

Appendix B

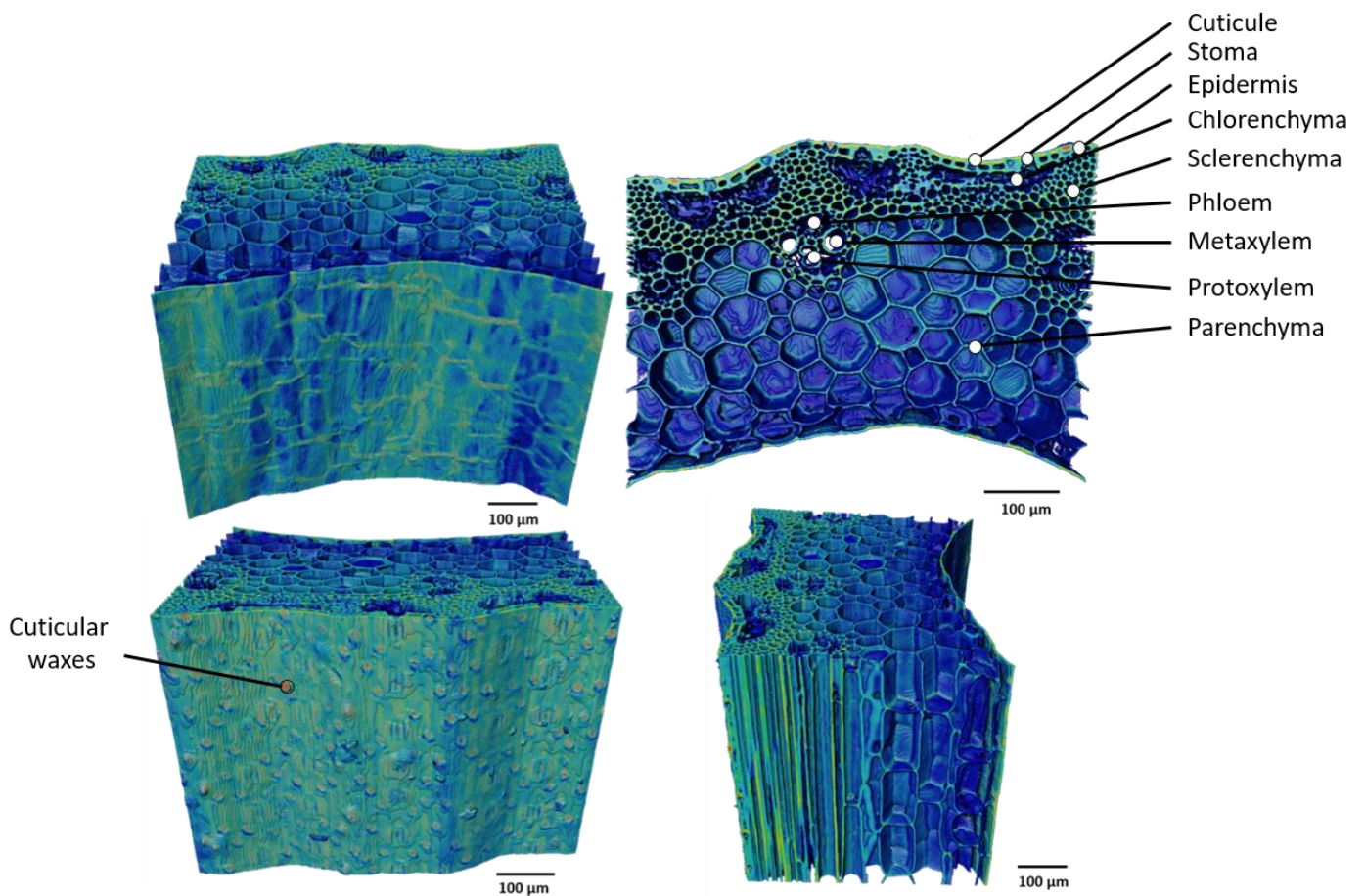
Additional results

In this part, we compare details of wheat straw images using different imaging modalities (X-ray tomography, confocal microscopy and scanning electron microscopy) and present additional results.

B.1 3D volume renderings of native samples: additional images

The following 3D images, shown on Figure B.1, notably highlights anatomical details described on the literature.

Figure B.1: Sub-volumes of raw wheat straw with different points of view: front (left up), back (left bottom), top (right up) and left side (right bottom). Several anatomical features are indicated.



B.2 High spatial resolution images of native and digested sample obtained by microscopy

Wheat straws were incubated in buffer (50 mM citrate buffer pH 5.4) or in presence of enzymes (buffer supplemented with 0.1 g/L of Novozymes Cellic HTEC2). After treatment, for optical microscopy imaging, samples were fixed with 2.5% glutaraldehyde in a 0.1 M sodium cacodylate solution, pH 7. Samples were dehydrated by incubation in 20, 40, 60, 70, 80, 90% ethanol baths for 1h each and then in 100% ethanol (2 times 24h). Wheat straws were infiltrated by resin (LRWhite) in ethanol: resin 2:1, 1:1 then 1:2 solutions (≈ 12 h each at room temperature) then 2 times in pure resin (24h each). Samples were put in resin capsules and incubated at 65°C for polymerization. Wheat straws were cut in half with a diamond knife with an ultramicrotome (UltraCut E Leica-Reichert) and then 1 μm slices were made from the sliced side of the wheat straw. Slices were fixed on glass slides, dried at 50°C and treated with 0.2% calcofluor. Samples were observed with confocal microscopy (Leica SP2 AOBS) with a monochromatic diode laser at 405 nm. Fluorescence emitted by calcofluor is collected between 415 and 500 nm. All observations were made with the same acquisition parameters. Zoomed images are made of maximal projections of x and y confocal images that have been captured with 40x objective and stacked on a z axis.

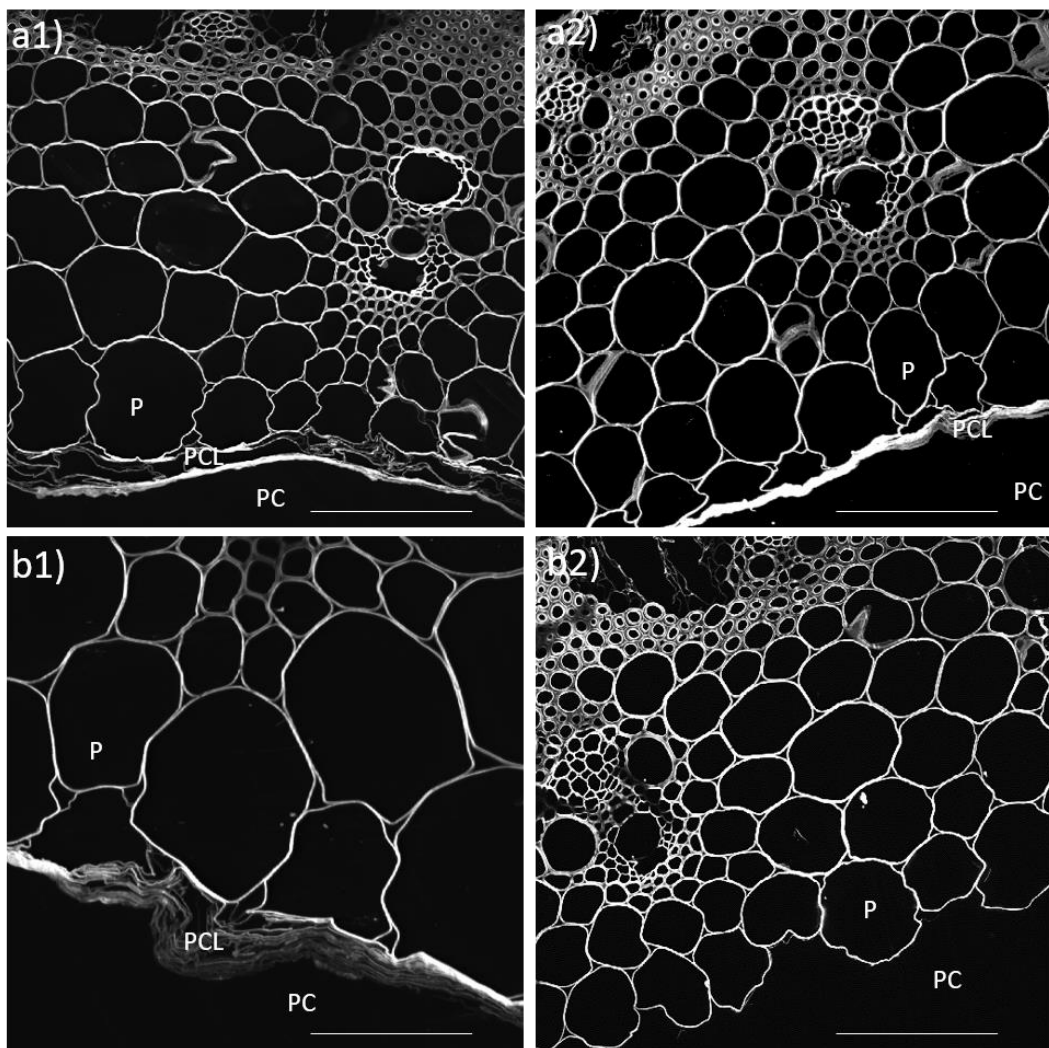


Figure B.2: Confocal images of wheat straw parenchyma. a) Control samples of wheat straw before (a1) and after incubation in presence of buffer 24 h (a2). b) Samples of wheat straw incubated in presence of enzymatic cocktail

during 90 min (a1) and 24 h (b2). Scale bar represents 100 μm , except for b1, 30 μm . P, parenchyma; PCL, pith cavity lining; PC, pith cavity.

From this imaging, the PCL is clearly observable as a bright and compact structure (Fig. B.2, a1), even if delamination may be observed occasionally (Fig. B.2, a2), no matter how long the samples is exposed to buffer. However, after 90 min exposure to enzymatic activity, PCL is much less bright, presenting a high level of delamination (Fig. B.2 b1). After 24 h, PCL was totally removed, exposing the first cells of the parenchyma (Fig. B.2 b2).

B.3 Mesh-like structure observed on the surface of the PCL

In order to confirm and deeper characterise the mesh-like pattern observed on the surface of the PCL, scanning electron microscope (SEM) and confocal microscopy were used. The SEM images were acquired using a FEI Quanta 250 FEG scanning electron microscope at an accelerating voltage of 10kV. The sample was hand-cut lengthwise using a sharp blade and mounted on microscope stubs and then coated by platinum sputtering (Leica EM MED 020). Microscopy observations were performed by confocal microscopy (Leica TCS SP8 AOBS confocal laser scanning microscope). Wheat straw sample was hand-cut lengthwise using a sharp blade and treated with 0.2% calcofluor. Calcofluor was excited at 405nm and detected in 415-465 nm range.

SEM observations (Fig. B.3, a1) clearly demonstrate the presence of the mesh-like pattern that appears in relief at the surface of the PCL as an organized structure. A closer view was realized at the location where the sample was cut, revealing different layers of the PCL (Fig. B.3, a2-a3). It is thus possible to reveal parallelepiped-shape structures on the inner surface of the PCL, apparently not always related to the cells forming the sub-basement of the PCL or to the first cells of the parenchyma. An even greater enlargement of the contours of such structure (Fig. B.3, a4) highlights its fibrous aspect. A quick attempt to define the composition of this structure was performed using cellulose dedicated strainer and autofluorescence of lignin, observed by confocal microscopy at a spatial resolution comparable to that of X-ray μCT . In both cases (Fig. B.3, b1-b2), the images revealed the same mesh pattern as the one seen on the volume rendering of the 3D images. This structure is clearly not an artefact, probably composed of cellulose and lignin, thus providing a dense material observable by the X-ray tomography. Fig. B.3, c1-c3 show some tomographic slice along the mesh pattern. For the case shown in Fig. B.3, c2 the local higher density in the PCL is associated with the presence of the cellular roof of a neighbouring parenchyma cell but this is not the case in Fig. B.3, c3.

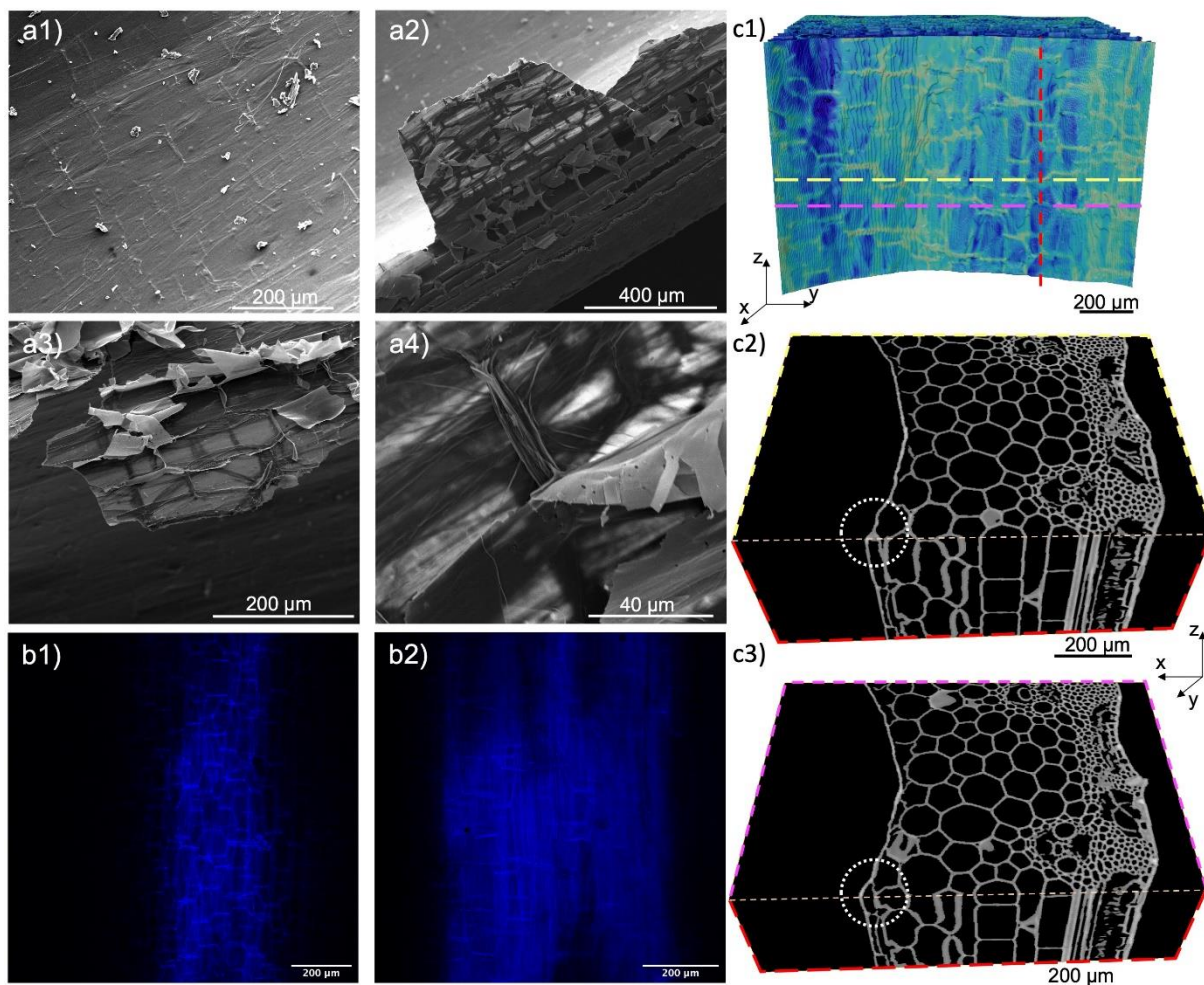


Figure B.3: a) SEM images of the PCL. b) Confocal microscopy of raw wheat straw: autofluorescence which indicates the presence of lignin (b1), sample marked with calcofluor to highlight cellulose (b2). c) X-ray μ CT images. Tomographic slices along the coloured dashed lines highlighted in c1 are shown in c2 and c3.

B.4 72-hour long enzymatic attack: protocol, additional SEM and μ CT images

As described in the Method section, the sample was first washed and dried. Then, enzymatic attack was performed as for the others samples, during 24h. After 24 h, the sample was removed from the first bath to be placed in a second fresh one for 24h. Finally, a third fresh bath was used for another 24h. After a total of 72 h of attack, the reaction was stop by washing the sample in three deionized water baths for 5 minutes, 10 minutes and one hour. Then the sample was dried in a ventilated oven at 45°C for 6 hours.

The SEM images shown in Figure B.4 were obtained as explained in §B.3 of this Supplementary information while the X-ray μ CT images are similar to those presented earlier in the paper. SEM images fully confirm the conclusion drawn from the μ CT images, with a total digestion of the PCL and of the cells located at the immediate vicinity of the PCL after the enzymatic attack: see subfigures a2 and b4, b6.

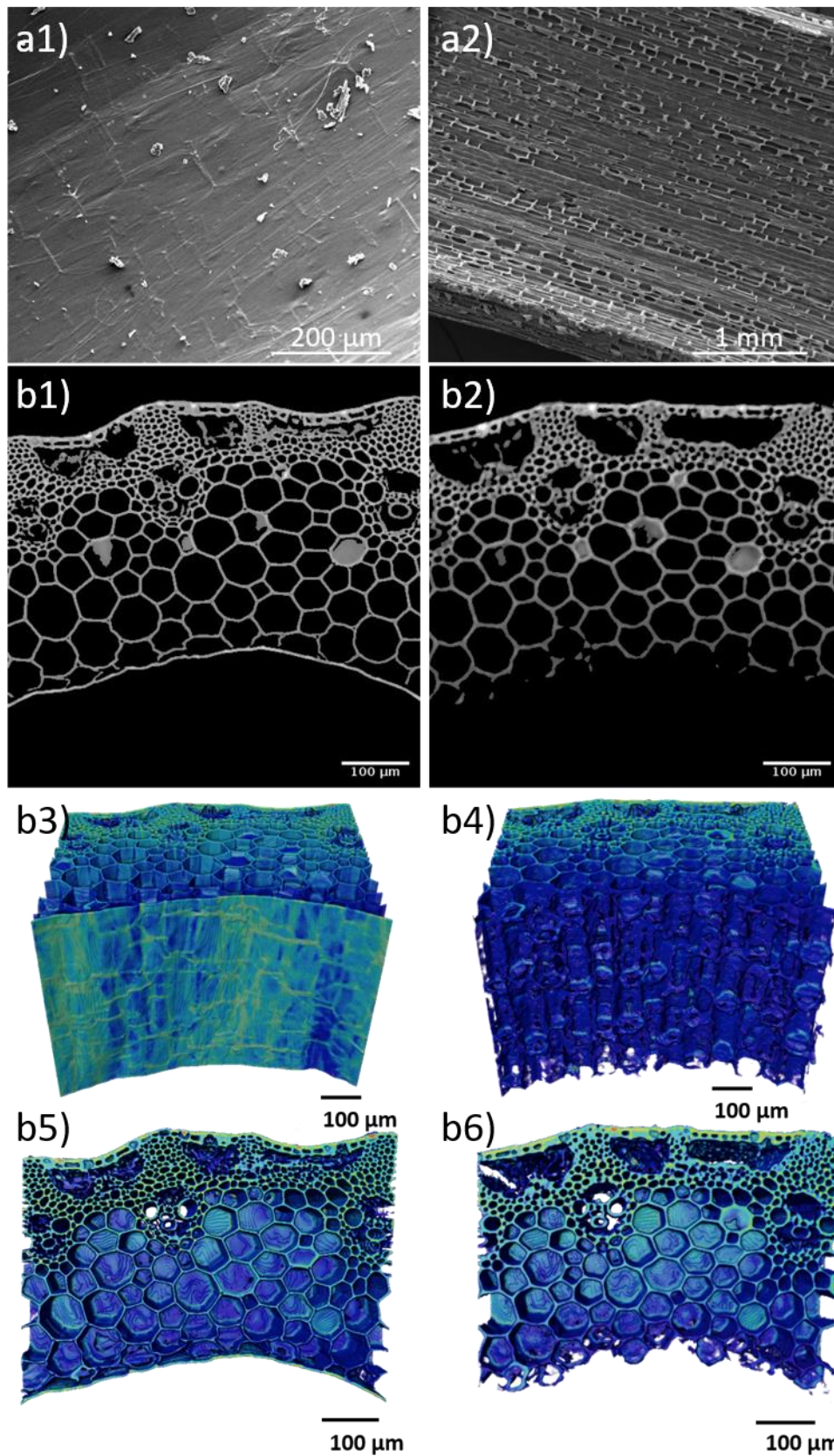


Figure B.4: a) SEM images of the internal plant cell wall before (a1) and after (a2) a 72h-long enzymatic attack. b) Images from X-ray tomography: tomographic slice and 3D volume rendering before (b1,3,5) and after (b2,4,6) the attack.

B.5 3D volume renderings of digested samples, for different durations of exposure to the enzymatic cocktail

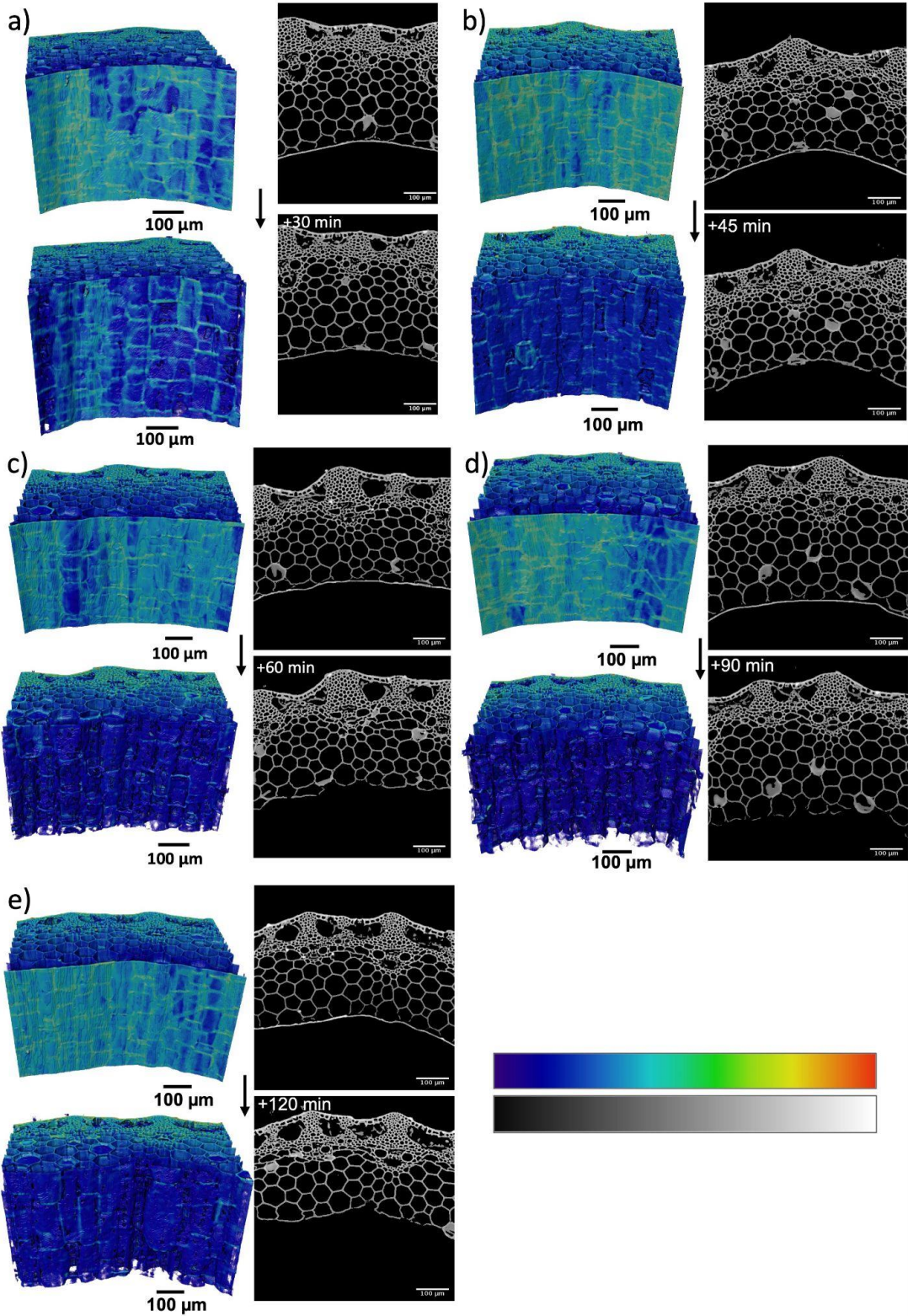


Figure B.5: Tomographic slice and 3D volume rendering before and after enzymatic attack. The duration of the enzymatic attack varies from one sample to another. The correspondence between the grey levels of the 2D images and the colormap of the 3D volumes is given on the right.

B.6 ROI-based analysis for a control sample

For the purpose of obtaining a control sample, a native wheat straw sample was washed in three successive baths of deionised water for 5 minutes, 10 minutes and one hour, and then dried for 6 hours in a ventilated oven at 45°C. The sample was soaked in a 50 mM citrate buffer at pH 5.4 supplemented with 0.1 mg/mL BSA for 120 minutes at 45°C under 1000 rpm agitation: this is similar to the protocol used for enzymatic attack but without the enzymatic cocktail. After the incubation time, the wheat straw sample was washed and dried again in a ventilated oven at 45°C. Finally, then the sample was scanned using X-ray μ CT.

In Figure B.6 we compare the grey levels (GL) distribution obtained for a set of ROIs positioned across the PCL for the control sample and native ones. The data for the control sample (dashed black line highlighted by black dots) are similar to those obtained from native samples. In particular, they display GL values in the range [2000-6000] above threshold, representative of the bright voxels of the PCL which disappear after digestion (data for a representative digested sample are given in red). Such a result proves that the alterations seen on the PCL can be attributed to the enzymatic cocktail effect, and not to some others manipulations of the sample during the protocol.

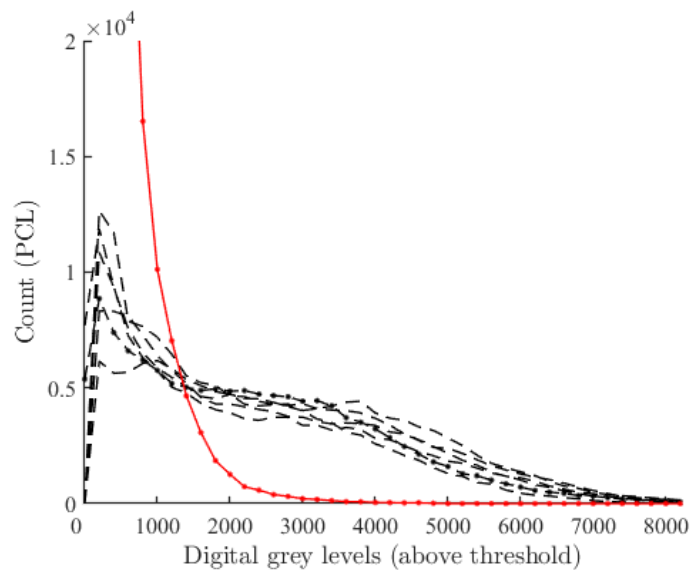


Figure B.6: Grey level distribution obtained for the set of ROIs positioned located across the PCL. The control sample is given by the black, dashed line with black dots. The 4 others black dashed lines are for 4 different native samples. A typical GL distribution for a digested sample is shown in red.