

Appendix A

Image processing

In this part, we detail how the tomographic slices are processed to get 3D volume rendering using Avizo (A.1) and how quantitative information are extracted using Matlab (A.2).

A.1 Image processing and 3D volume rendering using Avizo

The full image processing workflow using Avizo is summed up the Figure A.1. The effect of each successive operation can be appreciated on close-ups of a given tomographic slice. The first step consists in noise removal using the non-local mean filtering (NLMF) function implemented in Avizo. NLMF is the denoising procedure of choice to preserve the textures on an image (Buades et al., 2005). This filter was used in a 3D formulation and parameters (e.g. size of the search window, etc.) were optimized by measuring the actual noise reduction along intensity profiles taken on the images. The 3D volume rendering procedure requires a segmentation of the image, i.e. separation between the two constitutive materials, air and plant cell walls (PCW), which is made using a hysteresis thresholding algorithm. It creates a binary image using two thresholds: a voxel is said to belong to the air phase if its grey level (GL) value is lower than the lower threshold, and to the solid phase if it is superior to the higher threshold. A voxel with GL in between the two thresholds is selected as part of the solid material only if at least one neighbouring voxel (in 3D) already belongs to the solid material. The two thresholds are determined by careful inspection of the images (a discussion on the determination of such thresholds follows in §A.2.4). Finally, the binary image obtained is used as a mask. The mask function of Avizo overlaps the binary image and the original GL image. Voxels belonging to both the mask and the GL image are retained, together with their GLs, the other are set to zero. It is important to note that this procedure does not lead to segmented images, as it is classically understood i.e. images where each material is associated with a given GL value (e.g. 0, 1,...,n, when n materials are present). Here, only air is set to zero while the dynamic of GLs within the plant cell walls material is retained. Finally, Avizo volume-rendering tool is used to obtain the 3D volume renderings shown in this paper.

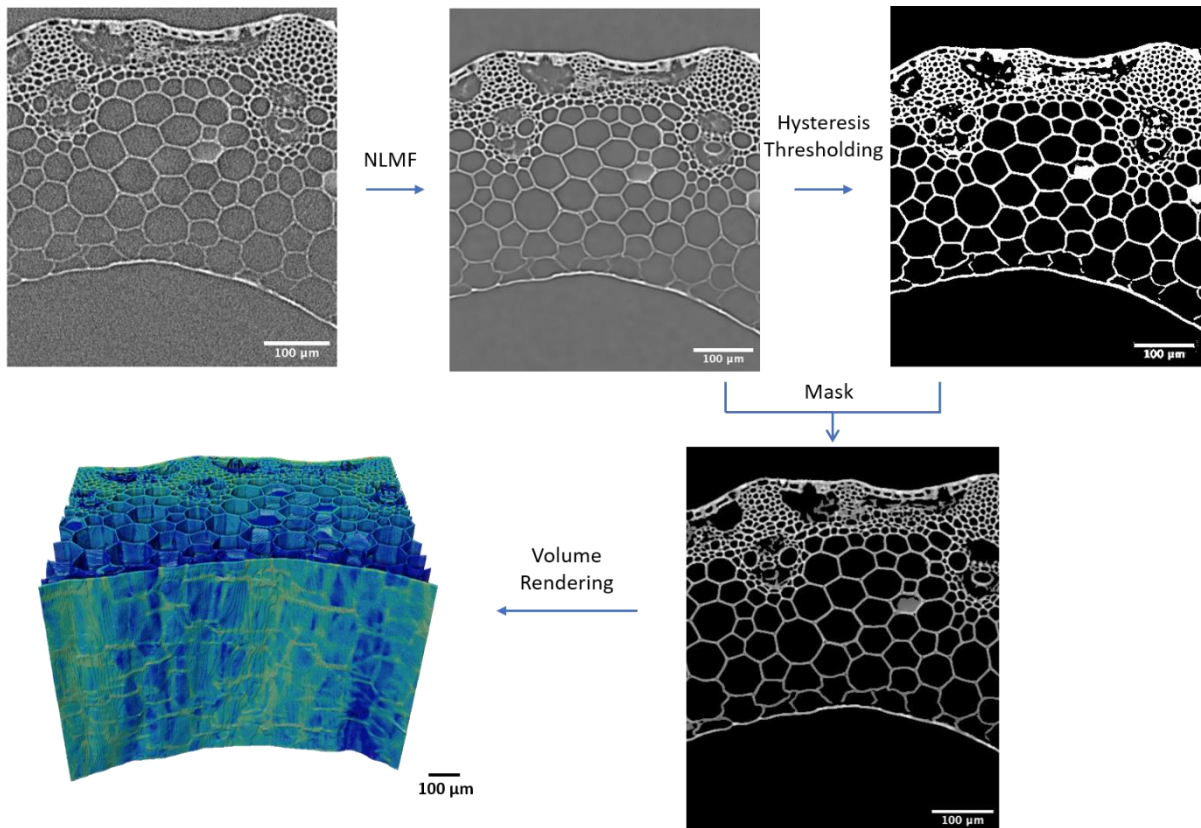


Figure A.1: Image processing workflow.

A.2 Image processing using Matlab

This section is divided into two parts. First, we present the preliminary operations that are necessary to be able to compare two different 3D images, obtained for a given sample before and after the enzymatic attack. Second, we give details about obtaining quantitative information from these images (3D-ROIs based analysis, 2D maps of the wheat straw internal wall) and discuss the determination of the grey level (GL) threshold above which a voxel is considered to be part of the PCW material. In the following, the distribution of GLs on a tomographic slice are plotted as histograms, where the y-axis gives the number of occurrences (count) of the GL values shown in the x-axis.

A.2.1 Histogram matching

Even if all 3D scans are performed with the same settings of the X-ray tomograph (voltage and intensity), leading to similar GL histograms on 2D radiographs from one sample to another, significant differences in image histograms are seen when tomographic slices coming from different samples are considered (after the 3D reconstruction process and the non-local mean filtering). Actually, the histograms shapes are similar but their width (that is the range of GL covered by the histogram) and mean value can be different.

To allow for a direct comparison of GL values between different samples (as is performed for the statistical analysis using ROIs detailed in the bulk of the paper), all the histograms are matched to a single reference histogram using Matlab command *imhistmatch* (from the Image Processing toolbox). As the histogram shapes are globally similar from one sample to another and because enzymatic degradation has a small effect on the image (Cf. article), we assume that this operation has a limited effect and does not induce any bias on the performed analysis. Basically, the effect of this operation is

to displace the histogram along the GL range and to decrease its width, as the reference image was chosen among the tomographic slices of a sample for which the signal dynamic range was the smallest.

To illustrate the histogram matching process, Figure A.2a shows two histograms obtained considering all the voxels on two tomographic slices (before and after digestion). As mentioned above, the histograms look similar, but with slightly different width and location over the 16-bit range. Note most of the histogram consists of “dark” voxels, i.e. indicating the presence of the background material, that is air (see the huge central peak of the histograms). The voxels giving information on plant cell walls material (PCW) are in the right tail of the histogram (this will be further discussed in §A.2.4). Note that, as there is not a clear separation between the range of GL values for air and the range of GL values for the PCW material, an unambiguous segmentation is not straightforward (again, see §A.2.4).

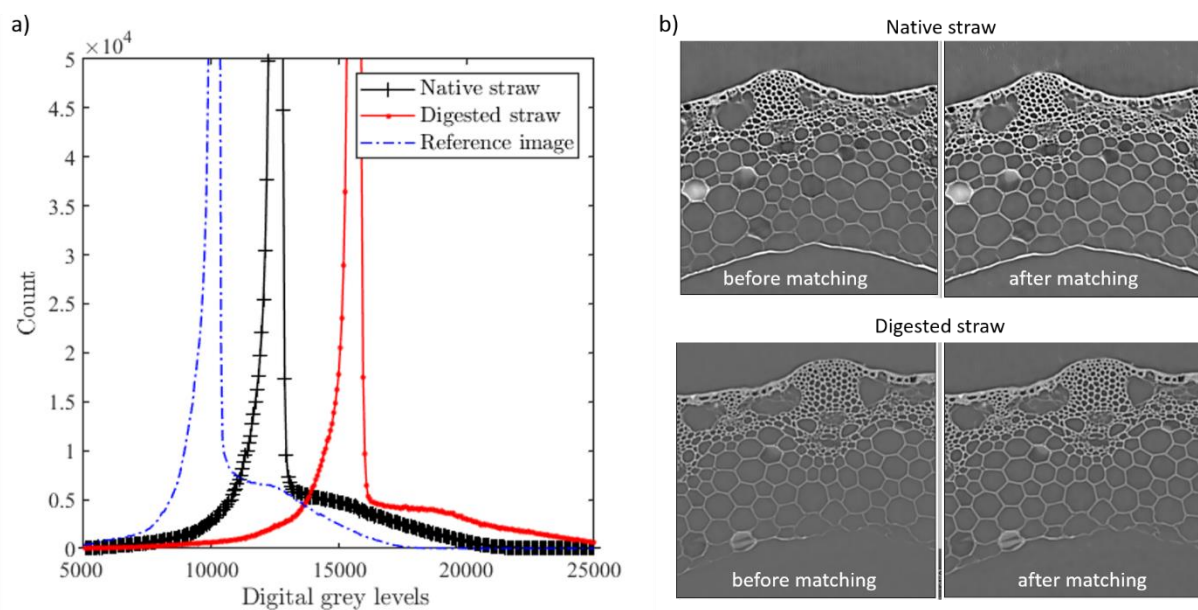


Figure A.2: a) Three histograms of the grey levels distribution for three tomographic slices. The reference histogram is the blue one. b) Details of tomographic slices for a native (top) and a digested (bottom) sample; before histogram matching (on the left, see the black and red histograms on a) and after histogram matching (on the right, see the blue histogram in both cases).

The histograms obtained after the matching procedure are perfectly superimposed with the reference histogram used for matching (in blue on Figure A.2a). We also display in Figure A.2b a detail of each tomographic slice, before and after the matching procedure. Careful inspection of images before and after matching reveals that no bias is induced by the matching, e.g. a significant modification of the GL values for some voxels, which would transform “air voxels” to “PCW material voxels”. Therefore, for each sample, all the tomographic slices are matched to a single reference image. Consequently, slices or 3D rendering from different samples can be directly compared. Also, the threshold above which a voxel is considered to belong to the solid material is the same for all samples.

In order to test the impact of the matching procedure on the statistical analysis performed on the 3D images, the later was repeated using different sets of matched images, each obtained using a different reference image (picked from a stack of tomographic slices, for a native or a digested sample). Typically, this results in a few percent dispersion on the value of the markers for enzymatic degradation used in the present study, less than the uncertainty associated with the determination of the threshold above which a voxel is considered to be part of the PCW material (see §A.2.4).

A.2.2 Spatial adjustment of native and digested 3D images

In order to be able to perform measurements at the very same location on 3D images for native and digested samples, the following procedure is followed. As a preliminary step, it must be mentioned that we took care to scan roughly the same part of a given sample, before and after digestion. The top-bottom orientation of a given sample is retained from the first (native) to the second (digested) scan. Once in the scanner, we use the top end of the sample as a reference and typically centre the sample 4 mm below the top.

The exact shift in z is determined manually by scrolling through the stack of transverse slices. Typical patterns formed by the cell walls in the transverse tomographic plane are detected on the two sets of images, see Figure A.3 as an illustration. The z -shift can slightly vary depending on the angular location and position along the stack. It is averaged on 10 values. The angular shift is determined by extracting the external contour of the wheat straw, at a given z , for the native and digested samples. Then, cross-correlation on the contours allows to determine the angle of rotation. This operation can be repeated along different z (typically the stack bottom, middle and top) but this typically leads to very close angular shift values (less than 0.1 degree in difference).

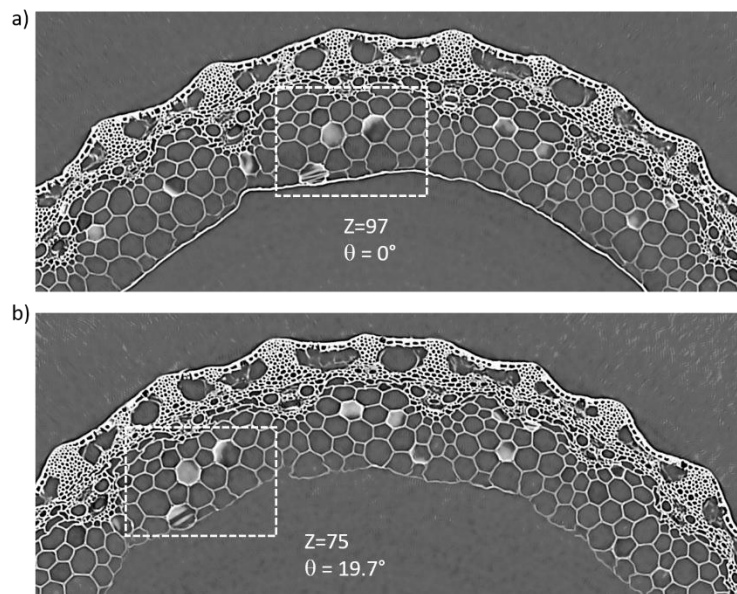


Figure A.3: a) A distinctive pattern formed by three in-plane plant cell walls is found on slice 97 within the stack of tomographic slices corresponding to the native sample (highlighted by the dotted white rectangle). b) The same pattern is recovered on slide 75 within the stack obtained after enzymatic attack, with an angular shift of 19.7° with respect to the initial slice.

Despite this adjustment, some local discrepancies (e.g. in cells shape) can be noticed when looking at the same location before and after digestion. They are attributed to the various mechanical solicitations of the sample during the protocol (imbibition / drying, positioning in the capillary tube for scanning) and possibly the release of internal mechanical stresses induced by the modification of the plant cell walls architecture caused by the enzymatic attack. Typically, a slight (i.e. a few voxels in amplitude) shrinking or dilatation of cells close to the PCL can be observed, as shown in Figure A.4.

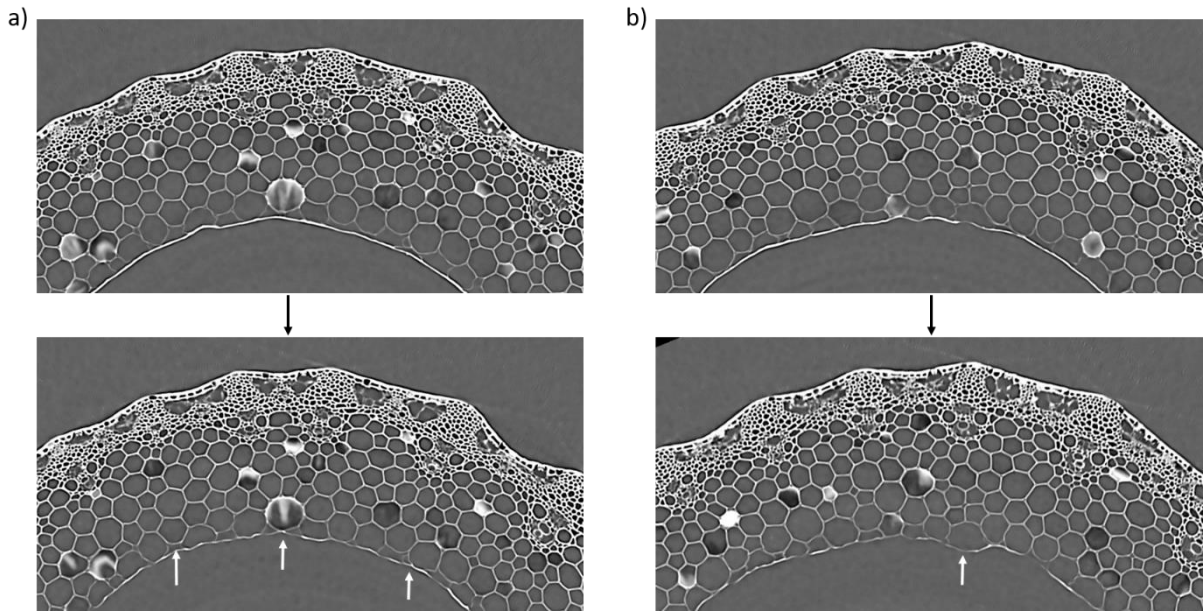


Figure A.4: Example of local shrinking (a) or dilatation (b) of cells close to the PCL after enzymatic attack (bottom images, top ones are native samples). The white arrows indicate the regions of interest. Here, the enzymatic attack duration is 30 minutes.

A.2.3 ROIs positioning and ROI-based calculations

Here we detail how the set of ROIs used for the statistical analysis is obtained. First, a set of 1000 values for (z, θ) locations is drawn at random. To consider non-overlapping ROIs only, a minimum distance (40 voxels) is imposed between each ROI centres of mass (whose location is given by z and a typical r , taken as the internal radius of the sample considered). Then, ROI-based calculations, which are the core of the statistical analysis presented in the paper, are typically performed as follows.

- each ROI is considered successively. The tomographic slice corresponding to the ROI z -location is rotated by the angle θ . A intensity profile, from the centre of mass of the sample to its edge (i.e. along the vertical direction of the image, as the image has been rotated), is extracted. The PCL and epidermis locations are respectively given by the position along this intensity profile of the first and last voxels belonging to the PCW material (they are highlighted with red crosses in Figure A.5a).

- the ROIs are positioned in order to lie across the PCL (red rectangle in Figure A.5b, with a 5 voxels x 31 voxels extent), across the epidermis (blue, 5 voxels x 31 voxels) or to be centred 100 voxels away from the PCL (green, 51 voxels x 51 voxels).

- voxels belonging to the ROIs are numerically extracted from the 3D image. As the vertical extent (i.e. in the z direction, perpendicular to the tomographic plane displayed in Figure A.5) of each ROI is 5 voxels, this is performed for 5 slices (with locations in the range $[z-2 z+2]$).

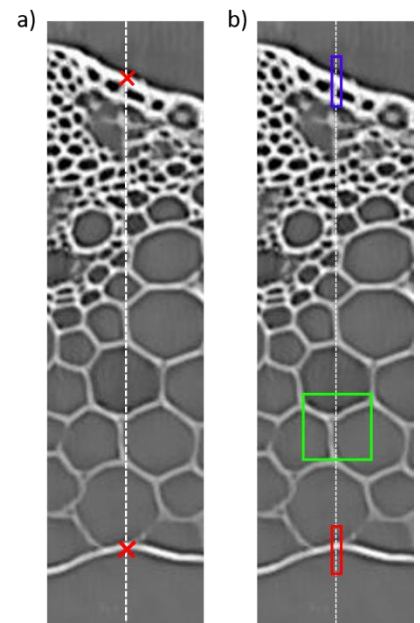


Figure A.5: see text for legend

Finally, all the voxels belonging to ROIs with the same location are grouped together and their GL distributions and/or mean values can be computed.

There is one main difficulty associated with such a method. Automatic detection of the PCL location is straightforward for native sample as the bright PCL voxels stand out. It may become tricky for digested samples because, as emphasized within the core of the paper, the PCL voxels fades away and even completely disappear for the longest durations of the enzymatic attack. In this latter case, the first PCW voxel found along the profile may belong to a parenchyma cell wall (see the red cross on Figure A.6b, where the digested counterpart of A.6a is shown).

To cope with such cases, the following correction scheme can be applied: the location of the PCL at a given location is then taken as the location of the PCL measured at the same location for the native sample (the PCL location on the native sample is denoted by a red cross on Figure A.6a and is shown as a white cross on A1.6b).

The results obtained with this correction significantly differ from those obtained without, particularly for long duration of enzymatic attack, when local disappearance of the PCL are observed (see Figures 3 and 4 of the paper).

For the shortest durations of enzymatic attack, the correction does not lead to significant differences. Note the PCL location found on a digested sample (at a location where the PCL is still clearly detectable) may slightly differ from the location found on the native sample (for a given ROI), due to the PCL displacement provoked by the shrinkage/dilatation discussed above. However, as the ROI width in the radial direction (31 voxels) is larger than the typical magnitude in voxels of the shrinkage/dilatation that may be observed, the GL distribution for the PCW material extracted from the ROI is the same with or without correction.

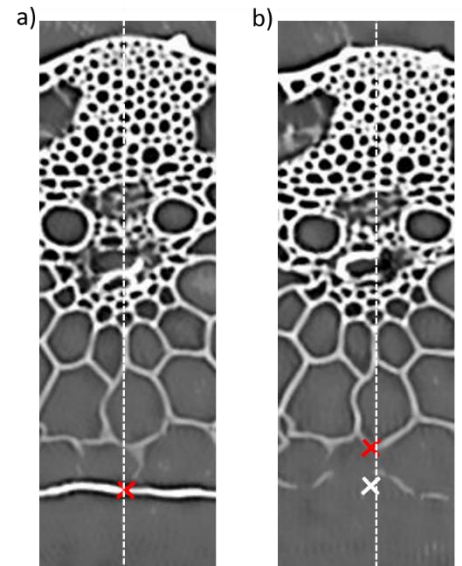


Figure A.6: see text for legend. a

A.2.4 Determination of the grey level threshold for PCW material

We now turn to a discussion about the choice of the grey level (GL) threshold chosen to separate the PCW material from air (the background material). For the following generic discussion, we use a detail of a tomographic slice as a reference image. Figure A.7 shows the distribution of GLs for the reference image which is shown as an insert. Some images obtained after binarization (voxels below the threshold are set to 0 -black-, voxels above the threshold are set to 1 -white-) are shown below the image histogram. The various thresholds used are shown as vertical dotted lines on the histogram.

When the threshold is too low, noisy air voxels clearly ends up being considered as PCW. When the threshold is too large, some voxels belonging to the PCW material are wrongly discarded: the PCW material is gradually trimmed when the threshold is increased.

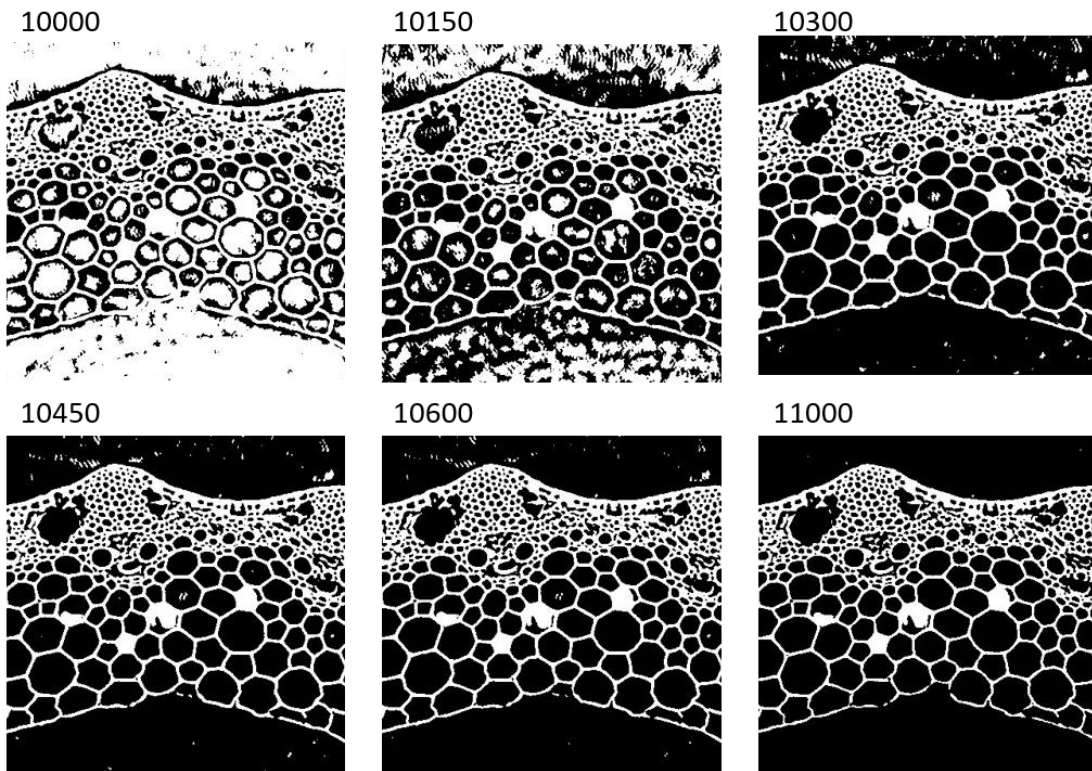
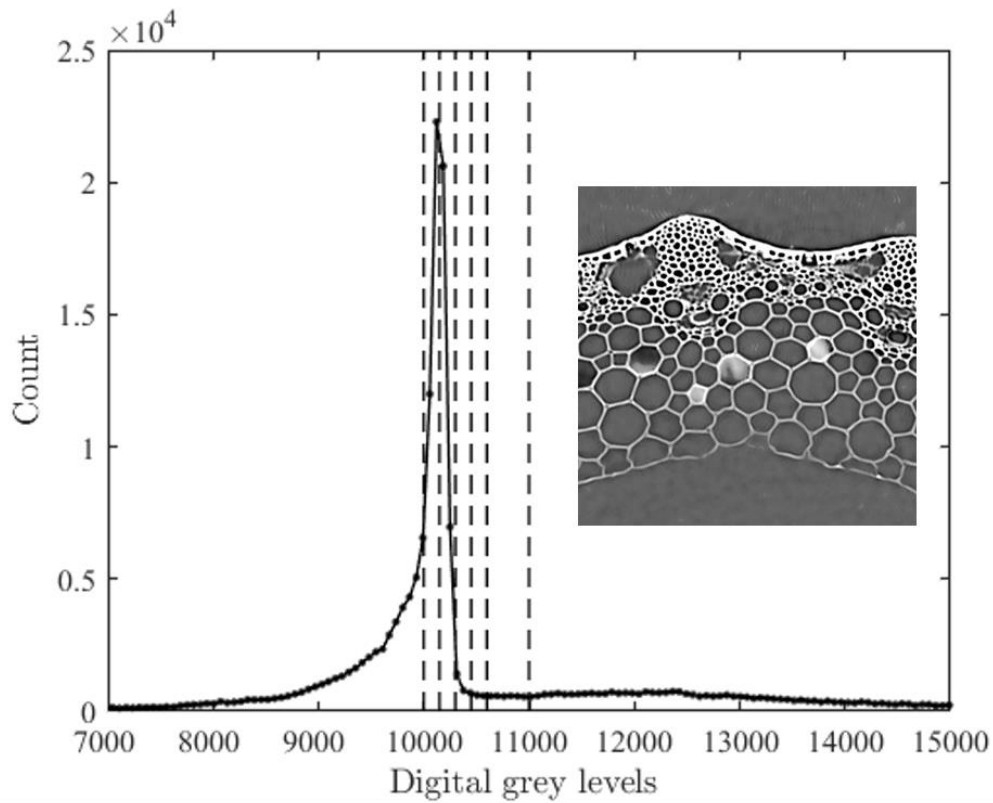


Figure A.7: Top: image histogram of the detail of a transvers tomographic slice shown as an insert. The vertical dotted lines highlight the thresholds used in the bottom part of the figure. Bottom: binarized images, for various values of the binarization threshold.

Figure A.8 shows the fraction of white voxels as a function of the binarization threshold. There is a sharp transition between a situation where the threshold is too low so that most of the voxels are considered as PCW material (threshold below 10000) and a situation where the trimming effect is dominating, see the gradual decrease of the fraction of white voxels for threshold above 10500. Consequently, the threshold is chosen to be 10400, just at the transition from a noise-dominated image to an image without trimming, with an uncertainty of +/- 100.

The above discussion and conclusion regarding the choice of the binarization threshold not only apply when considering the detail of a tomographic slice but also when restricting the analysis to the voxels picked in the ROIs defined for the statistical analysis. The histogram matching procedure presented in §A1.2.1 allows to consider a unique threshold for all the data. It is important to note that in the present study, we discuss in Figure 5 image histograms obtained considering only voxels having GLs above the threshold. Also, in Figure 6, markers are extracted by considering the voxels above threshold only.

The uncertainty in the choice of the threshold propagates into uncertainties on the markers. The uncertainty on the number of voxels is larger when considering the ROIs located at the PCL (see the red data points in Figure 6b) and this is all the more true than the duration of the enzymatic attack is long. For such cases, as the intensity of the PCW material voxels decreases a lot, these voxels become hardly discernible, close to the background level. Then, a slight change on the threshold can have an important impact on the number of voxels selected as PCW. Note that, on the contrary, the impact on the mean value of the grey levels for the PCW voxels is much lower (compare the errors bars for the red data points in Figure 6a and 6b).

As explained in §A1.1, the hysteresis thresholding algorithm used in Avizo to generate the 3D mask requires 2 thresholds. As expected from the above discussion, the choice of the lower threshold is the more critical, while the higher threshold does not have a significant impact on the number of voxels being identified as PCW material. The two thresholds were chosen as 10300 and 11000 respectively.

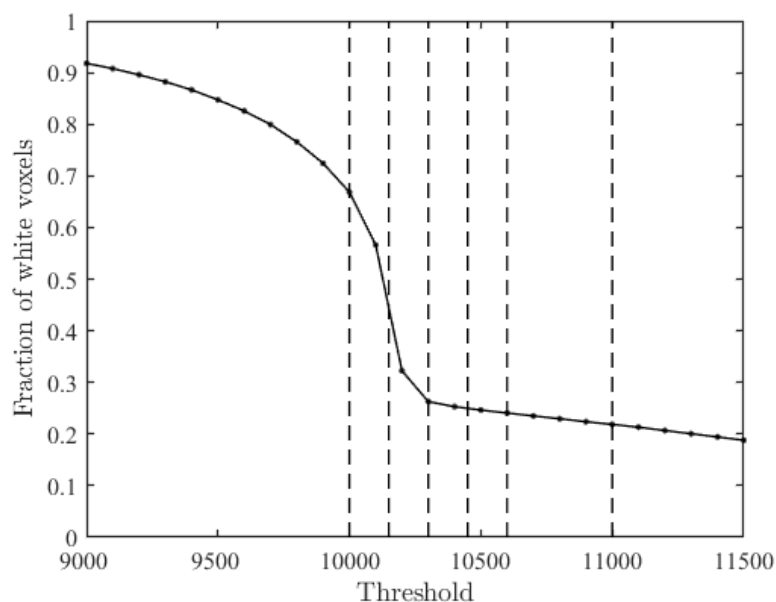


Figure A.8: Fraction of white voxels found in the image shown in Figure A.7, as a function of the binarization threshold. The vertical dotted lines highlight the thresholds used in the bottom part of Figure A.7.

A.2.5 From tomographic slices to 2D maps of the wheat straw PCL

The main difference between the ROI-based operations for statistical analysis and those used in order to obtain a 2D rendering of the wheat straw PCL lies in the ROIs spatial distribution. In the latter case, ROIs are located in order to approximately “pave” the internal surface. Each ROI has the same vertical (z direction), “lateral” (θ direction) extent and depth (radial direction). For a given z, the tomographic slice is rotated over 360 degrees by step $\Delta\theta$. The angular step value ($\Delta\theta = 0.5^\circ$) is obtained from an estimate of the internal perimeter of the sample divided by the ROI lateral extent. Then, as explained above in §A.2.3, the PCL radial location is automatically detected and voxels belonging to the ROIs are numerically extracted from the 3D image (i.e. over slices with locations in the range $[z-2 \ z+2]$ as the vertical extent is taken to be 5 voxels). The depth of the ROIs is a key parameter: it is taken to be 4 voxels only so that the ROIs contains solely voxels belonging to the PCL (whose thickness is a few voxels). On the 2D images, each voxel¹ value is obtained by computing the mean value of the GL of the voxels belonging to the corresponding ROI. Note this paving operation is not perfect as the internal wall is not perfectly circular and exhibits some corrugations as seen, e.g., from Figure 1, but one nonetheless end ups with 2D images where similar heterogeneities can be identified at similar locations on native and digested samples, at large or mesh-like structure scales (see Figure 7). This operation can be performed for the samples with the shortest durations of enzymatic attack (30 and 45 minutes), with a PCL that is still detectable (see discussion in §A1.2.3). One-third of the full stack of transverse tomographic slices has been used for Figure 7 and the subsequent analysis in Figure 8.

References

A. Buades, B. Coll and J. -M. Morel, "A non-local algorithm for image denoising," 2005 IEEE Computer Society Conference on Computer Vision and Pattern Recognition (CVPR'05), San Diego, CA, USA, 2005, pp. 60-65 vol. 2, doi: 10.1109/CVPR.2005.38.

¹ The term “voxel” is used throughout the paper, even if “pixel” may be used instead when 2D images, such as tomographic slices or 2D PCL maps, are considered.