doi:10.1017/S1431927620013525
Microsc. Microanal. 26 (Suppl 2), 2020
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The Versatility of X-ray Microscopy for Imaging Intact Plant Structures

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X-ray microscopy (XRM) is becoming a valuable technique for generating three dimensional (3D) image data to complement existing laser-based and electron microscopy. This is particularly true for imaging floral and inflorescence structures that are often delicate and complicated. Examples of this in the literature used traditional electron microscopy sample preparation methods and contrast agents to produce detailed 3D volume data of a variety of floral samples [1-4]. We have modified these procedures for multiple plant tissues and organs and can regularly generate data-rich 3D volumes over a range of scales, often with significantly shorter sample preparation times.

We have used soybean (*Glycine max*) to illustrate the wide range of plant structures that can be visualized and studied in ways that are not practical with other imaging technologies. We have documented the developmental cycle of flowering from axial bud growth to pollination and seed development (Fig 1). We have generated detailed 3D images of intact nodules formed by nitrogen-fixing symbiotic bacteria (Fig 2a). We have also imaged mature soybean seeds at multiple scales with no fixation or contrast agents. We are using microwave tissue processing to significantly accelerate sample fixation and contrast enhancement protocols, which can reduce sample preparation times from weeks to hours in some cases [5,6]. We have extended these imaging techniques to other floral and inflorescence samples as well.

We are using these data sets to develop computational segmentation protocols, both manual and machine learning models, that will allow us to identify and therefore measure plant organs, tissues, and individual cells from a wide range of plant samples (Fig 2b). The ability to visualize and measure biologically relevant plant traits that are not readily accessible with other 2D or 3D imaging systems is a major advantage of using XRM imaging. Combining XRM with laser and electron microscopies will provide researchers with significant insight into not only plant morphology and development, but provide valuable 3D spatial information for gene expression, as well as physiological and biochemical studies.

https://doi.org/10.1017/S1431927620013525 Published online by Cambridge University Press

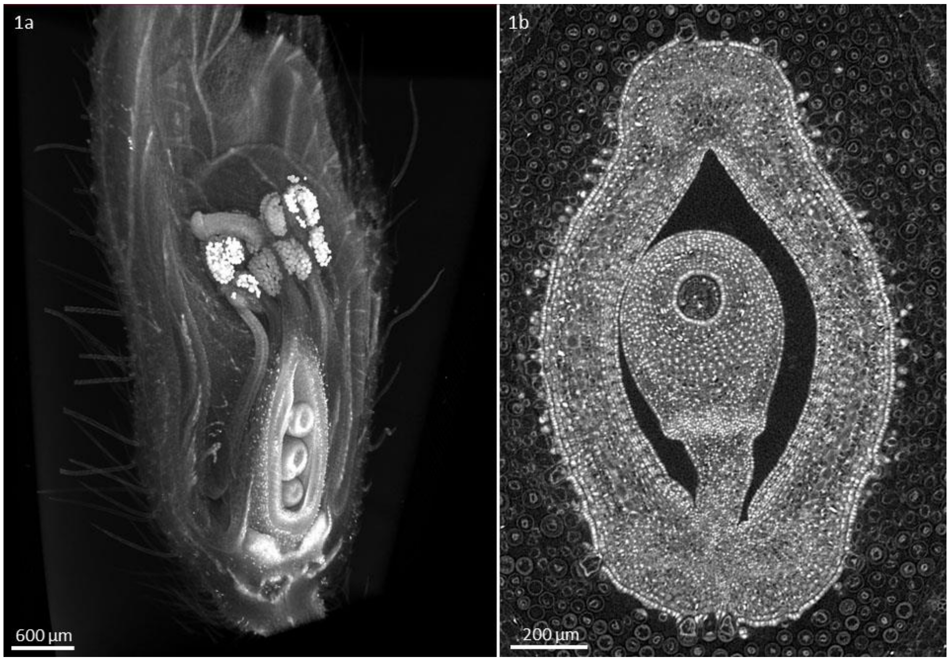

Figure 1. Fig 1. a. Volume rendering of a developing soybean flower contrasted with phosphotungstic acid and scanned with XRM at 2µm voxel resolution. b. Clipping plane 2D image from XRM scan of developing soybean seed at 1µm voxel resolution.

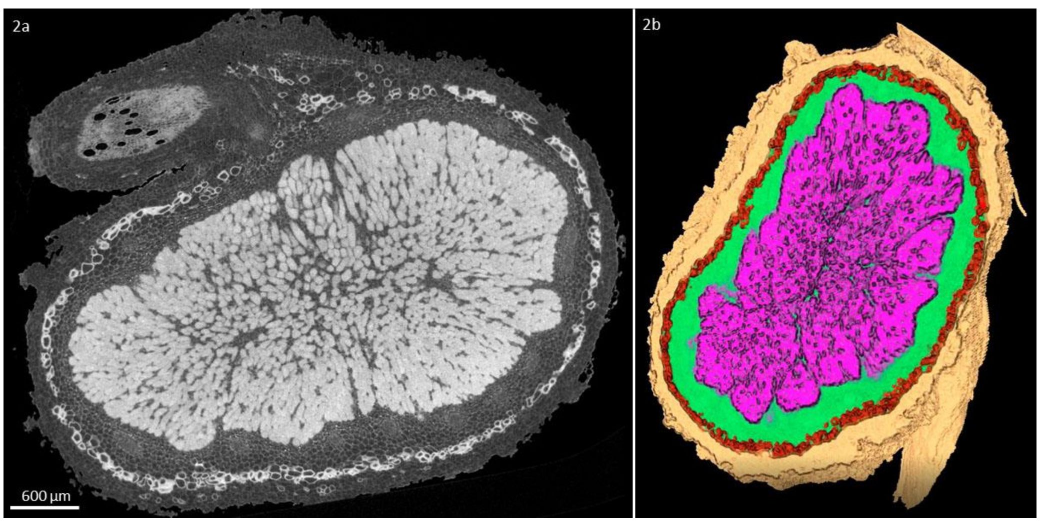

Figure 2. Figs 2. a. Clipping plane 2D image of a soybean nodule scanned with XRM at 2µm voxel resolution. b. Computational segmentation of soybean nodule into regions.

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[7] This work is supported by collaborative agreements with Valent BioSciences, LLC, and Sumitomo Chemical Company.

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