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Diffraction-limited IR Microspectroscopy with IRENI

J. Sedlmair[1,1b,,2], B. Illman[1,2]
M. Unger[2,3], C. Hirschmugl[2,3]

[1] US Forest Service, Forest Products Laboratory, One Gifford Pinchot Dr, Madison, WI 53726
[1b] College of Agricultural Sciences, The Pennsylvania State University, 109 Agricultural Engineering Bldg, University Park, PA 16802
[2] Synchrotron Radiation Center, 3731 Schneider Dr, Stoughton, WI 53589-3097
[3] Department of Physics, UW Milwaukee, 1900 E Kenwood Blvd, Milwaukee, WI 53211

In a unique way, IRENI (infrared environmental imaging), operated at the Synchrotron Radiation Center in Madison, combines IR spectroscopy and IR imaging, revealing the chemical morphology of a sample. Most storage ring based IR confocal microscopes have to overcome a trade-off between spatial resolution versus signal-to-noise ratio (SNR). For IRENI, this problem has been addressed by introducing a beam-matrix that is comprised of 3x4 IR-beams, generated from the fan of a bending magnet extracted over a range of 320x27mrad^2 by employing a set of suitable mirrors, see Fig. 1 [1]. By this a usable sample area of 40x60μm^2 is formed to illuminate a focal plane detector (FPA). Slight defocussing leads to a homogeneous illumination, which allows for investigation of environmental samples, biological samples, polymers, etc. [2]. To provide a rough estimate and explanation of how to get to a diffraction-limited resolution of xμm, the Rayleigh criterion, $d = \frac{0.61\lambda}{NA}$, shall be used here, with the minimal distance d that can be resolved with wavelength λ and numerical aperture NA. It needs to be stressed though that for IRENI Schwarzschild optics are used, which need a slightly different description than the Rayleigh criterion. For IRENI, a 74x-objective with NA=0.65 is used, in combination with a condenser of similar NA. With approx. 8 pixels needed to resolve an Airy pattern [3] this leads to a pixel size of 0.59μm for the smallest wavelength in mid-IR range λ=2.5μm. The FPA used here has a pixel size of 0.54μm, leading to adequate oversampling allowing for optimal resolution [1]. Therefore, IRENI is capable of providing a resolution on the order of μm [1], but with the benefit of the additional chemical information of a full IR-spectrum stored in each pixel and without the need of chemical staining. The average time to acquire microspectroscopic data is in the range of several minutes. This enables monitoring of temporal changes, e.g. phase separation, adaption of cells, or alteration of materials due to changes in their environment, at ambient temperature and pressure.

To demonstrate the capabilities of IRENI, a series of experiments on poplar wood is shown in Fig. 2. Wood is a composite material composed of approximately 30% lignin, 25% hemicellulose, and up to 50% cellulose. Wood structure is characterized by long fibrous cells with thick cell walls surrounding the void space of a lumen. Molecular assembly of lignocellulose and hemicellulose in cell walls provides the natural strength, toughness and utilitarian properties of wood [4]. Lignin is a complex phenolic biopolymer almost exclusively made of phenylpropanoid units (Fig. 3). Cellulose is a long polymer of repeated D-glucose monomers linked by β-1,4 glycosidic bonds (Fig. 3) intertwined to form microfibrils. The goal is to obtain FTIR chemical images of lignin and cellulose distribution in wood cell walls with the high spatial resolution offered by IRENI, as shown on the example of poplar wood in Fig. 2 and 3.

References:

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[6] Acknowledgments: IRENI was developed under grant MRI-DMR-0619759. The SRC is funded by UW-Madison and UW-Milwaukee.

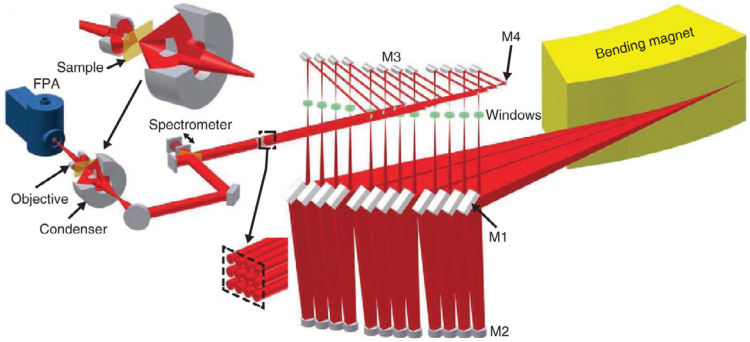

Fig. 1: Schematic of the generation of the beam-matrix for IRENI at SRC (not to scale), together with the setup of the microscope.

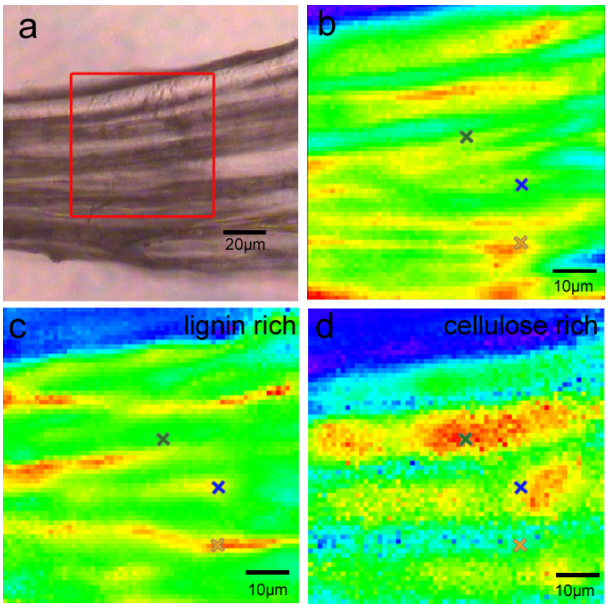

Fig. 2: Imaging of poplar wood. **a)** Visible image with IR sample area indicated, **b)** chemigram of sample area (integrated spectrum over entire spectral range, 700-4000cm^{-1}), **c)** integrated sample area spectrum over spectral range of **lignin** peak (1697-1762cm^{-1}), **d)** integrated sample area spectrum over **cellulose** peak (1018-1092cm^{-1}). The spectra of the points indicated by crosses are plotted in Fig. 3. The colour code goes from low (blue) to high intensity (red).

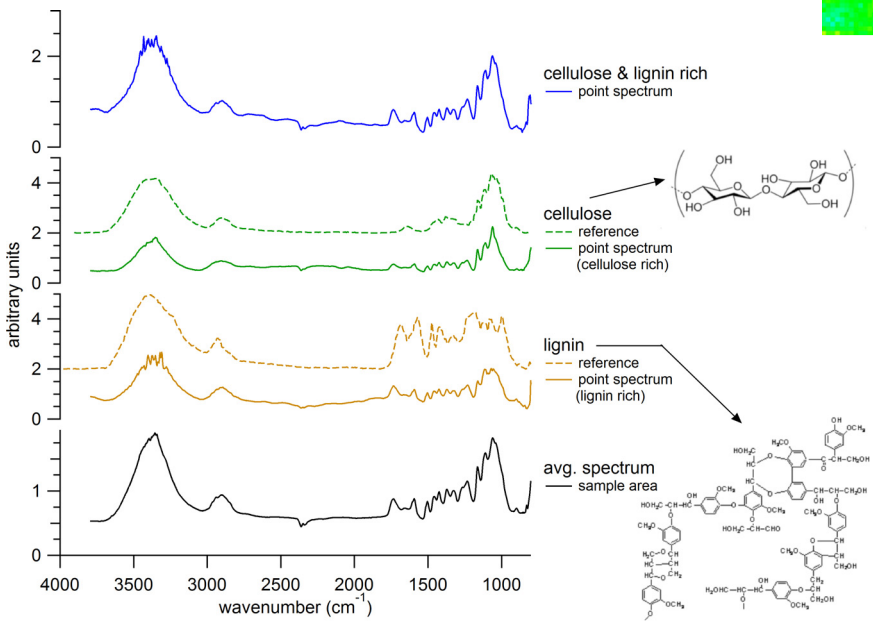

Fig. 3: Spectra of reference measurements (adapted from [5]) and point spectra of the positions indicated in Fig. 2 c-e. Additionally, proposed chemical formulas of cellulose and lignin are displayed [3].