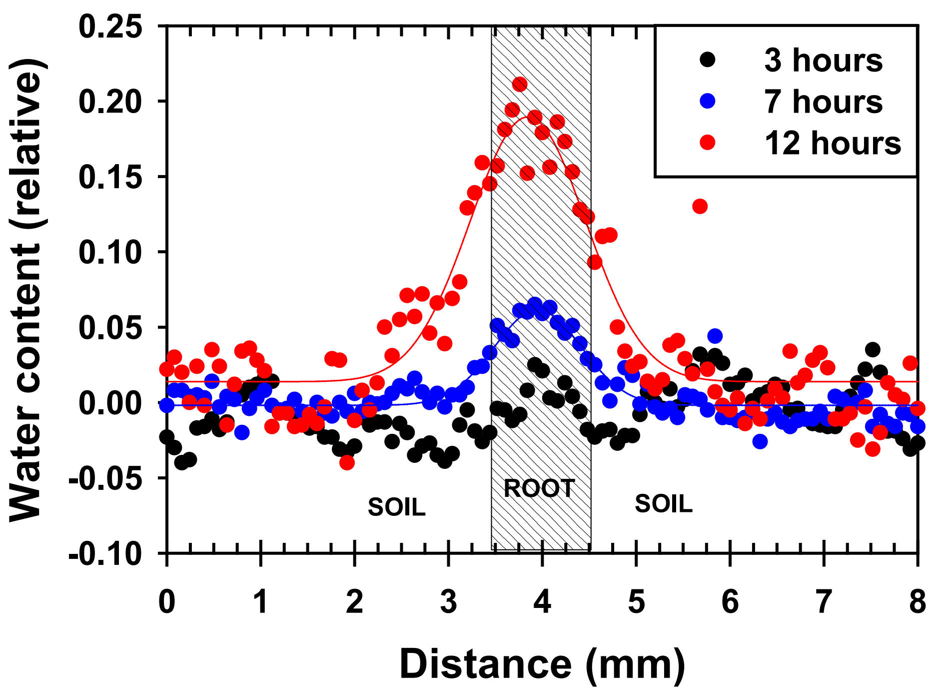
Pulse of H2O injected
at base of container

Difference between two images shows change in contrast (white) indicating water uptake and flow

Blue arrows shows opposite change in contrast (black) where water was removed from the system

New root hydration of rhizosphere





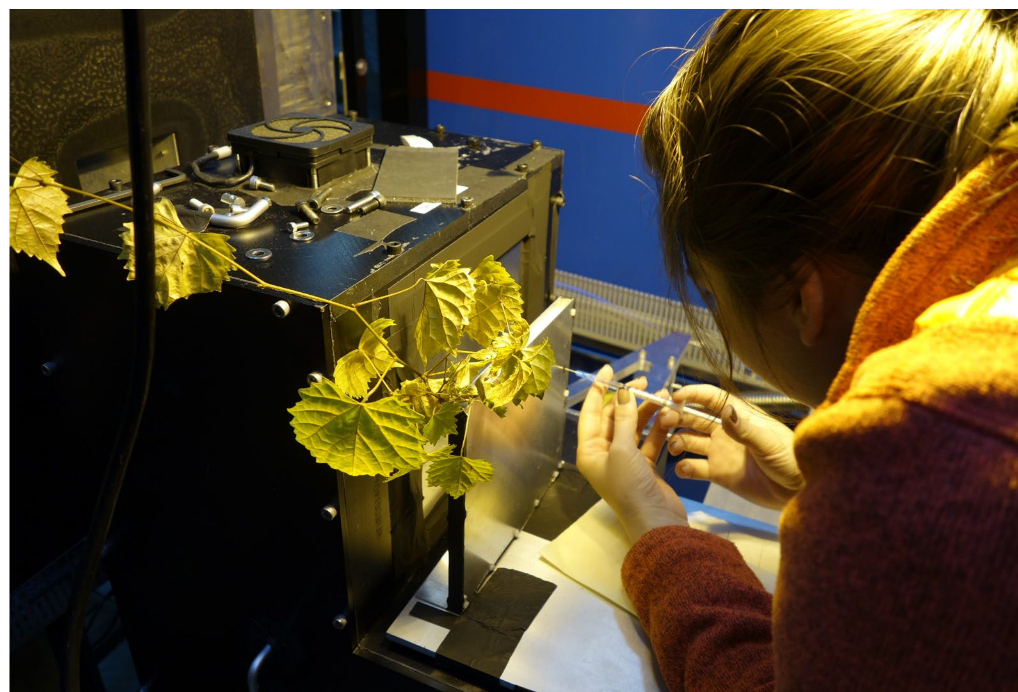
Rhizosphere development over time, root and hyphal water and exudate release

Glomalin, surfactants & organic matter change soil hydraulic, physical, chemical and biological properties – <u>Dynamics!</u>

Dynamics!

Injecting water near targeted roots

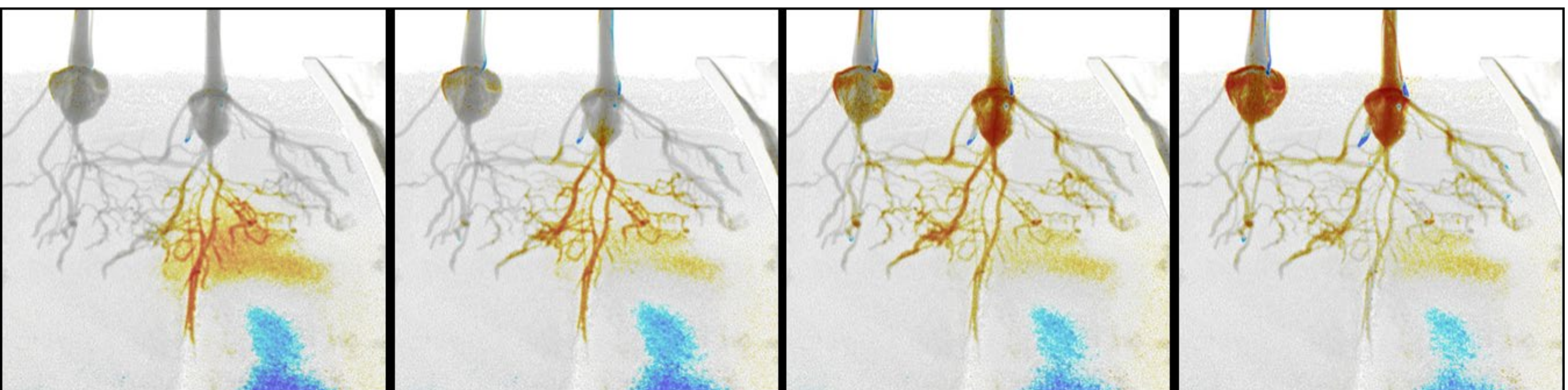
Track water vapor and saturated/unsaturated flow through the soil

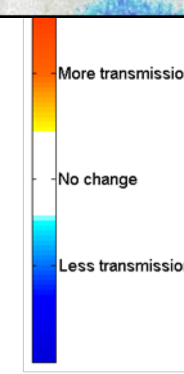


Water Uptake by Roots and Stem
- ability to assess individual roots in situ
- leverage contrast difference in D vs H attenuation

Time after 6 ml of D_2O injection 7 cm below deepest roots

100 min **200min** **460 min** **850 min**





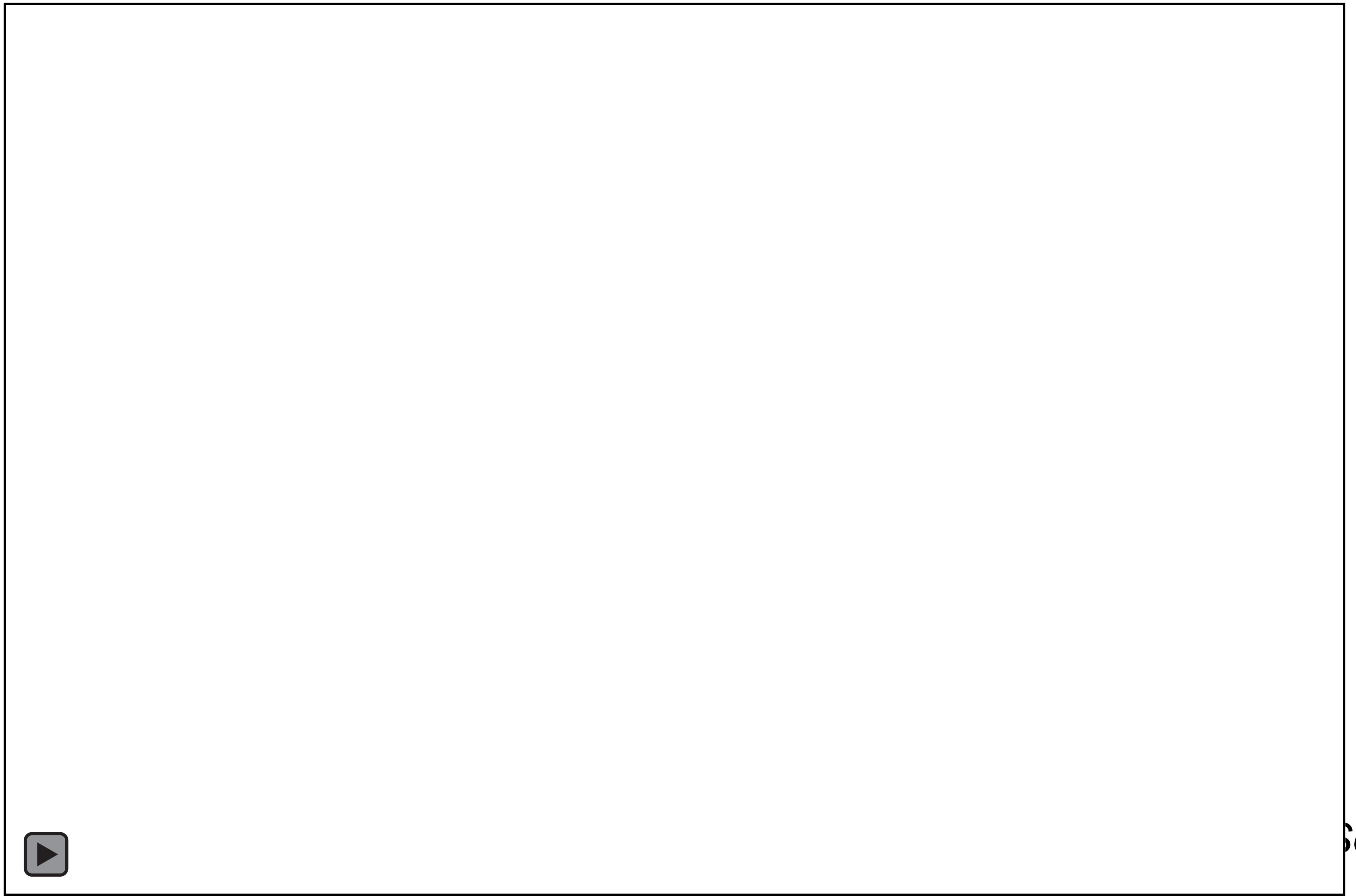
approximate
injection site

X

D2O uptake by maize over 14 h - HFIR

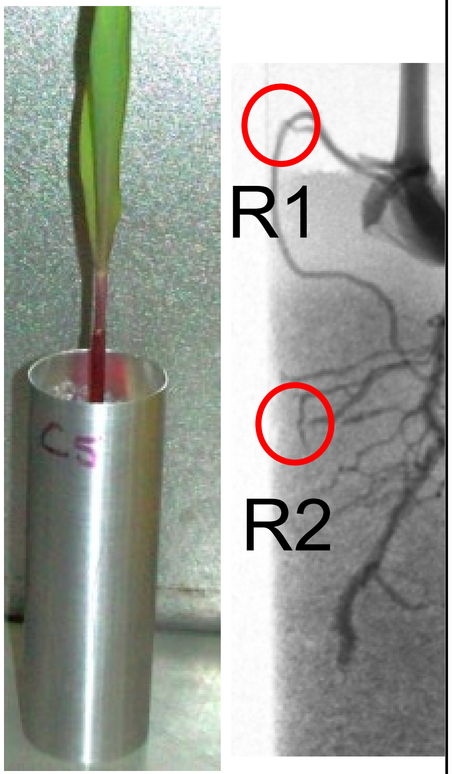
Water Uptake by Roots and Stem

- Pulse of deuterium (D_2O) added to surface of soil
- Uptake and replacement of existing water within the system illustrated by changes in contrast through time.



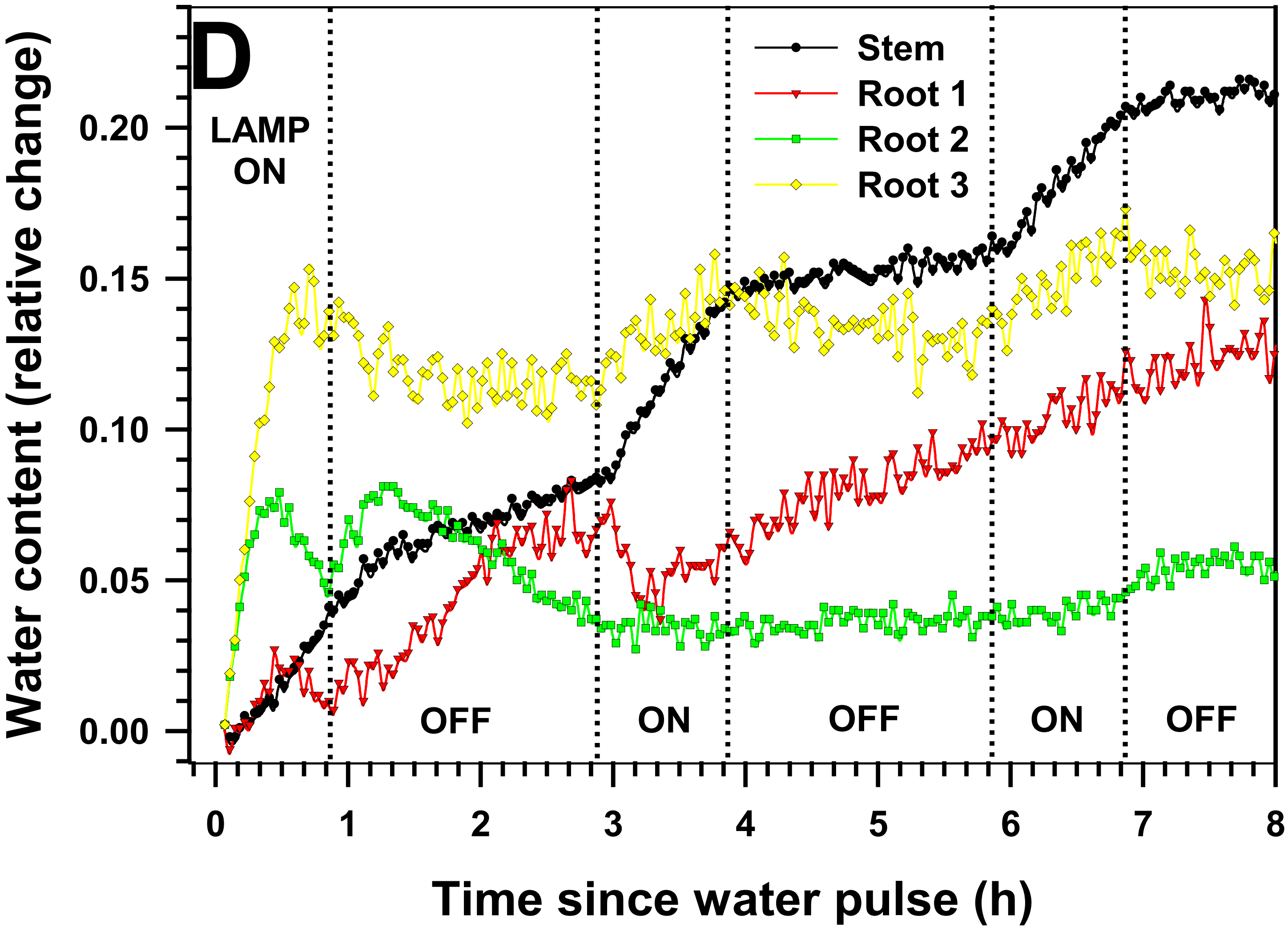
Soil (2013)

3D Tomography



10-d old maize seedl

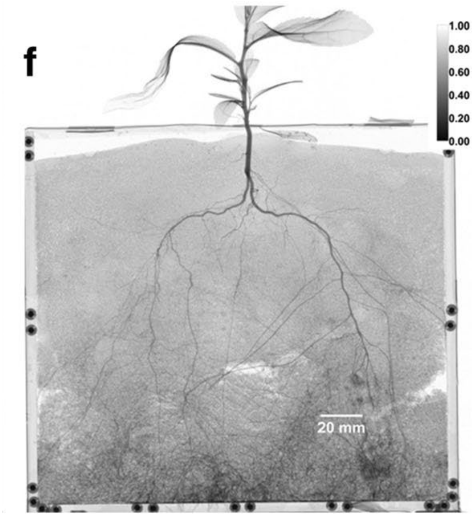
- Neutron radiograph at 100 µm pixel resolution illustrating root distribution (0.2-1.6 mm)
- Track water flow through three roots

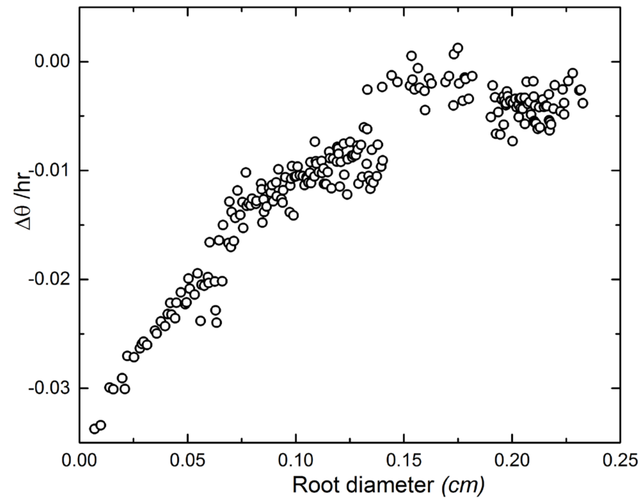


Timing of water uptake and transport illustrating impact of solar radiation on rate of water flux in stem, and ~0.5 mm first and second order roots.

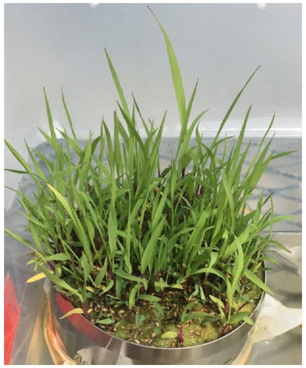
Warren et al. Plant and Soil (2013)

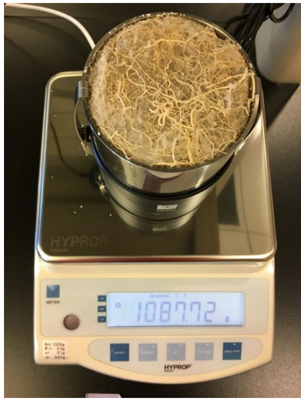
Quantifying and modeling water movement and extraction patterns



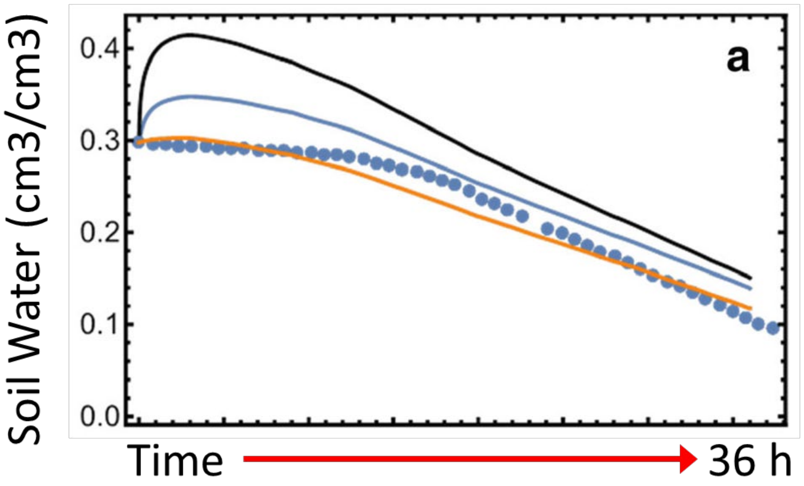


Smaller roots, greater water extraction rates, but also greater dehydration rates





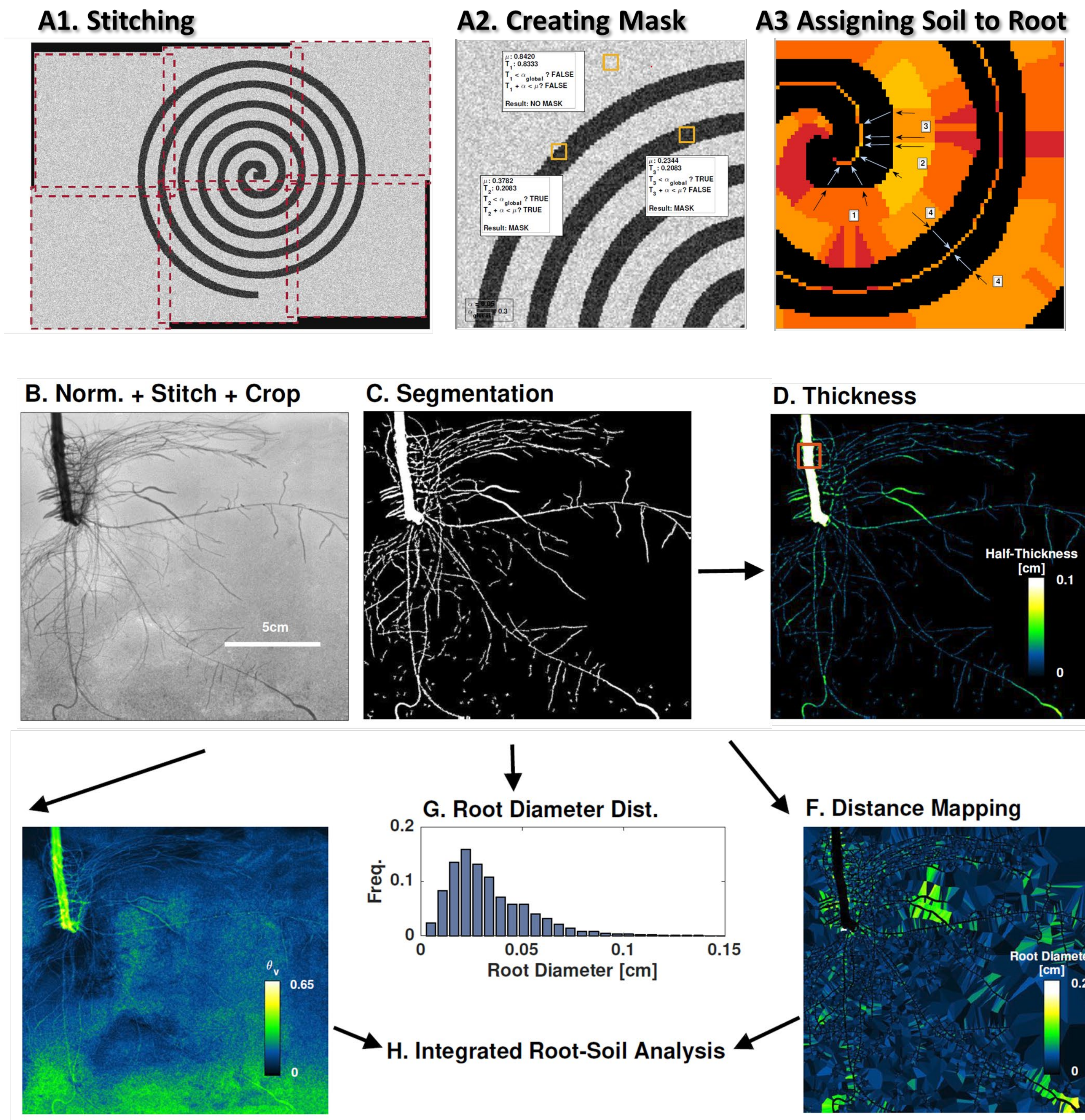
Use of root-free soil hydraulic properties does not fit data – role of roots/hyphae



Root uptake, hydraulic redistribution and soil drainage all contribute to the uncertainty in near surface modeling with roots, indicating new research needs.

Dhiman et al. 2018. Quantifying root water extraction after drought recovery using sub-mm in situ empirical data. Plant and Soil 424:73-89.

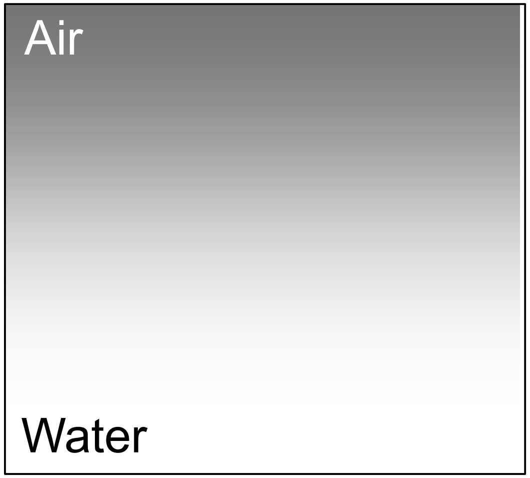
Automated Image Processing



DeCarlo et al. 2019 (under review)

Pore Water Distribution

Idealized Distribution



Measured Distribution

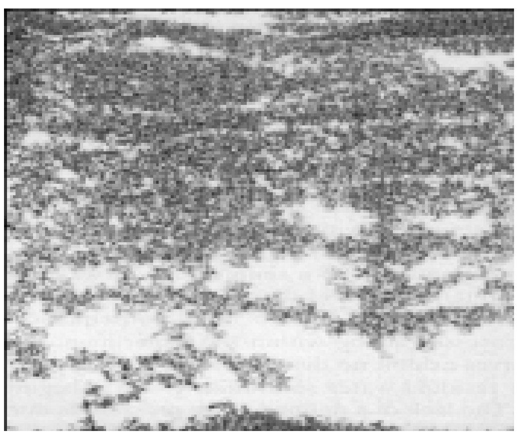
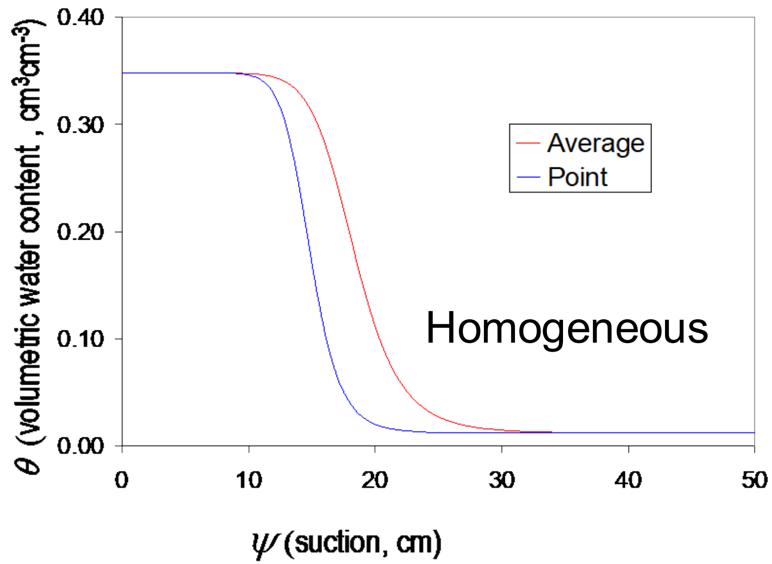
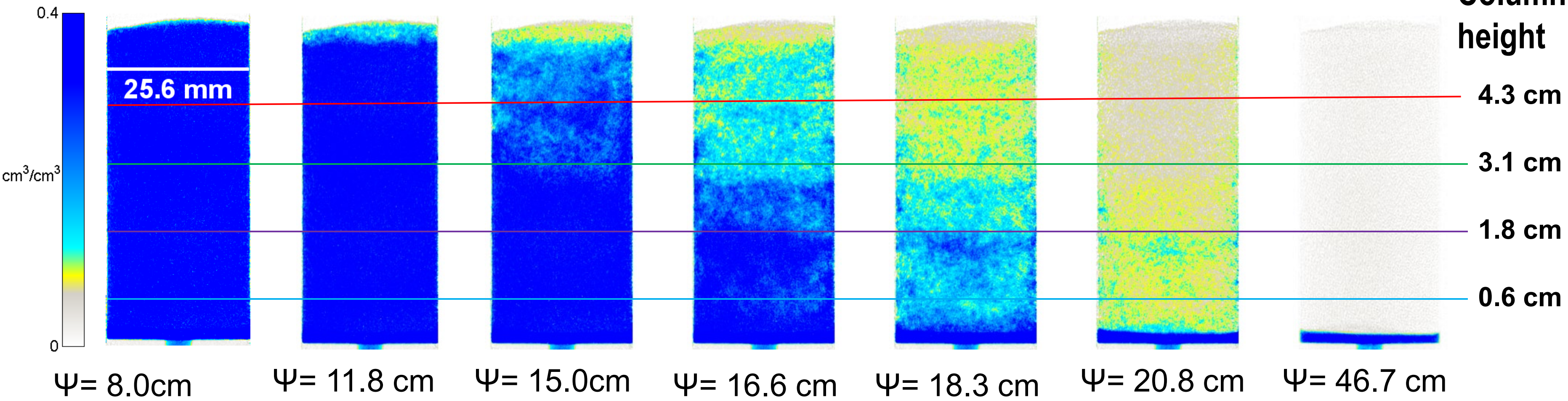


Image from Mortensen et al. (2001)

- Need to understand flow and transport in variably-saturated porous media
- Water, contaminants, dissolved ions, multiphase liquids
- Numerical modeling often assumes idealized distribution and boundary conditions, black box
- In reality, soils and rocks are extremely heterogeneous, requiring novel techniques

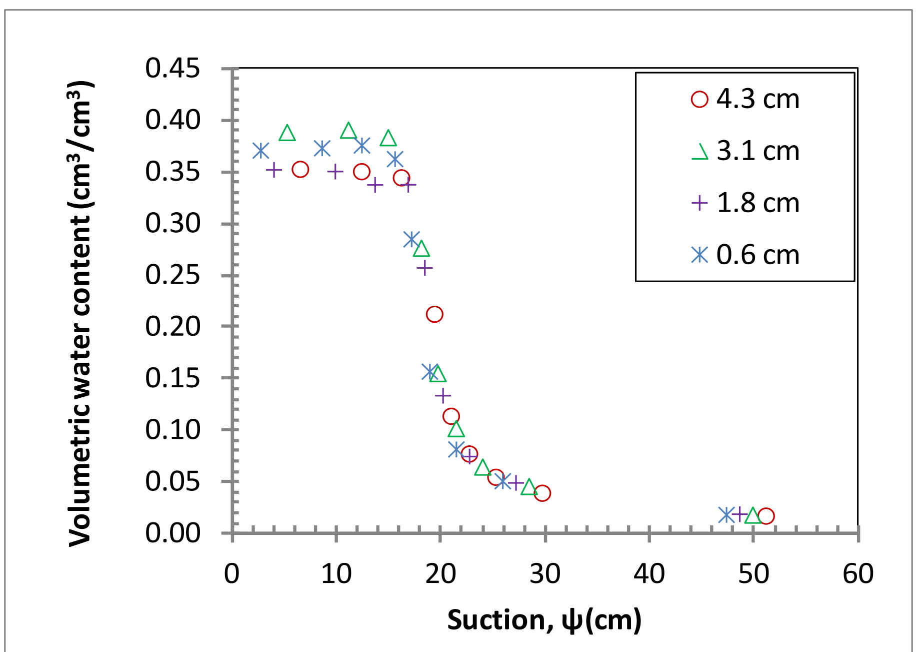


Point water retention curves



(Blue: water)

Flint Sand Drying Process (HFIR /CG1-D)

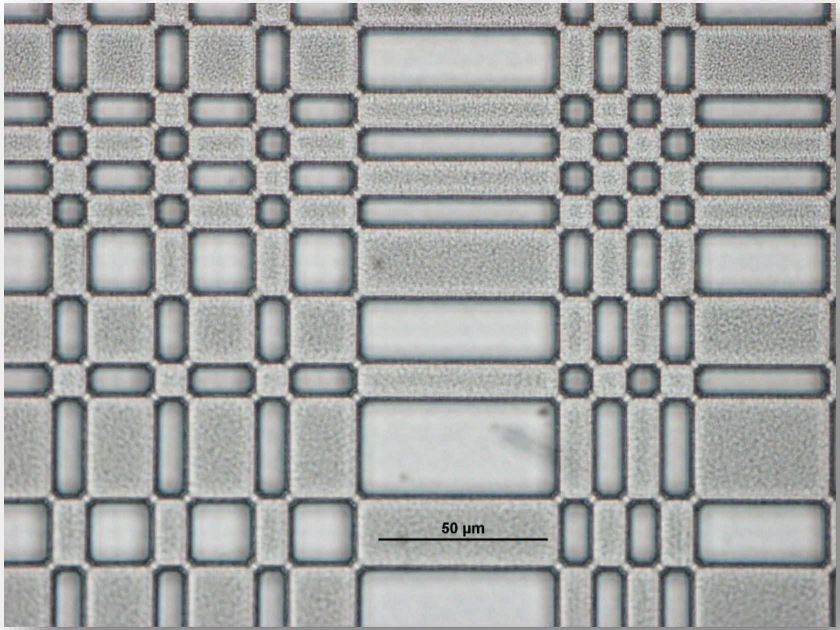


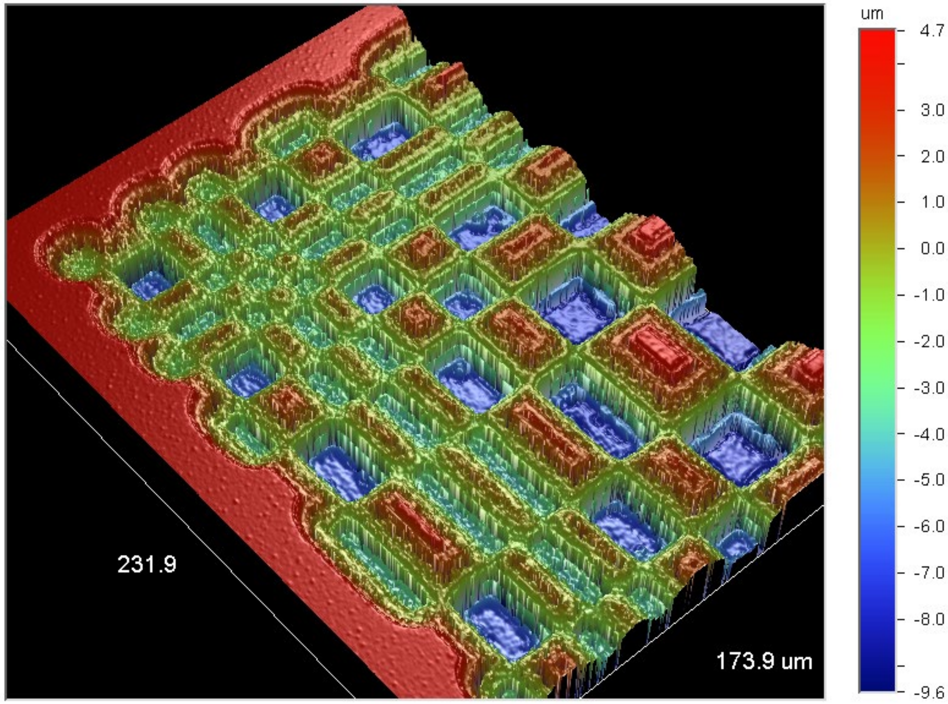
- Used hanging water column
- Measured point functions varied with column height
- Heterogeneity due to packing procedure
- Input parameters for numerical model

Advanced techniques to improve resolution

Gadolinium coded mask

Wafer Gd704 with 10.5 µm thick SU8: Example of the pattern before Gd etch

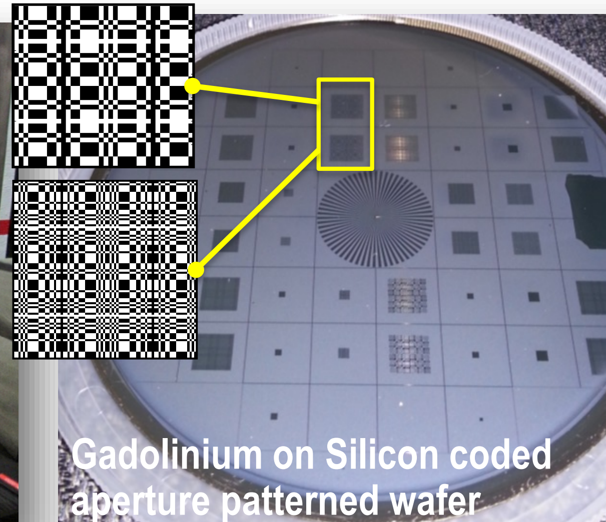


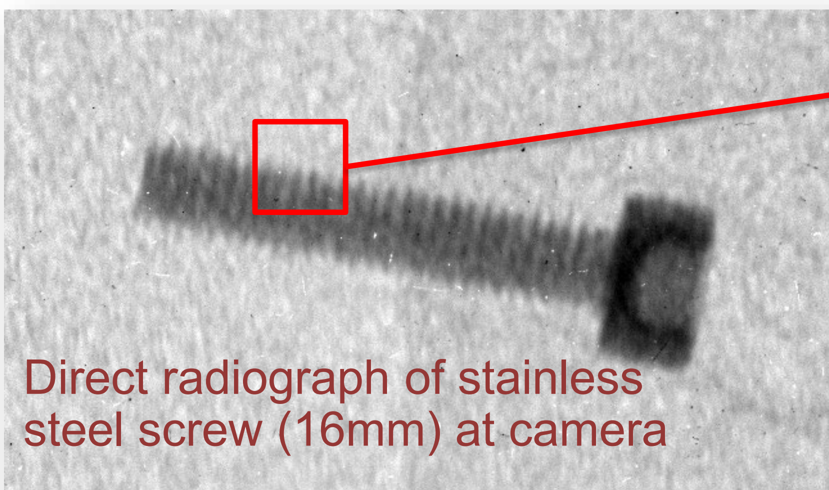


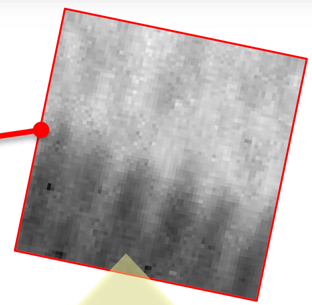
Gd704 after Gd patterning: 3D map of a 10µm 293x293 aperture fragment

Neutron Microscopy – Improved Resolution with Coded Aperture

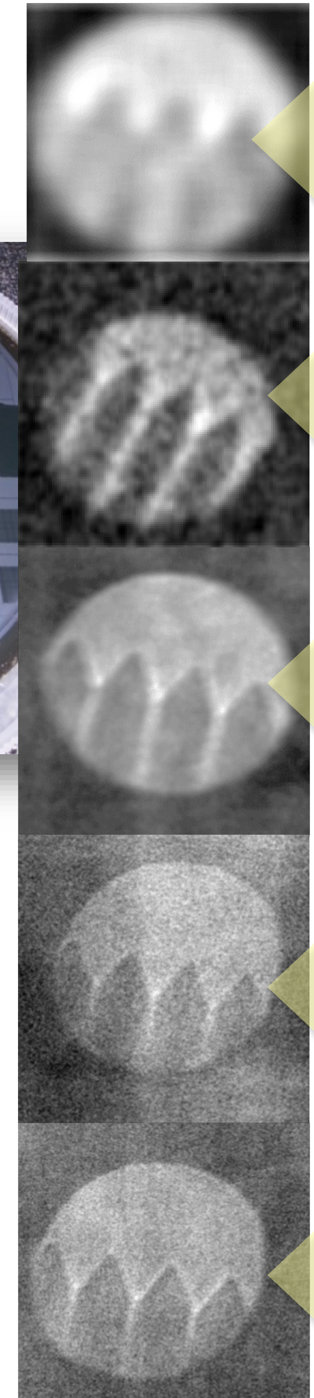
Measurements at CG1 – Philip Bingham







2.5x2.5mm sub-image from direct radiograph



200um mask
11x11 base
5.5 µm thick Gd
Mag 18x

100um mask
31x31 base
5.5 µm thick Gd
Mag 16.6x

50um mask
61x61 base
5.5 µm thick Gd
Mag 20.5x

20um mask
151x151 base
9 µm thick Gd
Mag 24.3x

10um mask
293x293 base
9 µm thick Gd
Mag 24.3x

Energy Selective Imaging

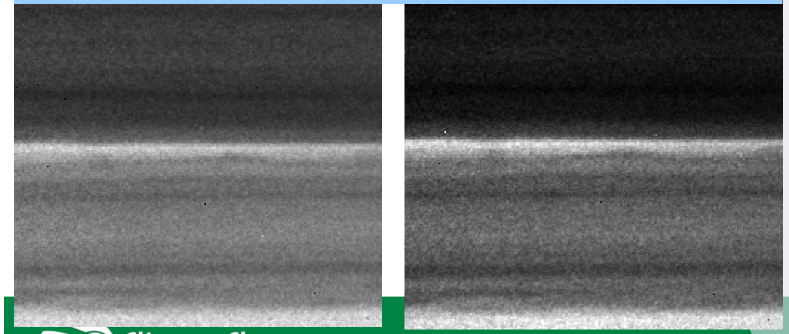
Energy-selective neutrons provide the ability to measure differential neutron attenuation interactions such as **Bragg edge phenomena**

Contrast for various elements are differentially enhanced, revealing additional information on material characteristics

Below – Grapevine stem tissue imaged at ORNL SNS SNAP beam line. Moist (top) or partially-dry (bottom)

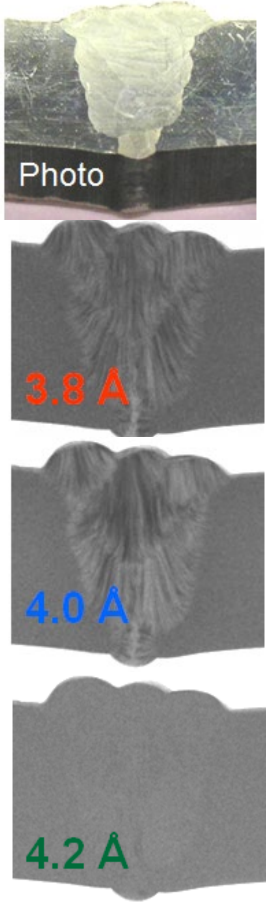
Neutron Wavelength Selection

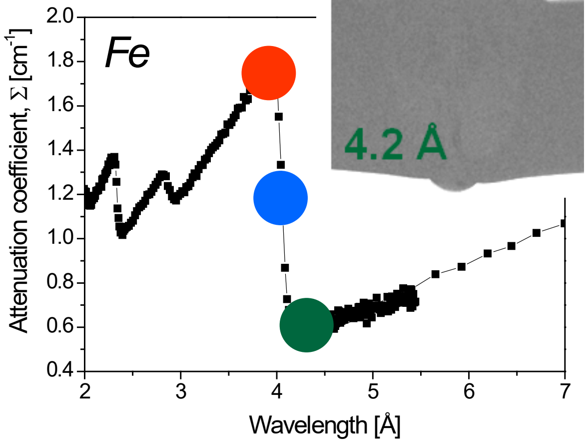
0.3 – 1.0 Å | **3 – 4 Å**



Better contrast was achieved using lower energy neutron wavelengths

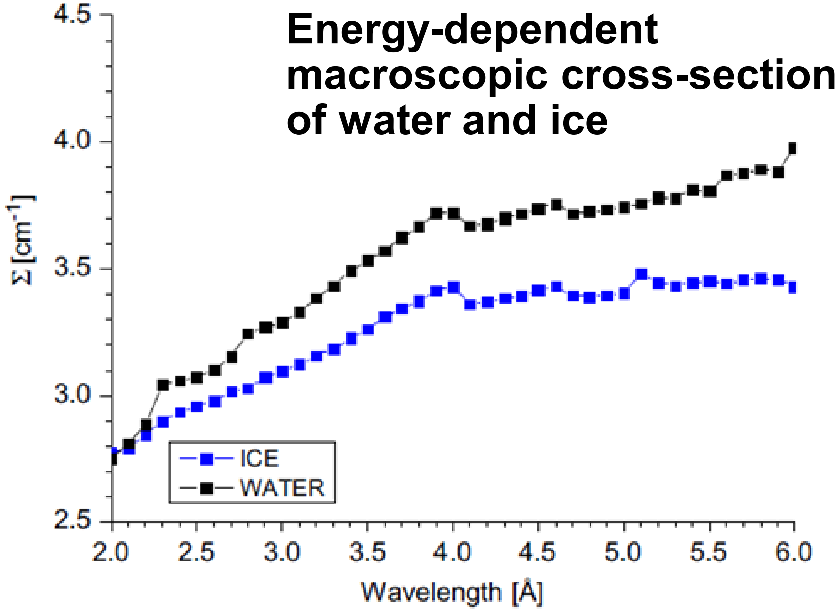
Measure of residual stress/strain characteristics of metals can be achieved using Bragg edge imaging





Kockelmann et al., NIM A, Vol. 578 (2007) 421

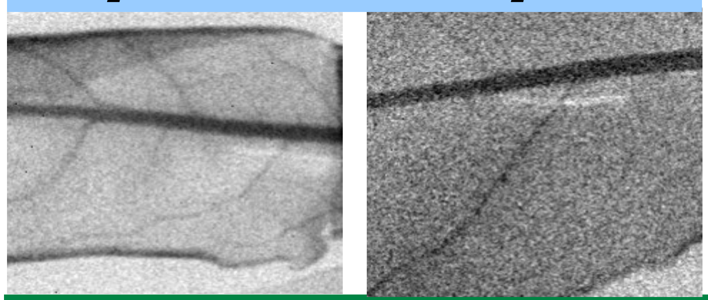
Material Phase



Energy-dependent macroscopic cross-section of water and ice

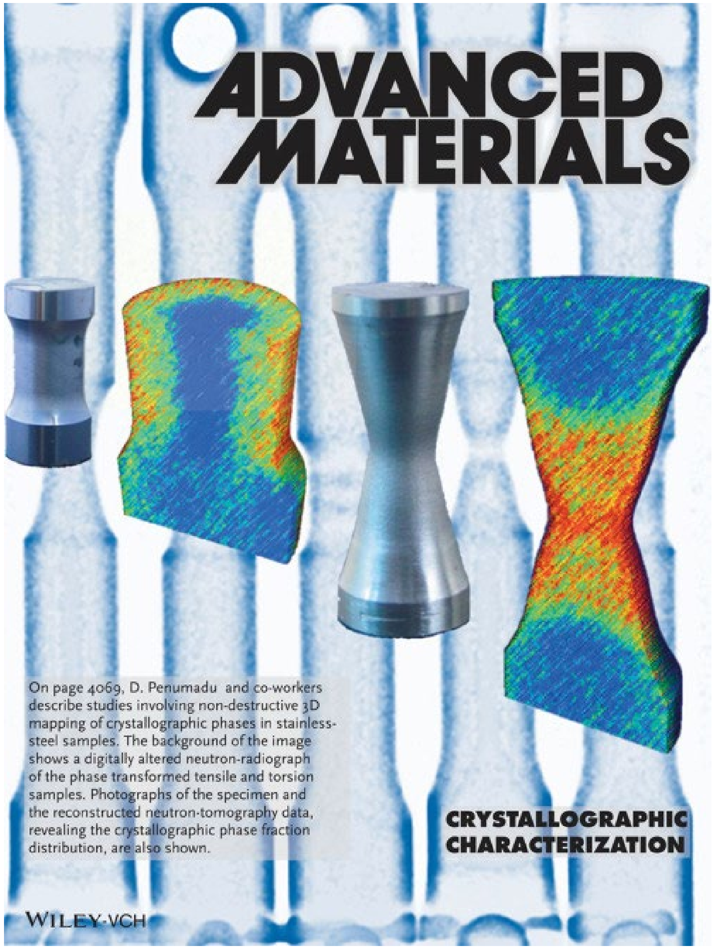
E.H. Lehmann, et al., Nucl. Instr. and Meth. A (2010), doi:10.1016/j.nima.2010.11.191

H_2O	***D_2O***



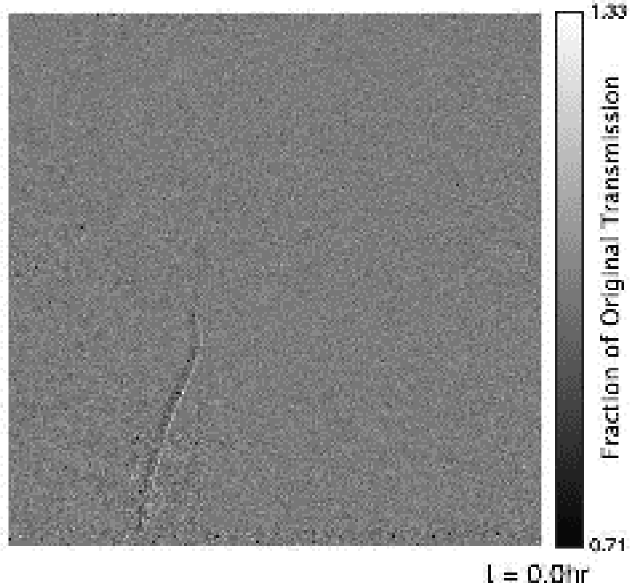
Isotopes also have different neutron attenuation

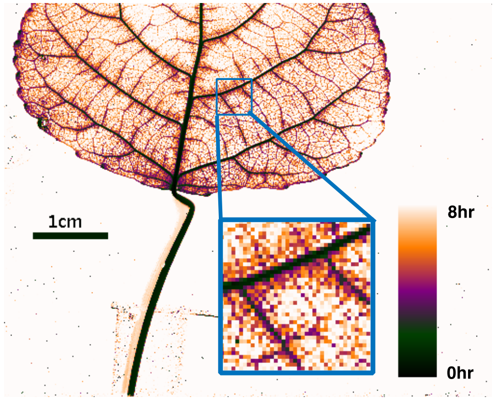
3D Map of Material Phase Using Energy Selective Neutron Tomography



R. Woracek et al. Adv. Mat. (2014) 26:4069-4073

Using contrast agents – Heavy Metal Uptake





Scientific Achievement

Solid uptake dynamics were traced in a transpiring leaf using Gadolinium (Gd) compounds and imaged using neutron radiography.

Significance and Impact

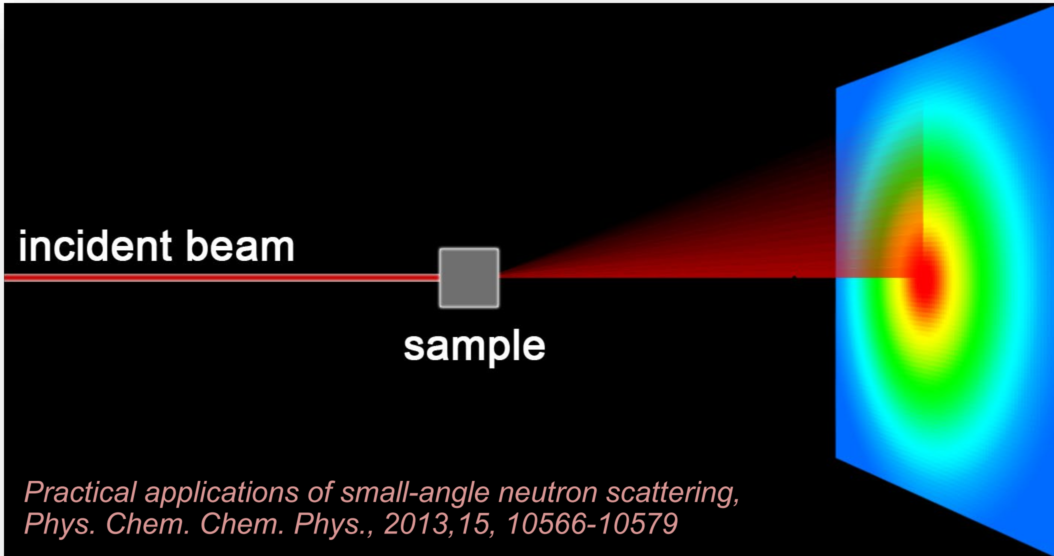
- Unlike heavy water (D_2O) which provides a lesser image contrast, the use of Gd compounds as tracers provide a strong neutron attenuation (i.e. contrast) that can be followed as a function of time in a plant system
- Gd-compounds are good candidates to serve as tracers for solid movements – possible use as a tracer for nutrients (e.g. phosphorus, nitrogen).

Top: Video shows uptake of Gadolinium in leaf over time. **Bottom:** Static image shows temporal map of leaf at 50% original transmission.

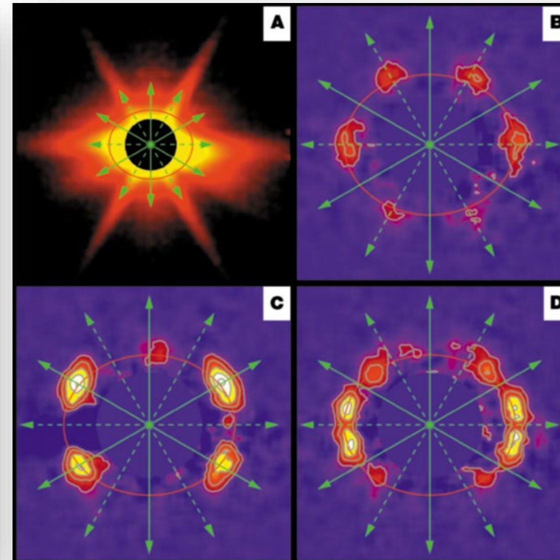
User team: K. DeCarlo and Sylvia Jacobson, Princeton University
Instrument team: H. Bilheux, Indu Dhiman, ORNL

Work performed at ORNL's High Flux Isotope Reactor CG-1D was supported by Scientific User Facilities Division, Office of Basic Energy Sciences, US Department of Energy.

Small Angle Neutron Scattering



Practical applications of small-angle neutron scattering, Phys. Chem. Chem. Phys., 2013,15, 10566-10579

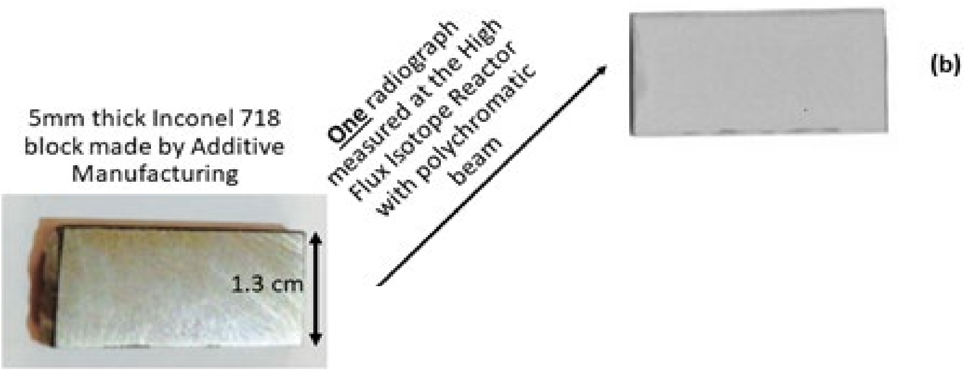


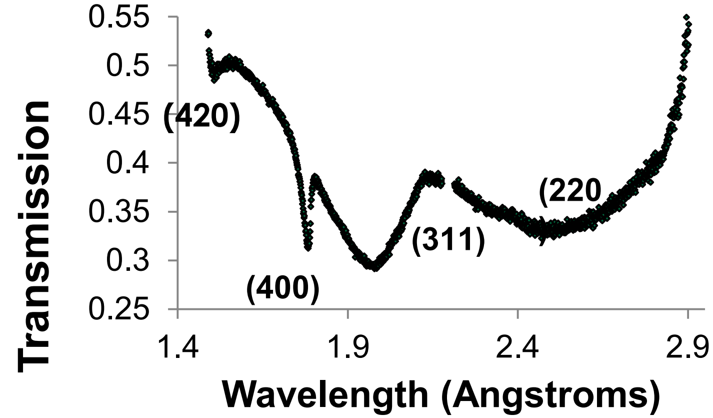
Realignment of the flux-line lattice by a change in the symmetry of superconductivity in UPt_3 Nature 406, 160-164(13 July 2000)

- SANS uses elastic neutron scattering, σ_e , at small angles to investigate material structure at the 1-100nm scale
- Measures the scattering length of neutrons, differentiating materials, isotopes, complex magnetic structures, and the structure and formation of polymers, etc.

Future NI at the SNS VENUS Beamline

Neutron Bragg edge imaging provides microstructure information of crystalline structures (lattice spacing, strain, preferred grain orientations, etc.)

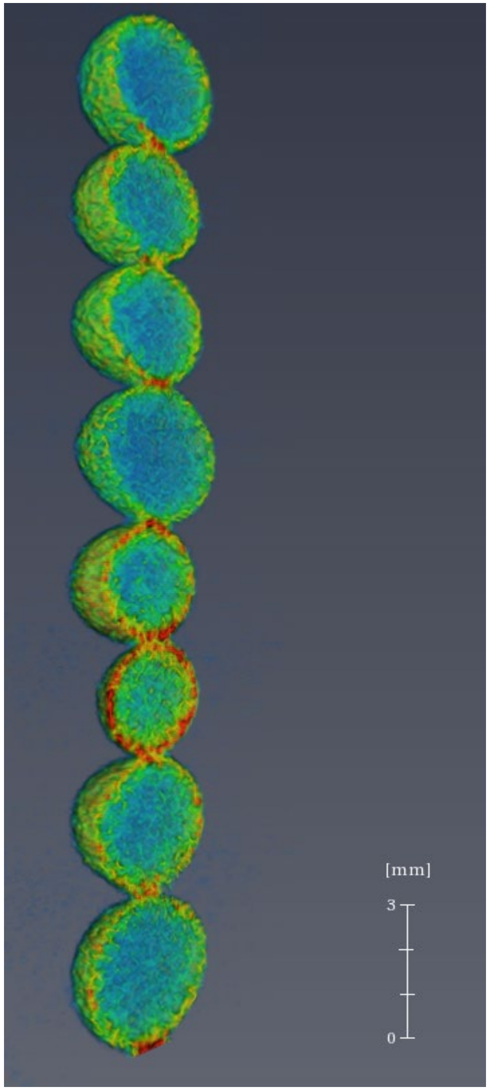




(a) A photograph shows an apparently featureless piece of AM Inconel 718 metal, fabricated using additive manufacturing techniques at the Manufacturing Demonstration Facility (MDF) of the Oak Ridge National Laboratory. **(b)** A polychromatic neutron radiograph measured at the High Flux Isotope Reactor (HFIR) CG-1D imaging beamline of the same metal does not show any feature either. **(c)-(e)** Wavelength-dependent or Bragg-edge neutron radiography reveals regions of preferred crystallographic orientation, which were intentionally produced to form the letters DOE (the U.S. Department of Energy sponsored this project).

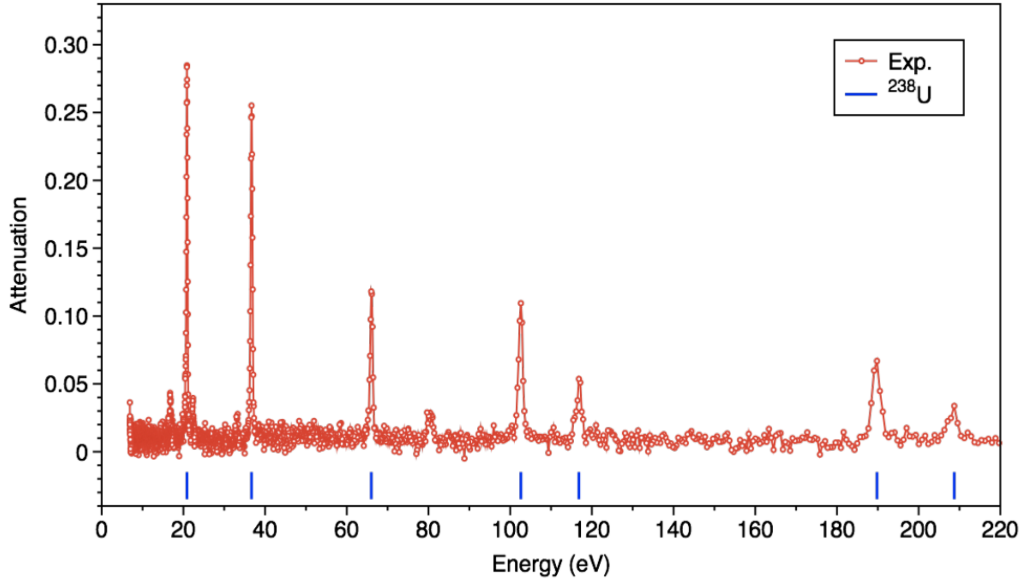
Future NI at the SNS VENUS Beamline

Neutron Resonance Imaging provides 3D isotope density mapping



Left: False color rendered volume of ^{238}U nuclear fuel TRISO spheres.

Bottom: corresponding ^{238}U resonances measured at the SNS SNAP high pressure diffractometer.



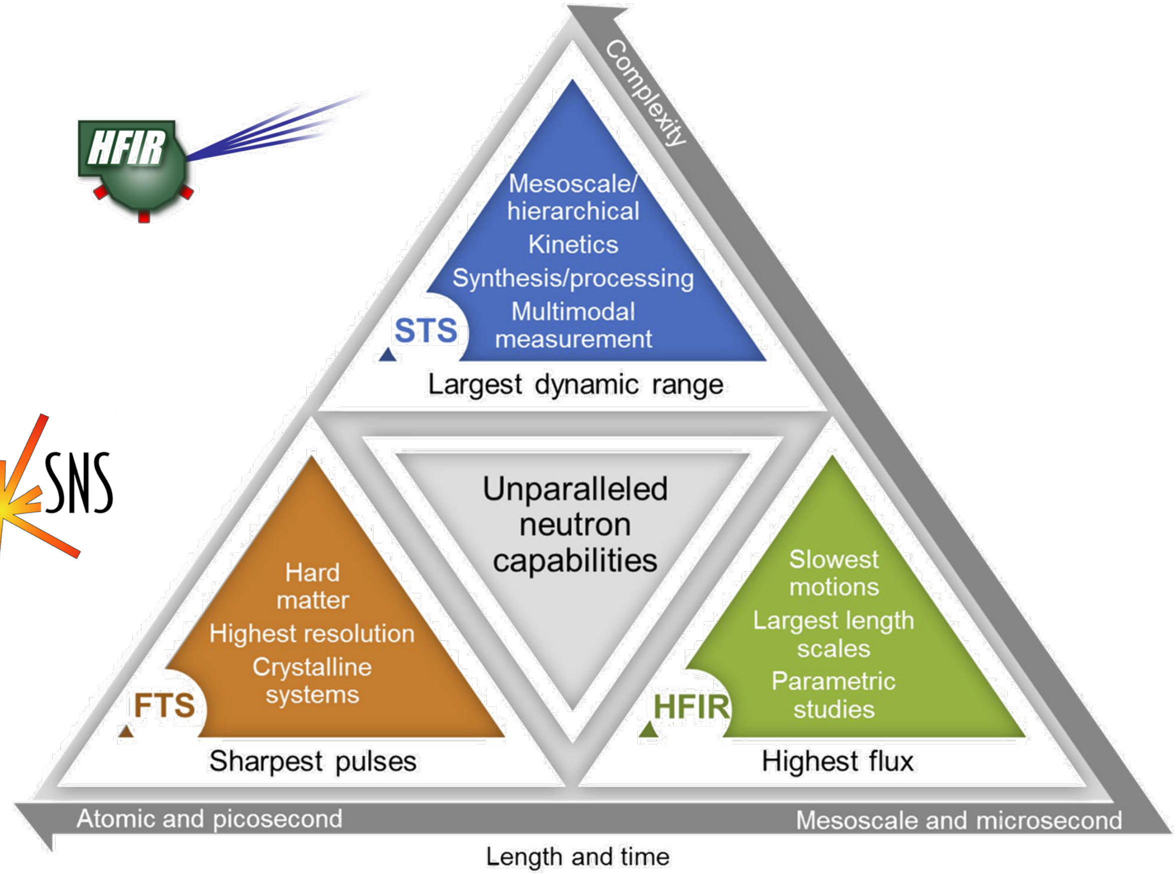
The CT scan represents the density distribution of 238U. We used the images corresponding to the peaks on the right side. At SNS, you can collect neutrons at different energies so you can measure images at different energies. If you collect the images corresponding to the 238U, then you can map in 3D where the specific isotope is located.

Oak Ridge National Laboratory | Spallation Neutron Source

Advanced Neutron Imaging

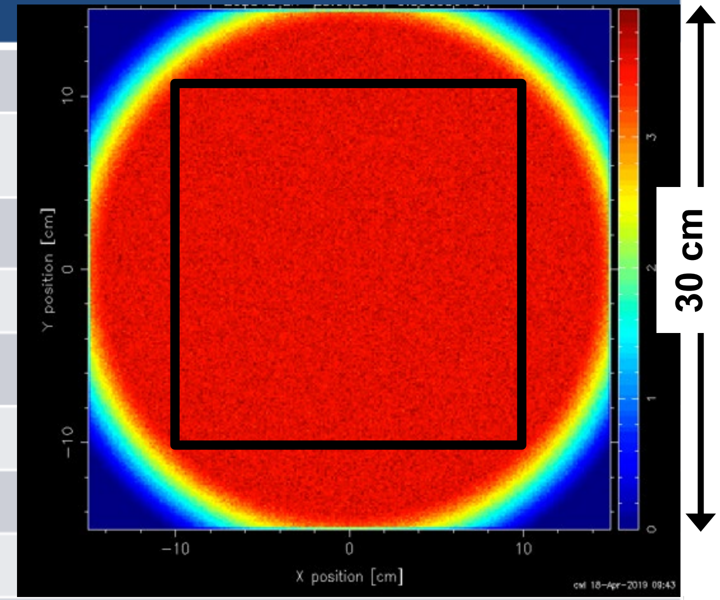
- Leverage three ORNL Neutron Sources

- **HFIR (current)**
  - Continuous neutron flux
  - 15 instruments
- **FTS (planned)**
  - Pulsed source (60 Hz)
  - High resolution
  - 24 instruments
- **STS (proposed)**
  - Pulsed source (15 Hz)
  - Large dynamic range
  - 22 instruments

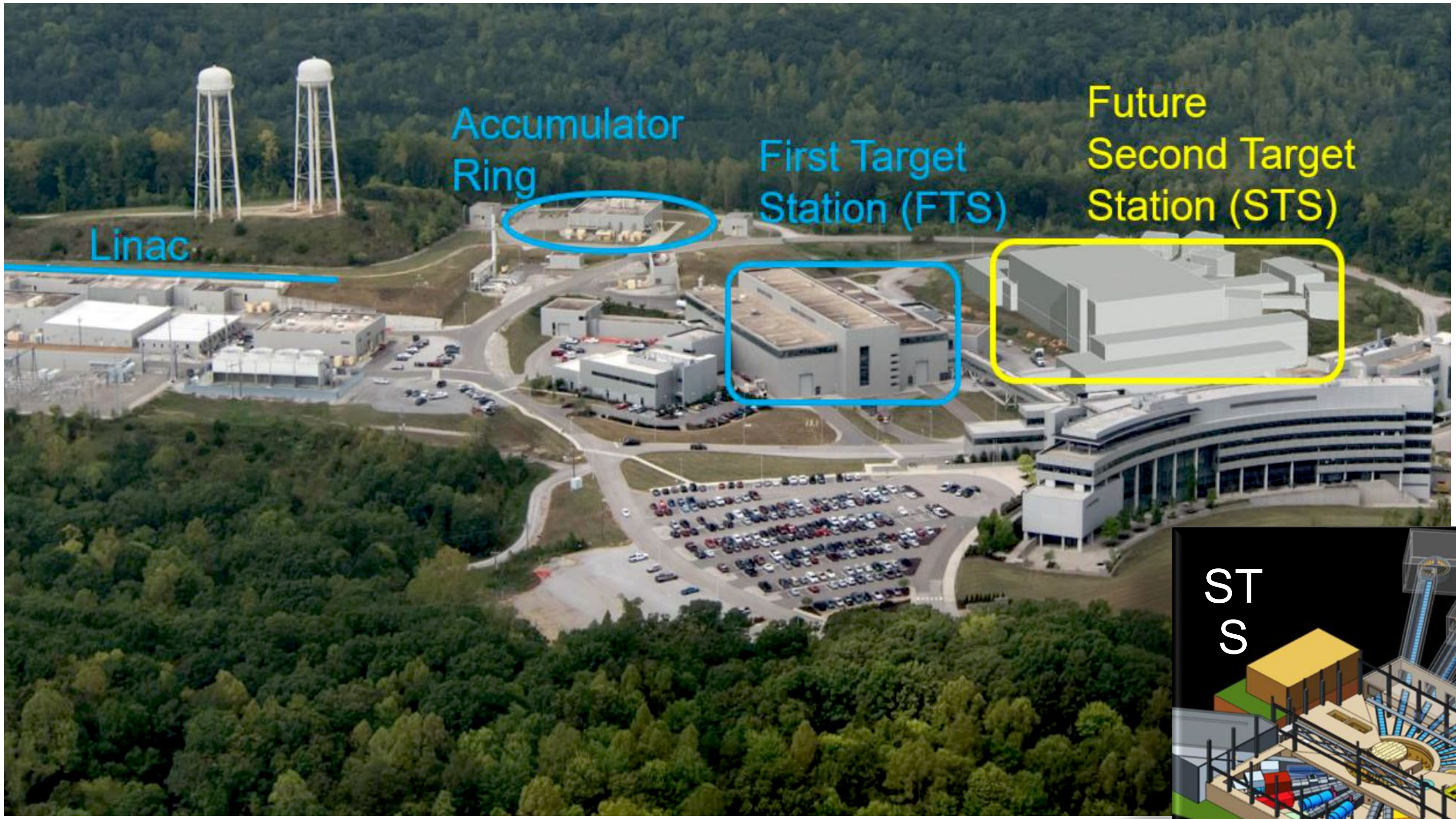


Planned ORNL SNS VENUS Beamline at FTS

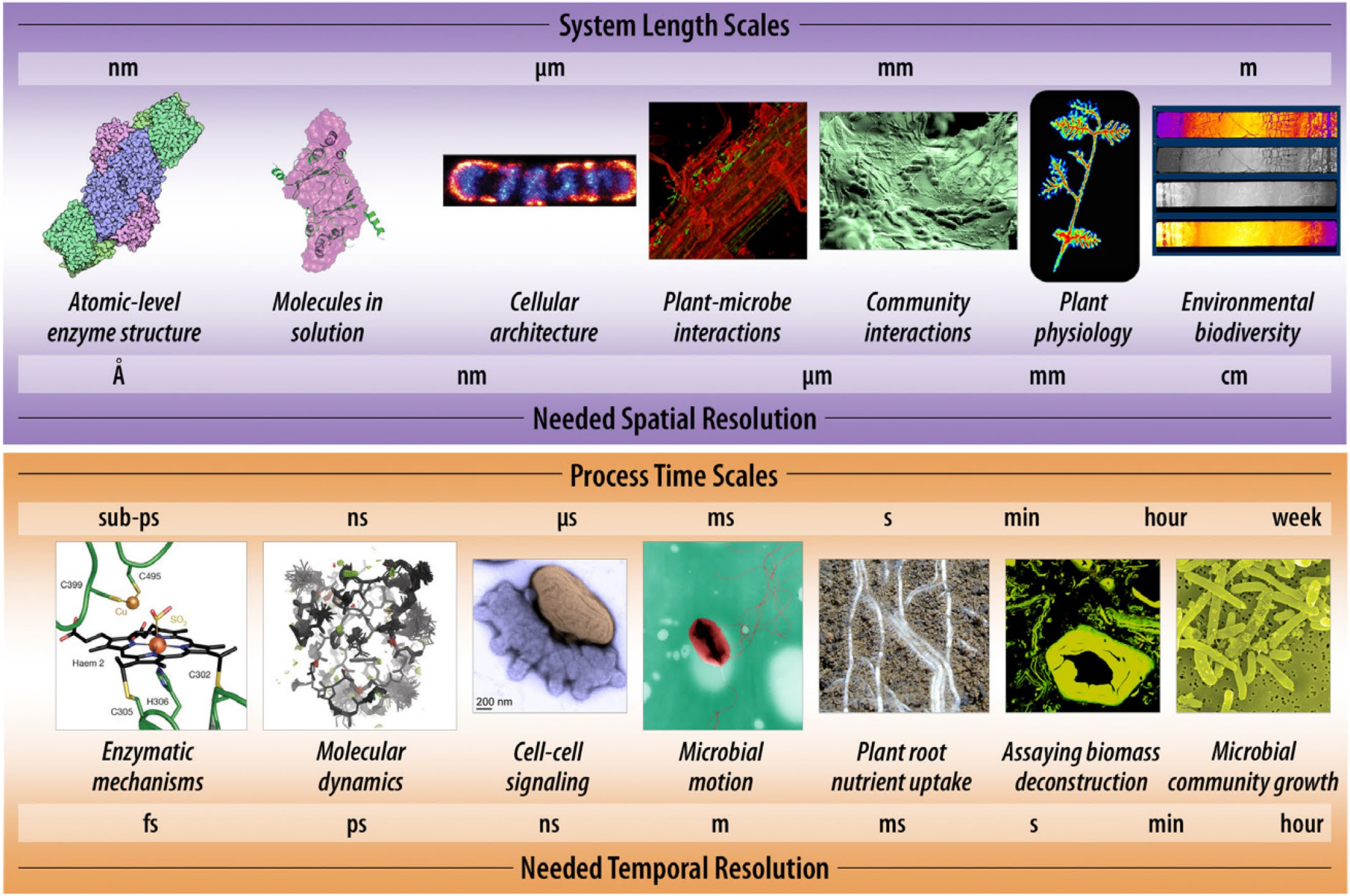
VENUS (Beamline 10)	
Beam Spectrum	Epithermal, Thermal, Cold
Moderator	H2 decoupled poisoned
Repetition rate	60 Hz
Wavelength bandwidth	~ 2.5 Å (Time-Of-Flight mode)
Spatial resolution	~ 50-100 microns
Resolution $\Delta\lambda/\lambda$	~ 0.12 % (at ~ 1Å)
Source-to-detector distance	25 m
L/D ratios	300 to 2000
Sample-to-detector distance	As close as possible to detector
Sample stage capability	500 kg maximum weight load, 1 m translation normal to beam, ~ 85 cm vertical travel from beam center, 0.5 m translation in the beam direction (provided by SNAP imaging project)
Detection system and resolution	CCD and Micro-Channel Plate (to be provided by K. Herwig's group) detectors
Maximum field of view	20 cm x 20 cm



STS – The Second Target Station at SNS

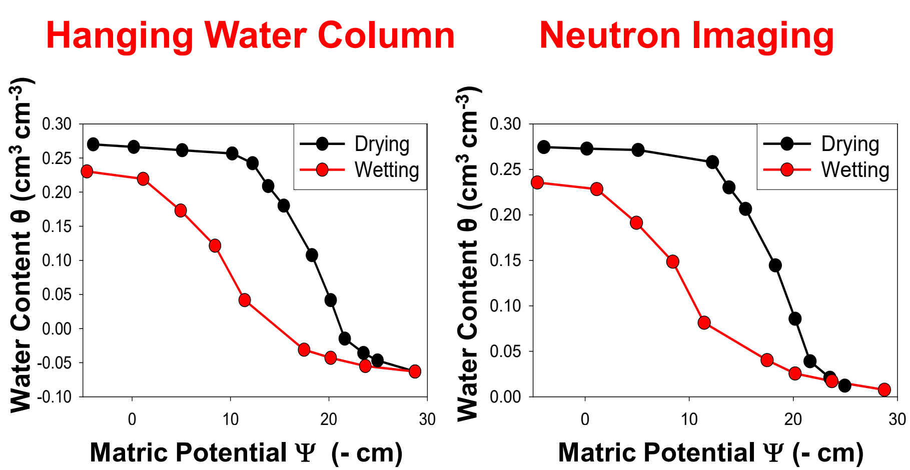


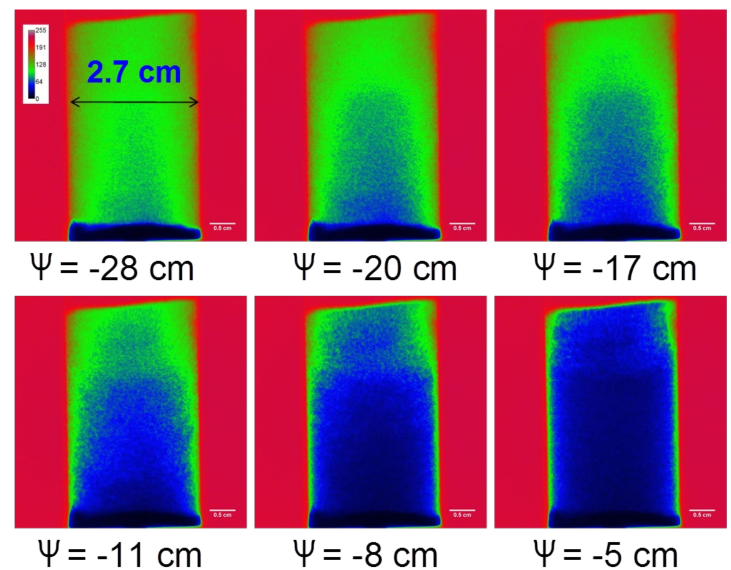
Complexity – leverage multiple techniques



Future Soil-Plant Neutron Imaging Research

- Root and mycorrhizal/bacterial interactions
- Plant or rhizosphere water dynamics – Carbon? Nutrients?
- Root – soil – water physical or chemical interactions
- Water/Chemical flow through porous media, mixed phases
- Soil development, Carbon sequestration
- Belowground competition
- Plant-stress dynamics





Thanks! Questions?

The HFIR CG1D and SNS beamlines are Public User Facilities: *http://neutrons.ornl.gov/users/*

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The ORNL Neutron Facilities High Flux Isotope Reactor and the Spallation Neutron Source are funded by DOE Basic Energy Sciences.