Nondestructive chemical imaging of wood at the micro-scale: advanced technology to complement macro-scale evaluations

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Abstract

Chemical images help clarify understanding of wood properties, durability, and cell wall deconstruction for conversion of lignocellulose to biofuels, nanocellulose and other value added chemicals in forest biorefineries. We describe here a new method for nondestructive chemical imaging of wood and wood-based materials at the micro-scale to complement macro-scale methods based on X-rays, acoustics, and georadar. Chemical detection, mapping and digital image construction were obtained with a high resolution Fourier transformed infrared microspectroscopy facility that obtains infrared light from a recently constructed beamline at the University of Wisconsin Synchrotron Radiation Center, Madison, Wisconsin USA. This pioneering Infrared Environmental Imaging (IRENI) facility is equipped with a unique design for illuminating and analyzing samples, enabling rapid FTIR data collection, high spatial resolution (pixel size of 0.54×0.54 μm^2), and computer-generated images of lignin, hemicellulose and cellulose in wood cell wall layers of commercially important *Pinus taeda* L. and *Populus deltoides* Bartr.

Keywords: wood, poplar, pine, chemical imaging, FTIR, lignocellulose, IRENI, synchrotron

Introduction

Nondestructive infrared based chemical images can replace time consuming destructive handling and fixative procedures that alter the nature of wood architecture and chemistry. Technological advances in microspectroscopy, computer capacity and specialized imaging software provide a unique opportunity for *in situ* and *in vivo* analysis, revealing wood chemistry and architecture at the micro- and nano-scale levels. Our approach has been to develop procedures with x-ray and infrared spectroscopy to image internal chemistry of solid materials (Hirschmugl and Gough 2012; Nasse et al. 2011a; Illman and Bajt 2003:

Illman and Dowd 1999). Here we describe chemical images of commercially and environmentally important pine and poplar wood using the one of-a-kind synchrotron-based IRENI (Infrared Environmental Imaging) high resolution Fourier transformed infrared (FTIR) microspectroscopy beamline facility at the University of Wisconsin Synchrotron Radiation Center (SRC). Infrared radiation interacts with the dipoles and functional groups of molecules of a sample in a non-invasive and non-destructive way, resulting in detectable absorbance spectra for construction of chemical images with micro-scale resolution.

Chemical images will provide information about wood weathering, fasteners, adhesives, coatings, fungal decay and artifacts. Additionally, chemical images of wall deconstruction will aid the design of protocols to breakdown cell walls for conversion of lignocellulose to liquid biofuels, nanocellulose and chemical feedstock. The objective here is to image lignin, hemicellulose and cellulose in wood of two important model softwood and hardwood species, pine and poplar.

Methods

Commercial kiln dried pine (*Pinus taeda* L.) and poplar wood (*Populus deltoides* Bartr.) were sectioned (10μm thick) with a Reichert sliding (sledge) microtome, stored at room temperature between two standard glass microscope slides and transferred to BaF_2 disks (PIKE Technologies, Madison, WI, USA) for data collection.

Wide-field infrared microspectroscopy was performed at the IRENI beamline facility (Figure 1) using a Bruker Hyperion 3000 and a focal plane array detector (FPA) with a programmable mapping stage coupled to a Fourier Transform Infrared (FTIR) vertex 70 spectrometer. The novel IRENI beamline configuration collects a section of synchrotron radiation (320 mrad × 25 mrad) generated by a bending magnet, collimates 12 light beams that are arranged in a 3×4 matrix side by side to illuminate the sample, creating a virtually homogeneously illuminated sample area of 40 μm × 60 μm using a 74x objective (Nasse et al. 2011a; Nasse et al. 2011b).

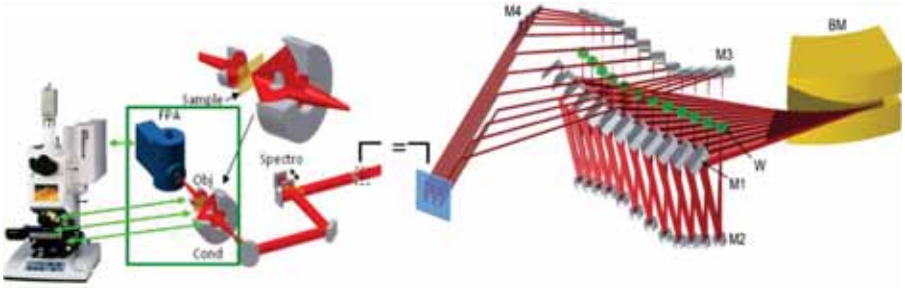

Figure 1. Schematic of the IRENI synchrotron beamline facility, going from right to left: From a bending magnet a section is extracted and subdivided into 12 beams that are collimated into a 3x4 matrix via a system of mirrors (M1-M4). The condenser is used to defocus the beams, resulting in homogeneous illumination at the sample plane. A 74x objective images the sample onto a 128x128 FPA with an effective pixel size of $0.54\mu m^2$ x $0.54\mu m^2$. The end station microscope is a Bruker 3000.

Infrared spectra were collected in transmission mode with a 74x-objective (NA = 0.65) in combination with a condenser of similar NA, determining that the geometric pixel size limit of the detector should not exceed 0.59 μm^2. The FPA (grid of up to 128×128 detectors) has a pixel size of $0.54\mu m^2$ in transmission mode that results in slight oversampling, enabling high spatial resolution at all wavelengths within minutes. Concurrent collection of thousands of infrared spectra in a single shot FPA image covering an area up to 50 μm^2 (96 x 96 pixels) results in a total of 9, 216 spectra. The spectral bandwidth ranged from 4000 cm^{-1} to 800 cm^{-1}.

Analysis of the data was carried out using OPUS (http://www.brukeroptics.cz/downloads.html) and IRidys (www.iryidys.com) running on IGOR Pro (www.wavemetrics.com). Wavemetrics' IGOR Pro was used to extract the spectra, integrate over spectral peaks and display 2-dimensional chemical maps.

Results and Discussion

Cellulose, lignin and hemicellulose were identified by characteristic absorption bands (Pandey1999, Pandey2003, Marchessault1962, Fackler2010, Fengel1992, Kumar2009, Gorzsás 2011) within the wood fingerprint region and illustrated with chemical imaging. A spectral signal was selected from multiple signals for each polymer in order to simplify the demonstration of the process and benefits of IRENI chemical images. At the assignment for cellulose (1372 cm^{-1}), hemicellulose also absorbs but the signal is weak. Absorption at 1506 cm^{-1} was selected for lignin, and 1740 cm^{-1} for hemicellulose. The absorption band assignments represent the most probable and most commonly made classifications. In contrast to spectra of chemical standards, the spectra of lignin, cellulose, hemicellulose and other wood chemicals overlap in cell walls and even the extracted polymers are not pure. The intertwined polymer fibers and chemical linkages make heterogeneous wood one of the most difficult specimen to study.

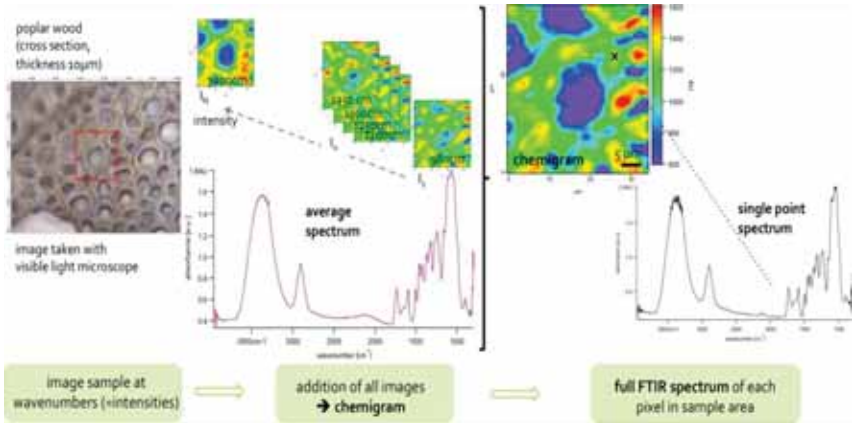

Figure 2. Acquisition of a full FTIR spectral image of poplar. Far left panel: sample area is indicated with a red square (34µm side length) on the light micrograph. Middle: images over entire mid-IR spectrum are obtained in one measurement and added to form chemigram. Right: chemigram with full FTIR spectrum in each pixel, and a single point spectrum from cell wall.

The process for generating IRENI micro-spectro-chemical images from an average spectrum and from a single point spectrum is shown in Figure 2.

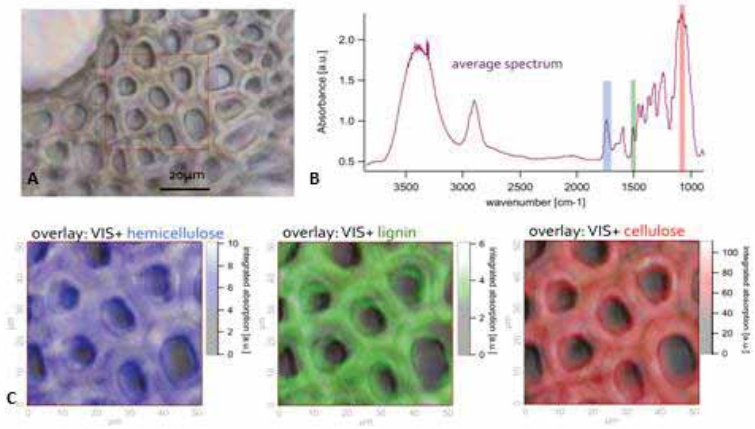

Figure 3. Chemical images of lignin, hemicellulose and cellulose in transverse section of poplar.

Figure 3 shows a light micrograph (A) of a transverse section of poplar wood with the sample region designated by a red square box (34µm side length). Specific spectral regions assigned to hemicellulose (blue), lignin (green) and cellulose (red), are shown in the average FTIR spectrum (B) of the sample, and used to generate chemical images (C). Transparencies (65%) of the images were overlaid on the sample region of the light micrograph. The lumen is gray. The color intensities illustrate the distribution and relative concentration of the polymers in cell walls of pine tracheids. Differences in the relative spectral intensity of cellulose, hemicellulose and lignin in pine and poplar are being examined elsewhere. In Figure 4, similar images for pine wood are shown over twice the sample area as Figure 3 to demonstrate the potential mapping capabilities of this system.

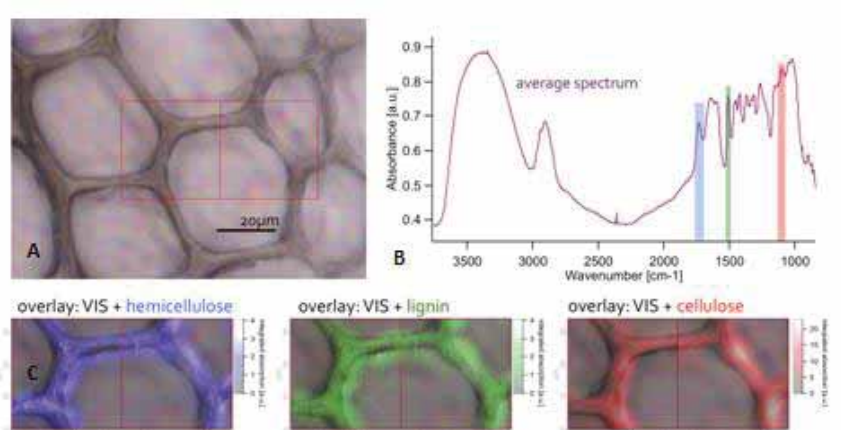

Figure 4. Chemical images of pine wood. A) Light micrograph of a transverse section of pine wood with the FTIR sample region outlined by a red box. B) The average FTIR spectrum with the integration of specfic spectral regions assigned to hemicellulose (blue), lignin (green) and cellulose (red). (C) Chemical images are displayed as transparencies (63%) overlaid on the red box sample region of the light micrograph.

Clearly, the results presented here are a small fraction of the 9, 216 (poplar) and 8,192 (pine) spectra collected for each spectral area of these samples. Data mining for multiple absorption bands promises to reveal new chemical and structural information at the micron scale. Preliminary experiments characterizing adhesive and chemical modifications are encouraging.

Conclusions

The multiple beam FTIR synchrotron beamline IRENI nondestructively measured chemical components of wood, allowing the extraction of specific spectra for hemicellulose, lignin and cellulose and the generation of chemical images with high spatial resolution. Preparation of samples was easy, cost effective, and required no special treatment, staining or storage. Micro-chemical-spectroscopy at IRENI offers many advantages over other techniques. Pixel size at IRENI is 0.54 x 0.54 μm^2 whereas typical table top FPA and synchrotron IR imaging systems are only 5 x 5 μm^2. Collection of full spectra over large sample area gives more accurate data than traditional techniques where a single spectrum from a sample is collected and averaged. Our current research includes fungal decay, adhesive bonding, chemical modification and moisture effects. This presentation will include examples of wood cell wall degradation and treatment of archeological wood.

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Proceedings

18th International Nondestructive Testing and Evaluation of Wood Symposium

Madison, Wisconsin, USA 2013

Abstract

The 18th International Nondestructive Testing and Evaluation of Wood Symposium was hosted by the USDA Forest Service's Forest Products Laboratory (FPL) in Madison, Wisconsin, on September 24–27, 2013. This Symposium was a forum for those involved in nondestructive testing and evaluation (NDT/NDE) of wood and brought together many NDT/NDE users, suppliers, international researchers, representatives from various government agencies, and other groups to share research results, products, and technology for evaluating a wide range of wood products, including standing trees, logs, lumber, and wood structures. Networking among participants encouraged international collaborative efforts and fostered the implementation of NDT/NDE technologies around the world. The technical content of the 18th Symposium is captured in this proceedings.

Keywords: International Nondestructive Testing and Evaluation of Wood Symposium, nondestructive testing, nondestructive evaluation, wood, wood products

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