Chemistry of wood in 3D: new infrared imaging

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Abstract

Chemical detection, mapping and imaging in three dimensions will help refine our understanding of wood properties and durability. We describe here a pioneering infrared method to create visual 3D images of the chemicals in wood, providing for the first time, spatial and architectural information at the cellular level without liquid extraction or prior fixation. Analysis utilized high-resolution Fourier transform infrared (FTIR) microspectroscopy that obtains infrared light from a synchrotron beamline facility at the University of Wisconsin Synchrotron Radiation Center that is equipped with a unique design for illuminating samples. An advanced detector allows multiple mid-infrared spectra to be collected simultaneously. Chemical images were generated in 3D for cell wall layers of *Populus deltoides* Bartr. A complete mid-infrared spectrum was obtained for each data point in the 3D image.

Keywords**:** wood, tomography, Populus, infrared, FTIR, synchrotron, 3D imaging

Introduction

A nondestructive three dimensional (3D) infrared imaging technique has been developed that allows generation of color images depicting spatial distribution of the chemical constituents of wood. The tomographic images provide structural and chemical information that can be missed by a monolayer approach that may result from manual slicing and staining. We published the first infrared tomographic images of wood in *Nature Methods* (Martin et al. 2013).

This infrared tomography technique was developed at the specialized synchrotron beamline IRENI (InfraRed Environmental Imaging) at the Synchrotron Radiation Center (SRC), University of Wisconsin,

Madison, WI USA. The technique couples the IRENI rapid 2D spectral image acquisition capability (Hirschmugl and Gough 2012) to a system that enables acquisition of transmission images as a sample is precisely rotated. A tomographic data set corresponds to projection images as a function of sample angle, and includes the *complete mid-infrared spectrum for each data point*. A full 3D image of the sample can be reconstructed for one or more infrared wavelengths. The combined Fourier transform infrared (FTIR) spectroscopy and tomography create color images where colors can be assigned by specific spectral identification or spectral changes. Each data point on the 3D grid (voxel) provides a wealth of information for advanced spectral analysis. A 3D image of the object can be visualized by a number of methods, including volume rendering or digitally slicing through the sample along any arbitrary plane.

Tomographic chemical images will provide information about wood artifacts, adhesives, weathering, coatings, and fungal decay. Knowledge about chemical identification and distribution will aid the design of protocols to breakdown cell walls for conversion of lignocellulose to bioplastics, liquid biofuels, nanocellulose and chemical feedstock for industry (Illman et al. 2013).

We describe here the nondestructive 3D infrared imaging approach, revealing distribution of the chemicals throughout an intact solid wood sample. The objective was to determine the architectural distribution of lignin, hemicellulose and cellulose in a sample of Populus wood.

Methods

Commercial kiln dried poplar wood (*Populus deltoides* Bartr.) was hand sectioned (50-100 μm thick) longitudinally with a scalpel or cross-sectioned with a Reichert sliding microtome (5-10 μm thick), stored at room temperature and transferred to the microscope stage for infrared data collection.

The novel IRENI synchrotron beamline configuration collects a section of synchrotron radiation (320 x 27 mrad) generated by a bending magnet, collimates 12 light beams that are arranged side by side to illuminate the sample (Nasse et al. 2011a; Nasse et al. 2011b). Infrared microspectroscopy was performed at the IRENI beamline facility using a Bruker Optics Hyperion 3000 and a Santa Barbara (Santa Barbara Infrared, Inc., Santa Barbara, CA) focal plane array detector (FPA) coupled to a Fourier Transform Infrared (FTIR) vertex 70 spectrometer. The FTIR spectra from every pixel in the 128 x 128 pixel detector array was collected and processed by OPUS 6.5 software (http://www.brukeroptics.cz/downloads.html). The infrared microscope stage with sample rotation mount is described in Figure 1.

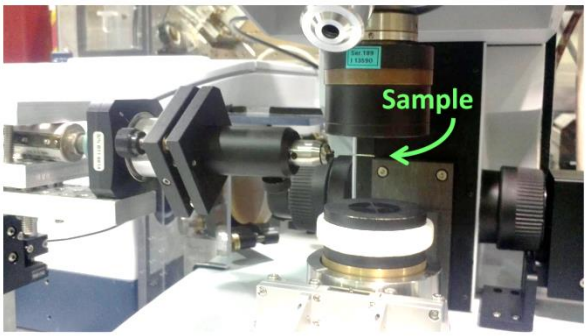

Figure 1 - Photograph of infrared microscope where the sample is held by a pin on a tip-tilt alignment stage at the focus position of the 15x condenser (NA=0.5). Sample and stage are rotated by a computer controlled Agilis rotation mount. The angle of rotation is measured by a potentiometer. Sample and stage above are mounted on an x-z-y stage to position and focus the point of interest on the axis of rotation and at center of the field of view of the FPA detector.

Computed tomography, sample rotation, and tomographic and spectral reconstructions are described by Martin et al. (2013). Briefly, spectral images were acquired in transmission mode with a 15× (numerical aperture (NA) = 0.5) condenser and a 36× (NA = 0.5) objective resulting in an effective projected sample pixel size of 1.1 × 1.1 μm. A series of Java scripts were written for reconstruction of one (or all) mid-IR wavelengths. Reconstruction of the transmission image of an infrared wavelength is similar to x-ray tomography, using Open Source imaging software such as Fiji.

Wood cellulose, lignin and hemicellulose were identified by characteristic absorption bands (Pandey 1999, Marchessault 1962, Fengel 1992, Gorzsás 2011).

Results and Discussion

The full mid-IR spectrum was collected for each data point in poplar wood, giving more accurate data than traditional techniques where a single spectrum is collected and averaged. A representative spectrum for lignin, hemicellulose and cellulose were selected from the full spectrum for analysis. Color images were generated to reveal the distribution of lignin, hemicellulose and cellulose in 3D.

Computer assisted tomography (CAT) scans based on x-ray energy sources do not provide the range of chemical detection that are obtained with infrared. This new ability to rapidly obtain high quality FTIR spectral images provides complementary information to the density differences of chemicals in x-ray tomography (Illman and Dowd 1999).

Representative FTIR images of Populus wood are given in Figure 2, where three virtual slices of wood are taken at different locations along the sample (d-f).

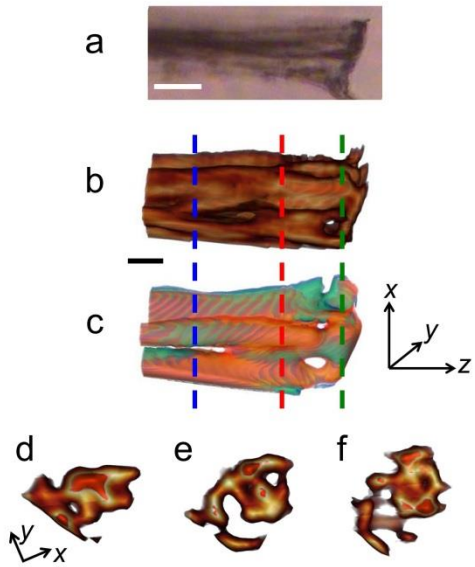

Figure 2 - Populus wood: 2D representations of 3D reconstructions. (a) Visible microscope image of a longitudinal sliver of wood (80 μm diameter). (b through f) Tomographic images, where red and blue-green colors are spectrally associated with lignin (1523-1627 cm^{-1}) and hemicellulose (1689-1781 cm^{-1}), respectively. d and f show digital cuts through the middle of the wood cells. e and f show two views of reconstructions of hydrocarbon stretching absorption modes between 2804-3023 cm^{-1}, similar to views a and b (b - c in Martin et al., 2013).

Conclusions

Tomographic mid-infrared chemical images were obtained with the IRENI synchrotron beamline facility. Sample preparation required no fixative, staining or special storage. This FTIR spectro-microtomography provided spectrally rich 3D visualizations of lignin, cellulose and hemicellulose in wood samples. The large data sets contain the full mid-infrared spectrum for each data point, allowing future comprehensive analysis of all chemicals in the sample. This technique addresses the challenging problem of determining the 3D molecular architecture of wood cell walls, and promises to be a tool for analysis of wood decay, chemical modification of wood and many other applications.

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