Development of Methods for Visualising Root-Particle Interactions

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Introduction

Root systems are essential in providing stability for plants and allowing uptake of water and nutrients. However, root systems are inherently difficult to study because they are concealed beneath soil and are sometimes referred to as the "hidden half" of the plant (Waisel et al., 2002).

Historically, methods of studying plant roots were largely destructive, using various types of excavations. This allowed measurements of morphological characteristics of root systems. Basic methods of *in situ* observation of roots have been developed, including the use of rhizotrons and the needleboard method (Goff, 1897). These approaches have provided the greatest insight into the architecture of root systems, but have numerous limitations.

Our objective is to develop high resolution imaging and visualization techniques to track root trajectories in heterogeneous soil and artificial granular media. The project will use the latest technological advances in microscopy and X-ray tomography to generate numerical descriptions of root growth.

Optical Projection Tomography (OPT)

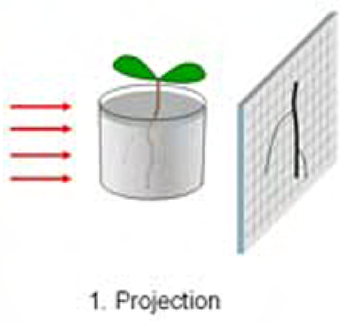

Figure 1 . Light is projected through transparent medium with growing roots. Sample is rotated whilst projected images are captured. These images are reconstructed to create a 3D data set.

* OPT microscopy is a technique used to image biological specimens in 3D, developed in Edinburgh by James Sharpe (Sharpe et al., 2002) for studying animal embryonic development. Transmitted / emitted light from the sample, is projected while the sample is rotated (Fig.1). Projections are then reconstructed using specialised software to form the 3D image.

* In previous studies it has been shown that the method can be applied to image biological samples (Fig.2), including plants (Lee et al., 2006).

* OPT will be used to collect information combining growth patterns and markers of biological activity of roots (Fig.3).

Figure 2. Reconstructed image of OPT scan of HH stage 26 chick embryo showing expression of Tbx3.

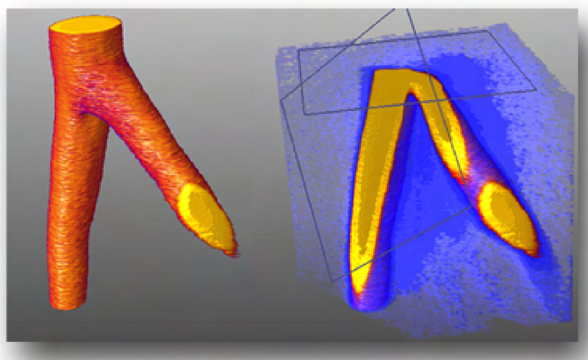

Figure 3. 3D reconstruction of OPT scan of roots obtained during the Aman Gill MRes project (SCRI-SIMBIOS) using the system built at SCRI.

X-ray Micro CT (X CT)

* X-ray Micro CT is a non-invasive 3D visualisation approach to image in depth root-soil processes (Fig.4). It has become an important tool for studying soil constituents, including plant roots *in situ* (Taina et al., 2008, Gregory et al., 2003).

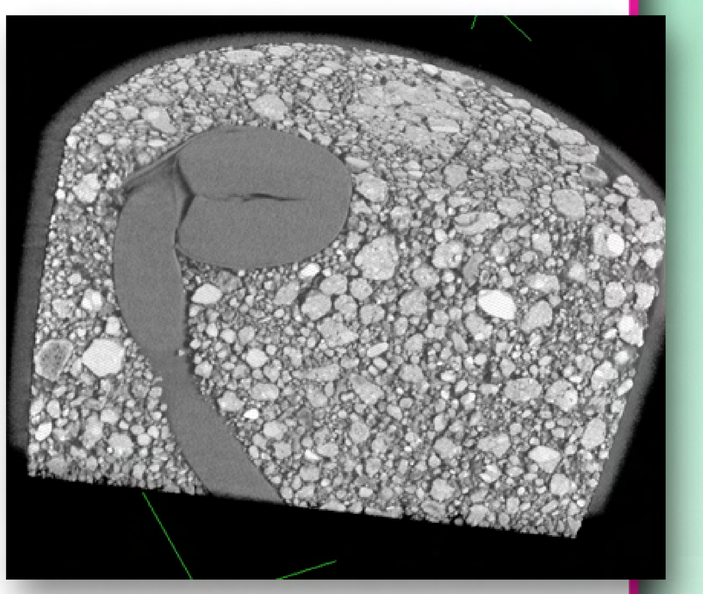

Figure 4. Reconstructed image of x-ray micro CT scan of *Lupinus* seed with root growing in soil. Image courtesy of Sonja Schmidt.

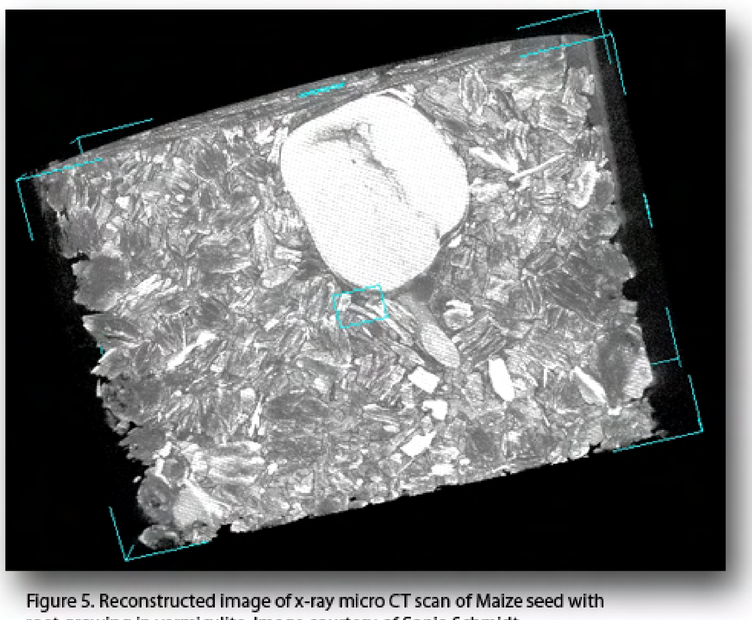

Figure 5. Reconstructed image of x-ray micro CT scan of Maize seed with root growing in vermiculite. Image courtesy of Sonja Schmidt.

* We will use X CT to observe root growth in 3D over time, along with the roots' interactions with soil or artificial particles (Fig.5).

Challenges and Objectives

1. Create reconstituted, isorefractive, heterogeneous medium for OPT microscopy of plant roots. This will allow us to control the properties of the growth medium (e.g. particle size) and will involve using **refractive index matching** techniques.

2. Cross validate approach using established X CT technique.

3. Use of image analysis techniques to obtain numerical descriptions of the evolution of root geometry in relation to the distribution of particles.

Refractive index matching is a technique used in optics to negate the visibility of boundaries between two or more different, transparent materials caused by a discrepancy in their refractive index. By using materials with matching refractive indices, the boundaries become invisible. This allows production of heterogeneous media with homogenous optical properties.

This research has applications in predicting the distribution of roots in complex soil environments.

References

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