FIB-SEM Serial Sectioning Nanotomography of Flax Fibres

Tan Sui, Hongjia Zhang, Siqi Ying, Patrick O'Brien, and Alexander M. Korsunsky*

Abstract— **Flax fibre has a complex hierarchical structure that ensures its high specific stiffness and strength. However, flax fibres also contain defects known as nodal markings, slip lines or dislocations that limit their strength. In order to promote the possibility of using flax fibre as alternative reinforcement for composite materials to replace synthetic fibres, it is important to develop experimental approaches that allow the characterization of their multi-scale structure at nanoscale resolution.**

Focused Ion Beam - Scanning Electron Microscopy (FIB-SEM) serial sectioning was used to visualize the inner micro-structure of a fibre bundle and of a single flax fibre. A series of high resolution cross-section visualisations of flax fibres were obtained. A cluster of two fibers was studied. The inner pore (lumen) running through the centre of the individual fibre was distinguished. The S2 secondary wall cell layer could be identified by considering the "etching" effect during FIB milling caused by the fact the in this part of the fibre wall the fibrils are oriented almost parallel to the axial direction. The inner microstructure visualization of the flax fibre along its length offers a significant basis for cross-correlating their structural features with mechanical properties.

***Index Terms*—flax fibre, FIB-SEM nano-tomography, microstructure**

I. Introduction

FLAX fibres are being extensively considered as a potentially environmentally friendly and cost-effective replacement for synthetic glass fibres reinforcement in polymer matrix composites that are being widely used in numerous branches of modern technology. The critical challenge to broader application of these composites lies in the natural variability of the mechanical properties of natural fibres, specifically their strength and stiffness. To overcome the difficulties that this presents both for designers and end users, technologically reliable solutions for property evaluation and control is required. The present investigation of the fibre micro- and nano-scale structure is aimed to give better insight into the link between internal architecture and performance [1-3].

Combining scanning electron microscopy with focused ion beam serial sectioning (FIB-SEM) allows micro- and nano-scale visualization of the inner structure of a bundle of fibres and or of a single fibre. We report a FIB-SEM serial sectioning. We show selected cross-sectional images and discuss the features of the fibre structure that can be identified using this imaging mode. In addition to revealing how fibers are assembled into bundles, the secondary cell wall dominating S2 layers could be identified. Further steps that can be taken to improve insight into the inner structure at the micro- and nano-micro scale are discussed.

Manuscript received Dec 26, 2014; revised Jan 27, 2015.

This work was supported in part by EU FP7project iSTRESS ''Pre-standardisation of incremental FIB micro-milling for intrinsic stress evaluation at the sub-micron scale", and by EPSRC through grants EP/I020691 "Multi-disciplinary Centre for In-situ Processing Studies (CIPS)", EP/G004676 "Micromechanical Modelling and Experimentation", and EP/H003215 "New Dimensions of Engineering Science at Large Facilities".

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II. Materials and Methods

A. Material structure and sample preparation

The plant fibre used in this study was obtained from common flax plant belonging to the Linum usitatissimum L. variety 'Hera' obtained in January 2014 from the Centre for Genetic Resources, Netherlands. Flax seeds were sown and grown in the plant growth rooms of the Plant Science Department, School of Biological, Earth and Environmental Sciences (BEES), University College Cork, Ireland, and the Centre for Biological Sciences, University of Southampton, U.K. Following the plant growth schedule given in [4], greenhouse conditions were maintained, with daytime illumination provided by 400WQ Philips mercury fluorescent lamps giving a total light intensity at bench level of 20 klx, and the temperatures of 22 °C by day and 16 °C by night. The crops were harvested at maturity circa 14 weeks in late May 2014.

The schematic diagram of the architecture of a single flax fibre is illustrated in Fig. 1 [1]. Key features can be identified as follows. The inner channel known as lumen is surrounded by the secondary wall that is several microns thick and consists of three sub-layers labeled S1, S2 and S3 in the outward direction. Each of the sub-layers is characterized by a particular angle that the aligned fibrils make with the fibre axis. Fig.1 illustrates that while the S1 and S3 layers contain cross-wound fibril arrangement running at a large angle to the fibre axis, the thickest secondary wall layer S2 contains parallel bundles of fibres that make a small angle of about 10°

ISBN: 978-988-19253-9-8
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

with the axis. Finally, the outer primary cell wall (labeled P) is composed of a mat of unaligned fibrils providing a superficial protective layer.

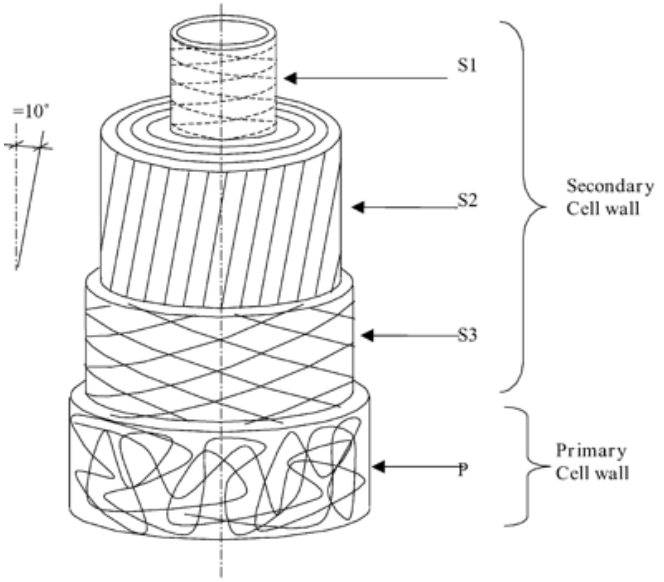
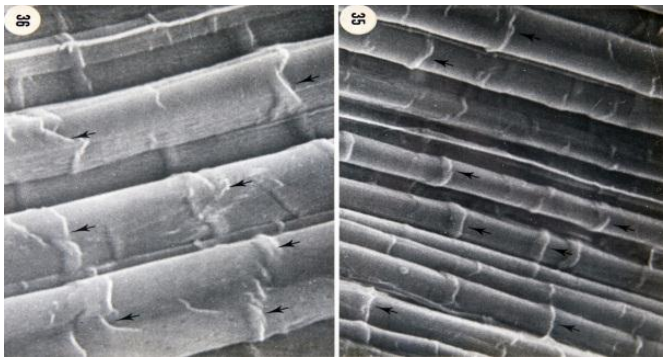


Fig. 1. Schematic diagram of flax fibre architecture (reproduced from [1] by permission of Elsevier Science publishing).

A bundle of flax fibers was separated carefully from the stem using tweezers under the optical microscope. In order to eliminate the charging effects by electron imaging, the bundle was mounted on an adhesive carbon disc and coated with a few nanometre-thin film of Au-Pd by the mini sputter coater (SC7620, Quorum Technologies).



a) b)

Fig. 2. Archival SEM images from [4] illustrating the systematic presence of nodal markings on individual flax fibres (shown by black arrows) that were obtained at the magnification of a) ×1645 and b) ×871. Typical diameter of the flax fibre lies in the range 19 μm to 20 μm.

Of particular interest in the microstructural analysis of flax fibres is the elucidation of the structure of nodal markings (also known as dislocations, slip lines, etc.) that are a prominent feature of flax fibres, but also of other natural fibres such as hemp and cotton. An illustration of prior SEM imaging work [4] is shown in Fig.2. Arrows indicate nodal marking features that are an inherent characteristic of natural flax fibre. Since the typical individual fibre diameter is approximately 20 μm, the detailed investigation of these features requires nanoscale resolution external and internal imaging of their structure.

Exterior SEM imaging of unretted flax fibres was carried out in the Multi-Beam Laboratory for Engineering Microscopy (MBLEM), Department of Engineering Science, University of Oxford, UK. SEM images of an individual flax fibre obtained using different imaging modes that are shown in Fig.3 reveal clear evidence that nodal markings are associated with the peripheral growth layers.

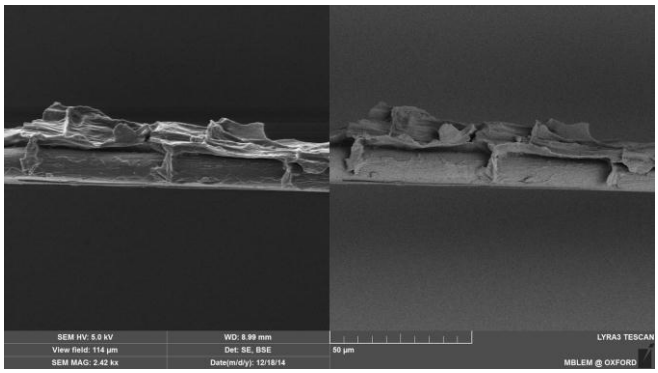


Fig. 3. Moderate magnification SEM images of unretted fibre obtained using secondary electron and back-scattered electron detectors.

Higher resolution imaging of the fibre surface shown in Fig. 4 reveals branched veins of typical width ~1 μm that form folds and knots around nodal markings.

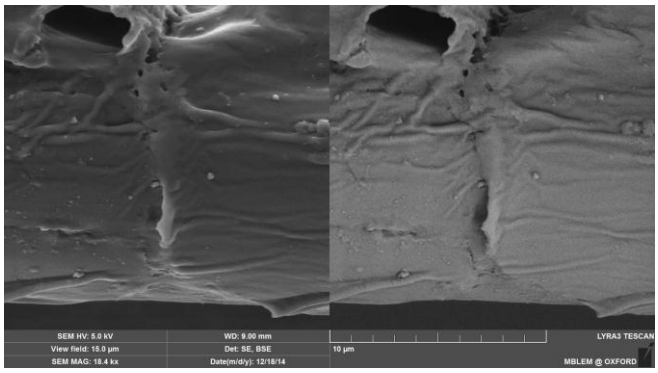


Fig. 4. Higher magnification SEM images of fibre surface in the vicinity of a nodal marking line viewed using SE and BSE detectors.

Two hypotheses can be put forward to explain the appearance of these vein-like surface features. They may reflect the surface bulges that appear due to the presence of subsurface bundles of fibrils. On the other hand, these features may be surface folds formed by creases in the outer layer that underneath could be either hollow, or filled with softer material.

Resolving such ambiguities, along with other detailed questions concerning the internal architecture, literally requires insight, i.e. internal imaging of the fibre structure that cannot be achieved at the required sub-micron resolution using e.g. the widely used optical techniques utilizing polarized light or fluorescence.

It is also worth noting at this point that it is precisely the features such as fibril folds that are likely to be associated with *dislocations* – regions that are of particular importance to understanding the properties of flax fibres. Dislocations

ISBN: 978-988-19253-9-8
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

are defined as irregular regions within the cell wall of natural fibres, and may also be called slip planes or nodes. From the load bearing point of you, the principal effect of such irregularities is associated with fibril kinking or splicing, that cause collective rearrangements of fibrils within bundles, and displaying strong effects on the stiffness and strength of the entire fibre.

In the next section we present the results of a preliminary nano-tomographic investigation of the internal structure of individual flax fibres using FIB-SEM serial sectioning. This is followed by a discussion of the challenges and prospects for this technique.

B. FIB-SEM serial sectioning procedure

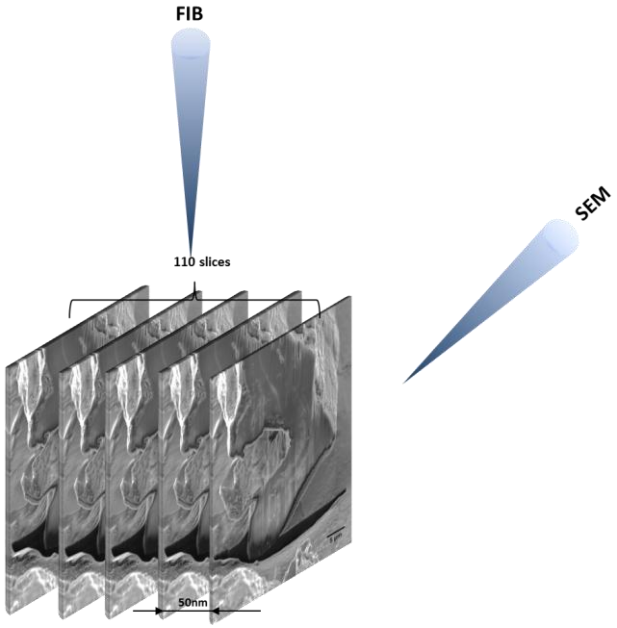


Fig. 5. Illustration of the FIB-SEM configuration for serial sectioning carried out on a cluster of two flax fibres.

The serial sectioning experiment was carried out using the FIB-SEM instrument LYRA3 XM (Tescan s.r.o., Brno, Czech Republic). Successive layers of material each of 50 nm thickness were removed by FIB milling, accompanied by SEM image acquisition of each exposed surface (Fig.4). Ion beam current of 128 pA was used in an attempt to achieve smooth condition of each consecutive section for optimal electron imaging. The FIB-SEM serial sectioning and imaging configuration is illustrated in Fig.5. The FIB milling depth (40 µm) and width (23.3 µm) were selected to ensure that at each milling step complete section through the entire fibre cluster was achieved. Precise alignment of the ion and electron beams was maintained to ensure stable centering of successive images and to avoid electron image drift or "jitter". In total, 110 sectional images were obtained, covering 5.45 µm in depth along the axial direction of the fibre cluster. Adequate SEM image resolution to reveal the internal microstructure of the fibre was selected to correspond to 25×25 nm^2 pixel size, with 1572×2240 pixel matrix chosen for each image frame. The parameters and settings used for the FIB and SEM milling and imaging are listed in Table 1.

TABLE I
SEM-FIB SERIAL SECTIONING SETTING PARAMETERS

	Parameters	Values
Milling volume	length	5.45 µm
	width	23.3 µm
	depth	40 µm
Slices	thickness	50 nm
	numbers	110
SEM image	pixel size	25×25 nm^2
	pixels	1572×2240

III. RESULTS

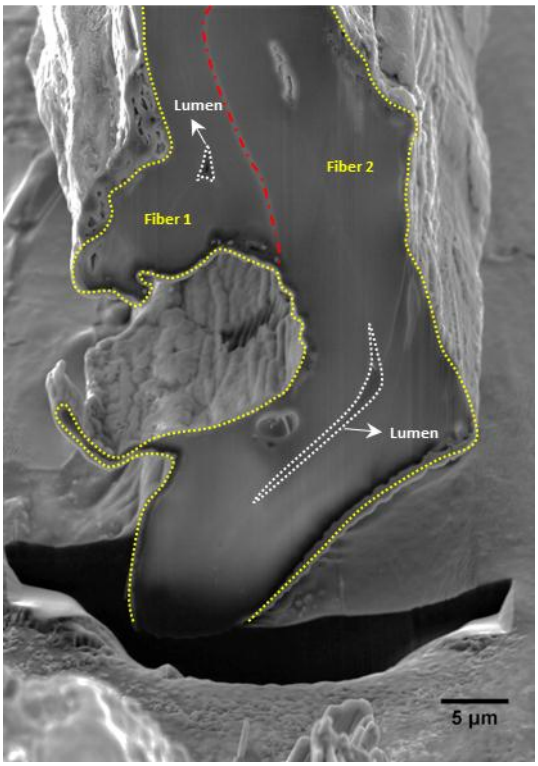


Fig. 6. SEM image of a particular FIB cross-section revealing the major features of a fibre cluster: the outer surface (yellow outer dotted line) and the darker primary wall adjacent to it, the junction between two fibres (red dash-dotted line), and lumen (white dotted lines at fibre core).

In order to discuss a representative cross-section SEM image, one image was chosen from 110 milling steps that allows a discussion of the fibre cluster internal structure. Figure 6 reveals that a cluster of two fibres was being processed. The interface between the two individual fibres is identified and indicated by the red dash-dot line. Identifying lumen in the two fibres is not straightforward due to the irregular shape taken by the fibres compressed into the cluster. To aid identification, the lumen line in the larger of the two fibres is highlighted using the white dotted line close to the fibre centre. The consideration of the smooth section reveals

ISBN: 978-988-19253-9-8
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that careful FIB sectioning allows the identification of such prominent structural features as lumen (that can also be traced as a faint central curve, even in regions when it is not open) and the primary outer wall adjacent to the fibre surface that is "etched" to appear a somewhat different shade of grey. However, the details of the sub-regions, in particular within the thicker secondary wall, do not become apparent from such imaging.

Figure 7 shows an SEM image of a different cross-section that is more affected by the so-called "curtaining" effect. This term has become used in the FIB-SEM jargon to refer to the shadowing effect that arises as a consequence of the variation in the material density in the regions closer to the incident ion beam, and the consequent unevenness of the milling rate of the material that lies further away. As a consequence, surface undulations are generated on the surface that can be seen in the image. The recognition of this phenomenon is important to avoid inappropriate association of the surface features that appear due to curtaining with some elements of material's internal structure.

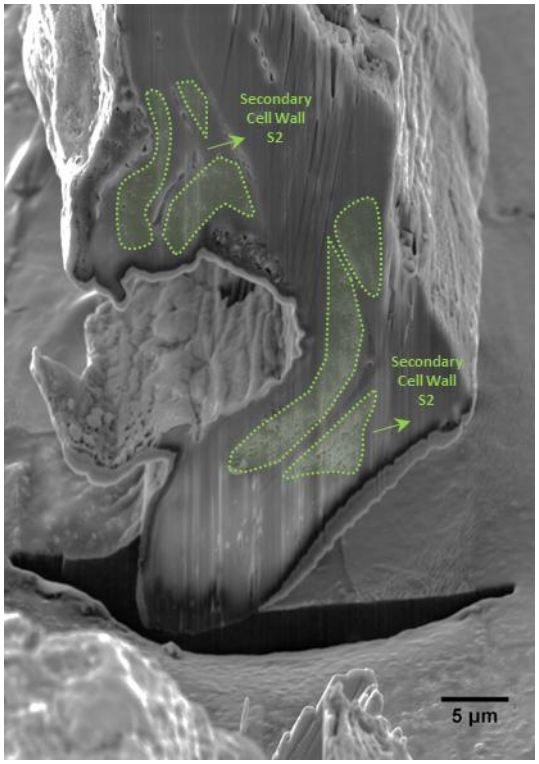

Fig. 7. FIB-SEM cross-sectional image that reveals some details of the secondary cell wall structure (S2) within the etched "islands" identified by the shaded regions surrounded by the green dotted lines.

Nevertheless, this uneven milling also produces an accompanying effect that aids the purposes of FIB-SEM sectioning for microstructural analysis. The shaded "islands" surrounded by the green dotted lines contain regions of "speckly" surface that arises as a consequence of ion beam scattering that leads to an "etching" effect at the surface. Since secondary walls S1 and S3 contain fibrils that are aligned to lie predominantly within the cross-sectional plane, their ion sectioning and mild etching produces a relatively smooth appearance. In contrast, the S2 secondary wall of the fibre contains fibrils that are aligned closely with the axial direction. Therefore, ion "etching" of an axial section leads to differential removal of the softer pectin matrix that provides the bonding that holds together the tougher fibrils that are removed more slowly. This contrast in the removal rate results in the surface roughening observed. We surmise that the islands highlighted in the image are likely to be associated with regions that lie within the S2 layer that forms part of the secondary fibre wall.

IV. Discussion and Conclusion

The preliminary trial of FIB-SEM serial sectioning reported in the present article suggests that this technique may become an interesting tool that takes the utility of electron microscopy out of the plane of surface imaging, and towards the possibilities of nano-scale tomography of internal fibre structures. Despite the very limited nature of the insights obtained, the feasibility of utilizing FIB-SEM to characterize the inner structure of natural flax fibres has been demonstrated.

However, significant challenges were also identified. Achieving contrast in identifying different regions within the fibre in the orientation used routinely for FIB-SEM serial sectioning (Fig.5) is not easy. This is due to the fact that good contrast usually arises from FIB milling at low ion currents performed at angles close to normal incidence [3]. This opens up the possibility of intermittent sample surface tilting to produce contrast in the course of data acquisition.

It is also worth mentioning that FIB sectioning, in combination with lamella preparation and nano-manipulator lift-out, offers the possibility of section preparation from regions associated with selected features, such as nodal markings (dislocations) for the purpose of subsequent multi-modal analysis using complementary techniques, e.g. EDX, tEBSD, etc.

Acknowledgment

The authors express their gratitude to Zora Strelcova and Jiri Dluhos at TESCAN Brno, s.r.o., Czech Republic, for their ongoing advice and support in the operation of FIB-SEM facilities in the Multi-Beam Laboratory for Engineering Microscopy (MBLEM), Department of Engineering Science, University of Oxford, UK.

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