

## **Supplementary Material 3: Water levels during enzymatic hydrolysis**

### **Spatiotemporal dynamics of cellulose during enzymatic hydrolysis studied by infrared spectromicroscopy**

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Water levels fluctuated during the course of observation of the enzymatic cellulose hydrolysis reaction (Figure S3. 1). There was an initial excess of moisture in the bottom left corner of the region of interest in the first half hour, followed by nearly 4.5 hours of minimal moisture levels ( $\sim 3 \mu\text{m}$ ). At approximately 5 hours into the experiment, buffer was pumped into the microfluidic device, flooding some parts of the region of interest with moisture levels up to  $\sim 12 \mu\text{m}$ .

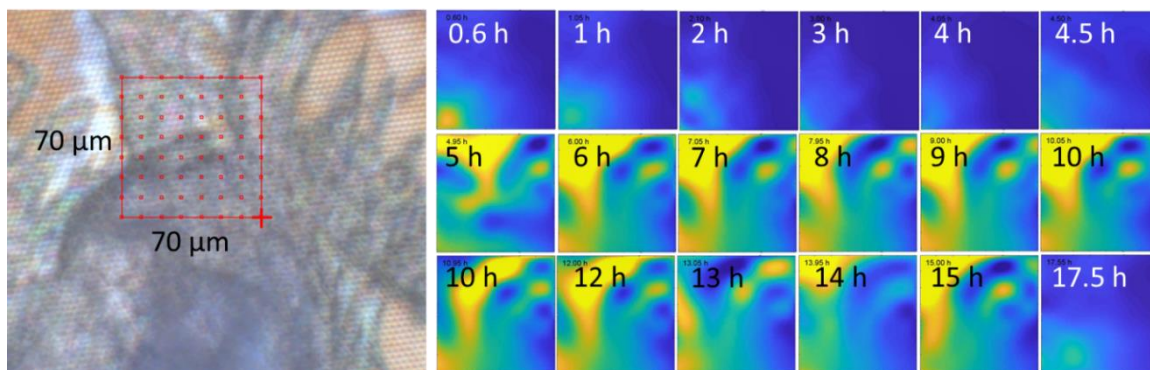


Figure S3. 1: *Brightfield image of the sample with points showing where IR spectra were collected during enzymatic hydrolysis. (Right) Heat maps at various times of the intensity of the  $2154 \text{ cm}^{-1}$  IR band indicating the presence of free water at various times during the course of enzyme hydrolysis. Darker (blue) regions contain minimal water ( $\sim 3 \mu\text{m}$  in thickness) while brighter (yellow) regions are flooded with water ( $\sim 12 \mu\text{m}$  in thickness). Each map covers the  $70 \times 70 \mu\text{m}$  area shown on the left.*