Electronic Supplementary Material (ESI) for Lab on a Chip.


# Supplementary information

## 1. Hydrodynamic characterization of the empty porous medium

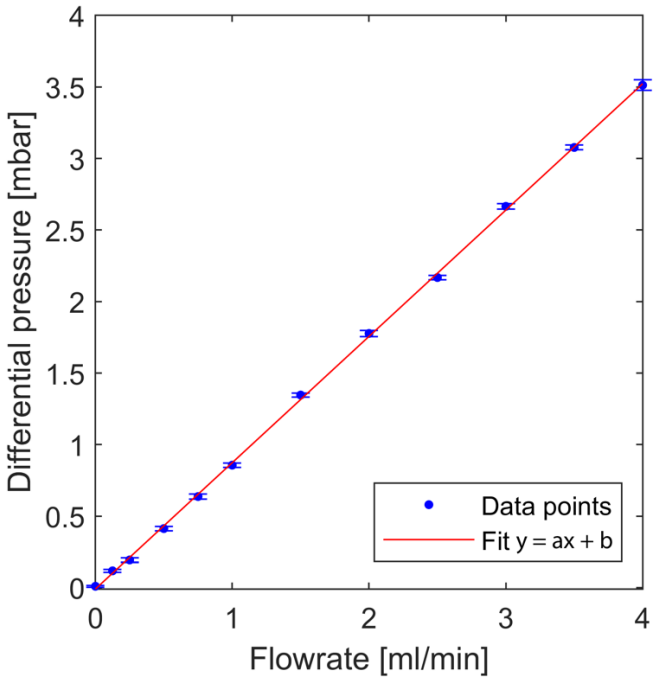


***Figure S1*** *The permeability of an empty porous medium was determined by imposing the flowrate using a gear pump and reading the differential pressure between the inlet and the outlet of the bioreactor. The obtained data were fitted onto a linear model and the initial permeability was extracted according to eq 3 (see Materials and Methods). We obtained a* $\simeq 0.88$, $b \simeq -0.01$ *and* $R^2 = 0.99968$. *The initial permeability of the system was found to be* $5.26 \cdot 10^{-11}$ $m^2$.

## 2. Camera module data analysis

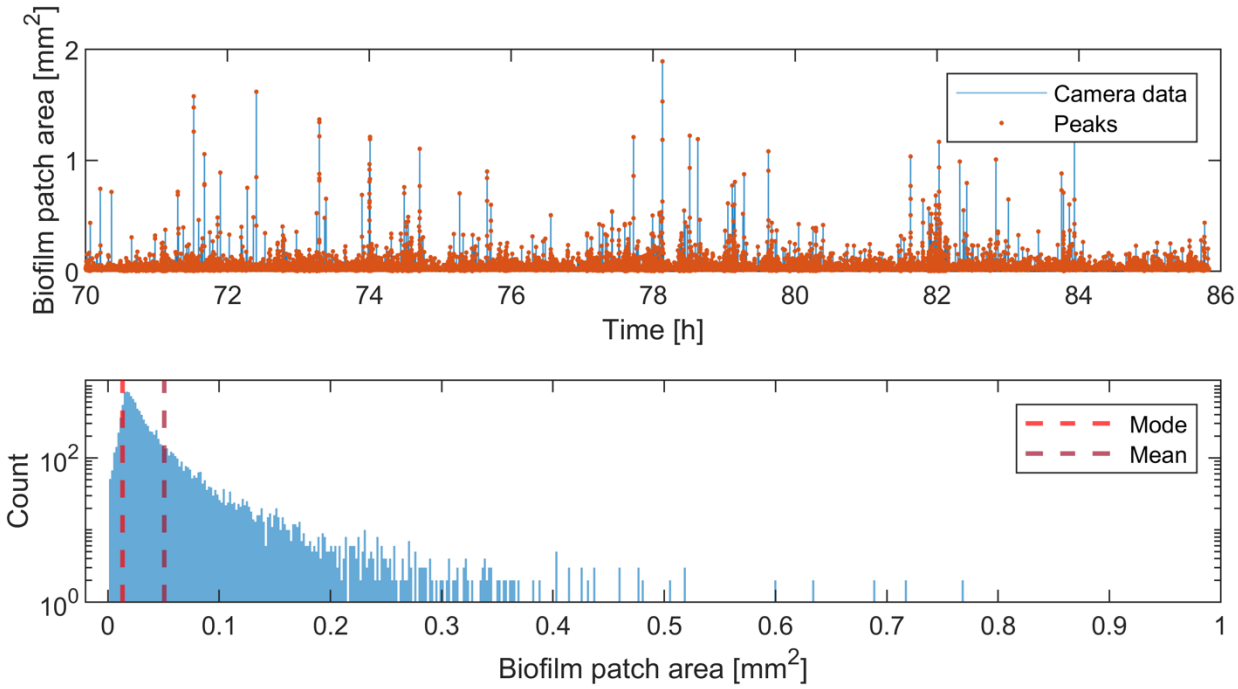


***Figure S2*** *(Top) The treated data obtained with the camera module are shown from time $t_1$ = 70 hours to $t_2$ = 86 hours with the locations of the identified peaks. (Bottom) The biofilm patch size distribution histogram of the corresponding peaks indicates an asymmetric, positively skewed distribution with patch sizes centered at mode 0.0138 mm², mean 0.0508 mm² and skewness of 8.03.*

## 3. Cross-correlation analysis of camera and spectrophotometric cell data

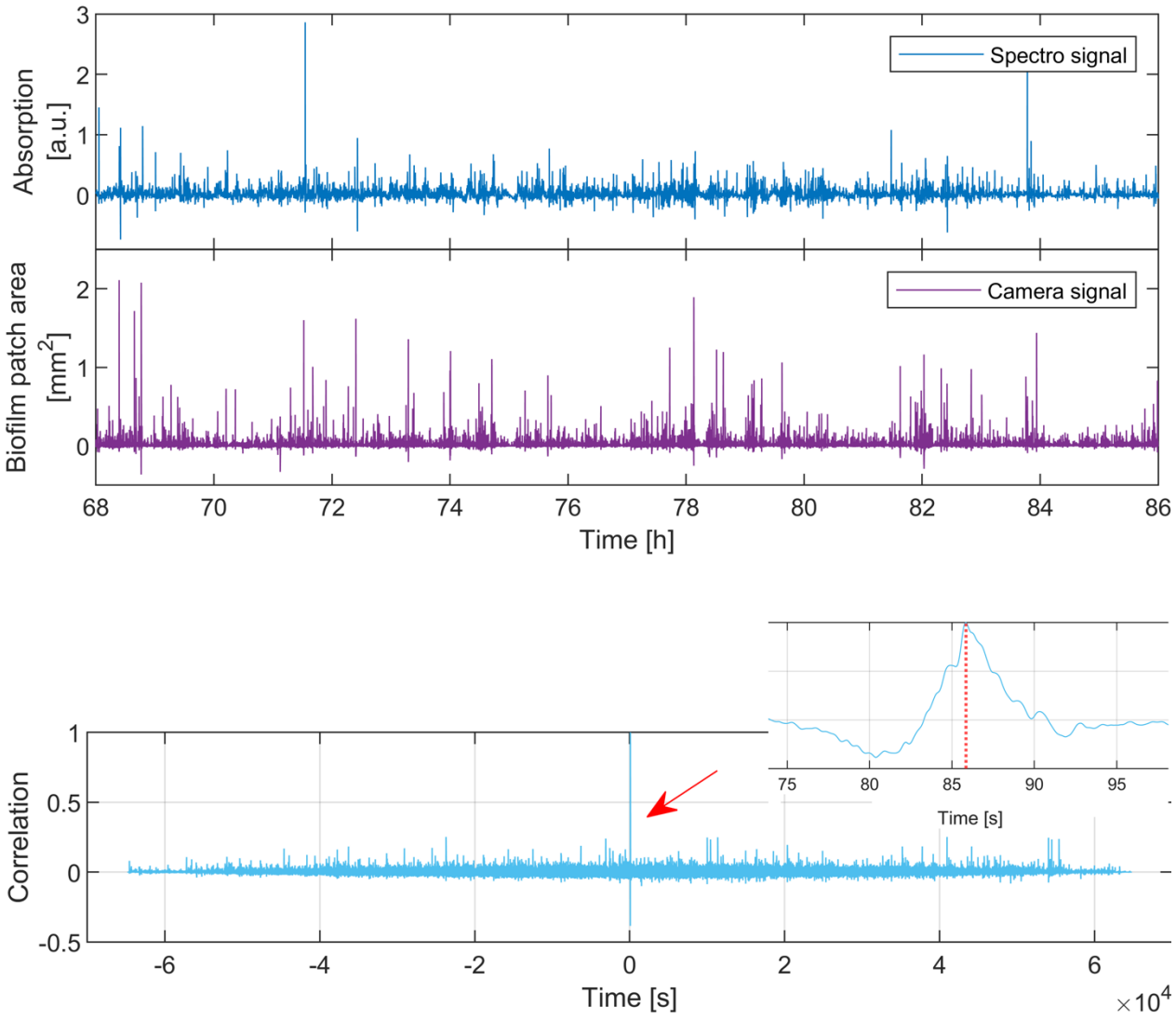


***Figure S3*** (A) Data obtained from the spectro (top) and camera (bottom) modules after treatment. (B) Cross-correlation analysis result of the above signals. The first 60 to 100 seconds are shown in the inset as both signals show high correlation for a time shift of 85.9 seconds.

## 4. Fourier analysis of camera and spectrophotometric measurements

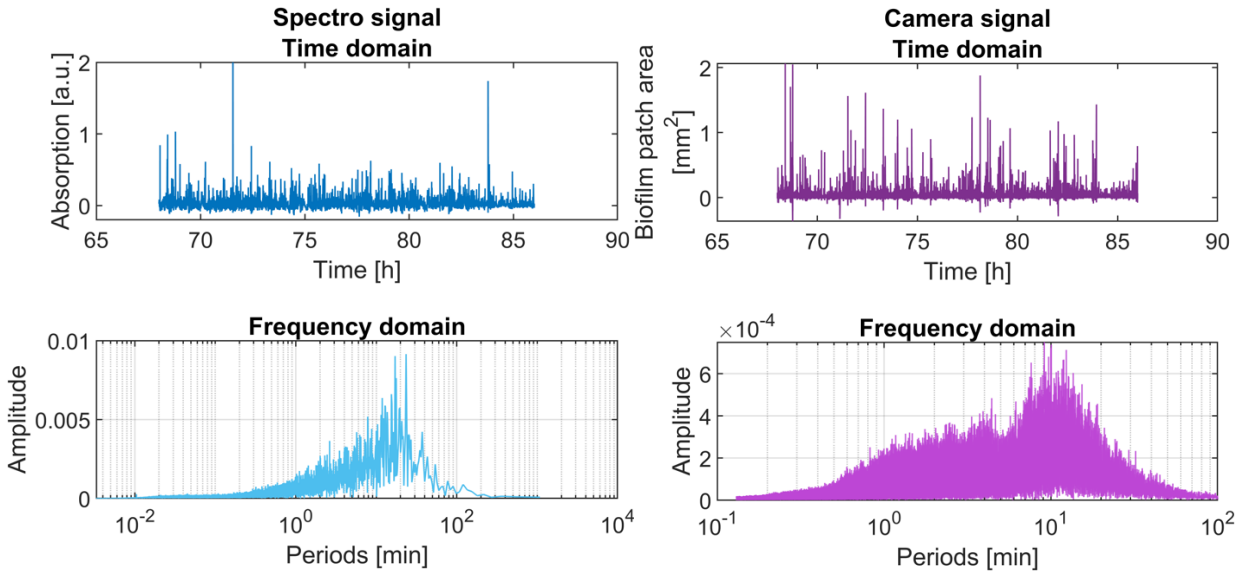


***Figure S4*** *Fourier transform of spectrophotometric and camera data from time $t_1$=68 hours to $t_2$=86 hours (Stage III of biofilm growth). Both sets of data showed predominant frequencies at the range of 10 to 20 minutes.*

## 5. CFD results

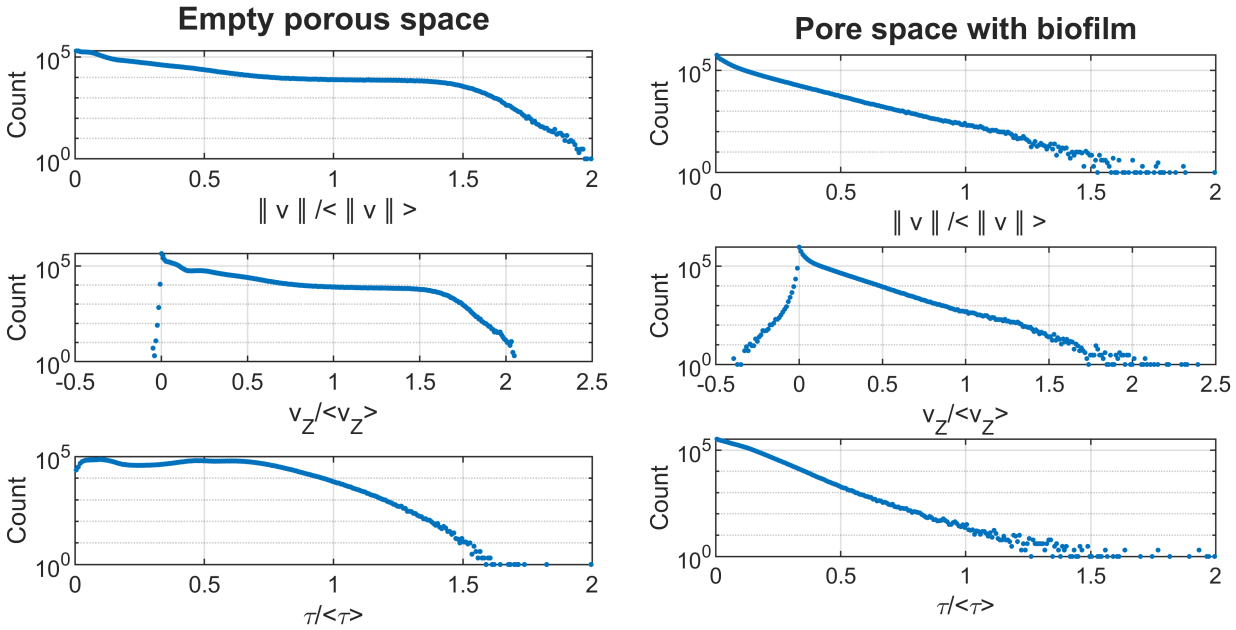


***Figure S5*** *Probability density functions of velocity magnitude (‖ $v$ ‖), velocity component on the Z axis ($v_Z$) and shear stress ($\tau$) extracted from CFD. The parameters were normalized by their mean value over the entire pore space, written as ⟨∅⟩ for any field ∅. On the left are the PDFs obtained from the dataset of the empty bioreactor while on the right are the PDFs from the dataset of the biofilm inoculated bioreactor that was imaged using µCT and $BaSO_4$ as a contrast agent.*

## 6. Empty porous medium characterization

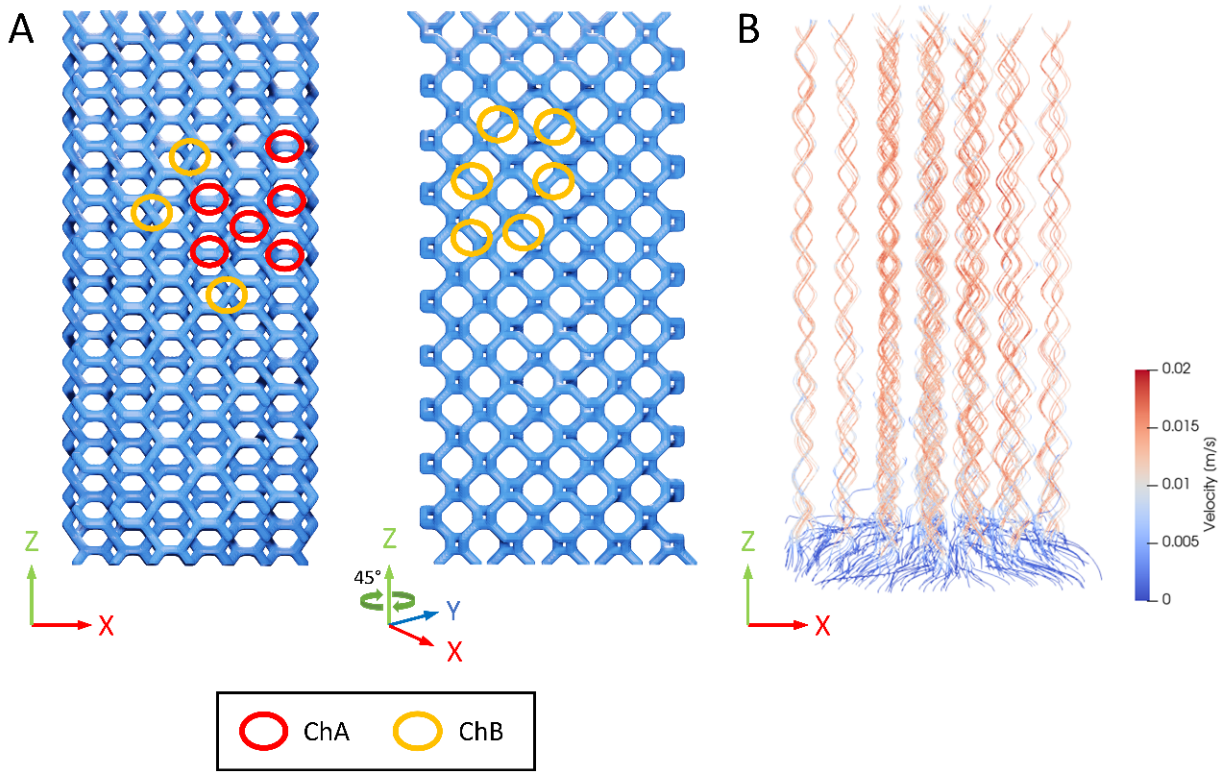


***Figure S6*** *(A) The porous structure contains two types of channels. Channels "ChA" are positioned perpendicularly to the main flow direction while channels "ChB" are at 45° from the main flow direction. The view on the right is rotated 45° around the Z axis in respect to the view on the left. (B) Streamlines obtained through post-processing of CFD data, with the magnitude of the velocity in color. These indicate that ChB channels provide a preferential flow path with elevated velocities compared to ChA channels.*

## 7. Control biofilm growth dynamics experiment

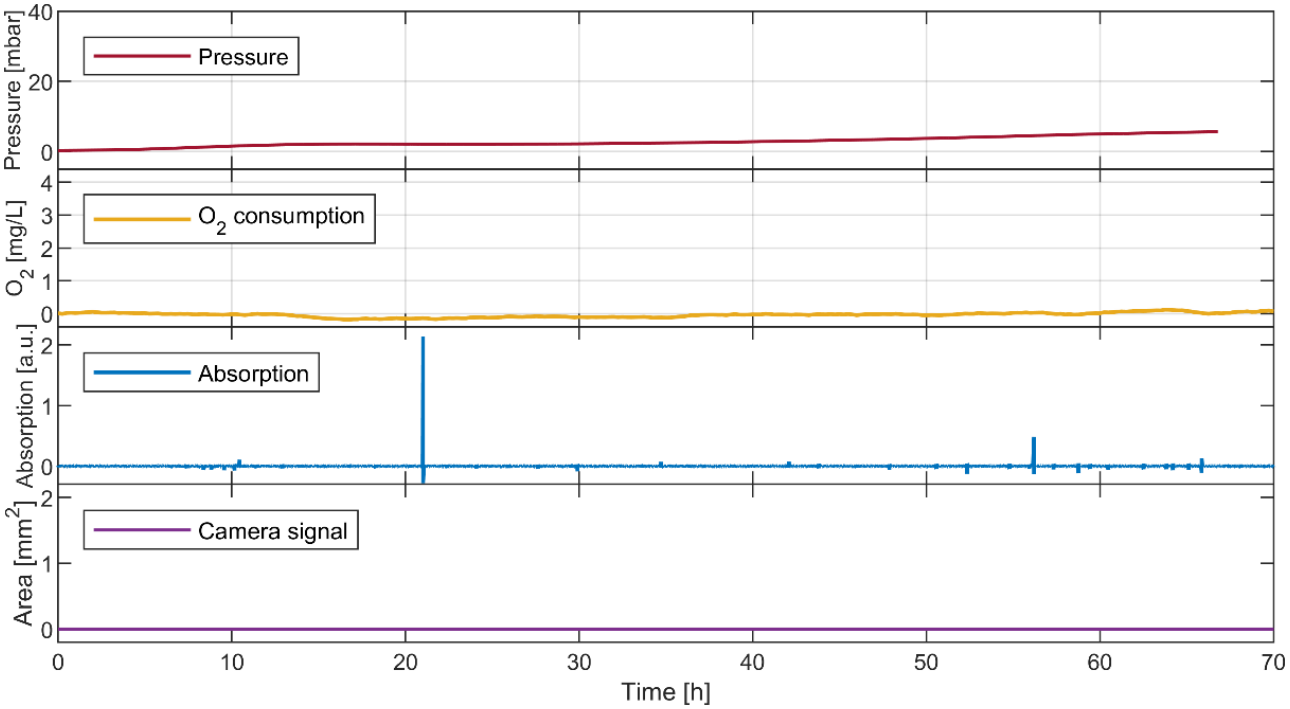


***Figure S7*** *Pressure/oxygen/spectrophotometric and camera module measurements of a control experiment where biofilm inoculation did not take place. The y axis scaling is kept the same as in fig. 4 for comparison. The few peaks present in the spectrophotometric measurements are thought to be air bubbles that made it through the bubble traps into the spectrophotometric module.*

## 8. Control $BaSO_4$ staining experiment

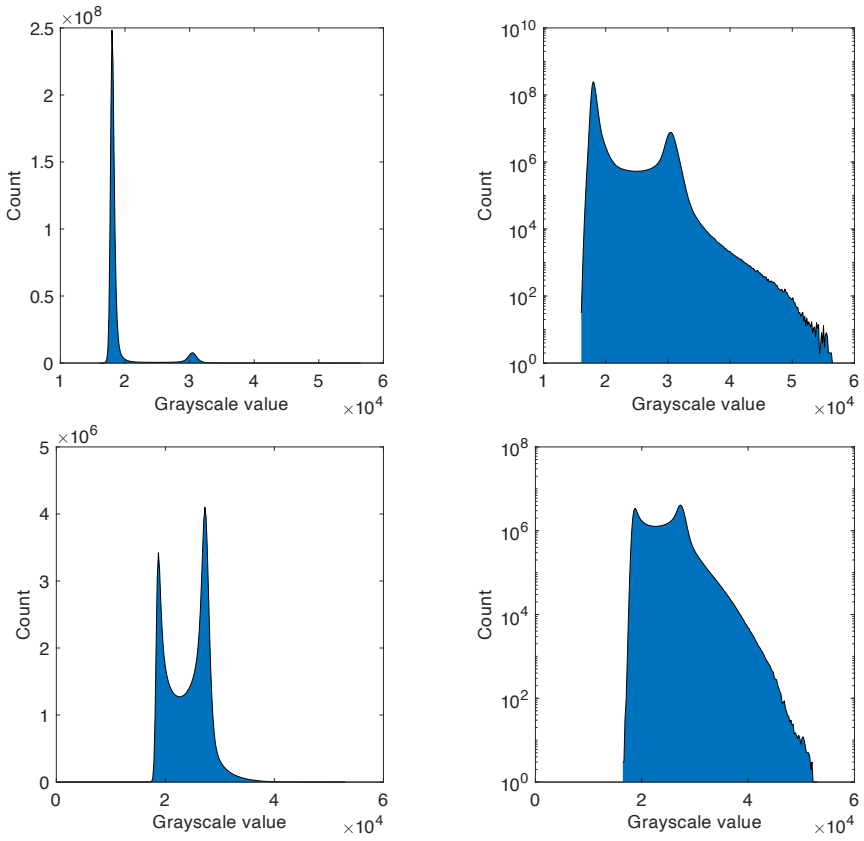


***Figure S8*** *Grayscale histograms of image stacks obtained through X-ray microtomography. Top left: grayscale histogram of a porous medium injected with $BaSO_4$ without previous inoculation with bacteria. The two peaks correspond to the bioreactor material and to the $BaSO_4$ filled pore space. The value 24500 was selected as the segmentation threshold. Top right: same plot in log y scale. Bottom left: grayscale histogram of a biofilm inoculated porous medium injected with $BaSO_4$. The image stack was masked to retain only the pore space according to the process described in materials and methods. The two peaks correspond to unstained biofilm and the $BaSO_4$ filled liquid phase. Segmentation was performed by using a trained machine learning model. Bottom right: same graph in logscale.*

## 9. Videos and stl files

- Short camera video showing a biofilm patch flow through
- Short video showing air bubble extraction from the system through a bubble trap
- Video of subvolume of segmented data rotating (in blender)
- STL files of different porous structures.