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## A 4D QUANTIFICATION PIPELINE TO CHARACTERIZE PLANT CELL WALL ENZYMATIC HYDROLYSIS IN HIGHLY DECONSTRUCTED SAMPLES

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Converting plant cell walls, as a renewable source of energy and materials, into bioproducts is an important step toward reducing dependence on fossil fuels. The primary obstacle in this conversion is overcoming the cell wall's inherent resistance to enzymatic breakdown called recalcitrance. Much of the research during past decades has concentrated on pinpointing markers of recalcitrance at the nanoscale. As a result, the enzymatic deconstruction of plant cell walls at the cell and tissue scales has been insufficiently studied. Our previous results have paved the way to fill this gap by connecting key parameters of poplar wood enzymatic deconstruction across nano and micro scales<sup>1</sup>. Building upon this work and to further investigate the recalcitrance across scales, we developed a protocol to acquire time-lapse images of spruce tree wood during enzymatic hydrolysis using fluorescence confocal imaging which generated images with highly deconstructed and deformed cell walls. Thus, it was necessary to develop a computational pipeline specifically designed to process this kind of images. The pipeline first segments the pre-hydrolysis image which can exhibit a tilt by applying spatial constraints on the watershed algorithm. The pipeline then employs an adapted spatial information propagation strategy to segment images of highly deconstructed samples by dividing the time-lapse images into sequential clusters where the final image of a cluster is also the first image of the subsequent cluster. Transformations are then computed within each cluster by registering the initial image of the cluster with the successive images within that cluster. Starting from the first cluster with the pre-hydrolysis image, these temporarily constrained transformations are then applied to the segmentation of the initial image of the cluster generating segmentations of the subsequent images. Overall, by limiting the registration to individual clusters, this approach successfully processes images of highly deconstructed samples (Figure 1). The quantification of cell and tissue scale morphological features using these segmentations sheds light on the underlying parameters of the enzymatic deconstruction. The pipeline also provides dynamics of cell wall autofluorescence intensity values to develop mathematical models of cell wall deconstruction.

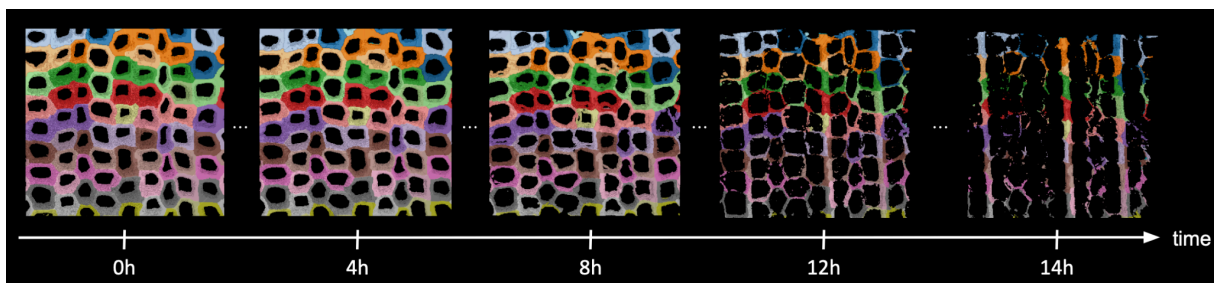


Figure 1 : 4D segmentation of spruce tree cell wall during enzymatic hydrolysis

### Références

[1] Refahi, Y., Zoghlami, A., Vine, T., Terryn, C., & Paës, G. (2024). Plant Cell Wall Enzymatic Deconstruction: Bridging the Gap Between Micro and Nano Scales. *bioRxiv*, 2024-01.