EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

# Experiment Report Form

**The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF**.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

***Reports supporting requests for additional beam time***

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

***Reports on experiments relating to long term projects***

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

***Published papers***

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

## Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

## Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

<table>
<tr><td> </td><td>Experiment title:<br>Phase-contrast tomography of Norway spruce phloem</td><td>Experiment number:<br>sc3569</td></tr>
<tr><td>Beamline:<br>ID19</td><td>Date of experiment:<br>from: 12 Sep 2012 to: 28 Mar 2013</td><td rowspan="2">Date of report:<br>30 Aug 2013<br>Received at ESRF:</td></tr>
<tr><td>Shifts:<br>6</td><td>Local contact(s):<br>Carmen Soriano / Paul Tafforeau</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):<br>Jussi-Petteri Suuronen[1]*, Tuula Jyske[2]*, Ritva Serimaa[1]*, Kari Pirkkalainen[1]*, Patrik Ahvenainen[1]*, Alexander Meaney[1]*, Aki Kallonen[1]*<br>[1]University of Helsinki, Department of Physics<br>[2]Finnish Forest Research Institute</td></tr>
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## Report:

The purpose of this ID19 experiment was to visualize conducting phloem anatomy in a number of Norway spruce (*Picea abies*) samples, and quantify the volume fractions and distributions of different types of phloem cells (i.e. sieve, albuminous, and parenchyma cells) as a function of sampling height, sampling time, tree age and tree size. Secondary aims were to visualize the sieve areas connecting adjacent sieve cells and to observe spatial variations in pathways for radial flow between phloem, cambium and xylem. From two trees, also a radial series of samples was scanned to obtain a comprehensive view of phloem structure from bark to cambium. For comparison with data produced with a home-lab x-ray microtomography system, a few samples were stained with osmium tetroxide and uranyl acetate, which tend to accumulate in nutrient-rich tissue such as parenchyma cells.

The experiment was initially awarded 3 shifts of beam time in September 2012; however, the scans taken during the first experimental session suffered from sample movement artifacts, and the manually corrected reconstructions were lost due to data transfer problems. The experiment was repeated in 3 additional shifts in March 2013 with weaker beam intensity. The second experimental session was very succesful, avoiding the sample movement problems nearly completely. All samples were scanned with a voxel size of 0.355 µm, using ~18 keV radiation and Paganin phase retrieval for enhanced contrast ($\delta/\beta$ = 1500, with the exception of the stained samples, for which $\delta/\beta$ = 200). Additionally, roughly half of the samples were imaged with a larger voxel size of 0.7 µm to obtain a larger field-of-view.

The results are presently being analyzed at the University of Helsinki, with the aim of publishing them in the form of a peer-reviewed article in an international scientific journal in early 2014. Initial analysis indicates that all of the required quantities can be calculated based on the reconstructions; sieve areas between adjoining sieve cells are visible (fig. 1), and the signal-to-noise ratio is sufficient for reliable segmentation of the conducting phloem area (fig. 2). According to preliminary results, the cell walls between sieve elements appear

thinner than previously indicated by home-lab x-ray microtomography, yielding higher values for the volume fraction of sieve cell lumina (ca. 60% vs. 40%). The results also confirm critical point drying as a relatively gentle sample preparation method: nuclei and other cell organelles are also seen preserved inside parenchyma (fig. 1). On the other hand, the good visiblility of nuclei and nutrient contents inside parenchyma cells also provides a challenge for data segmentation: parenchyma are not trivially separated from the cell walls, which have a similar grayvalue in the reconstruction. With further analysis, this could at least in part be solved by incorporating information from the regular absorption reconstructions from the same scans, in which the contents of parenchyma cells are much less clearly visible (fig. 3).

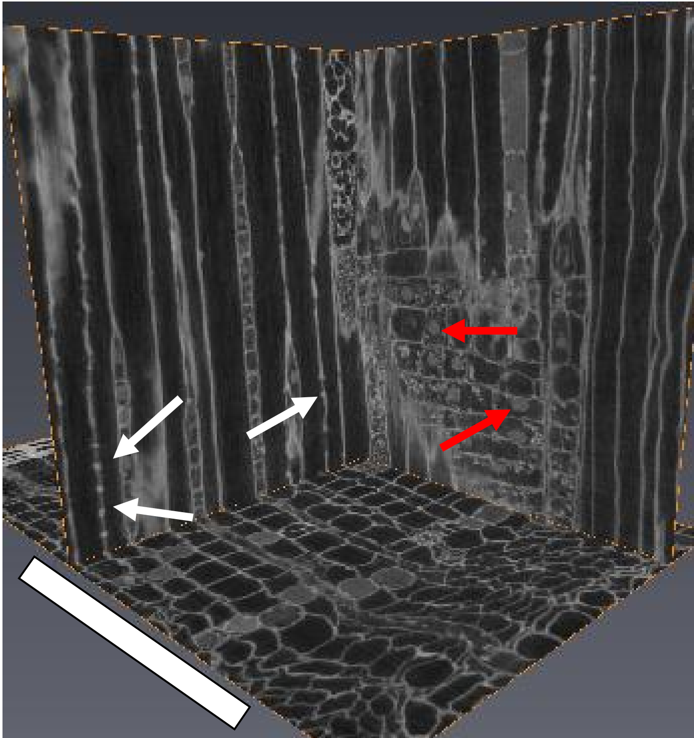

**Figure 1:** Three orthogonal views into a microtomographic reconstruction of a Norway spruce phloem sample. White arrows indicate lateral sieve areas between sieve elements, red arrows nuclei visible inside parenchyma cells. Scale bar is 300 µm.

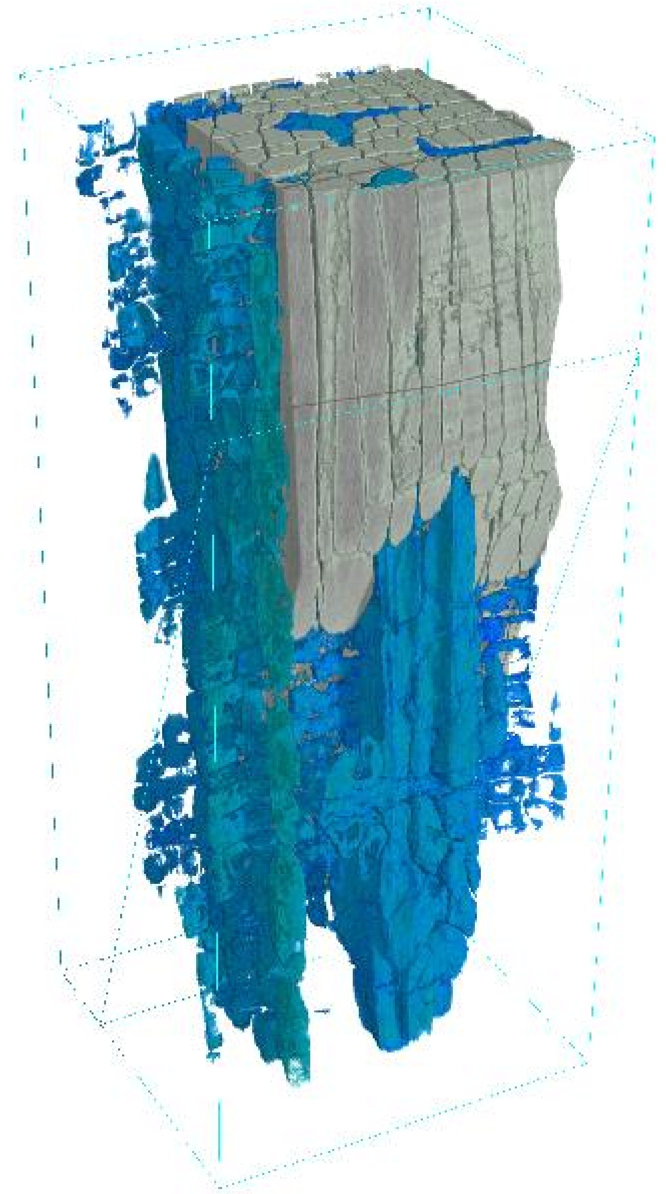

**Figure 2:** Segmentation of the conducting phloem in one sample, showing the lumina of sieve cells (59% of conducting phloem volume) rendered in green and clipped along two planes to show the lumina of albuminous and parenchyma cells (13%), rendered in blue. Only the cell lumina are rendered, with the cell walls and contents of parenchyma cells made transparent. Size of the bounding box is approximately 300 x 2280 x 730 $\mu m^3$.

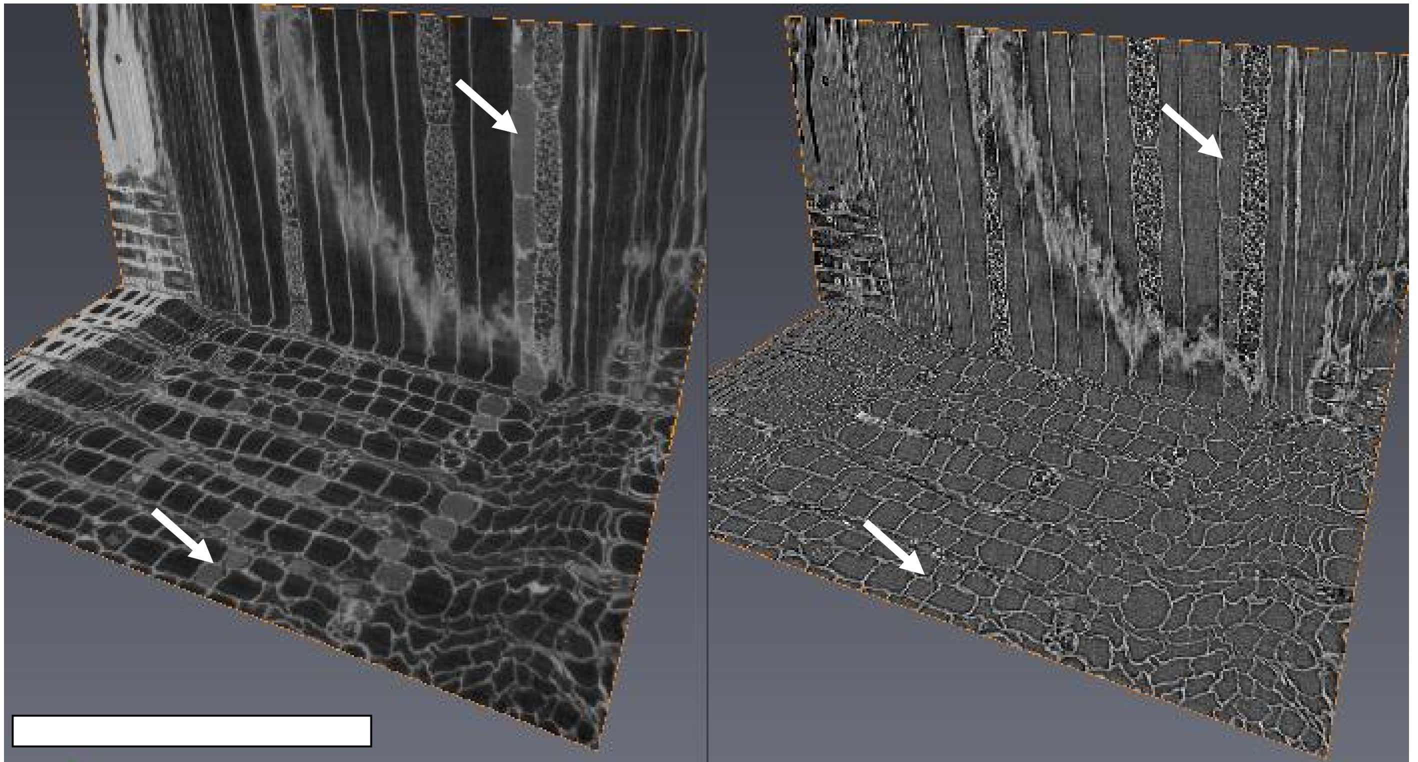

**Figure 3:** Comparison of reconstructions with Paganin phase retrieval (left) and with regular absorption images (right). Many features within axial parenchyma cells are not visualized without using phase contrast (arrows). Scale bar 300µm.