In situ microscopic investigation of plant cell walls deconstruction in biorefinery

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Plant biomass that primarily composed of polysaccharides and lignin constitutes a renewable energy source. The conversion of biopolymers into fuels, energy, and chemicals with a concept of biorefinery has been considered as a promising technology with the increasing energy supply concerns and the greenhouse effect on environment [1]. However, plant cell walls are generally heterogeneous composites, consisting principally of cellulose, hemicelluloses, and lignin components. Cellulose made up of D-glucose exists as semicrystalline nanofibrils that provide reinforcing scaffold for cell walls, while lignin and hemicelluloses act as the matrix materials that surround and encrust this biopolymer composite, contributing to the natural recalcitrance of biomass [2]. Therefore, one of the major technical barriers to commercialization of biomass conversion is the high cost of chemical/biological treatments to deconstruct the robust plant cell wall materials into soluble components [3]. To improve the above process and better understand its mechanism, deeper understanding of plant cell walls deconstruction is required and new imaging tools are essential to gain information on the dynamic changes in biomass structure and composition during treatments at multi-scale levels.

Optical microscopes with chemical staining and electron microscopy techniques have been proved to be valuable aids to study the ultrastructure and topochemistry of plant cell walls. The traditional in vitro methods of optical microscopic techniques are widely employed for visualization of multi-dimensional data of plant tissues. For instance, clear modifications of plant cell walls during ethanol organosolv pretreatment on Buddleja davidii have been observed by light microscopy combined with safranin staining [4]. With the increasing demands, microscopic methods should be conducted not only to characterize the gross anatomical structure of biomass, but also to visualize the spatial localization of polymers within plant cell walls. Electron microscopy techniques have emerged to characterize the morphological structure of biomaterials and track the distribution of biopolymers in plant cell walls. As one example of the use of transmission electron microscopy (TEM), the lignin density within morphologically distinct regions of cell walls can be vividly visualized [2, 5]. However, such traditional imaging modalities of light and electron microscopes are limited to localize the components distribution for high resolution images or perform quantitative measurements of compositional changes. Furthermore, the destructive sample preparation including drying, embedding, coating, and staining procedures may cause inevitable alterations, and thus losing the accurate and native traits of biomass. Therefore, non-destructive microscopic techniques are eagerly required that can be employed for timely monitoring the dynamic changes of biomass at multi-scale levels. Accordingly, confocal laser scanning microscopy (CLSM), due to its ability of high resolution records, has drawn much attention to elucidate the chemical characteristics of lignocellulosic materials. The autofluorescent groups mainly attributed to lignin support for imaging lignin distribution within cell walls near to a native state. Most recently, advanced confocal Raman microscopy (CRM) relying on the molecular vibration has shown to be well suited to give insights into both the chemical composition and the structure of lignocellulosic biomass with multi-phase sensitivity and label-free properties [6]. It enables the in situ topochemical study on the scale of single cell wall even the sub-cellular layer. By integrating over corresponding Raman bands, the spatial localization of lignin and carbohydrates as well as the cellulose microfibrils orientation can be simultaneously imaged and semi-quantitatively compared within different tissues or morphological regions [7]. Apart from CRM, the infrared absorption microspectroscopy, depends on changes in the intrinsic molecular dipole moments, can provide complementary information about the molecular vibrations [8]. FT-IR spectroscopic imaging microscopy therein has

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1. Introduction

Lignocellulosic biomass composed of plant cell walls represents an abundant and renewable carbon source. The conversion of biomass polymers into fuels, energy, and chemicals with a concept of biorefinery has been considered as a promising technology with the increasing energy supply concerns and the greenhouse effect on environment [1]. However, the accurate understanding of cellular architecture of plant cell walls remains unclear owing in part to complexity of biomass chemical structures as well as limitations of traditional analytical methods that may bring irreversible modifications. In situ microscopic techniques are readily required to visualize, characterize, and quantify the dynamic features of plant cell walls following treatments on multi-scale levels. This review gives insights into the recent contributions of a series of non-destructive techniques including confocal laser scanning microscopy, confocal Raman microscopy, FT-IR imaging microscopy, and atomic force microscopy to the field of biorefinery, focusing on the deconstruction of plant tissues. We also highlight the critical outlook and scientific challenges on the study of biomass utilization as well as microscopy applications.
been confirmed as another non-destructive method to visualize the components distribution. Considering the complex cell wall assembly, other high resolution techniques are also required for a detailed insight into the fibrillar texture of plant tissues and the surrounding cellular matrix which is related to the recalcitrance of biomass that ought to be overcome in the process of biomass utilization. Atomic force microscopy (AFM) has become one of the most important tools to image, manipulate, and quantify on the nanoscale level of biological tissues. The most outstanding feature of AFM is attributed to the fact that the interested regions can be probed and imaged in conditions very close to their natural state, but without any need of drying or freezing [9]. In current days, some alternative microscopes are developed which can simultaneously afford chemical and anatomical features with high resolution non-destructively. The combination of microscopy methods such as Raman and AFM has gained a significant impact in the field of biorefinery engineering for gathering ultrastructural and compositional information [10]. These instruments are allowed to reveal timely information on the morphology of lignocellulosic materials and spatial distribution of components within plant tissues at nano-scale.

In this review, the potential of various in situ microscopy techniques for revealing the morphological and topochemical characteristics of plant cell walls with a concept of biorefinery will be presented. We also highlight the critical outlook and scientific challenges on the study of biomass utilization as well as microscopy applications. The overview of currently known microscopic methods is meaningful in the bioconversion of renewable feedstocks and facilitates the optimized use of microscopic techniques as well as the development of microscopy techniques combination.

2. Application of microscopic techniques on plant cell walls deconstruction

2.1 Confocal laser scanning microscopy (CLSM)

Different microscopy techniques have been applied to characterize the chemical structures of lignocellulosic materials. CLSM with the capability to produce high resolution and serial optical sections of fluorescent samples is an excellent tool to track the dynamic features of plant cell walls. A particular advantage of CLSM is the provision of detailed information about the three dimensional internal structure of tissues in their native or near native state. In addition, CLSM imaging of the intrinsic autofluorescence of lignin enables a complete view of location and relative concentration of lignin in various wood species [11-13]. The autofluorescence intensity of lignin in cell wall of Caragana Korshinskii primarily accumulated in the cell corn regions, indicating high level of lignification in these areas [14]. In addition, this technique has been applied especially to quantify the compression wood severity in tracheids of Pinus radiata [15]. The differences between normal and compression wood revealed by CLSM imaging and spectral deconvolution indicated the presence of a higher p-hydroxy type lignin in the compression pine wood species. A pioneering study on the visualization of phenolics and flavonoids in plant cuticles confirmed the valuable ability of three-dimensional CLSM images to dig spatial localization information of biometers [16].

CLSM has been widely utilized to determine and estimate the cell wall structure present in tissue sections before and after digestion, giving insight into the mechanism of chemical pretreatments. Lee et al [17] successfully applied this method to uncover how the poplar changes during treatment with the ionic liquid 1-n-ethyl-3-methylimidazolium acetate (EMIMAC) at room temperature. Time lapse images of CLSM clearly visualized the reversible swelling of cell wall towards the empty lumen, but not the compound middle lamella. When the EMIMAC pretreatment was carried out at high temperature, the switchgrass cell walls swelling greatly followed by complete dissolution of the organized biomass structure, while after addition of anti-solvent water to this solution, fluorescent images visualized cellulose regeneration with rejection of lignin from the deliquescent products [18]. In EMIMAC treated corn stover, a direct correlation between changes in both the morphology and chemical composition of these plant cell walls was characterized by a series of optical sectioning images [19]. It enabled quantitative measurements of cell volume and mean cell wall thickness, finding selective dissolution in the secondary walls as a consequence of preferential swelling in these regions. Additionally, the potential of CLSM for quantification of cell wall degradability related to specific cell type information was also assessed in this study. When the renewable and economic cholinium amino acids ionic liquids ([Ch][AA] ILs)-water mixtures were used as impregnating agents, cell wall swelling mainly occurred in the sclerenchyma near the epidermal tissues of corn stover, which was opposite with the effects of EMIMAC [20]. A time course CLSM images of pretreated sections directly showed that with the elongation of pretreatment time, the fluorescent intensity decreased gradually in the lignin-rich cell corner and compound middle lumen of cell walls (Fig. 1), indicating the occurrence of selective removal of lignin from these regions. Furthermore, dramatic increase in xylan accessibility after pretreatment was in situ visualized by CLSM combined with immunolabelling techniques. These compositional and ultrastructural changes unlocked the biomass resistance and probably contributed to the enhancement in the enzymatic hydrolysis of polysaccharides. In terms of enzymatic degradation, CLSM has been utilized to estimate and compare the accessibility of cellulases and fungal cellulases to delignified cell walls [21]. Fluorescent images illustrated that cellulases primarily attached to the cells innermost surface, the cell corners, and the plasmodesmata, while fungal cellulases not only bind to these surfaces, but also penetrated inside the secondary wall from the cell lumen. Measurement of cell wall volume using CLSM and its application to studies of forage degradation was explored.
by Travis group [22]. The degradation profile of white rot fungus further confirmed that CLSM has sufficient resolution to track the degradation process of plant cell walls [23].

Fig. 1 CLSM images (488 nm excitation) of rice straw cells: untreated (a) and pretreated for 2 min (b), 5 min (c), 10 min (d) with 50% [Ch][Lys]-water mixture at 90 °C. All images were recorded at the same conditions.

Polymers dyed with fluorophores have been designed to selectively determine the spatial localization of target molecules, such as hemicelluloses and pectin. Recently, immunofluorescence labeling has greatly enhanced the investigation of polysaccharides distribution in plant cell walls [24-26]. The deposition of xylan in cell walls of wheat straw stem was investigated by using an immuno-microscopic method combined with monoclonal antibody LM11 specific to β-(1-4)-linked xylopyranosyl units [27]. Same protocol also succeeded in the localization of pectic galactan in tomato cell walls using CLSM [28]. Based on this method, Donaldson and co-workers [29] extensively studied the relationships between polysaccharides distribution and lignification as well as microfibril orientation, indicating that the latter two may be mediated by variations in the amount and distribution of noncellulosic polysaccharides. In addition, the changes in xylan distribution caused by dilute acid pretreatment in corn stover have been investigated using CLSM combined with immunofluorescence labeling. A time resolved series of CLSM images illustrated a dramatic loss of xylan fluorescence signal from the center of sclerenchyma cells, but an increase or retention of xylan at the cell lumen and middle lamella of sclerenchyma cells [24]. Biomass pretreatment results in a large variability of structural and compositional characteristics of plant cell walls. CLSM provides means to accurately image components distribution and to extensively quantify cell wall degradability in a simple and fast way. These traits are essential to uncover the mechanism of plant cell walls deconstruction in the field of biorefinery.

2.2 Confocal Raman microscopy (CRM)

CRM is a powerful method to provide spatially resolved information about the chemical composition of biological samples near to a native state [6]. With confocal collection optics, the method is well suited to localizing the components distribution within plant tissues at the cellular level, either semi-quantifying polymers content at the subcellular level by comparing average spectra of distinct cell wall layers. Compared to CLSM, the extracted information from Raman images can provide direct visualization of various main components distribution simultaneously, including carbohydrates, lignin, and silica [30]. The polymer chemistry and orientation within and between different cell walls of various wood species has been extensively investigated using this method [7, 31-33]. Generally, the lignin densely deposits in the cell corner regions, whereas the carbohydrates mainly localize in the secondary wall showing an opposite pattern with lignin. CRM also shows its advantages in studying the changes in topochemistry between normal and reaction wood [34], as well as the wild type and transgenic plants [35]. Our group focused on the Raman imaging of Miscanthus Sinensis internode and thus trapping the polymers localization in various plant tissues. Most importantly, Raman analysis for the first time demonstrated an accompanied trend between lignin and hydroxycinnamic acids distribution within rind and pith vascular bundles of Miscanthus Sinensis [36]. The complexity and heterogeneity of components distribution within plant cell walls may impede the enzymatic degradation of lignocellulosic materials. Thereby pretreatment is required to overcome the biomass recalcitrance and in turn, facilitating the sugar release performance.

CRM has been proposed as a valuable approach to assess the in situ effect of alkali pretreatment on Miscanthus X giganteus [37]. By combining the depth profiling and quantitative measurements, it was found that the pretreatment resulted in a preferential removal of lignin from the interior cell wall but left the morphology of cellulose largely undisturbed. Our group found similar pattern of delignification using the same pretreatment with poplar fiber walls, while excessive impregnation in alkali at high temperature would give rise to a rapid loss of carbohydrates (Fig. 2) [38]. As shown in Fig. 2a, cell wall swelling was clearly observed during alkali pretreatment. In addition, a reduction of lignin concentration was mainly noted in the secondary walls pretreated for 10 min, indicating that the solvent allowed penetration into cell walls from cell lumens and thus a preferential delignification in these regions (Fig. 2b). When the residence time was extended to 60 min, Raman images exhibited enhanced level of delignification, especially in the cell corner regions, whereas over the course from 90 min to 180 min a slower decrease of lignin concentration was obtained and the residual lignin signal in cell walls was hardly visible, suggesting the completion of delignification process. The dynamic changes in carbohydrates distribution following alkali pretreatment was also revealed by Raman images (Fig.
In the first 60 min pretreatment, as expected, there was no visible reduction of carbohydrates concentration in both the S and CCML, while by lengthening the residence time to 90 min a rapid loss of carbohydrates especially in the S regions was observed, which had a negative impact on pretreatment cost-effectiveness. In the process of ionic liquid (EMIMAC) pretreatment on switchgrass, there existed a synergistic mechanism of biomass deconstruction regarding the dissolution of cellulose and lignin as well as cell wall swelling [19]. According to the authors, Raman images revealed three changes as a consequence of this treatment: (i) preferential removal of lignin from the secondary wall, (ii) homogeneous cellulose dissolution within cell walls, (iii) tracheids possessing faster swelling and dissolution of lignin and cellulose compared to parenchyma. Another CRM study on the EMIMAC pretreated polar fiber walls by Zhang et al. [39] further stated the process of biomass deconstruction which can be clearly divided into two stages that slow penetration of EMIMAC and rapid dissolution of lignin and carbohydrates. Based on the Raman microspectroscopic analysis, a novel model was proposed to better understand how plant cell walls respond to the EMIMAC pretreatment. By mapping the biodegradation of native corn stover with fungal cellulases and cellulosomes, it showed that facile digestion of the non-lignified primary walls and minor digestion of parenchyma secondary walls were observed, while after delignification all cell walls were completely consumed, suggesting a strongly negative correlation between cellulose digestibility and lignin content [21]. Furthermore, the fungal cellulases acted about 5-fold faster than cellulosomes against either untreated or delignified cell walls based on the Raman spectral analysis.

Given the complementary nature and chemical sensitivity, CRM holds great potential for dynamic in situ imaging of plant cell walls deconstruction as a consequence of chemical/biological treatments in site-specific cell wall types within different plant tissues, which can form a linkage between the micro-level behaviors and the macro-level performances of biomass. This may ultimately contribute towards an understanding of the molecular biology of many plant traits and the mechanism of various chemical/biological treatments. Nevertheless, the impedance of fluorescent background produced from chemical pretreatments, which may constrain the analysis or quality of Raman images, should be taken into consideration.

2.3 FT-IR spectroscopic imaging microscopy

FT-IR spectroscopy has been widely utilized as a useful tool to characterize the composition of wood [40], the molecular interactions between wood polymers [41] as well as the hydrogen bonding of cellulose chains [42]. While the lack of high resolution and localization function was restricted for a long time primarily to academic research. In past decades, the advanced FT-IR imaging microscope that combines an infrared spectrometer with an optical microscope not only owns the overall functions mentioned above, but also allows for imaging large and heterogeneous regions of biomaterials. Compared with CRM, FT-IR imaging microscopy relying on infrared absorption successfully avoids the interference of fluorescent background, and thus acquiring higher quality images. Furthermore, it carries out the imaging of hemicelluloses and pectin respectively, which is unsolvable by CRM due to their low concentrations in plant tissues. With these advantages, FT-IR microspectroscopic imaging succeeded in localizing the primary polymers distribution within plant cell walls and exploring the relationship between chemical composition and morphology of native materials [43]. A similar detecting was also applied for monitoring the compositional vibrations between wild and transgenic aspen stem by comparing their features from the bark to the pith [44].

As well known, understanding structural dynamics of enzymatic lignocelluloses disintegration is crucial for making biofuel productions from lignocellulosic biomass. With the high spatial resolution, FT-IR imaging technique is considered to be suitable to visualize the microstructural changes of plant tissues during enzymatic degradation. Himmelsbach et al. [45] uncovered the process of enzymatic retting of flax stems by this approach, showing that the
fiber bundles were separated from each other but the morphology of cellulose remained undisturbed, whereas the pectic acids, esters, salts and hemicelluloses appeared to be completely degraded by the enzymes. Similarly, the feasibility of FT-IR imaging to monitor in situ enzymatic degradation of plant tissues was further confirmed by Gierlinger group [46]. The real-time imaging and spectral analysis of enzyme treated cross-sections from poplar normal and tension wood presented a rapid and selective degradation of the gel layer in tension wood, suggesting that the wood species with a high proportion of gel-like structure may represent an interesting cellulose resource for sugar fermentation. Microorganism treatment is considered as a promising approach to break down the recalcitrance of lignocellulosic biomass and improve the degradation of wood polysaccharides [47, 48]. As a precise tool in biodegradation analysis, FT-IR imaging microscopy was applied on brown-rot degraded spruce wood to track the fate of polysaccharides in plant tissues and visualize the trails of incipient decay. Yin et al [49] using FT-IR microscopy investigated the effect of steam treatment on the chemical structure of spruce, revealing a chain rupture of hemicelluloses accompanied by a slight cleavage of lignin in both earlywood and latewood tracheid walls. Combined with AFM nanoindentation results, a conclusion was safely deduced that the development of hygroscopic and micromechanical properties of the treated wood as increased steam temperature correlated well with the pattern of polymers degradation (hemicelluloses and lignin) [50]. Overall, FT-IR imaging microscopy permits the in situ investigation of chemical and structural features on the micrometer level in bio-materials and particularly, in the elucidation of relative loss or changes in the distribution of key chemical components during chemical/biological treatment with a concept of biorefinery.

2.4 Atomic force microscopy (AFM)

AFM allows measuring surface morphology and properties with a sharp probing tip through an interaction between the tip and surface. By monitoring the motion of the probe as it is scanned across the region, a nano-scale image of the surface can be constructed. In addition, AFM permits observations in ambient air and liquid environments to generate topography (height image) and elasticity (phase image) data simultaneously, as well as in situ tridimensional images. With these advantages, AFM has been proved to be a precise method to visualize the fibrilar structures of cellulose microfibrils in the field of biorefinery. Kirby et al. [51, 52] took the lead in studying the microfibrillar texture of parenchyma cell walls in the water chestnut, Amsterdamse bak carrot and Bintje potato by AFM. The arrangement of cellulose microfibrils in the wheat straw cell walls from different tissues was also compared using this method [53-55]. AFM images of sugarcane cell walls revealed the microfibrils assembly in a laminated fibrous structure with oblique orientation [2]. The detected average fiber diameter was about 25 nm, while in other studies the individual cellulose microfibril has a diameter of approximately 3 nm [21, 56, 57]. The obvious differences may be indicative of variable cell wall morphology between feedstocks. Direct imaging of AFM not only improves our ability to elucidate the native cell wall structures, but also provides new insights into understanding of its biosynthesis. In this regard, Ding et al. [21, 58] visualized the molecular architecture of parenchyma cell walls from maize stem pith, revealing the relationship between elementary fibrils, macrofibrils, and microfibrils (Fig. 4). Furthermore, from correlations of these AFM data with relatively published discoveries about plant cell walls biosynthesis, the authors proposed a new molecular model that each elementary fibril was made up of 36-glucan chains which consisted of both crystalline and subcrystalline structures. Under light tapping force conditions, Simola et al. [59] and Chernoff [60] demonstrated the microfibrils arrangement and fine lignin structure on wood fiber surface by differentiating materials with different hydrophilicity under AFM phase imaging. Direct visualization of the cellulose crystal using AFM has revealed higher resolution and more accurate measurements of microfibrils [61, 62].

![AFM images of the cellulose elementary fibrils on parenchyma-type secondary wall (pSW) surfaces. (A) An individual twisted cellulose elementary fibril shown in delignified pSW surfaces. (B) An untreated pSW showing macrofibrils and microfibrils. Color bars in (A) and (B) represent the scale of the AFM height images; the white arrow in (A) indicates macrofibril splitting. Scale bar: 10 nm.](image)

The nanoscale traits of biomass such as the porosity in cell wall matrix, the arrangement of cellulose microfibrils and crystallinity all convey the resistance of lignocelluloses to deconstruction [63]. Thereby the changes in filament organization of cell walls during chemical treatment have been widely imaged by AFM. As one example, Abud et al. [2] analyzed the effect of sulphuric acid pretreatment on sugarcane internodes by AFM images, revealing the loss of filaments and formation of globular structures that presumably containing lignin on the
parenchyma cell wall surface. After the sugarcane biogases was impregnated with oxalic acid, AFM scan revealed that the original smooth surfaces were changed into a non-homogeneous one appeared with irregularly shaped hydrophilic deposits that probably due to the exposure of cellulose [64]. Similar patterns were also observed after ammonium hydroxide and hydrothermal pretreatment [65, 66]. In our group, AFM was conducted to better understand the dynamic changes of cellulose microfibrils in poplar cell walls following alkali pretreatment. The AFM data revealed that poplar cell walls had undergone the swelling of microfibrils, the loss of matrix polymers, the increase of surface roughness, and the formation of cracks as the increased treatment severity, facilitating the subsequent enzymatic saccharification of polymers, the surface features of plant cell walls, and the nanoarrangement of cellulose microfibrils can be directly after chemical/biological degradation has already been demonstrated on various plant tissues. The spatial distribution of cell walls had undergone the swelling of microfibrils, the loss of matrix polymers, the increase of surface roughness, changes of cellulose microfibrils in poplar cell walls following alkali pretreatment. The AFM data revealed that poplar hydroxide and hydrothermal pretreatment [65, 66]. In our group, AFM was conducted to better understand the dynamic changes of cellulose microfibrils in poplar cell walls following alkali pretreatment. The AFM data revealed that poplar cell walls had undergone the swelling of microfibrils, the loss of matrix polymers, the increase of surface roughness, and the formation of cracks as the increased treatment severity, facilitating the subsequent enzymatic saccharification of treated biomass [38]. In addition, a detailed three dimensional measurement for the treated Agave americana L. fiber by NaOH and NaOCl captured the dissolution of non-cellulosic components and the delignification on fiber surface accompanied with a softening and subsequent flattening of the cell wall structure [67]. AFM images were also generated for revealing the effect of ammonia fiber explosion (AFEX) pretreatment on the thickened vascular bundle cell surfaces within corn stover [66].

The use of AFM imaging provides tremendous potential in degradability measurements to be performed on plant cell walls and even on single fibers in situ, which can track more immediate and accurate information than that obtained by chemical fractionation of the tissues. The direct visualization of the enzymatic digestion of a single fiber in an aqueous environment using AFM by Quirk et al. [68] is one of the strongest confirmations. It elaborated the mechanism of the enzymatic digestion of cellulose and further identified the most effective enzymes for the digestion of various cellulose structures or isomorphs. One highlight of this study was real-time imaging of enzymatic cellulose degradation using liquid-phase AFM imaging. This method was also successfully applied to investigate the cooperativity among cellulases [69]. Bubner et al. [70] demonstrated that the enzymatic degradation of cellulose was subjected to deceleration and acceleration process resulting from the specificity of individual cellulase for crystalline or amorphous regions. Ganner et al. [71] using AFM further revealed the synergistic action of endoglucanase enzyme on amorphous cellulose and cellobiohydrolase enzymes on crystalline cellulose respectively. Similar results were also confirmed by Liu et al. [69] in addition to the binding of enzyme particles to cellulose. Overall, the real time in situ AFM technique is particularly promising to understanding the mechanism of enzymatic degradation of cellulose, which is pivotal for biorefinery engineering.

3. Outlook

The potential of kinds of microscopy techniques for non-destructive in situ investigation of plant cell walls before and after chemical/biological degradation has already been demonstrated on various plant tissues. The spatial distribution of polymers, the surface features of plant cell walls, and the nano arrangement of cellulose microfibrils can be directly visualized by these chemical imaging methods. Results of such examinations are complementary to the wet chemical analysis, which will significantly promote the comprehension of cell wall deconstruction mechanism, and thus improving the efficiency of biomass conversion. In addition, the utilization of microscopy techniques on the chemical structure of plant tissues opens up several progressive perspectives, including upgrading of microscopic instruments for better evaluation of cell wall structures and developing new types of microscopes with combination of two or more techniques. Therefore, the progress in microscopic tools can provide more detailed and accurate observations of different structural and morphological phenomena in the field of biorefinery.

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