Abstract: Xylan constitutes a significant portion of biomass (e.g. 22% in corn stover used in this study). Xylan is also an important source of carbohydrates, besides cellulose, for renewable and sustainable energy applications. A currently used method for the localization of xylan in biomass is to use fluorescence confocal microscopy to image fluorescent dye labeled monoclonal antibodies that specifically bind to xylan. However, with the rapid adoption of Raman-based label-free chemical imaging techniques in biology, identifying Raman bands that are unique to xylan is critical for the implementation of the above label-free technique for in situ xylan imaging. Unlike lignin and cellulose that have long been assigned fingerprint Raman bands, specific Raman bands for xylan remain unclear. The major challenge is the cellulose in plant cell walls, which have chemical units highly similar to that of xylan. Here we report using xylanase enzymes to specifically remove xylan from the feedstock. Under varying degrees of xylan removal, with minimum impact to other major cell wall components, i.e. lignin and cellulose, we have identified Raman bands that could be further tested for chemical imaging of xylan in biomass in situ.

Structural Considerations for Raman Spectroscopy
Raman spectra of plant cell walls arise primarily from the three major components: lignin, cellulose and hemicellulose (mostly xylan). Lignin’s primary Raman contribution is located at 1600 cm⁻¹ with other small bands. Although cellulose and xylan have highly similar chemical substituent groups, the groups have different organization and local environments. Cellulose has highly ordered structures (such as commonly found cellulose Iα, Iβ and II). These highly ordered structures distribute energy among the vibrational modes differently than found in the disordered structures such as in xylan. To separate cellulose and xylan in the Raman spectrum, we developed an enzymatic assay that removes significant xylan with minimum impact on cellulose and lignin.

Impact of Cell Wall Xylan Concentration Change to Raman Spectrum

Raman Spectra of Xylan Model Compounds

Concluding the Amount of Xylan in Cell Wall

Xylan % is analyzed by compositional analysis. No significant digestion of cellulose/lignin has been detected.

Impact of Cell Wall Xylan Concentration Change to Raman Spectrum

Some Candidate Raman Bands

Conclusion: Our results have shown that there are several Raman bands that are sensitive to xylan concentration. They are highly promising for xylan detection. We have tested a few on pretreated CS, and further testing is in progress for more variety of samples.