24 August 2012

Dr. Nandita Singh  
Senior Science Editor  
[Journal of Visualized Experiments](http://www.jove.com)

Dear Dr. Singh:

Below please find a detailed list of our responses/revisions based on the reviewers’ comments. Our comments are in bold text below each reviewer comment. We hope these now make the manuscript acceptable for publication in JoVE.

Sincerely,

Andrew J. McElrone

Response to Reviewers' comments:  
  
Reviewer #1:   
  
*Summary:*   
The authors provided an overview of the utilization of HRCT to study vascular tissue in woody plants. Details of the protocols are given, and software options to produce 3D reconstructions are listed, being one of them Avizo. The images are illustrative on what is possible to image and the quantitative data that can be extracted from the reconstructions, i.e. cavitation.   
  
*Major Concerns:*  
I consider that the authors could compare the HRCT to other types of CT scans, or non-invasive technologies in plants. For instance Lee et al, 2006, Plant Cell 18:2145 showed 3D reconstructions of organs and gene expression labels, or Iyer-Pascuzzi et al Plant Phys, 2010, 152:1148 showed 3D reconstructions of rice roots. Also a comparison to other high resolution 3D scans could be done, i.e. Jahnke et al, 2009, Plant Journal, 59:634. This would give the readers the elements to assess the strengths and weaknesses of HRCT for research purposes.

**The space constraints of JoVE (particularly the methods/protocol section) limit how much we can talk about the details of these other techniques. While our research group has conducted work on other imaging techniques (e.g. NMR visualization of plant vascular function), we were invited to submit a manuscript based specifically on HRCT work at ALS- thus the focus of the current manuscript. However, we do acknowledge that there are numerous additional applications of advanced visualization techniques and have addressed the reviewer’s concern by incorporating text referring to these other techniques in the Discussion section where it was appropriate.**  
  
*Minor Concerns:*  
I would prefer the authors to stick to the international metric system. For instance, in the section Description of the ALS facility Beamline 8.3.2, in the second paragraph, it reads "on the 4'' diameter?". The measurement should be given in centimeters.

**Good catch- We have thoroughly proofread the manuscript again and have ensured that only international metric units are being used.**

The link of Movie 2 does not play.

**The text listed for the link was written in half regular text and half hyperlink text. The link does in fact work if the entire address is copied into a browser address bar. We have edited the text to ensure consistent formatting of the entire text. We assume that this will be taken care of during final manuscript preparation.**  
  
  
Reviewer #2:   
  
*Summary:*   
The invited Methods article "Utilization of high resolution computed tomography to visualize the three dimensional structure and function of plant vasculature" by McElrone et al. provides a detailed description of the use of HRCT to visualize the architecture of plant xylem vessels. It is a very specialized technique that cannot be employed in any laboratory in the world but needs specific facilities. Therefore it is not a drawback that the article describes the details of the techniques adapted in the authors' laboratory. The article is a welcome addition to the nice work of the authors' group published in other journals.  
  
*Major Concerns:*  
None.

*Minor Concerns:*  
\*) The imperative used in the protocol description is rather unusual, albeit, for this reviewer it is not disturbing.

**We followed the format required by this journal**

\*) This reviewer could not find any structure in the order of the reference list. This should certainly adjust according to the guidelines of the journal.

**We followed the format required by this journal**

\*) One article was cited as "in review". Also this needs to be adjusted in accordance with the journal guidelines.

**This manuscript has been in review since late June. We assume that we will hear about the status of this manuscript soon and will provide the JoVE editorial staff with this info as soon as we hear. If the timing of publication of this article requires, we could change this to an “unpublished data” reference.**

\*) typo: "Figure.1." -> "Figure 1."

**Good catch- This typo was corrected.**

\*) typo: "The vine is contained an acrylic tube (1)." -> Should read "The vine is contained in an acrylic tube (1).", no?

**The reviewer is correct and the text has been edited accordingly in the legend for Figure 2.**

\*) Some movies and Figures were published by other journals before. This reviewer is uncertain about the copyright issues in these cases.

**We were told by the JoVE editor that copyright issues for already published figures and movies will be taken care of prior to publication. We assume this is still the case.**