ESRF	**Experiment title:** Responsiveness of cellulose fibril orientation to artificial mechanical stimuli – nanodiffraction on single compression wood cell walls.	**Experiment number**: SC-4196
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Report:

Summary

We successfully managed to measure the microfibril angle (MFA) in single wood cell walls and fern cell walls and its response towards external stimuli. The experiments were carried out in cross-sectional fibre geometry, exploiting the curvature of the Ewald sphere. In this geometry, the XRD patterns originate from the intersection of the Ewald sphere with inclined rings from the equatorial reflection from cellulose fibrils. The MFA can be determined directly and in a highly spatially resolved fashion in cross sections of single cells.

Samples and Setup

The experiment aimed at the characterization of the MFA in single wood cells and its response to external stimuli applied during growth. In addition the unknown nanostructure of single fern cells was studied with high resolution.

Experiments were carried out at the Nanofocus endstation (EH3) of ID13, employing a setup of nanofocusing lenses to produce a beam of approx. 150x150nm at an energy of 14.85 keV. Data were acquired with the novel Dectris Eiger 4M detector, giving access not only to WAXS but as well SAXS data at the same time. The samples consisted of 10µm cross sections of fir tree branches (*Abies nordmanniana*). The branches were subject to artificial bending in order to induce artificial reaction wood. To vary the degree of response, 3 different bending radii as well as a bending with constant force was applied to the branches for three months, starting from August 2015. Following a cautious selection of influenced wood cells we scanned the interface between pre- and post-stressed cells to monitor the MFA.

An additional set of samples consisted of skelerenchymatic cells from fern (*Asplenium elliottii*) and (*Polypodium vulgare*), being model systems for two different secondary cell wall types. Whereas the lignified cell wall in *Asplenium eliottii* is expected to have a similar morphology to wood cell walls with a pronounced layered structured, not much is known on the structure of *Polypodium vulgare* that has a lignin-deficient cell

wall and is expected to show a poly-lamellate architecture with strong deviations in the MFA, akin to a Bouligand structure. From these samples also 10µm cross sections were prepared and measured.

Principal outcome

We successfully managed to scan an overview of regions of interest with 500 nm resolution as well as highly resolved single cells with 200 nm spatial resolution, without any obvious beam damage interfering with the data evaluation.
An example of sklerenchymatic fern cells (overview scan) and the higher resolved scan of single cell are presented in Figure 1.

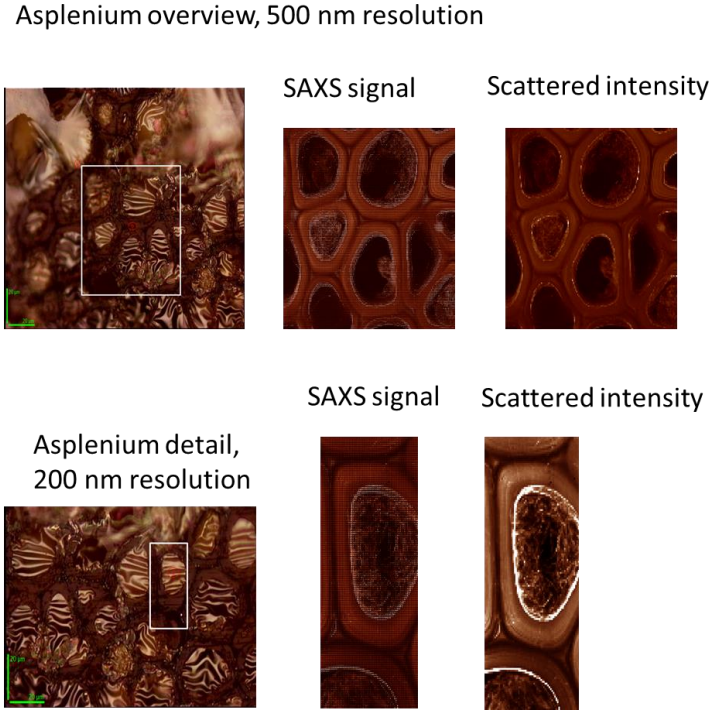

Figure 1 Scans of asplenium sclerenchyma. An overview with 500 nm resolution of 500 nm and a detailed scan of a single cell with 200 nm spatial resolution.

The lamellate nature of the cell wall is directly visible from the scattered intensity in Figure 1.
A closer analysis of the MFA is still pending but with data of this quality we expect to unveil even subtle details in the composition of the different cell types and draw meaningful conclusions on different types of cell architectures in relation to the chemical main constituents.

Conclusions and further proceedings

The experiment is regarded as highly successful. We were able to measure all intended wood samples and expand the dataset even on other types of cells (fern cells). The quality of the focused was very high and we were able to measure with a higher resolution than initially anticipated. We are currently in the process of deeper data evaluation and we are sure that this experiment will help us to gain significant insights into the nanostructure of plant cell walls and their response towards external stimuli, a point which will surely warrant publication. Given the impressive data quality we aim at collaborating further with ID13 on the topic and possibly find ways to increase the resolution even further to gain access to the important sub 100 nm size range.