EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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.

ESRF	**Experiment title:** Exploring the structural memory of plant fibre cell walls as a function of load	**Experiment number**: LS-2259
Beamline:	**Date of experiment**: from: 26 Feb 2014 to: 02 March 2014	**Date of report**: 26 June 2014 *Received at ESRF:*
Shifts: 12	**Local contact(s)**: Michael Sztucki	

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Report:

Please note that analysis of the data from this experiment is still ongoing, and that a manuscript based on the complete results will be submitted to a scientific journal at a later time. If accepted for publication, the final paper will be send to the ESRF library. This report simply describes the experiments performed and the data collected.

Introduction

The aim of the experiment was to study naturally occurring irregular zones in plant cell walls, so-called dislocations, especially to study their crystalline characteristics in the unloaded, humidified state, and during tensing (also in a humid state). The hypothesis is that in the wet state and at moderate stress levels, dislocations dissipate stresses rather than function as failure initiation points and thus are useful to the plant in maintaining cell wall integrity during the life span of the plant. The first step in testing this hypothesis is to study the structure of dislocations, specifically whether their structure differs from the surrounding cell wall and whether structural changes are induced by tensile stresses.

Initial tests

The experiment was carried out using hemp fibres (*Cannabis sativa* (L.)) as model plant fibres. These fibres are comparably long (5-10 mm and 10-30 µm in diameter), which facilitates handling. In addition, earlier studies focusing on dislocations carried out by the participants have been performed on hemp fibres. Before the start of the beam time we performed off-line tests on fibres with the tensile testing setup provided by ID13to get used to the setup, to confirm earlier tensile test results and especially to assemble the humidity cell around the samples and to monitor the achieved relative humidity. The stipulated 80% relative humidity were reached at room temperature, but later when mounting the assembly at the beamline, we observed that the temperature in the hutch was higher (~25 ˚C) and that the maximum relative humidity achievable was 73%. Consequently, experiments were carried out at this humidity level. In additional off-line tests fibres were strained under a light microscope with crossed polars in the user laboratory. These tests confirmed that dislocations gradually became less visible during straining in tension, and that they were no longer discernable at a force level of approximately 400 mN.

Data capture

Table 1 gives an overview of the data successfully captured, i.e. unsuccessful attempts are not shown. Since the light microscope at the beamline could not be used simultaneously with the tensile stage and the humidity cell, the original plan of performing line scans across a dislocation was deemed unviable, as the exact location of the dislocations was impossible to confirm once the fibre had been mounted. Instead mesh scans were performed. To avoid radiation damage influencing the second and later mesh scans captured for a given fibre, great care was taken not to re-measure any region. Due to the tensile strain and the lack of a microscope, this was not trivial. Two different approaches were used: Displacement in the longitudinal direction of the fibre, and displacement in the transversal direction of the fibre. In the first case the mesh position was moved in the opposite direction of the strain-induced movement of the fibre. For this approach fibres were chosen which were either filled with dislocations or nearly without dislocations. The rationale behind this choice was that different positions would not be too different from each other, and that any differences between meshes would be predominantly due to differences in tensile stress. In order to try to catch data from the exact same dislocation before and after tensing, an alternative transversal displacement approach was also attempted. For these fibres a distinct dislocation surrounded by normal cell wall was chosen, and the upper and lower half of the fibre was mesh-scanned before respectively after tensing, extending the lower mesh to compensate for the strain. Up till now it had been found that at ID13, fibres failed at much lower force levels than experienced in earlier tensile tests. ESRF staff confirmed that fibres could behave differently when tested in the line, and that X-ray radiation most likely damaged the fibres. Therefore modest tensile force levels were chosen. However, tensing these fibres to failure after the second mesh had been collected revealed that they were much stronger than the fibres tested previously, and the supposed 'after tensing' mesh scan therefore does not represent a state where the dislocations are no longer visible using polarized light. Unfortunately, when this was realised our beam time was over.

Table 1. Overview of successful measurements obtained at ID13 on hemp fibres during the allocated beamtime.

Sample ID	**Data collected**
A08	Mesh scan on untensed fibre okay, mesh scan after tensing only partially successful (partially on the frame)
A48	Mesh scan on untensed fibre okay, mesh scan after tensing only partially successful (partially outside fibre)
A50	Mesh scan on untensed fibre okay
A53 (many dislocations)	Mesh scan on untensed fibre okay, mesh scan after tensing okay for 2 levels of tensing, areas displaced in the longitudinal direction of the fibre between each mesh
A15 (many dislocations)	Mesh scan on untensed fibre okay, mesh scan after tensing okay for 7 levels of tensing, areas displaced in the longitudinal direction of the fibre between each mesh. 8th level of tensing partially on the frame
KU31 (no/few dislocations)	Mesh scan on untensed fibre okay
KU64 (no/few dislocations)	Mesh scan on untensed fibre okay, mesh scan after tensing okay for 4 levels of tensing, areas displaced in the longitudinal direction of the fibre between each mesh. Fibre broke during meshing of the 5th level of tensing.
KU06 (distinct dislocation)	Mesh of untensed upper half okay, mesh of lower half after tensing okay too. Dislocations not gone, tensing level low.
A74 (distinct dislocation)	Mesh of untensed upper half okay, mesh of lower half after tensing okay too. Dislocations not gone, tensing level low.

Data analysis

Figure 1 shows a colour coded image of one of the 24×63=1512 X-ray diffraction patters in the mesh scan obtained from sample A74 before tensing. The parameters to be compared between dislocations and normal cell wall, as well as between different tensing levels include: 1) the intensity of the 200 signals, 2) the angle compared to horizontal of a line connecting the two 200 signals, and 3) the length or angle between the upper and lower edges of the 200 signals. Analysis of these parameters will help clarify possible differences in crystallinity and microfibril angle between dislocations and the surrounding cell wall and how these characteristics differ between the tensed and the un-tensed state.

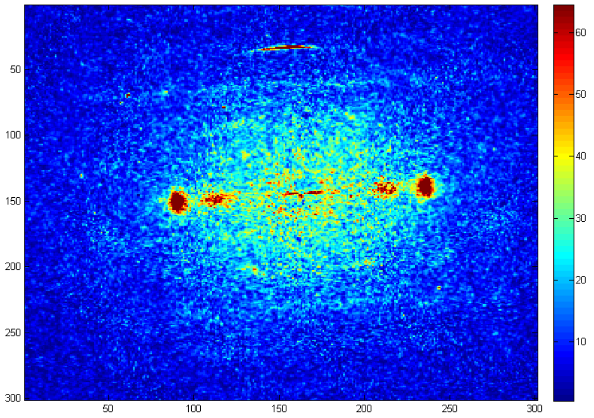

Figure 1. Example of X-ray diffraction data obtained at ID13 for hemp fibre sample A74.

Supplementary analysis

For the successfully analysed fibres the cross sectional area close to the failure will be measured using ESEM in order to calculate stress levels from the recorded force readings.