

## Quantification of monolignol ferulate conjugate in Zip-lignin poplar by stimulated Raman scattering microscopy

Wei Shen<sup>1,2\*</sup> ([weishen@msu.edu](mailto:weishen@msu.edu)), John Ralph<sup>1,3</sup>, Shawn D. Mansfield<sup>1,4</sup>, and Shi-You Ding<sup>1,2</sup>

<sup>1</sup>Department of Energy Great Lake Bioenergy Research Center, Michigan State University, East Lansing, MI, 48824 USA; <sup>2</sup>Department of Plant Biology, Michigan State University, East Lansing, MI, 48824 USA; <sup>3</sup>Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, USA; <sup>4</sup>Department of Wood Science, University of British Columbia, Vancouver, BC, Canada

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**Project Goals: To quantify and localize the monolignol ferulate conjugates (zip-lignin) in genetically modified poplar cell walls by *in situ* chemical imaging using non-destructive hyperspectral Stimulated Raman Scattering (hsSRS) microscopy, therefore provide deeper understanding of cell wall deconstructability affected by lignin chemistry.**

GLBRC has demonstrated that Zip-lignin poplar contains more abundant ester linkages by incorporating monolignol ferulate conjugates in lignin backbones. Ester linkages can be easily hydrolyzed under basic condition, which results in increased cell wall digestibility after mild alkaline pretreatment. Zip-lignin also shows increased digestibility under other oxidative pretreatment, suggesting there are complex chemical and topological factors affecting the zip-lignin poplar cell wall features. Among these factors the amount of ferulate moieties incorporated into lignin backbones are of particular interest. Up to this point, they are generally estimated by measuring the ferulate derivatives released through reactions that specifically cleave lignin  $\beta$ -ethers but leave the  $\gamma$ -esters intact. However, the low amount of ferulate derivatives released through this process makes the accurate quantification problematic. In addition, these destructive methods suffer from only delineating bulk structure, i.e., from materials that have been finely ground and thus lose all ultrastructural information, which is of critical importance to understand how cell wall deconstructability is affected by lignin chemistry. In this work, we develop an *in situ* chemical imaging system based on hyperspectral stimulated Raman scattering microscopy with high spectral and spatial resolution. Using this platform, hyper-spectra are taken between 1500–1700  $\text{cm}^{-1}$ , new algorithm is developed to analyze the fine chemistry of the  $\alpha$ - $\beta$  carbon double bonds that present in all type of lignin. We found a  $\sim 30 \text{ cm}^{-1}$  shift in their Raman spectrum due to the conjugation between carbon double bond and the carbon oxygen double bond in carbonyl group, and this shift is specific to zip-lignin and has been verified through reduction of ferulate to its corresponding alcohol. By multivariate curve resolution of the hyperspectral images, we uncovered a spatially distinct distribution of zip and native lignin in plant cell wall. These results contribute to a deeper understanding of zip lignin formation process and its effect on biomass deconstruction processes.

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