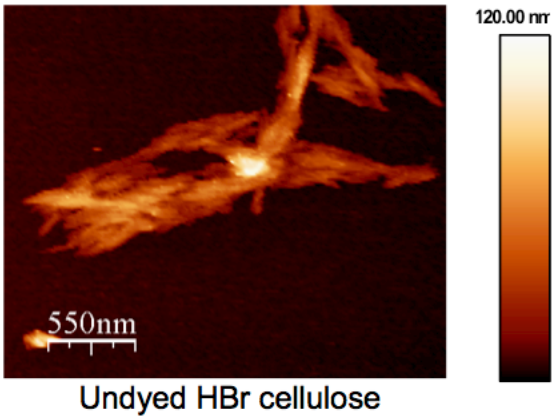
Supporting Information

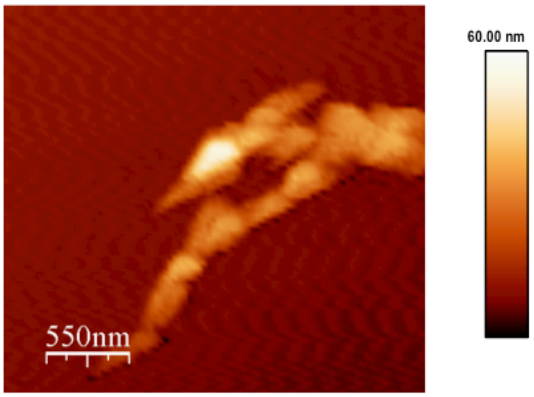
Alexa Fluor-labeled Fluorescent Cellulose Nanocrystals for Bioimaging Solid Cellulose in Spatially-Structured Microenvironments

Jay W. Grate,* Kai-For Mo, Yongsoon Shin, Andreas Vasdekis, Marvin G. Warner, Ryan T. Kelly, Galya Orr, Dehong Hu, Karl J. Dehoff, Fred J. Brockman, and Michael J. Wilkins

CONTENTS



Undyed HBr cellulose



Alexa 546 HBr cellulose

Figure S1. AFM images of cellulose nanocrystals, derived from an HBr acid hydrolysis method, before and after dyeing with Alexa Fluor 546.

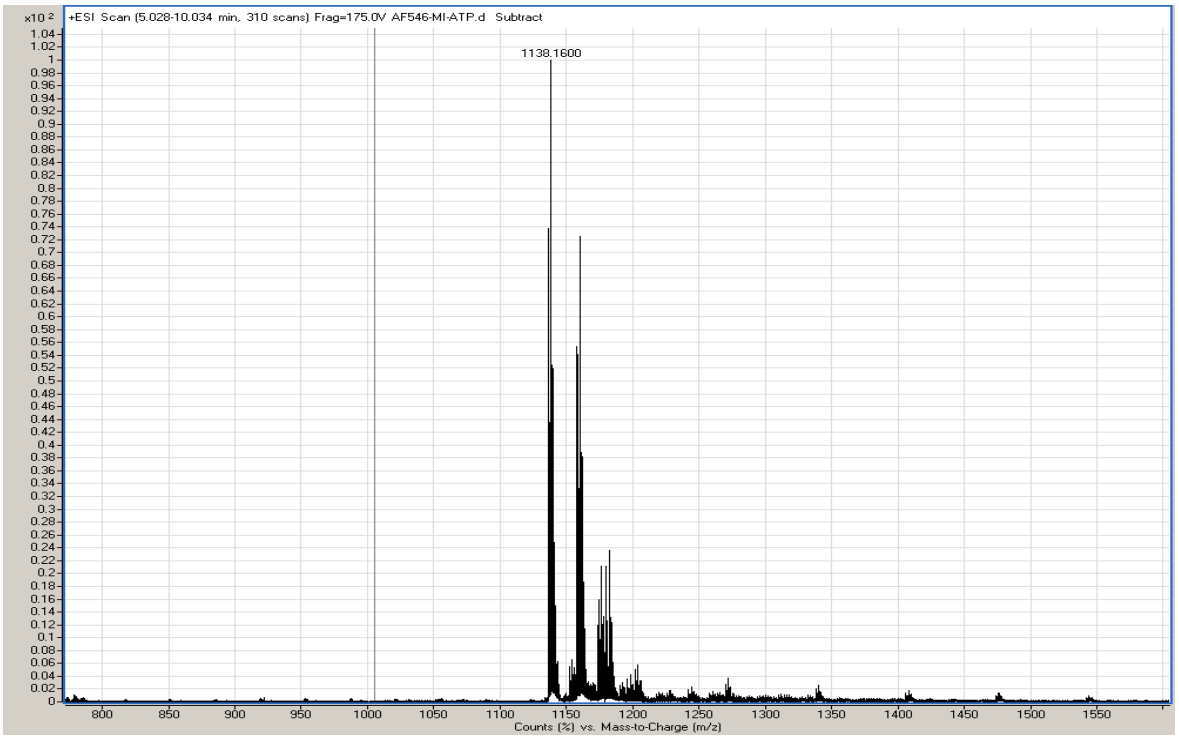


Figure S2. Mass spectrum of crude reaction mixture of **1**

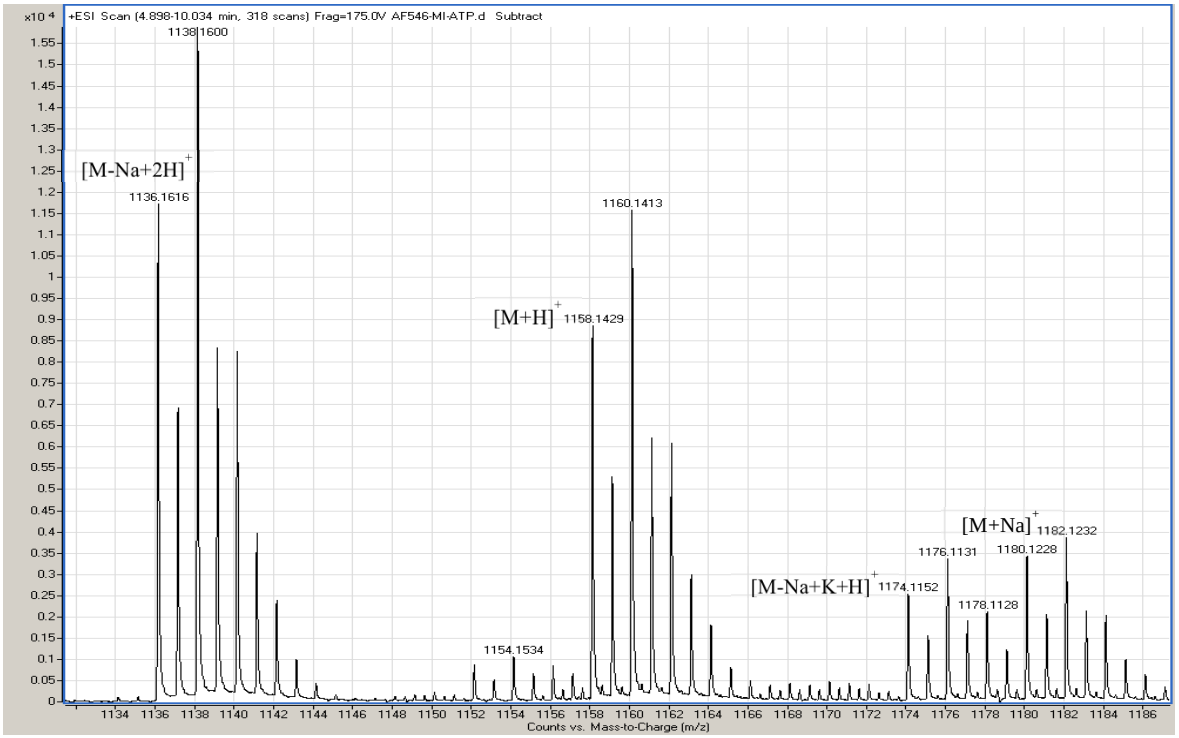


Figure S3. Mass spectrum of **1** after preparative TLC purification

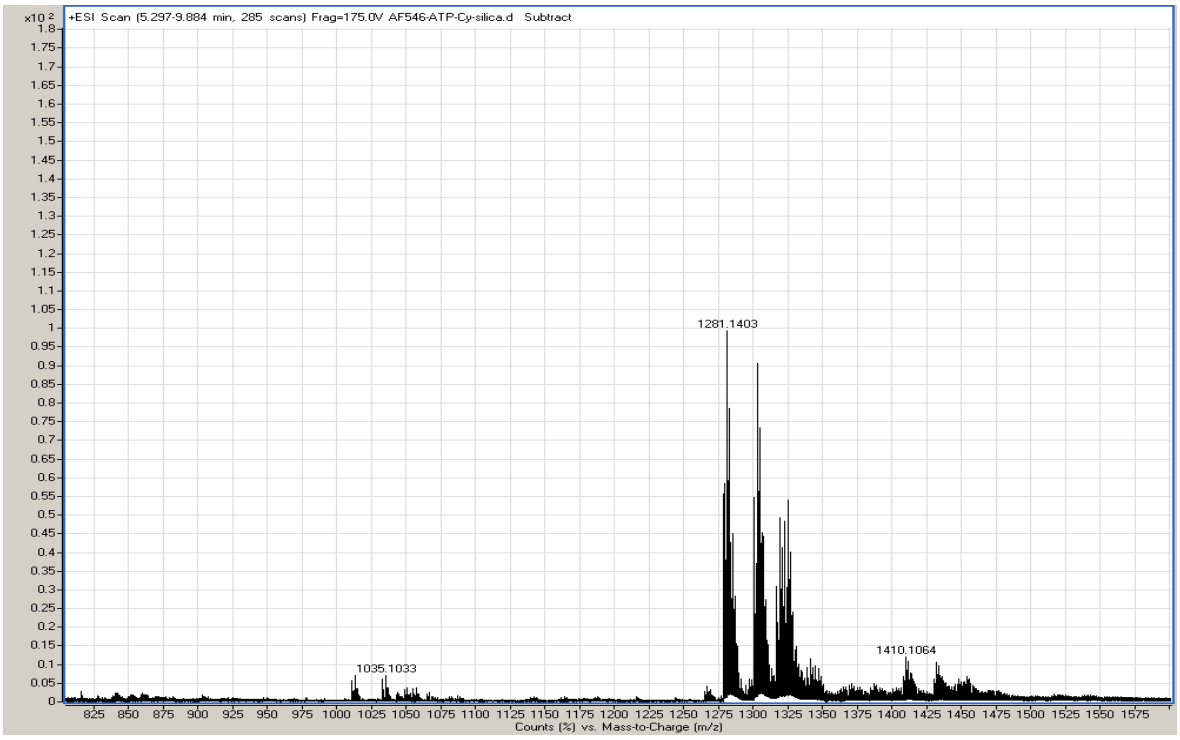


Figure S4. Mass spectrum of crude reaction mixture of **2**

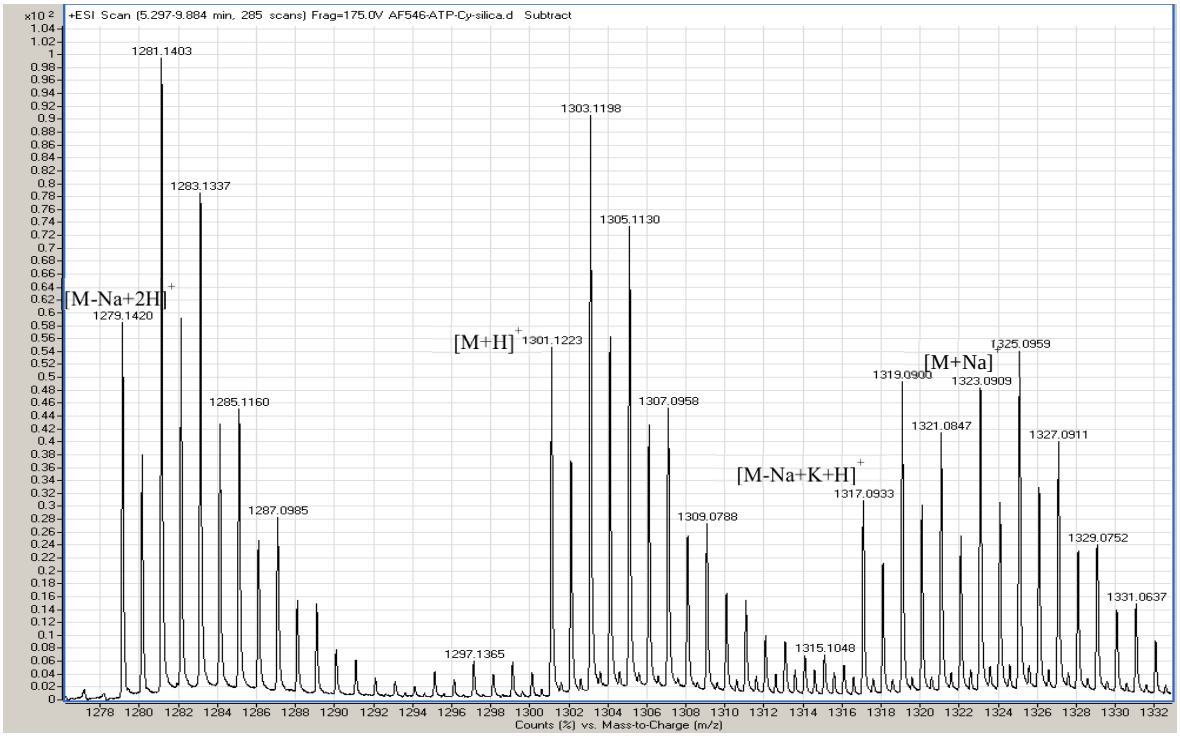


Figure S5. Mass spectrum of **2** after preparative TLC purification

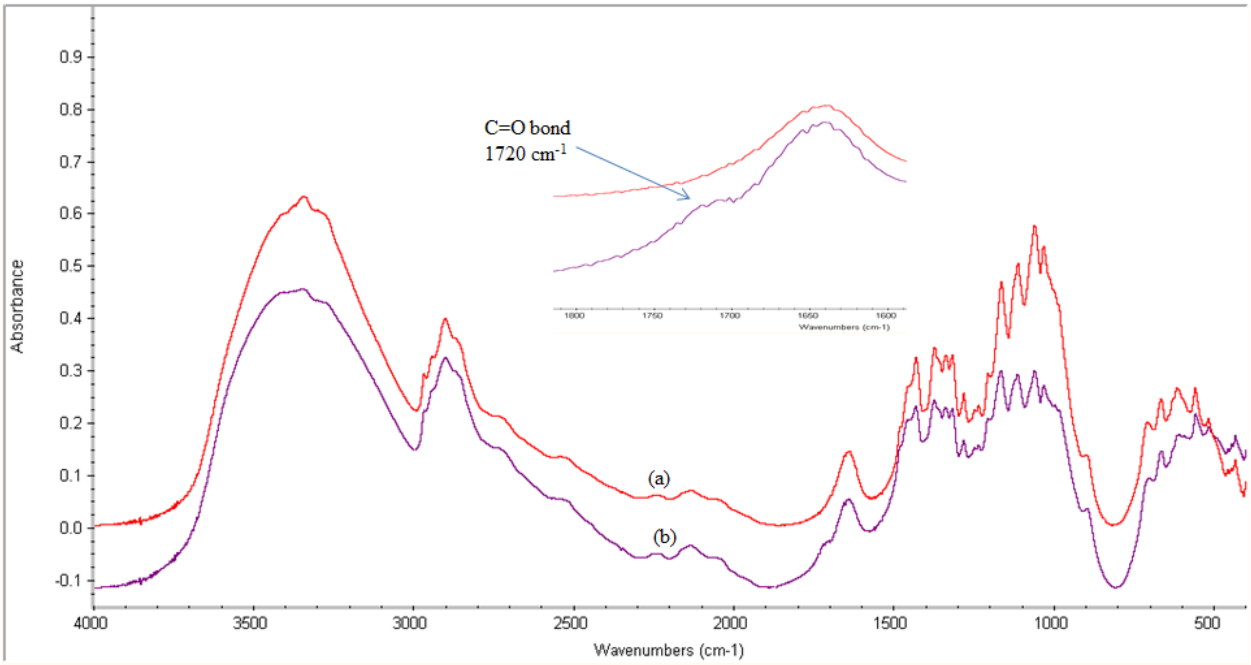


Figure S6. FTIR spectrum of (a) unmodified cellulose nanocrystals and (b) partially oxidized cellulose nanocrystals (1%DA-modified cnxtls).

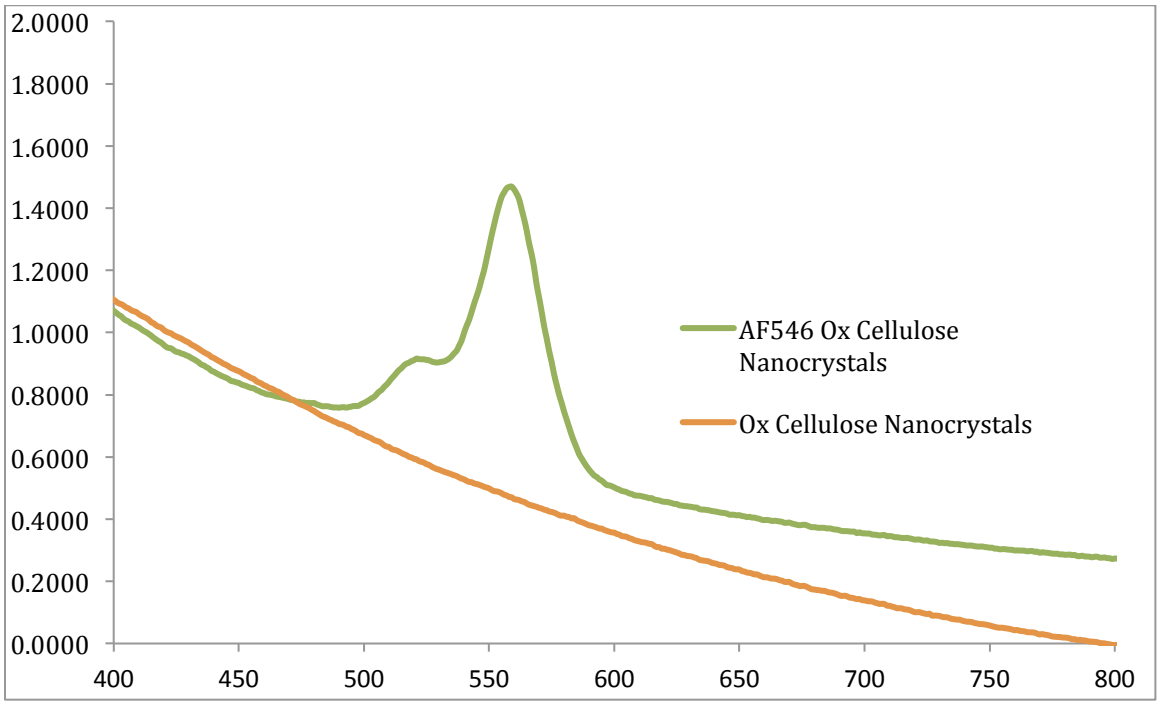


Figure S7. UV-vis absorbance spectroscopy of AF546 cellulose nanocrystals prepared by reductive amination of periodate-treated material. Traces show the material before and after dye conjugation.

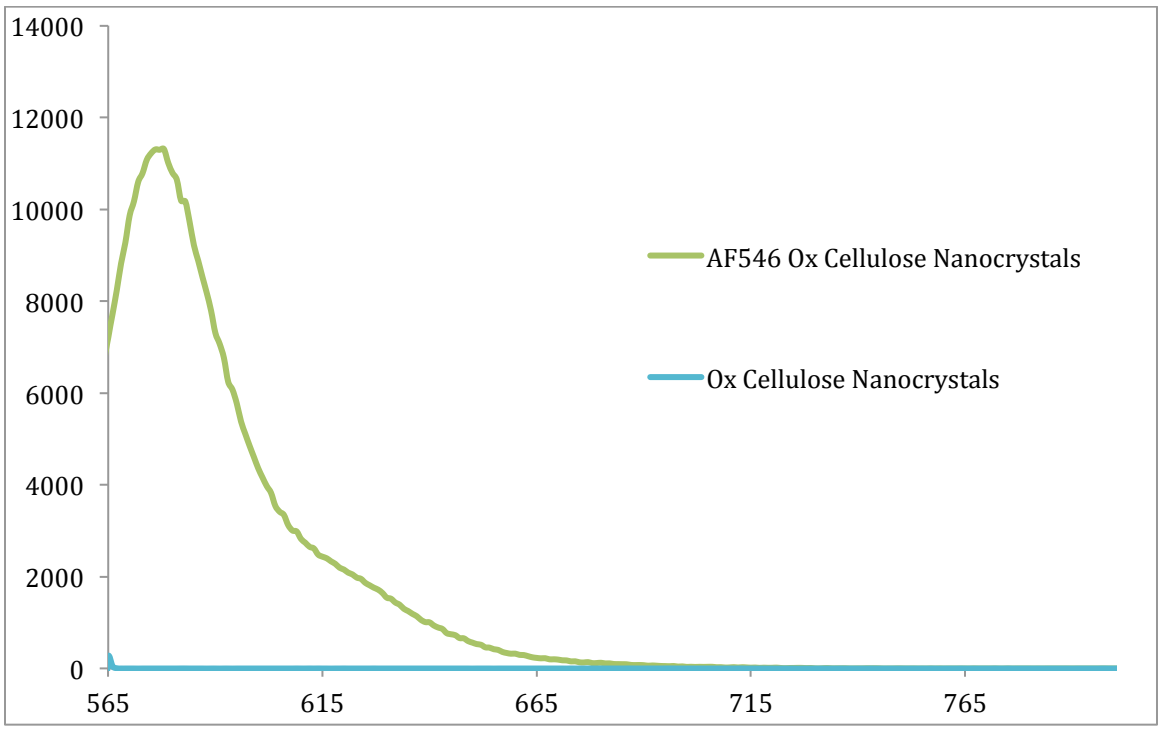


Figure S8. Fluorescence emission spectroscopy of AF546 cellulose nanocrystals prepared by reductive amination of periodate-treated material . Traces show the material before and after dye conjugation.

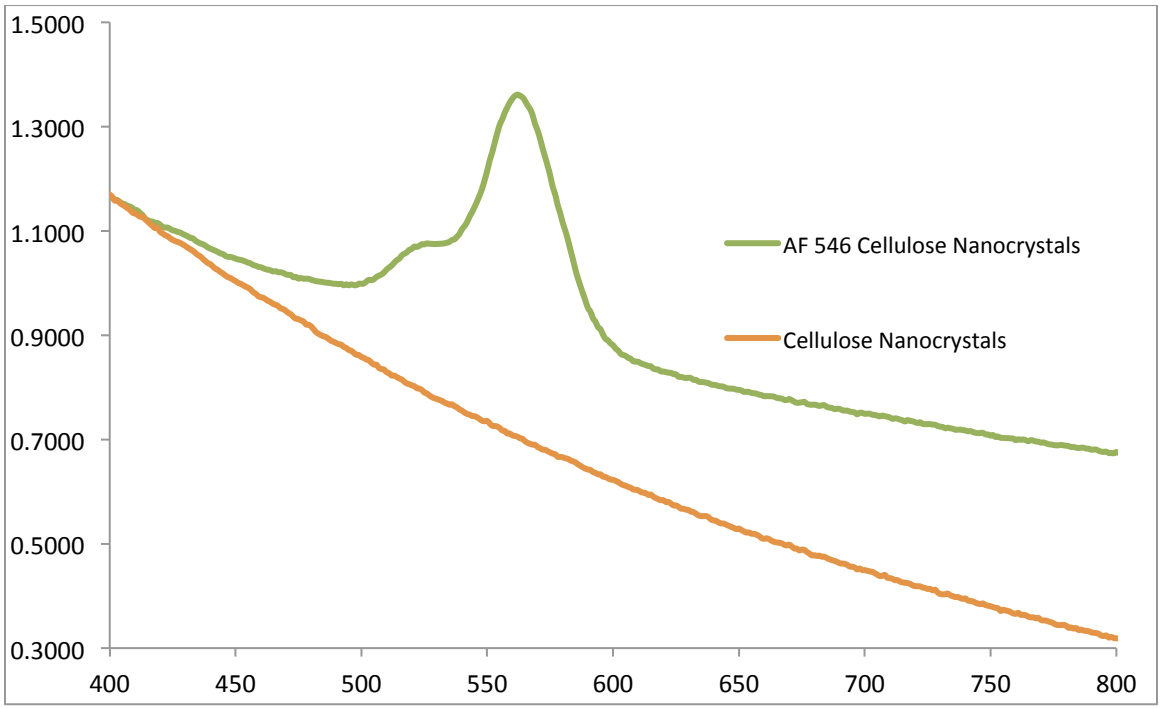


Figure S9. UV-vis absorbance spectroscopy of AF546 cellulose nanocrystals prepared with the triazine chemistry. Traces show the material before and after dye conjugation.

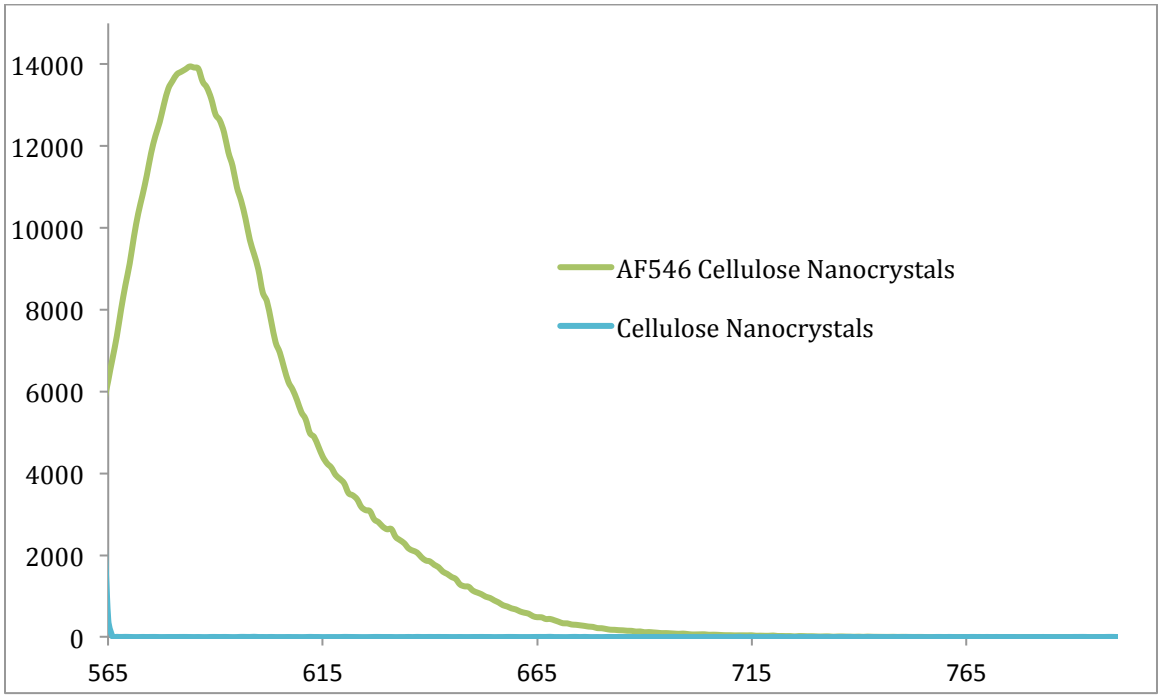


Figure S10. Fluorescence emission spectroscopy of AF546 cellulose nanocrystals prepared with the triazine chemistry. Traces show the material before and after dye conjugation.

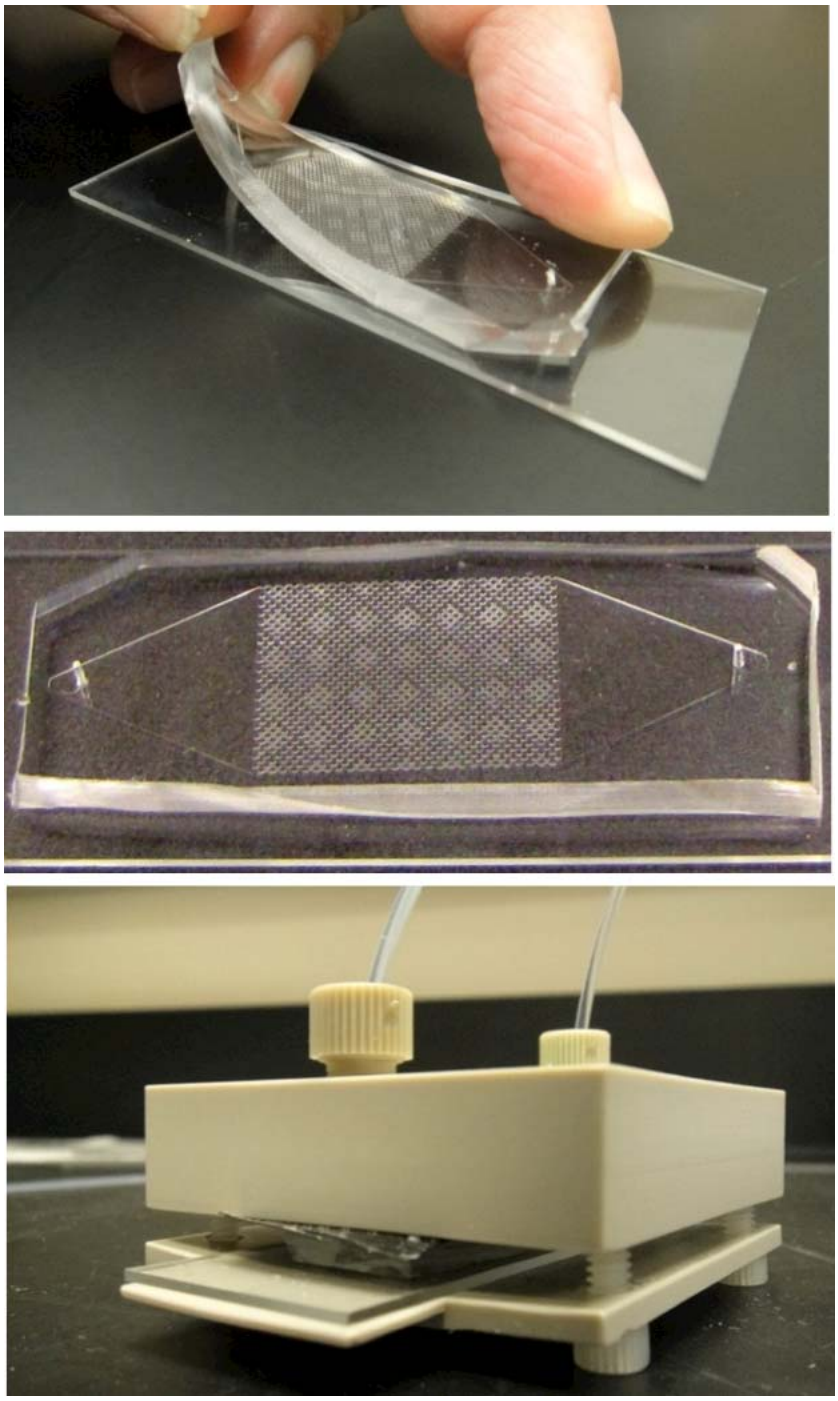


Figure S11. Micromodel created from PDMS, with a heterogeneous pore network in this case, with the holder for microfluidic experiments with fluid inputs and outputs

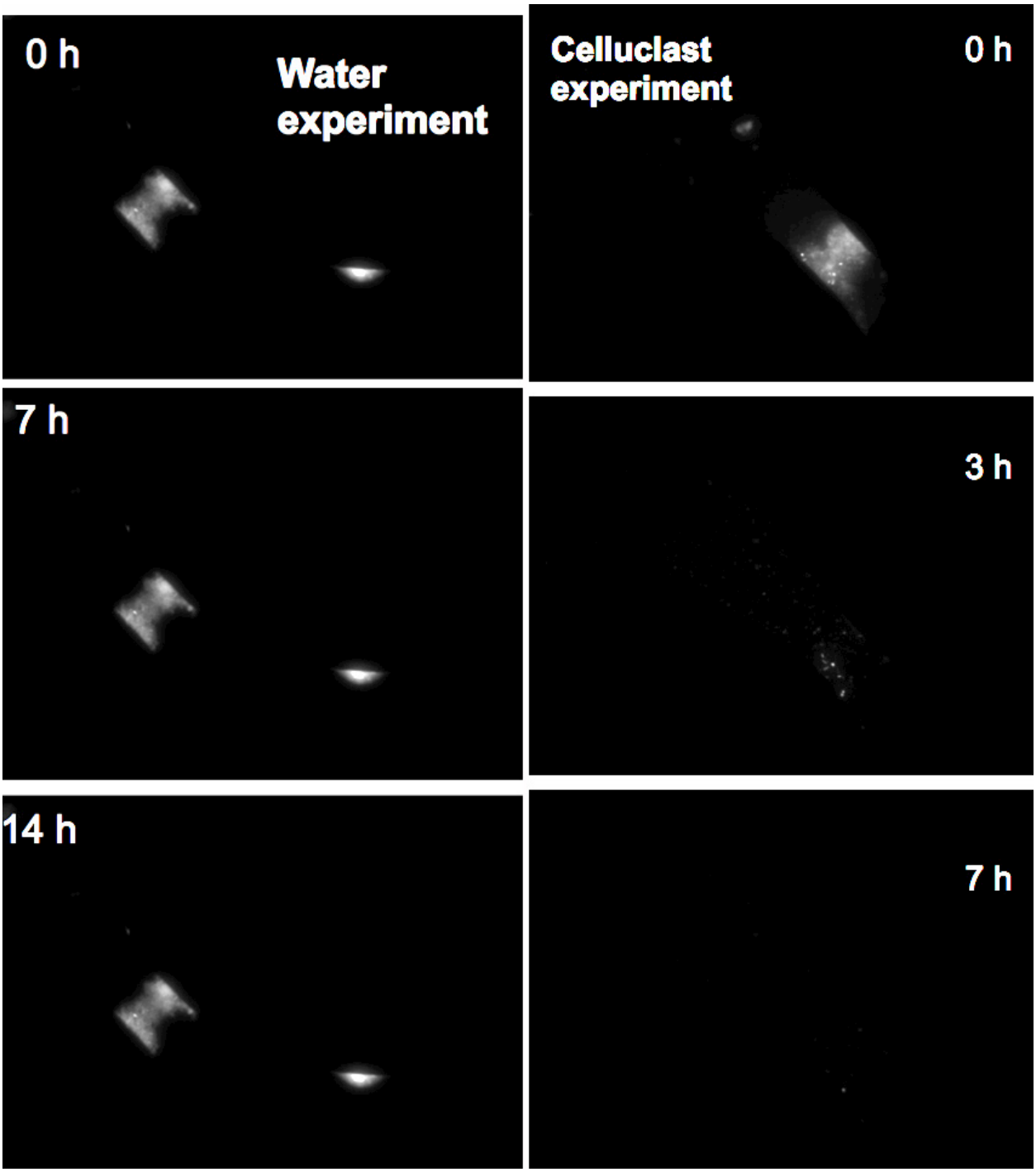


Figure S12. Fluorescent images of Alexa Fluor 546 labeled cnxtls in water in the micromodel, showing stability in water over at least 14 hours(left column), or in a 10x dilution of Celluclast enzyme mixture in the micromodel, showing hydrolytic degradation of the fluorescent cellulose(right column). (Excitation, 532 nm)

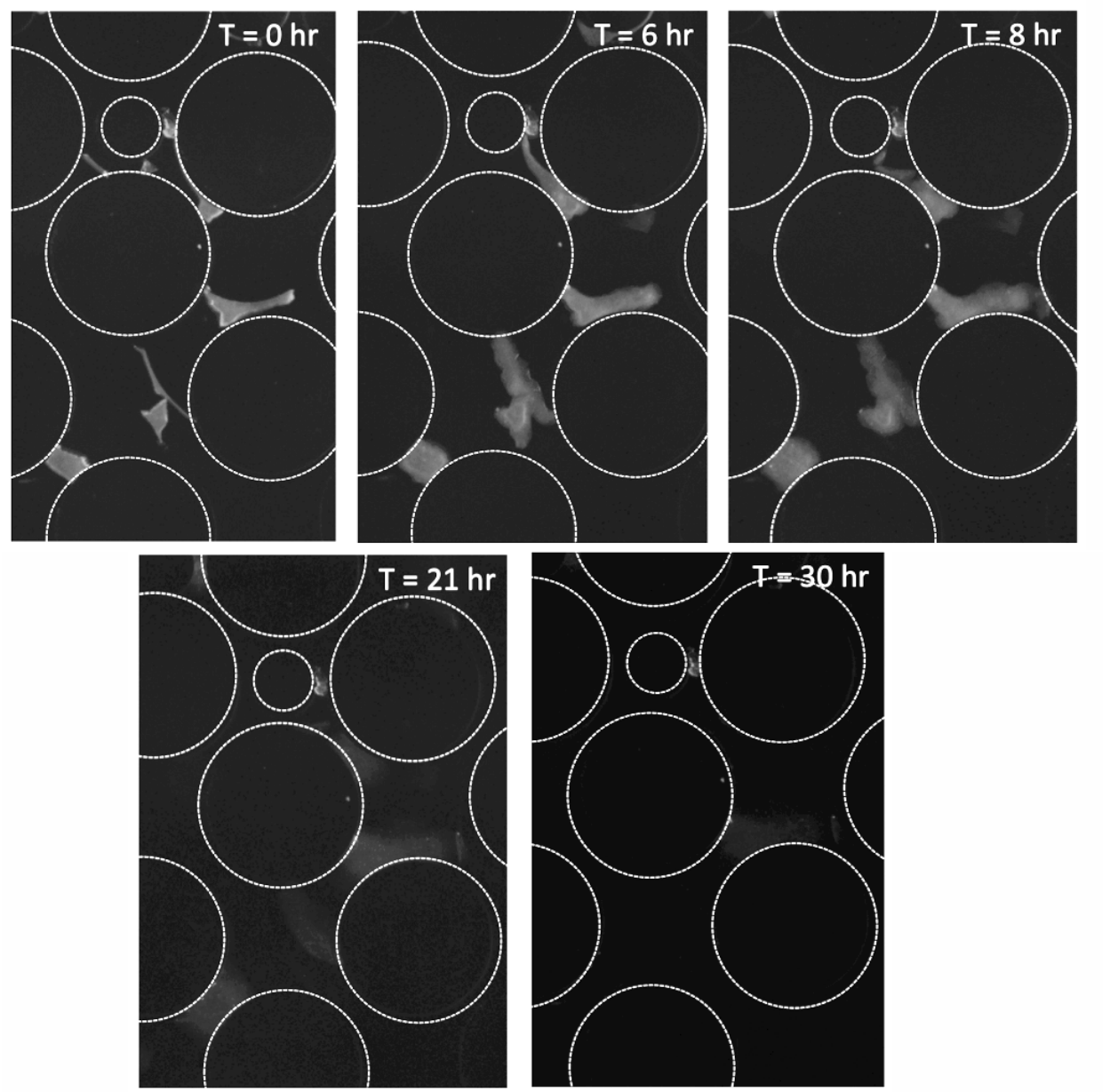


Figure S13. Hydrolysis of spatially-localized Alexa Fluor dyed cnxtls (prepared using triazine chemistry) by a flowing solution of Celluclast, as observed by epifluorescence microscopy. Pillar locations are drawn in for clarity in this heterogeneous pore network with two different pillar sizes and varying arrangement. The fluorescent solid cellulose domains initially swell under the action of the enzyme, and then fade in intensity and disappear.

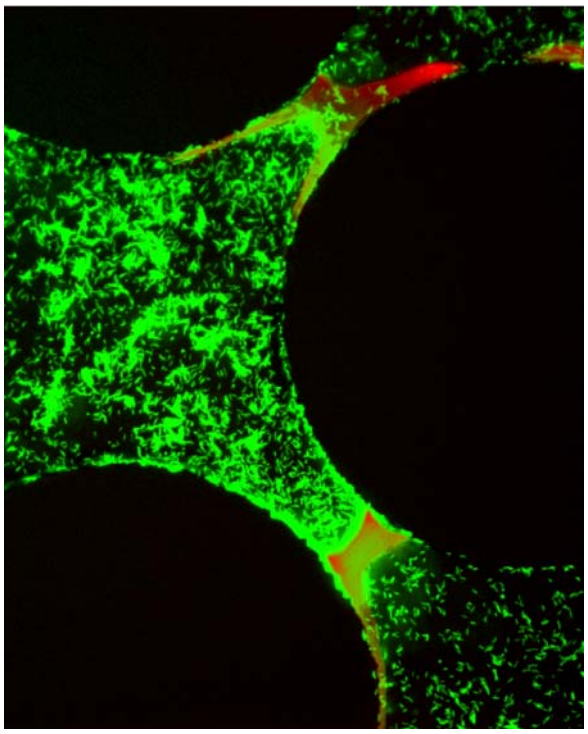


Figure S14. Composite image (green: mOrange-expressing Flavobacterium strain, red: immobilized fluorescent cnxtls (Alexa Fluor 647, triazine labeling method)) illustrating proliferation of the bacteria among the cellulose deposits and in the pore space of a pore network.

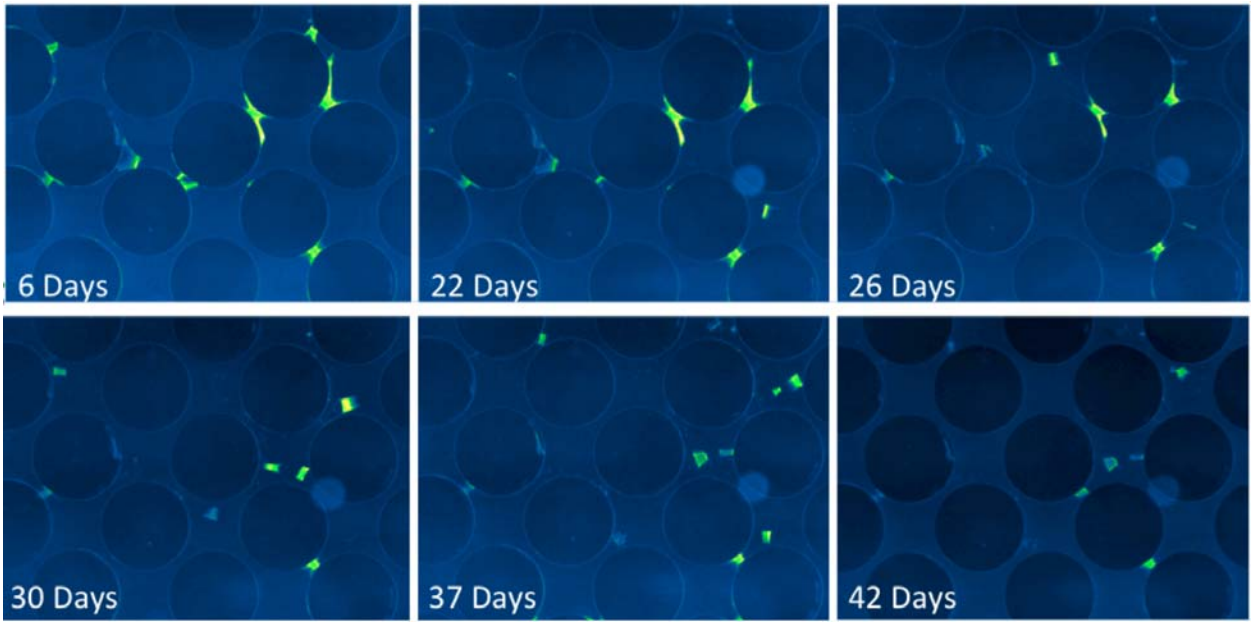


Figure S15. Disappearance of spatially-localized Alexa Fluor dyed cnxtls (Alexa Fluor 546 prepared using triazine chemistry) in a homogeneous pore network innoculated with a *Cytophaga hutchinsonii* species, as observed by epifluorescence microscopy. Medium was periodically refreshed by flow with quiescent periods in between. The fluorescent solid cellulose shown here as green is seen to decrease in fluorescent intensity and ares, and be physically displaced by flow events, as they are consumed.