HIGHER ACID-CHLORITE REACTIVITY OF CELL CORNER MIDDLE LAMELLA LIGNIN IN BLACK SPRUCE

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Abstract

To determine if there was a delignification behavior difference between secondary wall (S2) and middle lamella (cell corner or CC) lignin, black spruce cross-sections were acid-chlorite delignified and the tissue was evaluated *in-situ* by Raman imaging. Lignin concentration in S2 and CC was determined in numerous latewood cell areas in the two hour delignified cross section. In all instances, compared to the S2-lignin the CC-lignin reacted more rapidly. This result contradicted what has been reported previously. The Raman approach to study the topochemistry of delignification is better because not only whole lignin is analyzed, but isolation of morphological-region-enriched fractions of the fiber wall is not required.

Keywords: black spruce, wood, cell wall, cell corner, lignin, acid chlorite, delignification, Raman, imaging,

Introduction

The wood cell wall is a heterogeneous natural nano-composite of cellulose, lignin, and hemicelluloses. Lignin, a component of the cell wall, is present at 20-30% level. The cell wall is organized in several layers. The primary wall (P), secondary wall (S1, S2, and S3), and middle lamella (mL) have different structures. For example, it is known that compared to S2 lignin's concentration in cell corner (CC) middle lamella is ~2.5 times greater[1]. Delignification of wood is a very important reaction for a number of industries including pulp and paper industry. Because there are differences in the structures of middle lamella- and S2-lignins, it is expected that the lignin reaction rates will be different. Additionally, in diverse morphological regions of wood, the existence of carbohydrate polymers of varying structures and organization will have an effect on lignin reactivity. Such topochemical effects have been studied earlier but the findings have been controversial[2]. One of the main reasons seems to be the limitation of the methodology used. Here, a new technique, Raman imaging was used to study the differences in the delignification rates of the CC and S2 regions.

The latest Raman imaging instrumentation is well suited to permit compositional mapping of the woody tissue with chosen lateral resolutions. The imaging technique was recently used to analyze cell walls *in situ*[1,3]; the applications showed that information on both cellulose and lignin can be obtained.

The current study focuses on investigating the difference in the delignification of the S2 and middle lamella lignins. Using Raman microscopy, this was achieved by investigating directly S2 and CC regions of the intact woody tissue. Moreover, the sampling was carried out in presence of water to avoid possibility of artifacts due to anisotropic shrinkage of cell regions upon drying. This allowed for the delignification information that was not compromised by need for physical isolation of morphological regions or limitations associated with the embedding, staining, and chemically derivatizing procedures.

Experimental

Sample Preparation

The samples were taken from the main stem of a black spruce tree and no compression wood was present in the area of the samples. Transverse sections (CS), 1 cm by 0.5 cm by 30 μm thick, were prepared by microtome from segments between the 30th and 40th annual rings. Some of the cross sections were delignified for various durations at 70° C using the following acid chlorite treatment. Sampling in D_2O was performed using the previously published procedure[4,5].

A hot water bath was set up and maintained at 70°C ± 2°.A stock solution of 1200 mg of sodium chlorite and 1200 mg of sodium acetate in 20 ml of distilled water was prepared. Four ml of this stock solution was placed in a small glass vial with a teflon-lined cap and was placed in the bath. Four black spruce sections, 30

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μm thick, were added to the hot stock solution in the vial besides 260 μl of glacial acetic acid (15:1 volume). The vial was gently agitated several times an hour. After 60 minutes the vial was removed from the bath and one section was removed, thoroughly washed with distilled water, and stored in 95% ethanol. The vial solution was replaced with fresh stock solution and acetic acid. After 2 hours, 2 sections were removed from the vial, thoroughly washed with distilled water, and stored in 95% ethanol.. The remaining section was removed from the vial after 4 hours and treated as indicated above and stored in 95% ethanol. This work is currently ongoing and the results from 2 and 4 h delignified sections are presented here.

Raman Analysis

A Raman microprobe spectrometer, LabRam HR 800 (Horiba Jobin Yvon, Edison, NJ) was used in the present study. This spectrometer is equipped with a confocal microscope (Olympus BX40), a piezoelectric x,y stage, and a CCD detector. This instrument provides ultra-high spectroscopic resolution and unique polarized 633-nm laser (20-mW) excitation, which enables the system for woody tissue investigations. Woody tissue spectra from various morphological regions were obtained by using the serial (raster) mapping technique. An Olympus 100X water immersion objective was used for the Raman studies. Further details were the same as published earlier[1].

The lateral resolution of the confocal Raman microscope was ~1 μm. Considering the lateral resolution, the S2 and CC regions of cells could be sampled individually. LabSpec 4.08 software was used to obtain Raman spectra in specific spectral ranges (250 to 3200 cm^{-1}). This software was used to perform all spectrometer operations, process collected data, and generate Raman images. Such images were based on component-specific spectral regions and represented distribution and organization of the cell wall components. Unless stated otherwise, peak height of the Raman band at 1600 cm^{-1} and band area of region 1519-1712 cm^{-1} were used for investigating lignin. For the later region, area intensity was calculated by the sloping baseline method. Spectral data and Raman images were not processed or manipulated in any way. Experimental error was close to 10%. This calculation was based on spectra obtained sequentially at different times from the same sample location.

Results and Discussion

Two hour delignified

Earlier as well as recently[1], in our laboratory, cell walls of black spruce wood have been studied using a Raman microscope. It was found that bands at 1600 cm^{-1} was useful in investigating the distribution of lignin in woody tissue sections. A bright field image an area containing several latewood cells is shown in Fig. 1. This Fig shows the area that was selected for the present delignification study along with the sampling grid.

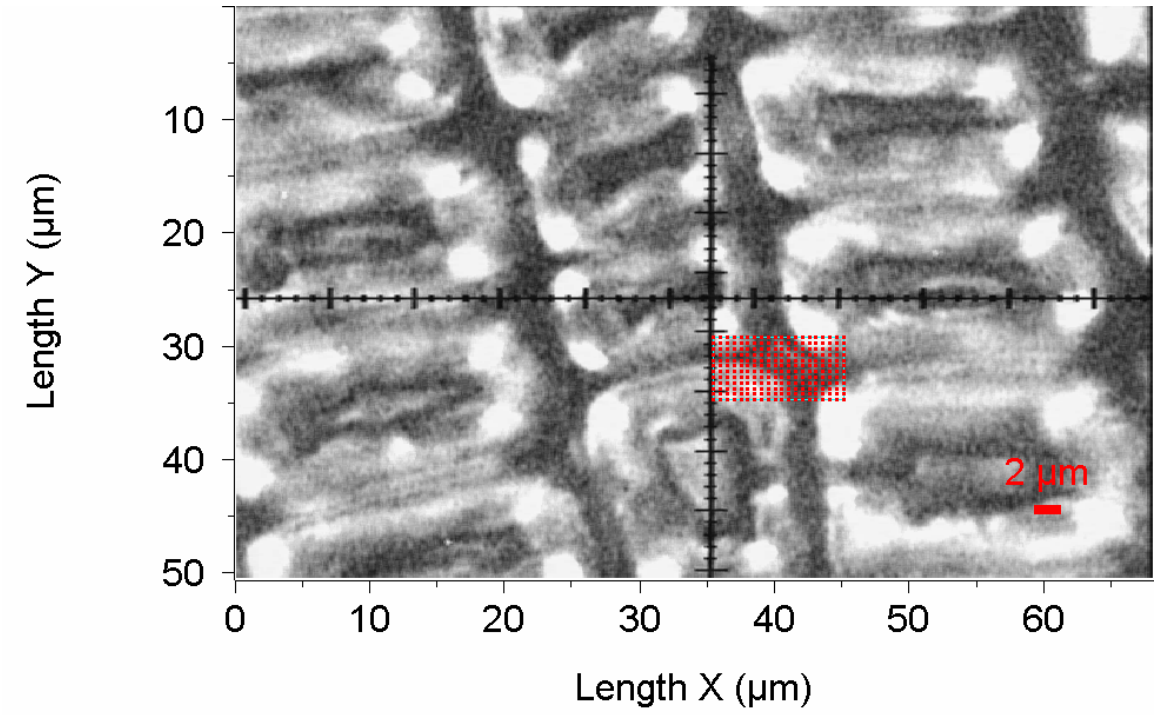

Figure 1—Bright field image of 2 h delignified woody tissue with superimposed sampling grid pattern. Sampling grid covers both S2 and CC regions. Spectra were obtained sequentially from a series of positions using point-by-point scanning.

The Raman images produced using the peak intensity of the1600 cm^{-1} band and the 1519-1712 cm^{-1} spectral region are shown, respectively, in Fig. 2 and Fig. 3. The lignin image in Fig. 2 clearly shows that the 2 CCs and the adjoining area showed the minimum intensity indicating that, compared to S2, the lignin concentration these regions was lower. After 2h of acid-chlorite delignification, middle lamella lignin concentration was about 75% of the remaining S2-lignin concentration. Considering that originally CC has ~2.5 times higher lignin concentration[1], if the rate of delignification were to be same for lignins in S2 and CC, the image intensity of lignin in CC should have been 1500 counts not 450 (table 1 column 2). This implies that the CC middle lamella lignin reacted at a rate that was about 3 times faster compared to the S2-lignin rate. This calculation assumes that during the entire 2 h delignification the reaction rate was uniform. Further experiments that involve investigation of shorter-duration delignified tissue are planned and are expected to address the reliability of this supposition.

The image plot (Fig. 3) was generated using the area spectral intensity, 1517-1712 cm^{-1}, rather than absolute height of the 1600 cm^{-1} band and showed that although the data was quite noisy, the S2-lignin concentration in S2 was indeed higher (red, magenta in S2 vs. mostly green in CC). This supported the observation made from Fig. 2.

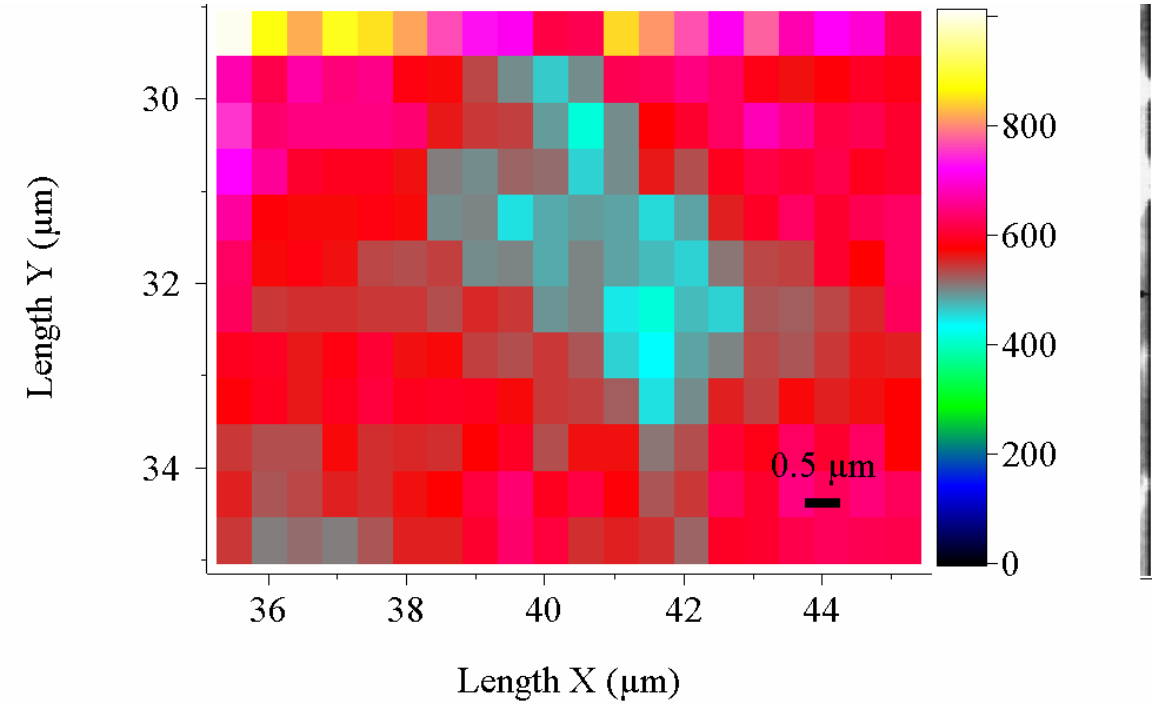

Figure 2—Raman image (false color) of lignin spatial distribution in the cell wall area selected in Fig. 1; Image was generated using the 1600 cm^{-1} peak intensity. Bright white/yellow locations indicate high lignin image intensity; light blue regions indicate low intensity, e.g., CC area. Intensity scale appears on the right.

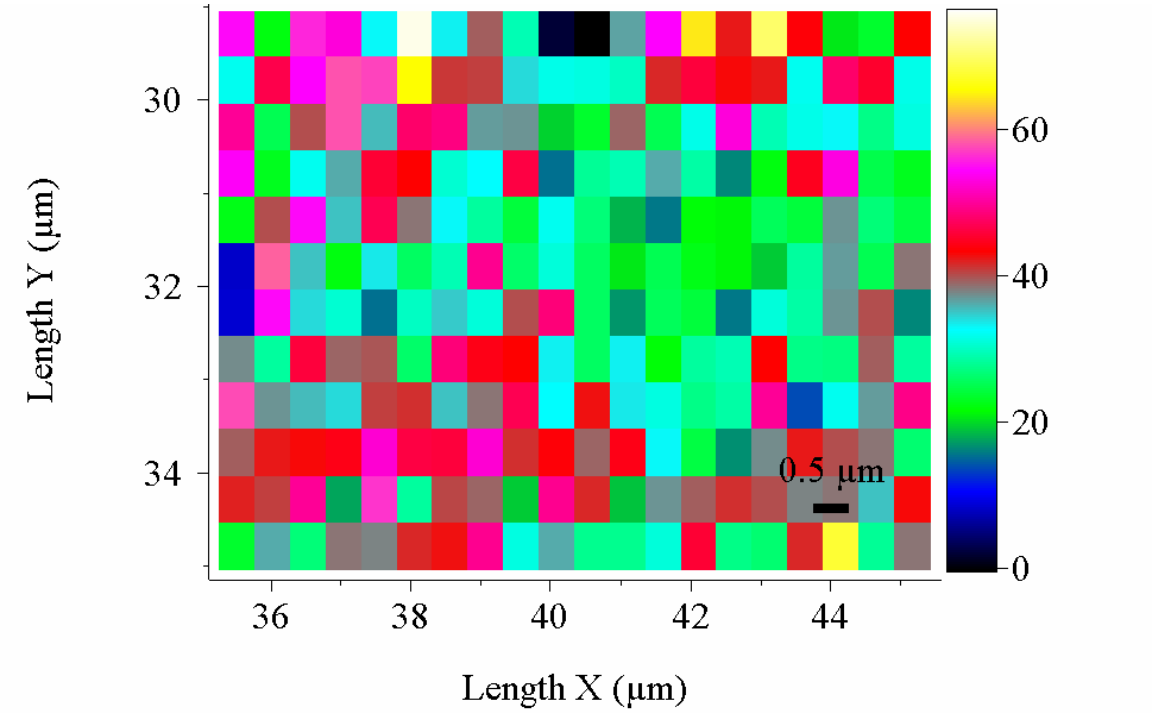

Figure 3—Raman image (false color) of lignin spatial distribution in the cell wall area selected in Fig. 1; Image was generated using the 1519-1712 cm^{-1} region intensity. Because of low signal intensity the counts of the integrated area are low. Bright white/yellow locations indicate high lignin image intensity; light blue/green regions indicate low intensity, e.g., CC area.

*Table 1: Acid-chlorite delignification comparison of S2 and CC (middle lamella) in black spruce**

Section ID	1600 cm^{-1} peak Intensity S2 CC middle lamella	1519-1712 cm^{-1} area Intensity S2 CC middle lamella
Control section	8000 20000	400 1000
2 hour delignified	600 450	50 25
4 hour delignified	400 400	35 30

* Typical data

Four hour delignified

Images, bright field and Raman, from a 4 h delignified section were generated. A typical set of Figures, similar to the 2 h delignified case, is shown in Fig. 4, Fig. 5, and Fig. 6. Lignin image plot, generated using the 1600 cm^{-1} peak intensity in Fig. 5, showed that upon additional delignification lignin concentration in S2 regions declined further and became comparable to the concentration in CC areas (~ 400 intensity units, Fig. 5). This meant that while the S2-lignin remained reactive after 2 h, the middle lamella lignin reacted only minimally upon further chemical treatment. This observation is in accordance with the conclusion from the 2 h delignification study that the S2-lignin reacted at a lower rate and consequently, compared to CC, had higher concentration in S2.

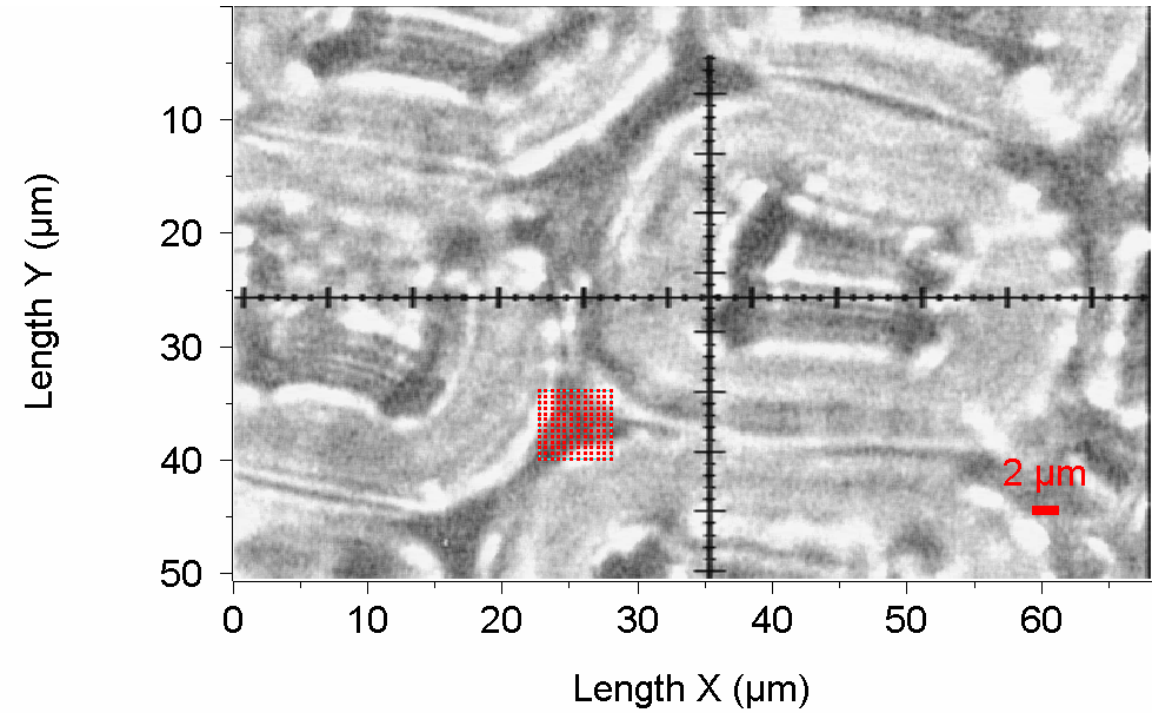

Figure 4—Bright field image of 4 h delignified woody tissue with superimposed sampling grid pattern. Sampling grid covers both S2 and CC regions. Spectra were obtained sequentially from a series of positions using point-by-point scanning.

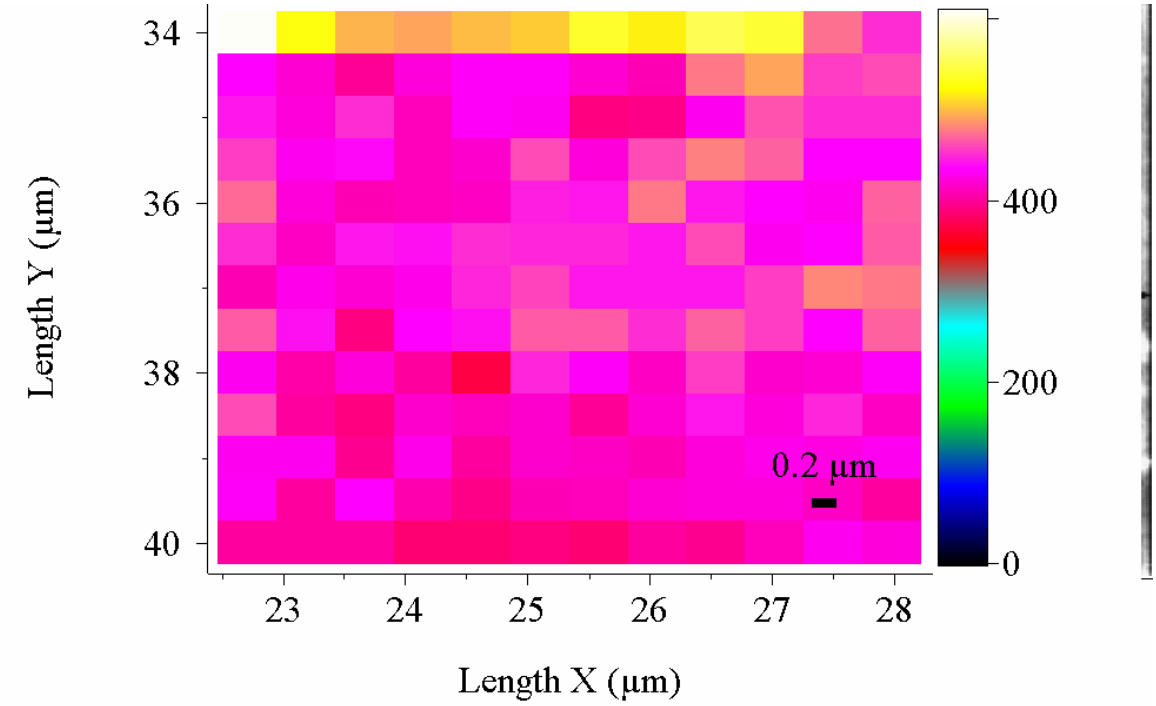

Figure 5—Raman image (false color) of lignin spatial distribution in the cell wall area selected in Fig. 4; Image was generated using the 1600 cm^{-1} peak intensity. Bright white/yellow locations indicate high lignin image intensity; light blue regions indicate low intensity. Both S2 and CC regions showed same image intensity. Intensity scale appears on the right.

Further support indicating additional reaction of S2-lignin came from the image plot created using the 1519-1712 cm^{-1} area intensity (Fig. 6). Although for the latter plot, the S/N was poor the advantage was that the sample fluorescence underlying the spectra could be excluded. Intensity distribution pattern in Fig. 6 appeared random and analogous to Fig. 5, no particular region showed higher lignin concentration.

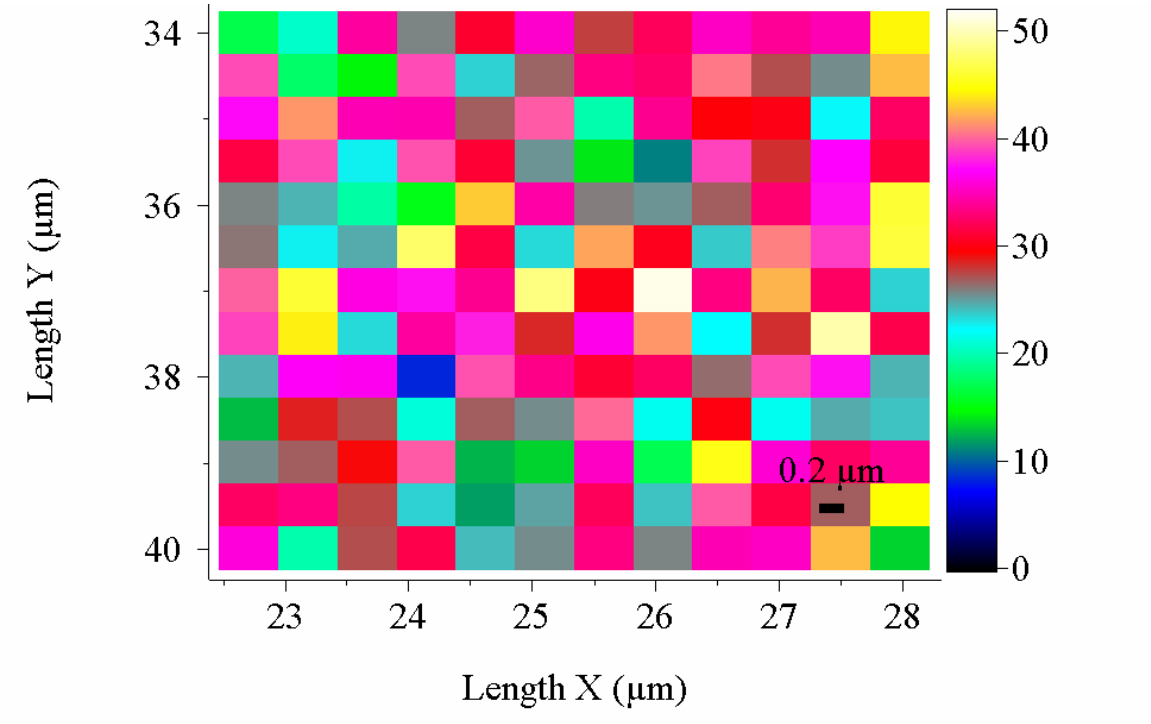

Figure 6—Raman image (false color) of lignin spatial distribution in the cell wall area selected in Fig.4; Image was generated using the 1519-1712 cm^{-1} region intensity. Because of low signal intensity the counts of the integrated area are low. Bright white/yellow locations indicate high lignin image intensity; light blue/green regions indicate low intensity.

The differences in lignin reactivity are likely to result from not only the chemical structural differences (e.g., middle lamella lignin has lower phenolic O-H, lower uncondensed β-O-4 aryl ethers, lower methoxyl content, and is highly cross linked), but also due to presence of fewer carbohydrate polymers. The latter will cause fewer interactions/associations with the middle lamella lignin and the delignification reaction kinetics may be expected to be less diffusion dependent compared to the S2-lignin. Wood fiber pre-treatments that will increase the access of delignifying chemicals to S2-lignin may be beneficial in reactions that are aimed at removing lignin.

Conclusions

Topochemical difference in the acid-chlorite reactivity of S2- and CC-middle lamella-lignins was detected. Compared to S2-lignin, the rate of delignification of the middle lamella lignin in CC was found to be 3 times higher. It is anticipated that there are chemical and physical factors that cause the difference in reactivity.

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