Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

In situ Infrared Imaging of the Local Orientation of Cellulose Fibrils in Plant Secondary Cell Walls

*Alexander Veber[1,2], Victor M.R. Zancajo[1], Ljiljana Puskar[2], Ulrich Schade[2], Janina Kneipp[1]**

[1] Department of Chemistry, Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany

[2] Institute for Electronic Structure Dynamics, Helmholtz-Zentrum Berlin für Materialien und Energie GmbH, Albert-Einstein-Str. 15, 12489 Berlin, Germany

Supporting Figures

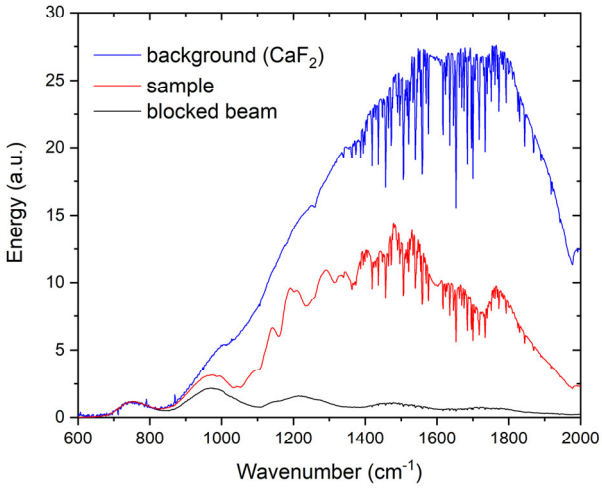

Figure S1. An example of single channel infrared light spectra collected from a clear (no sample) CaF_2 window region (upper/blue line – "background"), plant cross section (middle/red line – "sample") and optically blocked sample position of the microscope (bottom/black line – "blocked beam")

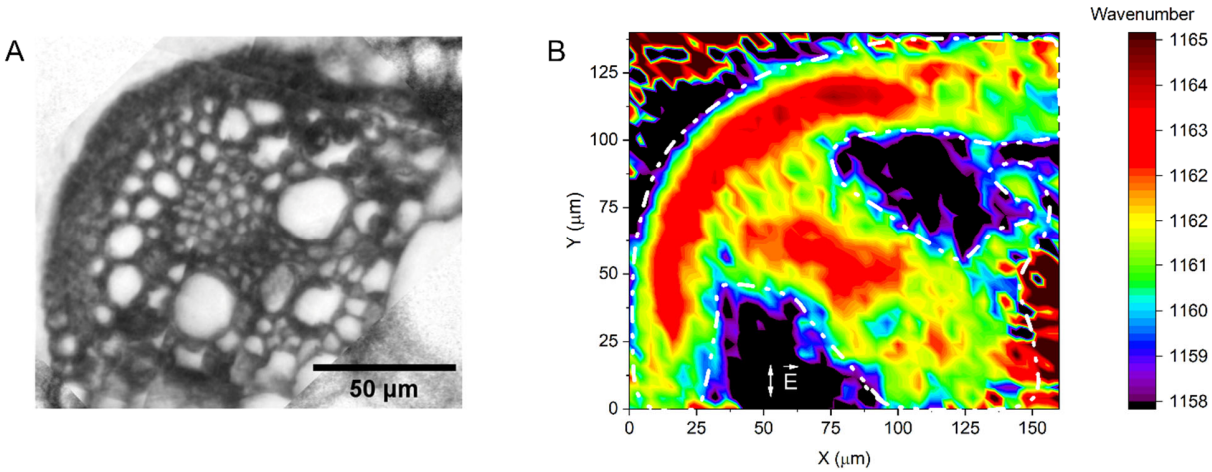

Figure S2. (A) Bright field image and (B) corresponding false color map of the position of the absorption maximum of the ~1160 cm^{-1} C-O-C vibrational band assigned to glycosidic bonds. The white dash-dot line in (B) shows the approximate border between peak position values below and above 1160 cm^{-1}. The maps were obtained from wide area measurement of the cross-section at 0° orientation of the sample. The electric field of the IR radiation is oriented in the vertical direction as indicated by the double-sided arrow in the panel (B). The color values of the map in (B) were interpolated and do not reflect the mapping spot size of 7 µm.

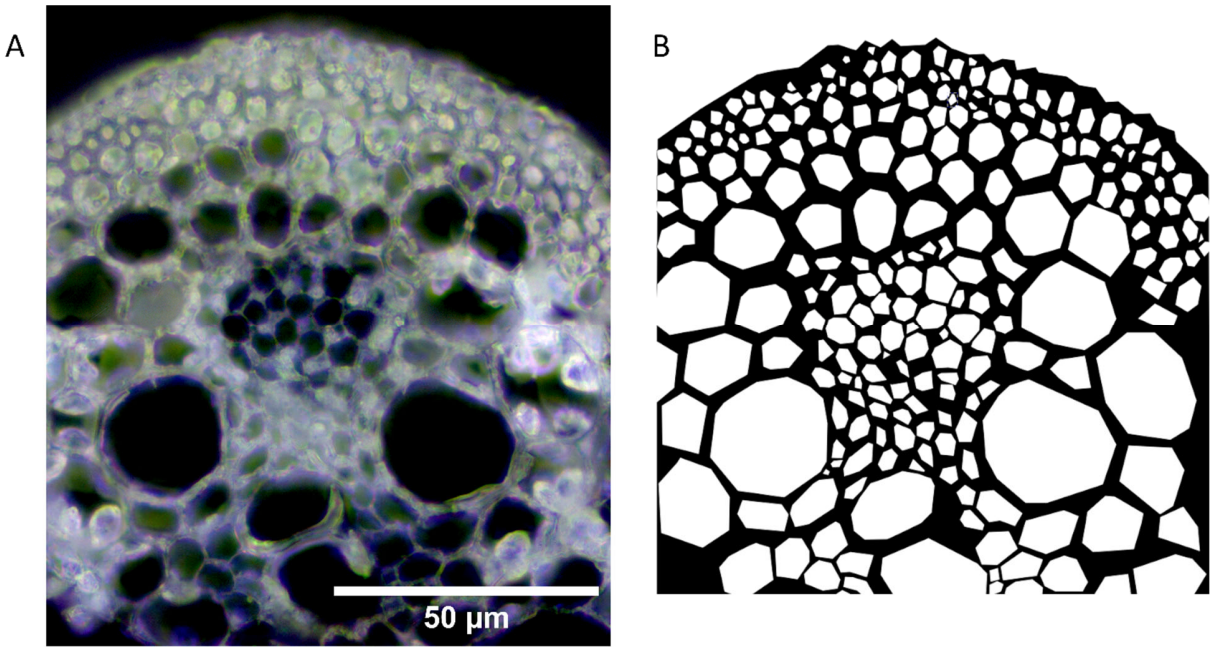

Figure S3. (A) Bright field microscope image of the investigated *Sorghum bicolor* cross-section and (B) corresponding generated binary image of the cell-wall skeleton.

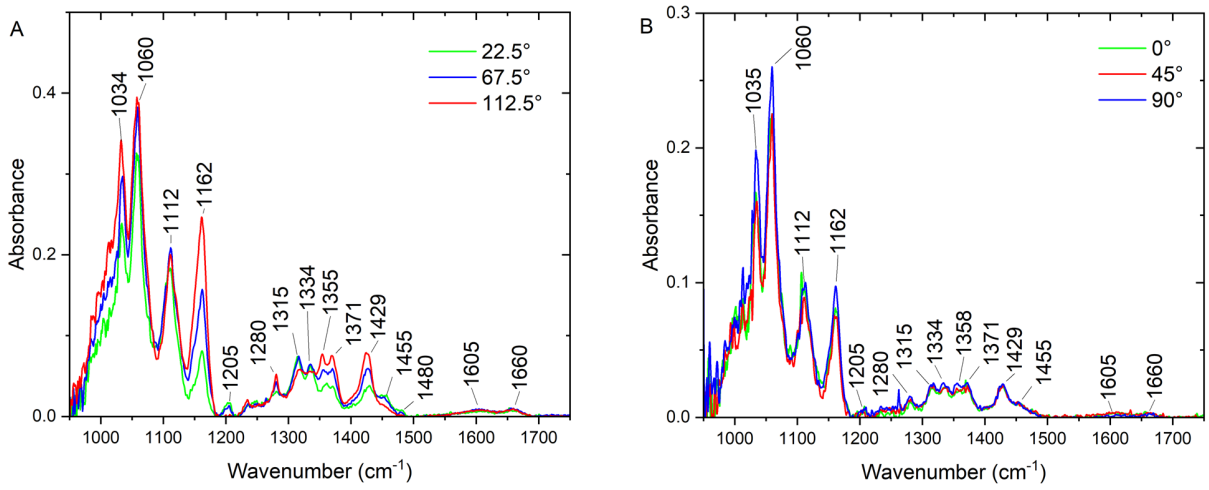

Figure S4. FTIR microspectra of a reference sample of nanocrystalline cellulose at different angular orientations of the sample. (A) Cellulose microcrystal, selected from the sample and (B) microscopically unoriented region of the nanocellulose layer. In panel (A) maximum and minimum of the absorption at 1162 cm^{-1} is observed for the spectra taken at 112.5° (red) and 22.5° (blue), respectively. Aperture size during the measurement was 5x5 μm^2.

Table S1. Assignment of the vibrational modes observed for a nanocrystalline cellulose (NCC) reference sample as well as for bands of lignin and hemicellulose found in the plant FTIR spectra. Only those bands that can be ascribed to these two compounds in the observed spectra and that are not assigned to cellulose are shown. Assignments are based on Refs. [29],[41], [49-52] of the main manuscript text.

Wavenumber (cm^{-1})	Assignment
Cellulose reference (NCC)	
1000, 1013, 1035	C-O stretching (C_6H_2–O_6H)
1060	C-O stretching (C_3-O_3H)
1115	C–O stretching (C_2–O_2H)
1163	C–O–C antisymmetric (glycoside)
1207	C–O–C symmetric stretching (glycoside)
1280	C-H deformations vibrations
1315	O–H in-plane bending (C–O–H)
1334	O–H in-plane bending (C–O–H)
1358	C-H deformations vibrations
1371	C-H deformations vibrations
1429	O–H in-plane bending (C–O–H)
1455	O–H in-plane bending (C–O–H)
Lignin	
990	C-O stretching or -CH=CH- out of plane bending
1028	C-O stretching
1230	C-C, C-O, C=O stretching
1140-1155	C-H of aromatic in-plane bending and C=O stretching
1230, 1265	C-O stretching
1420	C-H bending in CH_3
1460	C-H bending in CH_2 and CH_3
1510, 1600	Aromatic skeletal vibration
1660	C=O stretching
Hemicellulose	
1020	C-O stretching
1050	C-O stretching (C_3-O_3H)
1110	C-O stretching(C_2-O_2H)
1150	C–O–C antisymmetric (glycoside)
1240	C-O stretching (carboxylic acid)
1313	O–H in-plane bending (C–O–H)
1380, 1602	COO- stretching
1730	C=O stretching (–$COCH_3$ and carboxylic acid)