Dear Dr. Wu,

Please find enclosed manuscripts entitled " Xylem water distribution in woody plants visualized with a cryo-scanning electron microscope". We have revised the manuscript to address your comments. Your comments are in blue text and our response follows.

I am looking forward to your decision.

Sincerely yours,

Kenichi Yazaki

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Done.

2. Please use h, min, s for time units.

Done.

3. Step 5.1: Please write this step in the imperative tense.

We have revised the sentence in step 5 as follows:

"NOTE: Typical setting for the observation is described below. Some modifications are required depending on the vacuum condition or electron beam.

5.1. Set the SEM parameters for observation as follows:"

4. 7.1: Please write this step in the imperative tense.

We have added steps describing actions for metal coating to this paragraph. We have regarded original the sentence as "NOTE". We have added the materials for coating to the list.

" NOTE: Recent improvements to the SEM instrument and image processing can provide high quality images of electric insulating specimens without metal coating. However, non-conductive specimens, such as biological materials, are sometimes subject to charge; higher brightness at specific positions of the specimen due to accumulation of electrons (“charging”). Exposing the specimen to electron beams for a longer period of time or for a high magnification, increases the charging effects. Coating the surface of the specimen with electric-conductive metal materials prevents the occurrence of charging. Use the vacuum coating system which is installed within the cryo-SEM unit in order to prevent the temperature of the specimen from increasing during coating.

7.1. Ensure that coating material is installed at a designated evaporator head of the coating system.

7.2. Maintain the temperature of the cold stage in the coating system below −170 ˚C.

7.3. Place the specimen holder on the cold stage of the coating system after sufficient freeze-etching.

7.4. Open a partition between the cold stage and the evaporator head..

7.5. Set the current value and the voltage value of the evaporator head at ca. 30 mA and ca. 5 V, respectively.

7.6. Evaporate coating material for ca. 30 s to coat the surface of the specimen.

7.7. Set the current value of the evaporator head at zero, and close the partition.

7.8. Place the specimen holder on the cold stage of the specimen chamber for observation."

5. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol in yellow (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

We have marked essential steps to be filmed. Extracted steps have been described in a separate file named " 59154\_R2\_essential.docx".

6. Figure 1: Please add a short description in addition to the figure title in Figure Legend.

We have added the description of figure 1 as follows:

"The flow of procedures from sampling to SEM observation described in this paper is shown."

Additionally, we have collected both title of section 2 and the sentence in Step 3.7 as follows:

"2. Freeze fixation with LN2"

"3.7. Cut the surface of the sample only once or twice."