



## **Laboratory X-Ray Microtomography as a tool for 3D visualization of biofilm in porous media made up of zeolite**

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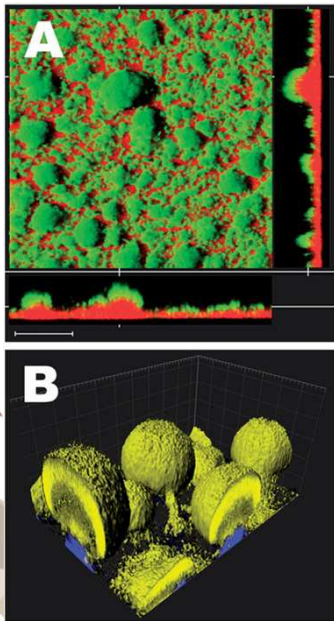
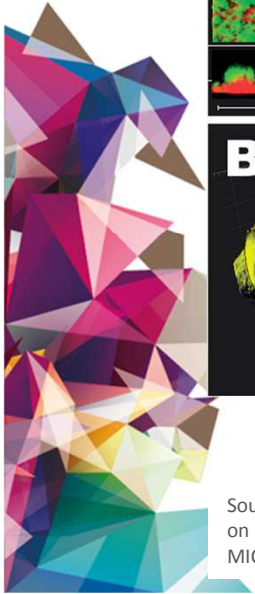
Attendance to this conference was financed by Croatian Science Foundation

# background

- **Bioclogging** - growth of biofilms in porous media, reduces permeability and affects hydrodynamic properties
- **Porous media** – i.e. soils or biofilters, very important in civil engineering, industry, water and wastewater treatment
  - **Numerical 3D models** of biofilm growth in porous media are desirable to improve industrial processes.
  - Ideally, models should be based on real experimental data at microscale, mesoscale and macroscale.
  - **Mesoscale** – biofilm structure and distribution at the pore size.

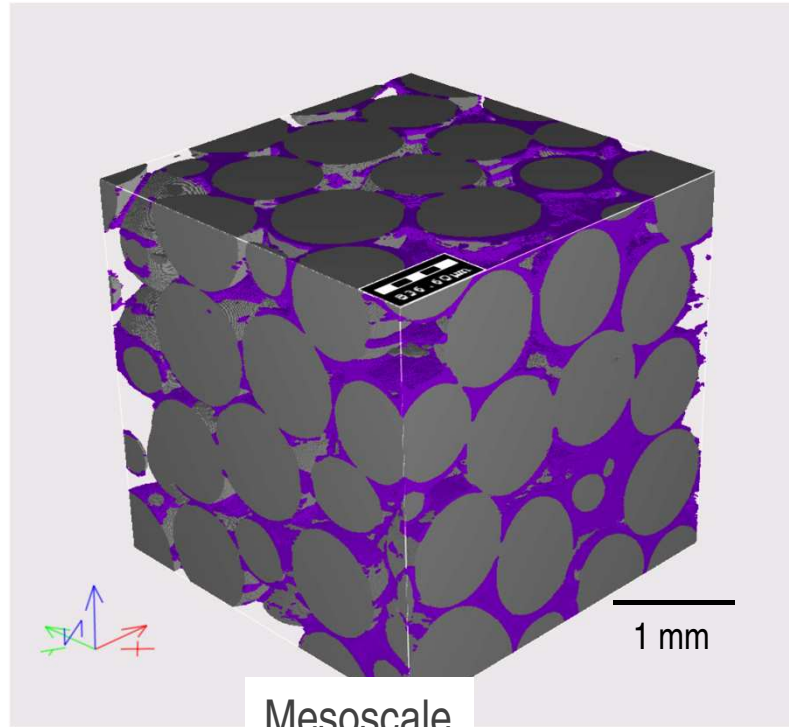


# background



Microscale

Source: Azeredo et al. 2016. Critical review on biofilm methods. CRITICAL REVIEWS IN MICROBIOLOGY



Mesoscale



Macroscale

# background

**X-Ray Microtomography** - imaging technique based on the absorption of X-ray by matter

Absorption is density dependent so the biofilm and bulk liquid (water, nutrient media) have almost identical absorption coefficient ☹️

but, with the use of suitable contrast agent it is possible to differentiate **support media, biofilm phase, liquid phase.**

First reports: Davit et al. 2011. – using BaSO<sub>4</sub> as contrast agent

Iltis et al. 2011. – using silver microspheres as contrast agent

Rolland du Roscoat et al. 2014. – using 1-chloronaphtalen

Davit Y, Iltis G, Debenest G, Veran-Tissoires S, Wildenschild D, Gerino M, Quintard M. 2011. Imaging biofilm in porous media using X-ray computed microtomography. J Microsc. 242:15-22.

Iltis GC, Armstrong RT, Jansik DP, Wood BD, Wildenschild D. 2011. Imaging biofilm architecture within porous media using synchrotron-based X-ray computed microtomography. Water Resour Res. 47. Doi:10.1029/2010WR009410.

Rolland du Roscoat S, Martins JMF, Sechet P, Vince E, Latil P, Geindreau C. 2014. Application of synchrotron X-ray microtomography for visualizing bacterial biofilms 3D microstructure in porous media. Biotech Bioeng. 111:1265-1271.



# background

Major issues of X-Ray Microtomography method:

## **ISSUE 1. REPRESENTATIVITY**

Is what is imaged as biofilm actual biofilm or something else?

## **ISSUE 2. REPRODUCIBILITY**

Are results (biofilm growth and imaging) reproducible in separate experiments?



# experiments

- **Biofilms** were grown in glass columns filled with grains of natural zeolite (aluminosilicate mineral) of 1-1.25 mm size fraction
- Model organism was *Bacillus cereus* 4080 LBK



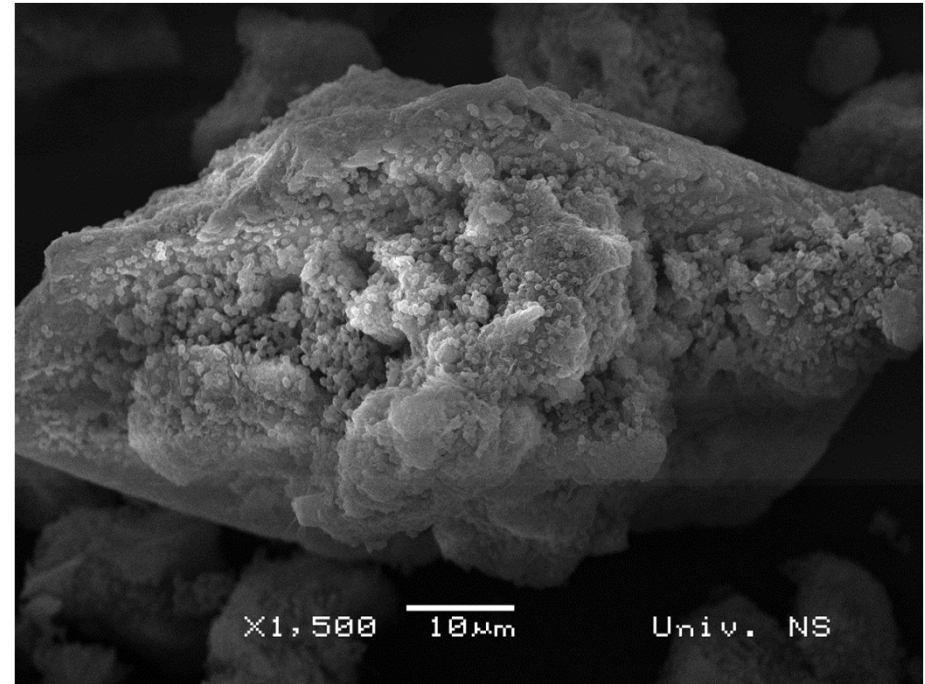
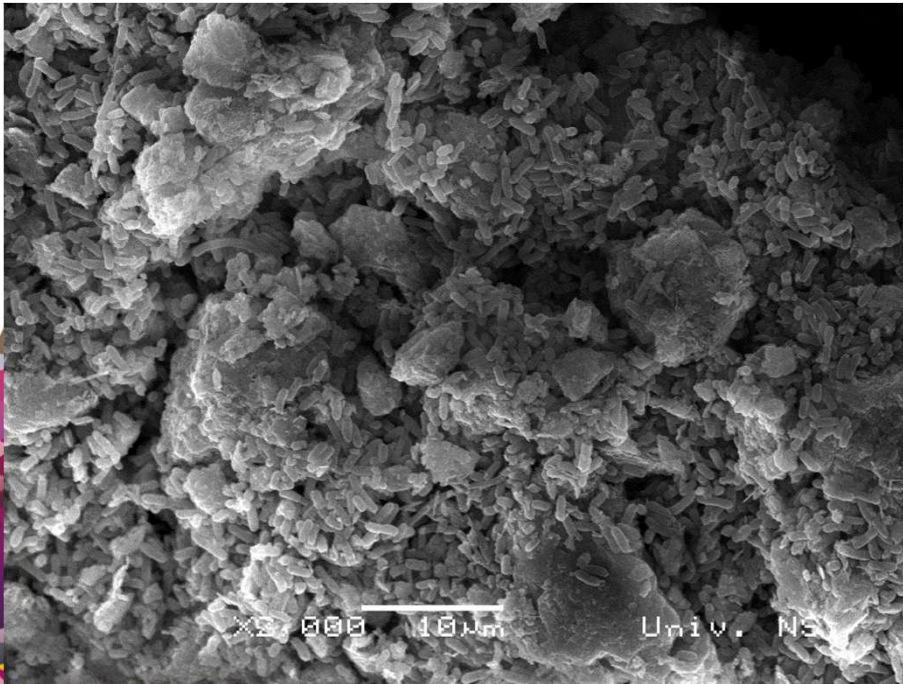
# experiments

- The columns were inoculated with precolonized zeolite grains: the zeolite was incubated in batch system for 24 h with aeration (LB media, 30°C) with pure culture of *B. cereus*
- After the incubation, the bacteria were attached to the zeolite surface

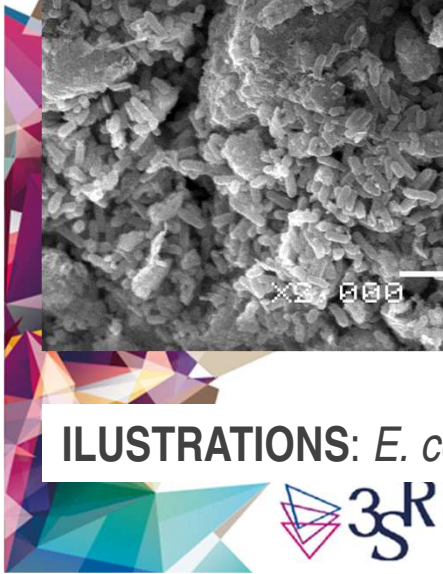




# experiments

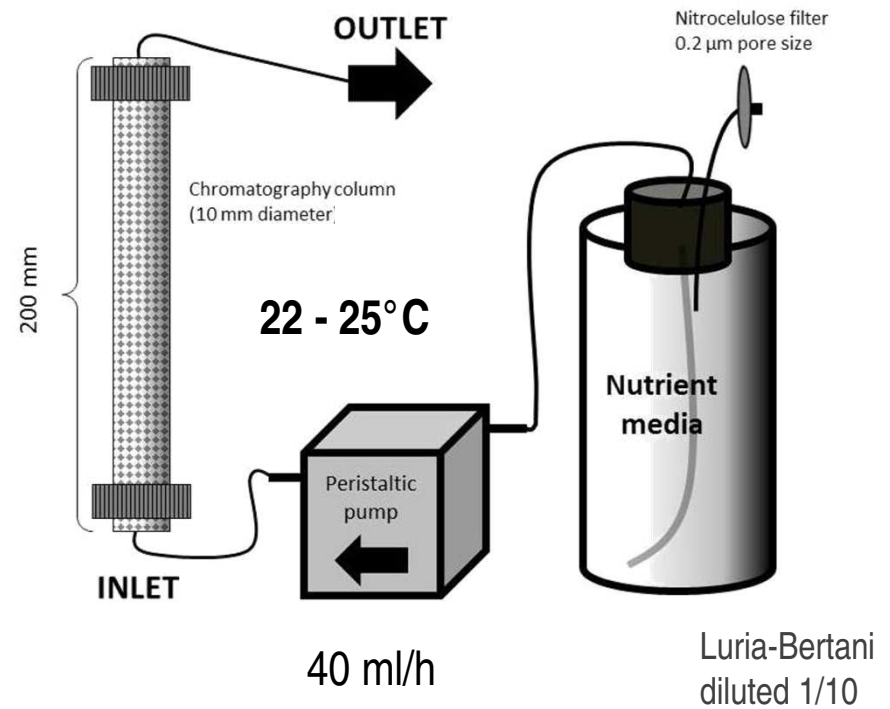


**ILLUSTRATIONS:** *E. coli* and *S. aureus* immobilized on zeolite after 24 h of incubation



# experiments

- Precolonized zeolite was transferred to the columns, connected to peristaltic pump and continuously fed with nutrient media

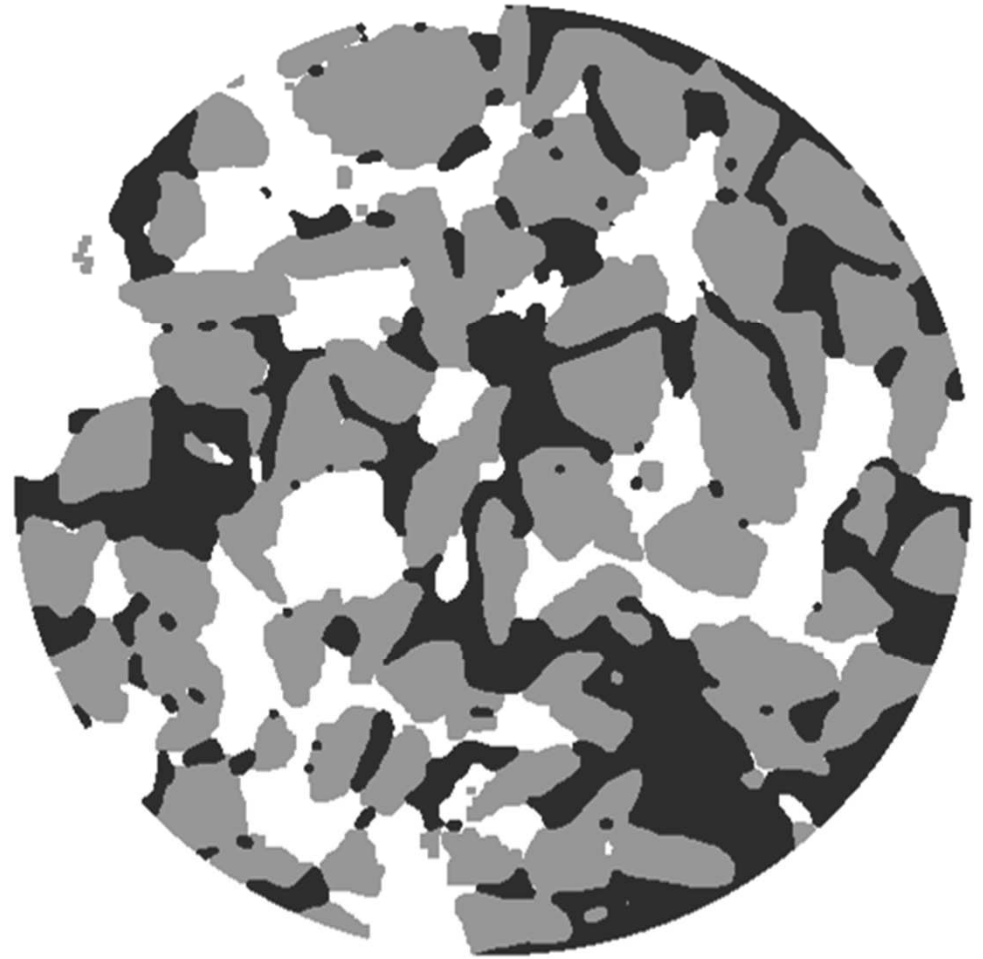
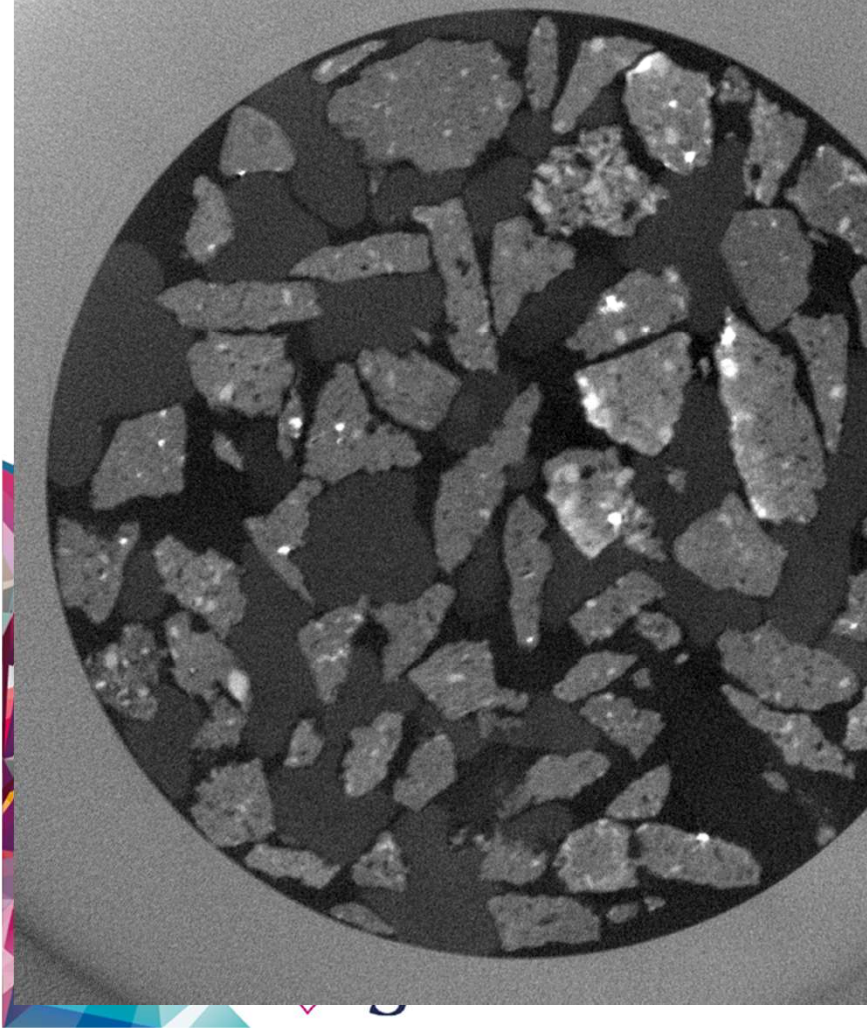


# experiments

- After desired time of growth, the columns were filled with 1-chloronaphtalen and scanned with custom made laboratory X-Ray Microtomograph (Laboratoire 3SR, Grenoble)

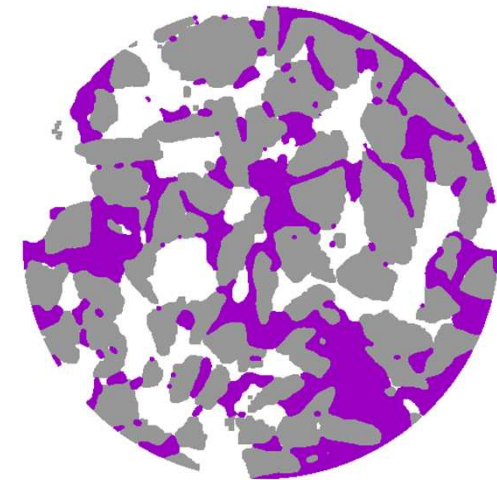
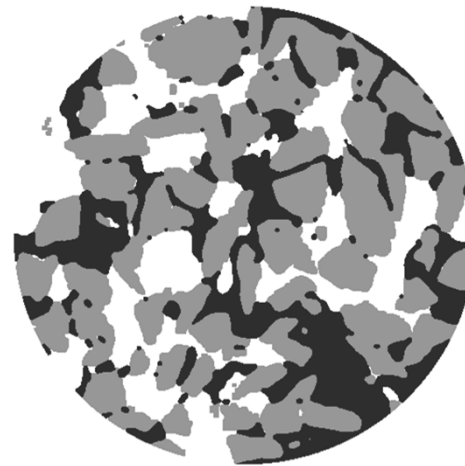
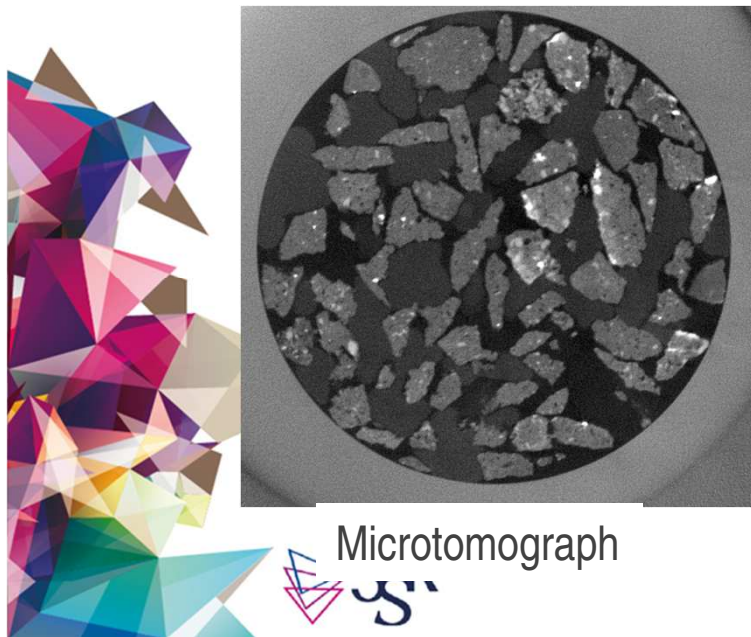


Resolution 10  $\mu\text{m}$



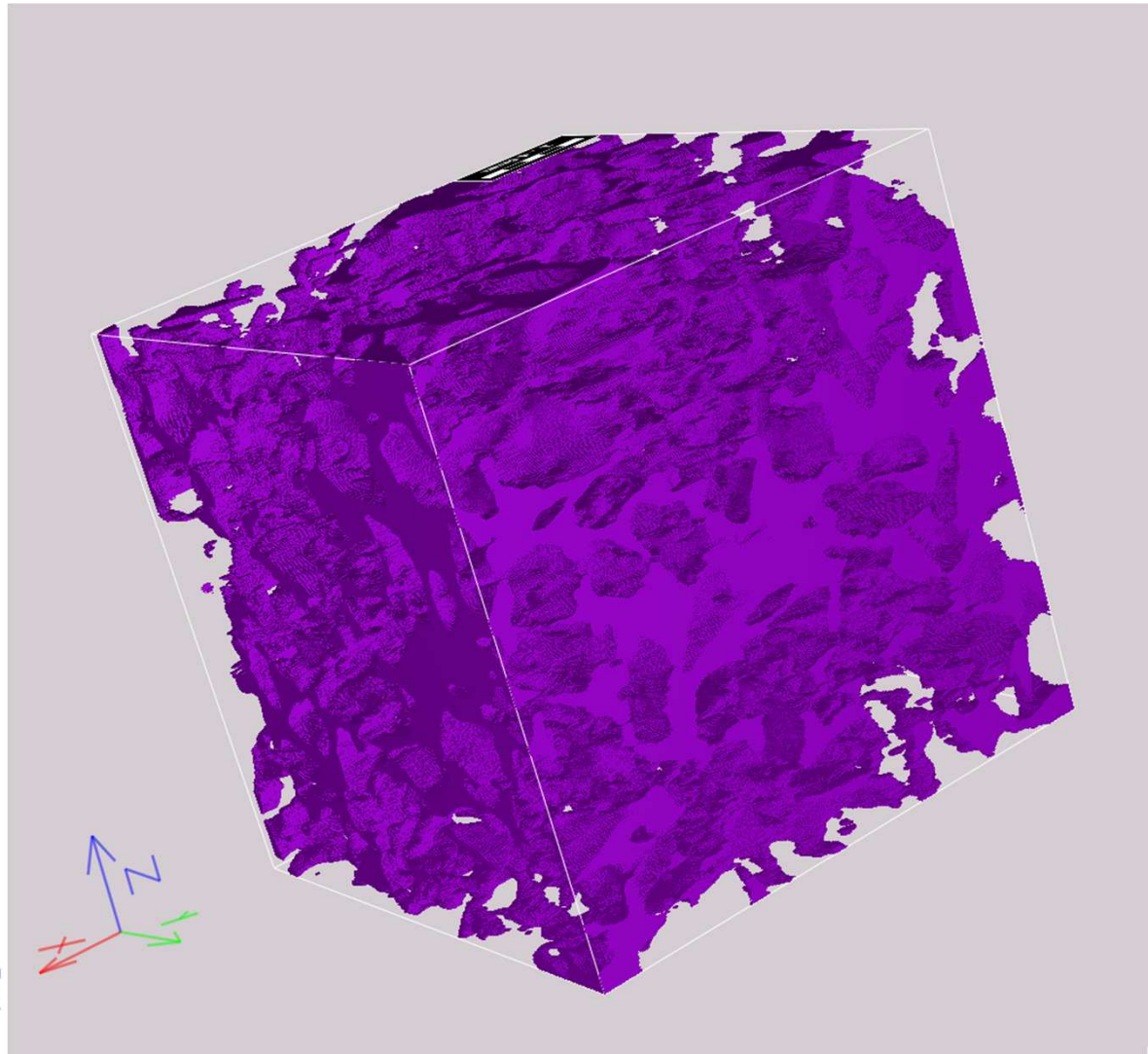
# results

- The .raw images were then imported to Geodict® software
- Geodict enables complete 3D visualization and quantification of the desired microtomographs.



# results

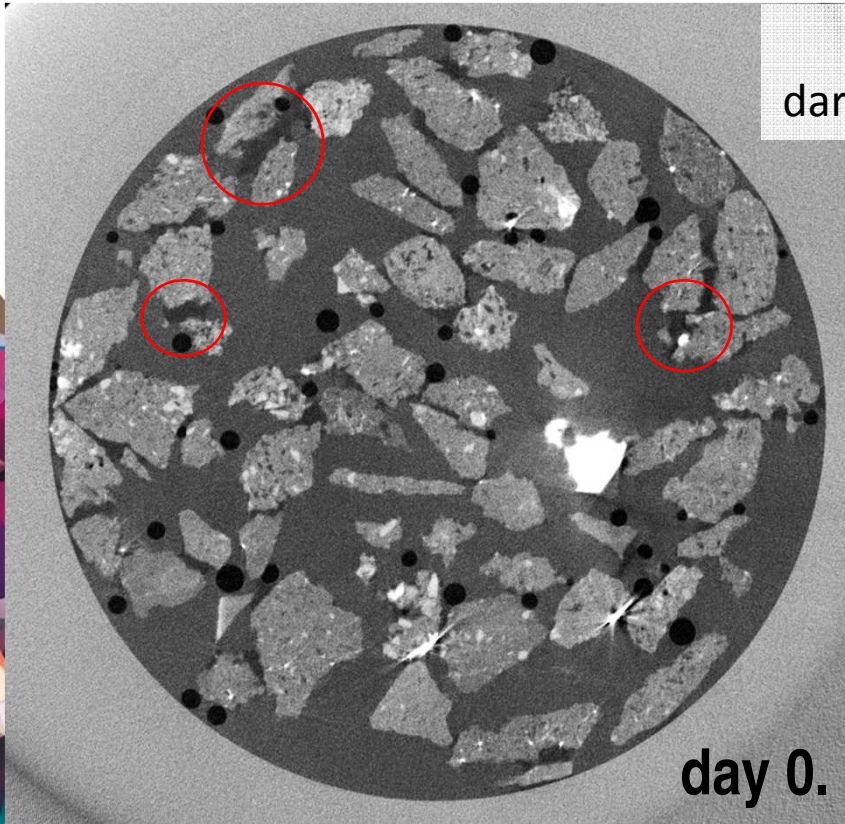
● biofilm



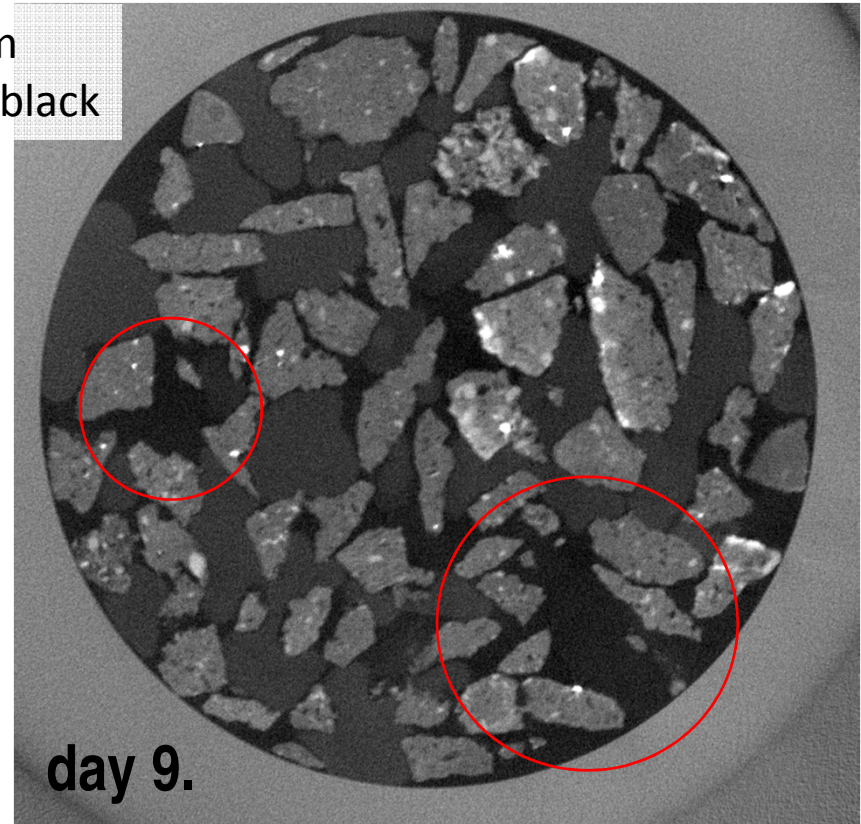
# results

## ISSUE 1. REPRESENTATIVITY

Is what is imaged as biofilm actual biofilm or something else?



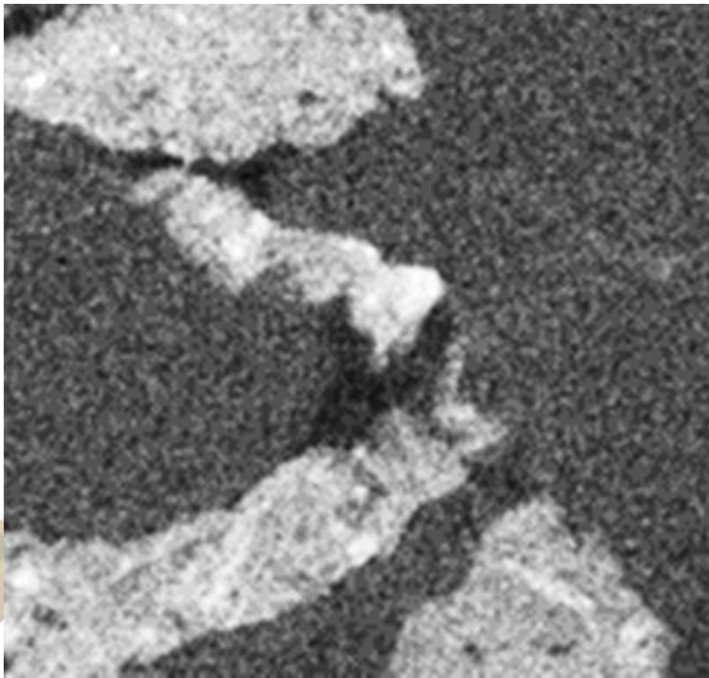
Biofilm  
dark grey/black



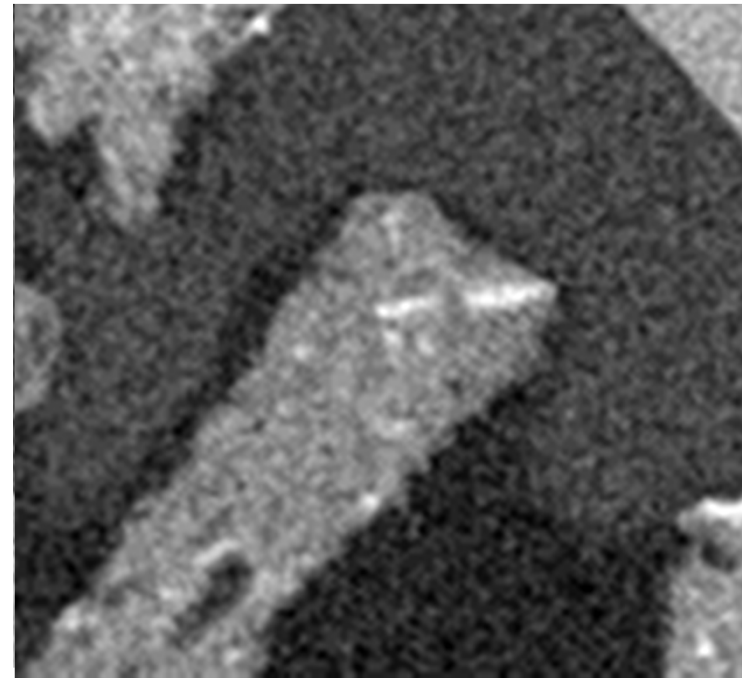
# results

## ISSUE 1. REPRESENTATIVITY

Is what is imaged as biofilm actual biofilm or something else?



day 0.



day 9.

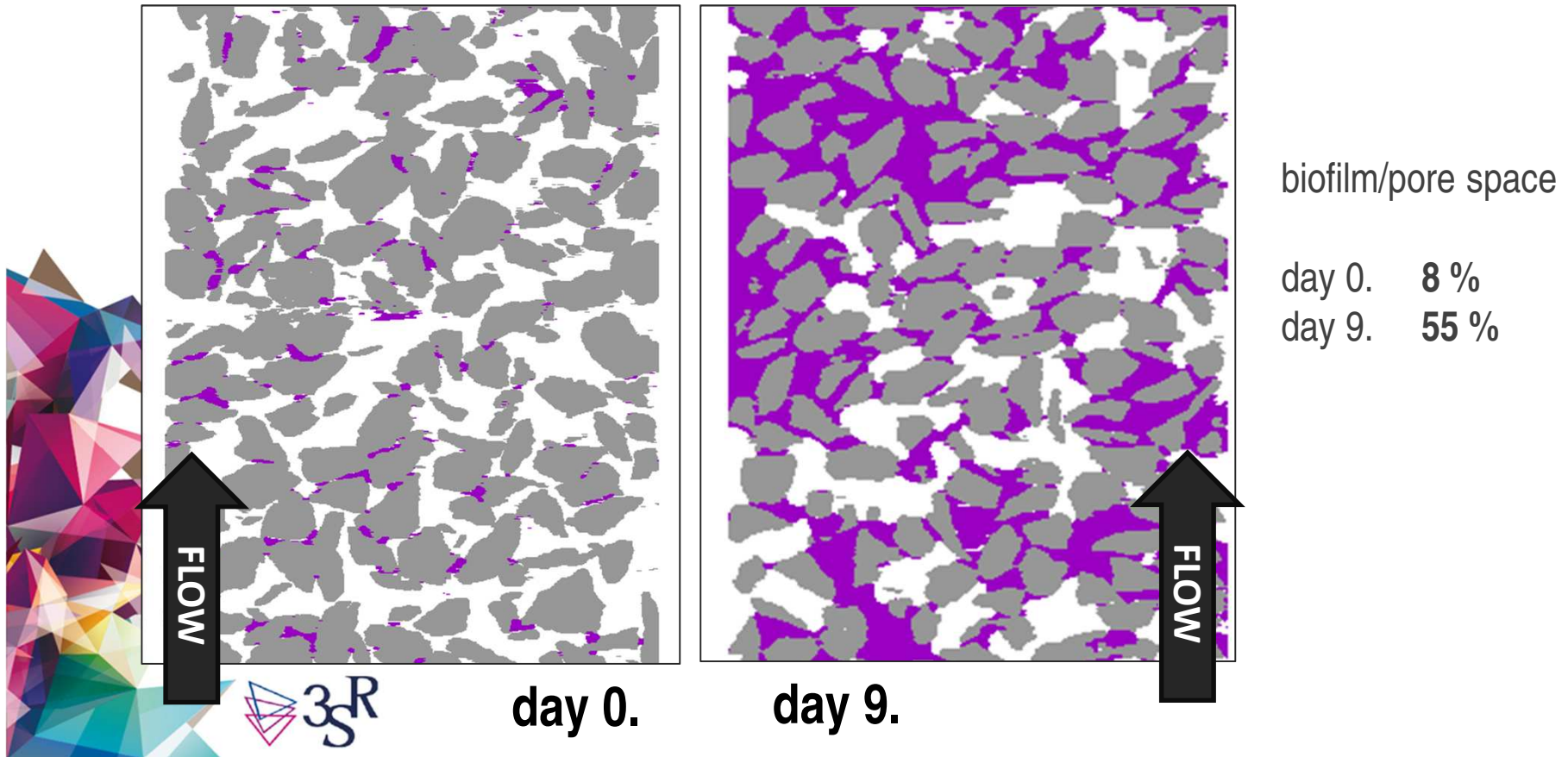




# results

## ISSUE 1. REPRESENTATIVITY

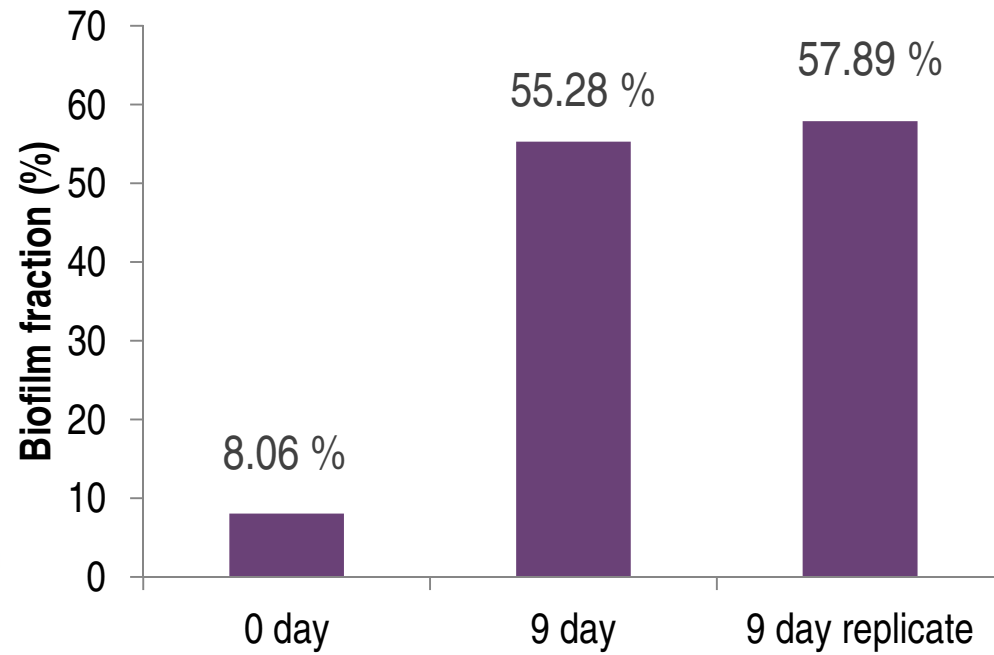
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# results

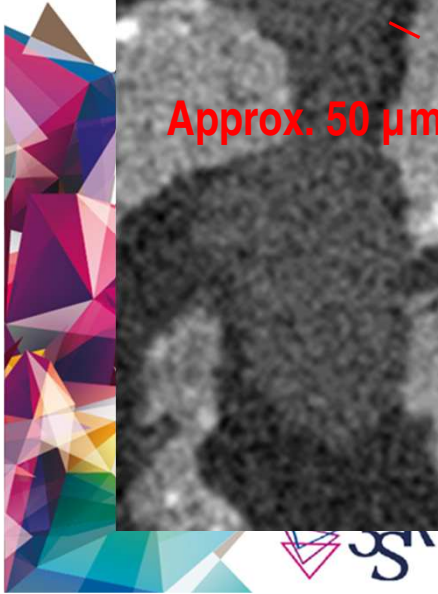
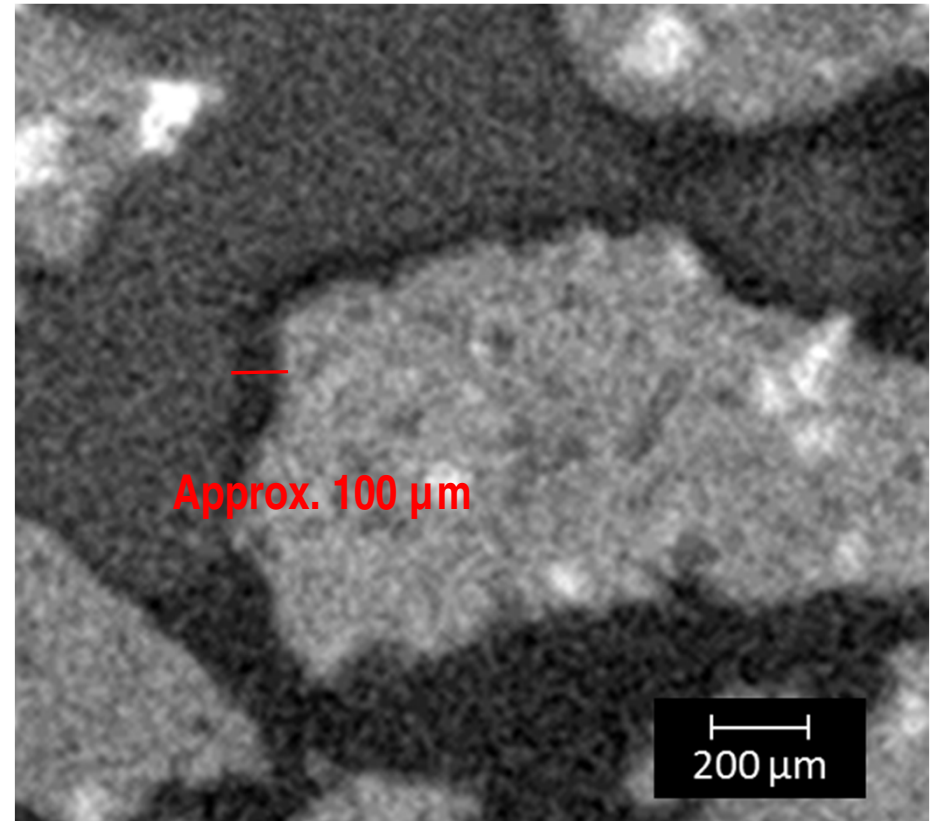
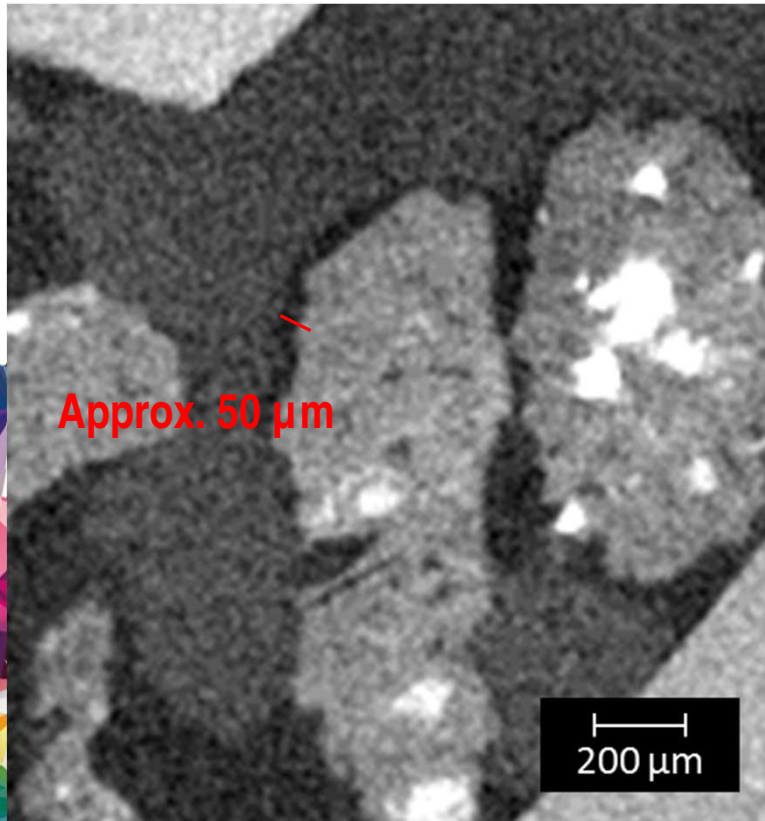
## ISSUE 2. REPRODUCIBILITY

Are results (biofilm growth and imaging) reproducible in separate experiments?



# results

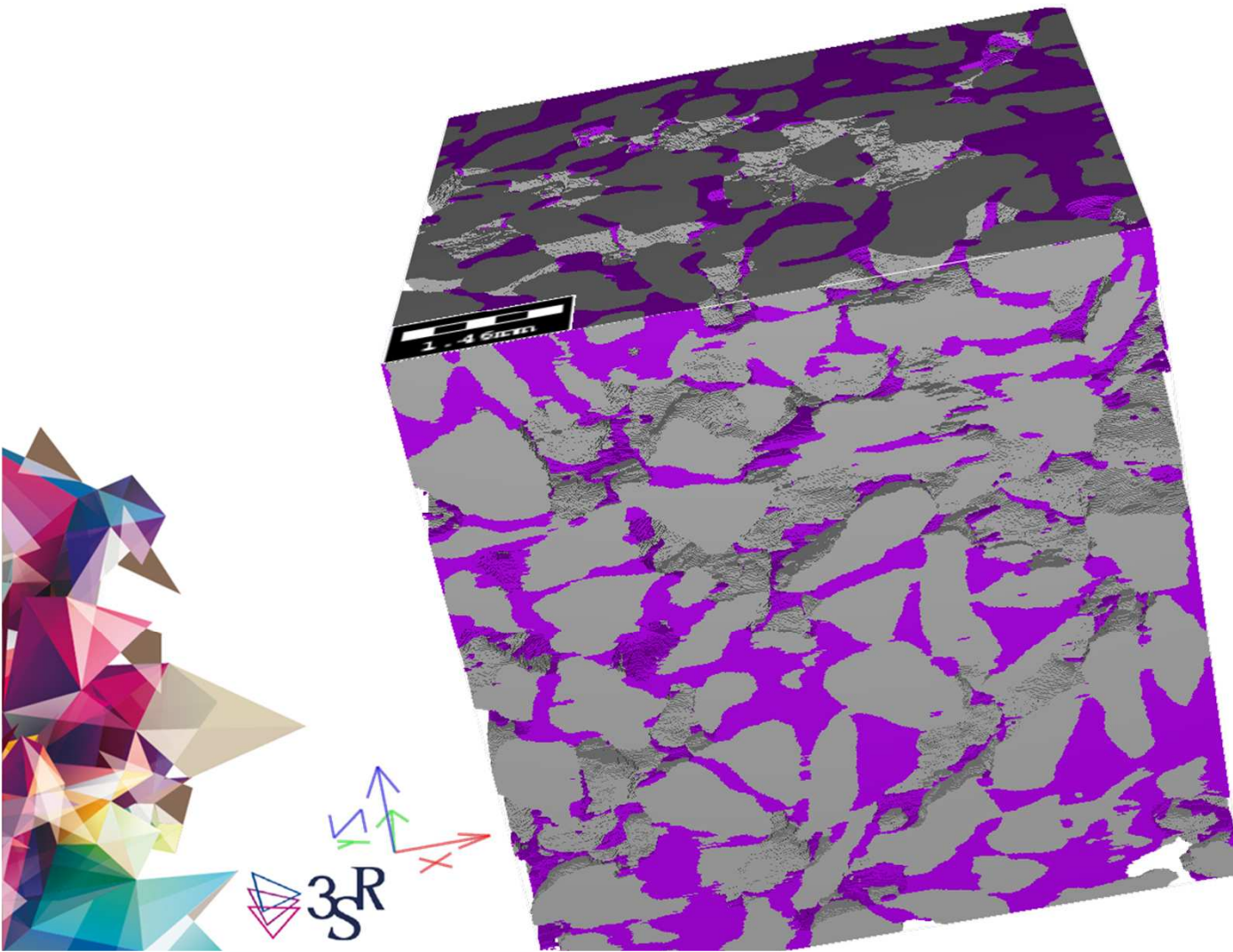
IN ADITION: Biofilm thickness can be roughly estimated from the images, and with finer resolution maybe even precisely determined.



# conclusion

- By using X-Ray Microtomography biofilm can be 3D visualized and quantified inside the porous media.
- **Is what is imaged as biofilm actual biofilm or something else?**  
Yes, it is biofilm, but at early stages of growth (< 5-7 days) the biofilm cannot be distinguished from nutrient media.
- **Are results (biofilm growth and imaging) reproducible in separate experiments?**  
Yes, despite heterogeneity of biofilm growth, the results obtained from two separate experiments were comparable, thus reproducible. This enables monitoring of biofilm growth through time.





Thank you for  
your attention!