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## Visualization and quantitative evaluation of functional structures of soybean root nodules using synchrotron-based X-ray mCT and X-ray fluorescence

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Legume-rhizobia symbiosis, the most efficient plant N<sub>2</sub> fixing system, has been long recognized as a sustainable alternative to the use of nitrogen (N) fertilizers. Previous histological studies have provided a detailed description of anatomical structure of root nodules in 2 dimensions. Nodules consist of two functionally important tissues: (1) a central infected zone (CIZ), colonized by rhizobia bacteria, which serves as the site of N<sub>2</sub>-fixation, and (2) vascular bundles (VB), connecting the nodule with the root and plant, serving as conduits for the transport of the water and nutrients from plant to nodules, also for the translocation of the fixed-N compounds from nodule to plant. Visualizing these tissues by traditional microscopic methods involves using destructive and labor-intensive approaches for thin sectioning of nodules and staining sections. Therefore, we used synchrotron-based X-ray micro-CT (S-XRCT) and X-ray fluorescence (S-XRF) techniques for non-invasive and fast visualization of these important structures in intact soybean root nodules in both 3D and 2D. The S-XRCT imaging of root nodules at 0.7 microns spatial resolution allowed us to visualize the nodule vessels (> 7 microns diameter), and to automatically segment the VB and CIZ in the 3D reconstructed images. The elemental maps, generated by S-XRF of root nodules at 20microns resolution, revealed the unique localization of Zn and Fe within the VB and CIZ tissues, and enabled the 2D visualization of these tissues. To better understand the physiological basis of the differences observed in N-fixation of soybean genotypes, a quantitative evaluation of these tissues is essential. This study, for the first time, introduces the application of S-XRCT for volume quantification of the CIZ and VB tissues in intact soybean root nodules. The proposed methods allow for high-throughput phenotyping of the functionally important nodular structures by simultaneously imaging of multiple root nodules, enhancing the applicability of these methods.

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