ESRF	**Experiment title:** Microfocus Analysis of Nitella and Chara Algae Cell Walls	**Experiment number:** SC-570
Beamline: ID 13	**Date of experiment:** from: 9/6/99 to: 11/6/99	**Date of report:** 6/10/00
Shifts: 6	**Local contact(s):** Martin Müller	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
Prof. Athene M. Donald
Mr. Owen M. Astley *

University of Cambridge

Report:

Introduction

The giant green algae *Chara* and *Nitella* (often called stoneworts or brittleworts) are useful materials to investigate the structure of plant cell walls due to their large size (up to 60 mm long) and similarity to the walls of higher plants.
Evidence from transmission electron microscopy (TEM) has suggested that the microfibrils in the walls of both *Chara* [1] and *Nitella* [2] have a helicoidal arrangement—successive layers of microfibrils through the wall have a gradually changing orientation angle.
Striations are also seen on the surface of the cells. The nature of these striations is unclear, although it appears that they may be involved with protoplasmic streaming, the movement of protoplasm within the cell.
The aim of this study was to investigate the helicoidal structure and the striations within the cell wall using microfocus X-ray diffraction. Microfocus diffraction is necessary when attempting to view the helicoids since the microfibrils are known to be straight over the beam size (several μm). This is not the case with the larger beam spot of conventional X-ray sources. A small beam size also allows the cell wall to be scanned across.

Experimental

Intact pieces of cell wall were removed from *Chara* and *Nitella* plants, washed with artificial pond water, and attached to washers before being placed in the beam.
X-ray diffraction was performed on station ID 13 in the wide-angle regime with a beam size of 2 μm and a wavelength of $\lambda = 0.787$ Å. Patterns were recorded on a CCD camera in time steps of 30 s and the wall was scanned across with step sizes of 10–20 μm.
The orientation of the cellulose microfibrils was determined by making circular azimuthal scans at a constant 2θ around the diffraction pattern using software produced in the laboratory.

Results

The predominant semi-crystalline material in the walls was found to be cellulose I. This has already been reported for *Nitella*, although it differs from previous findings for *Chara* [3].
Sadly, no evidence for helicoidal structure in the cell walls was found. It should be pointed out that this does not necessarily indicate no helicoidal structure, but could be due to too many separate cellulose layers making separation of the scattering signals from each layer impossible with the recorded signal:noise ratio. However, it was possible to show that the predominant microfibril direction was perpendicular to the long axis of the cell. This did not change at different points in the cell.
The striations seen by optical microscopy did not appear to influence the diffraction pattern and made no difference to the predominant microfibril orientation or the crystalline structure.
In some regions crystalline material was seen. This was attributed to the calcium carbonate (which gives the plants the common name of stonewort), and was conclusively identified as $CaCO_3$ and not a hydrate of calcium carbonate.

References

[1] A. C. Neville and S. Levy. Helicoidal orientation of cellulose microfibrils in *Nitella opaca* internode cells: ultrastructure and computed theoretical effects of strain reorientation during growth. *Planta*, 162(4):370–384, 1984.

[2] S. Levy. Two separate zones of helicoidally orientated microfibrils are present in the walls of *Nitella* internodes during growth. *Protoplasma*, 163(2–3):143–155, 1991.

[3] G. S. R. Krishnamurti. On the nature of cell wall in *Chara* sp. *Phykos*, 4:17–19, 1965.