

VISUALIZING BIOFILMS WITHIN POROUS MEDIA USING CONTRAST-ENHANCING STAINING AGENTS

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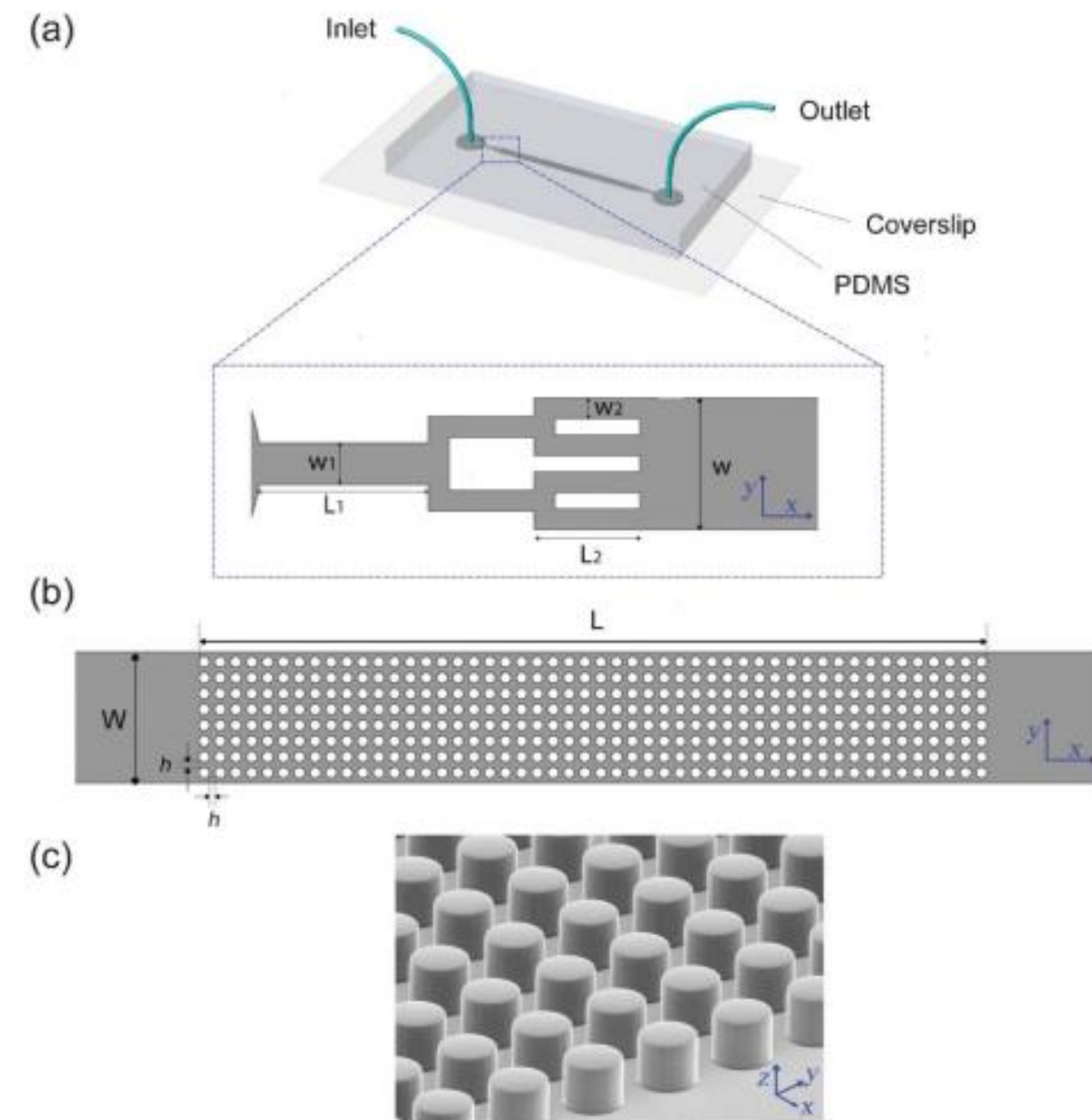
BIOFILMS COLONIZE THE PORE SPACE AFFECTING THE MATERIAL PROPERTIES AND WATER TRANSPORT

Biofilms induce:

- Bioclogging
- Microbially induced precipitation
- Gas formation
- Dissolution
- Etc.

Their effect is often indirectly determined or studied in 2D using microfluidics

Schematic example of a microfluidic setup



μCT CAN VISUALIZE THE INTERIOR OF OPAQUE MATERIALS, BUT CANNOT DISTINGUISH BIOFILMS FROM WATER

Biofilm = 99% water

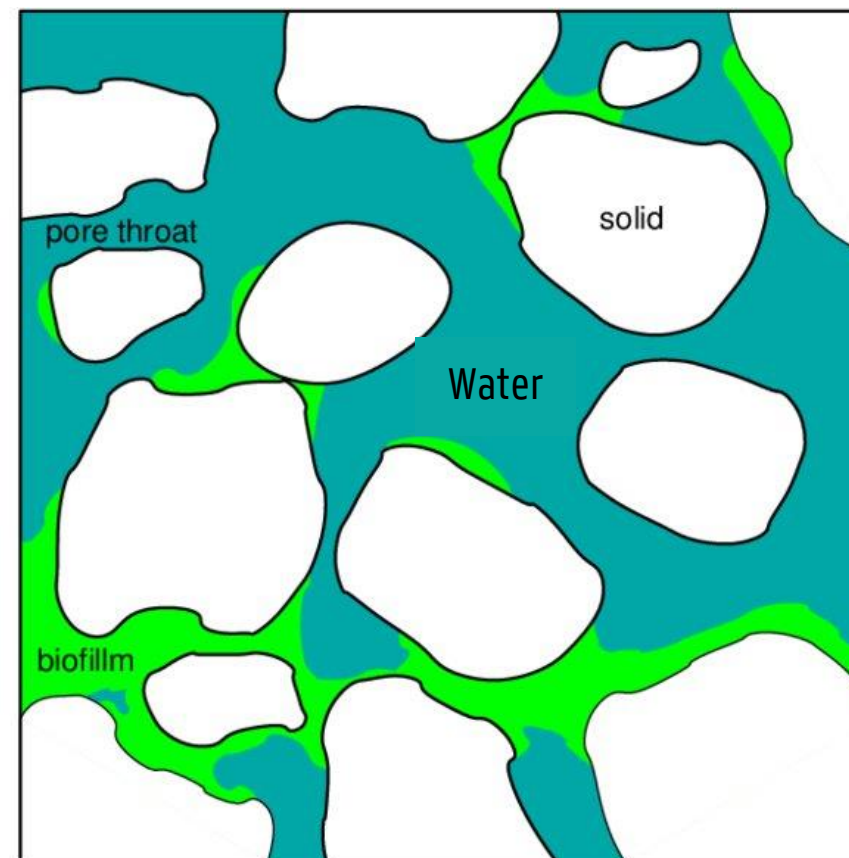
Positive contrast agents

- BaSO_4
- silver-coated microspheres
- 1-chloronaphthalene

Drawbacks:

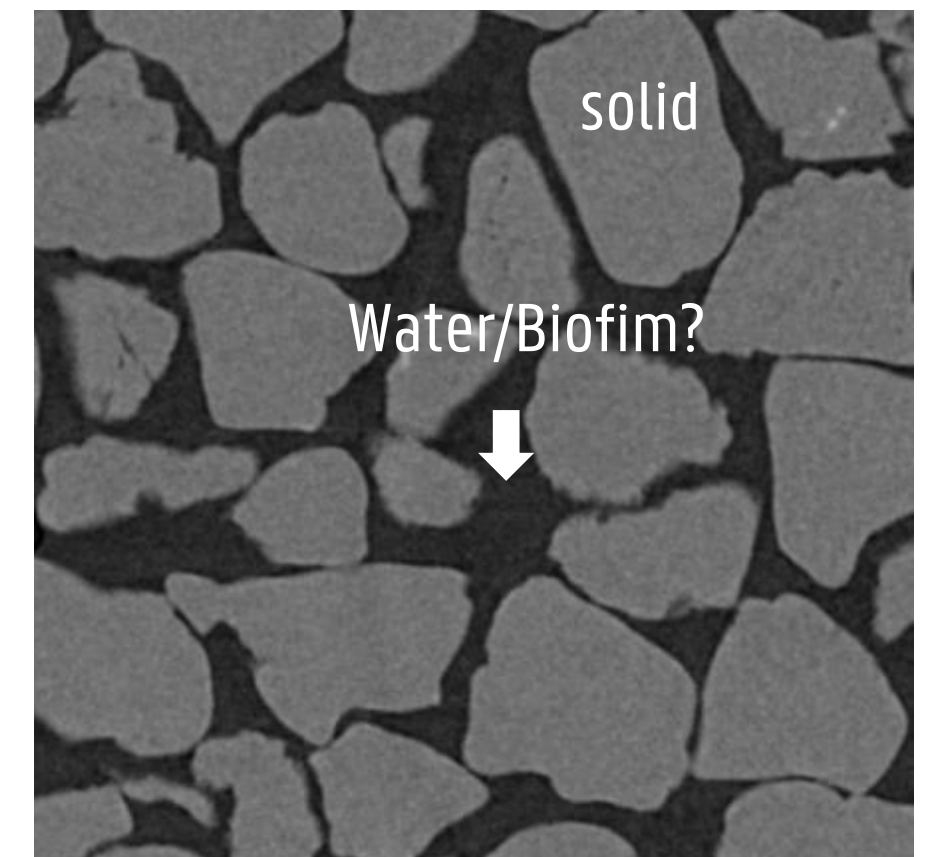
- Alters fluid properties
- Heterogeneous distribution
- Sedimentation

Schematic model biofilm
in porous medium



Anozie Ebigbo, 2009

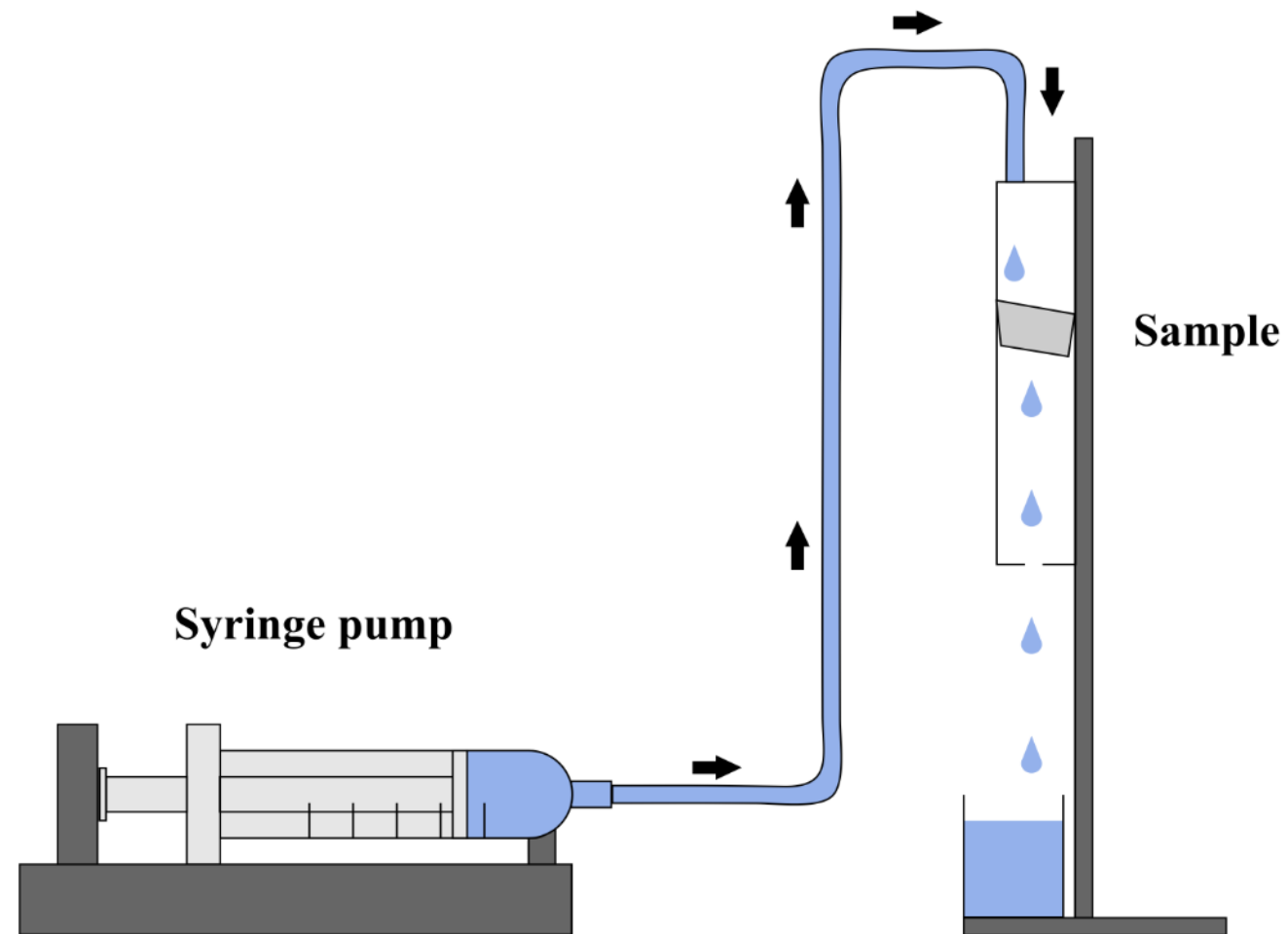
μCT cross-section



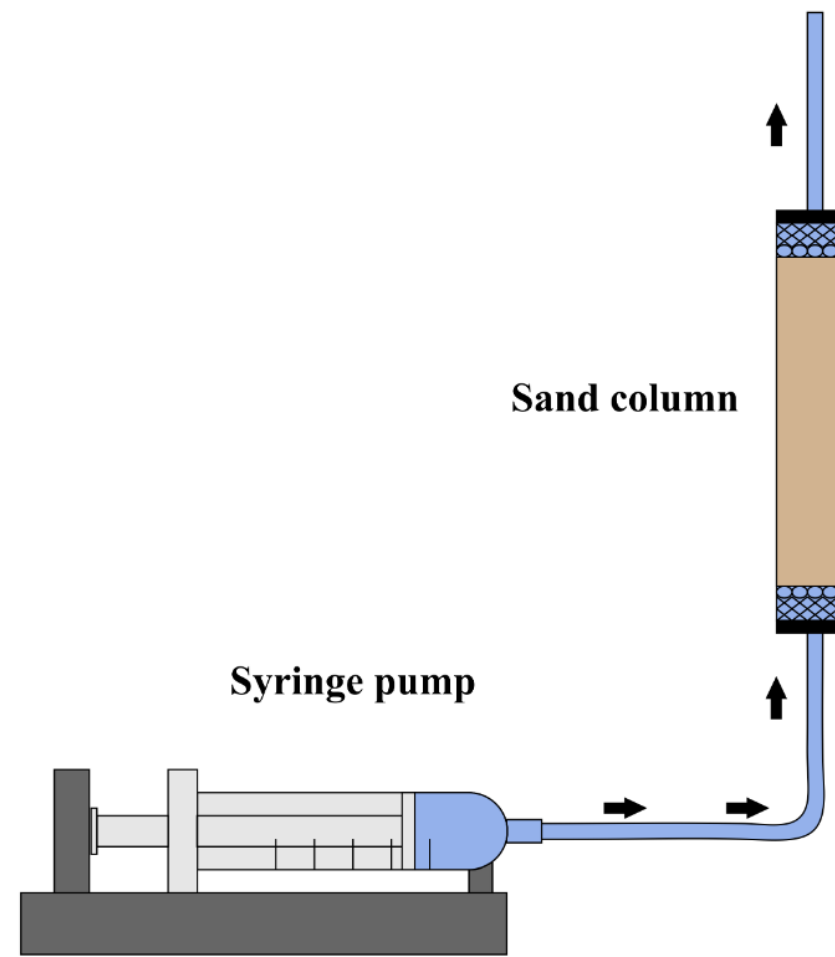
Aim: Identify other negative contrast agents to visualize biofilms in 3D with μCT.

BIOFILMS WERE CULTURED ON STONES AND WITHIN A SAND COLUMN

Dripflow - Visualization
“big” biofilms on stones



Flow - Visualization
biofilms within pores



General workflow

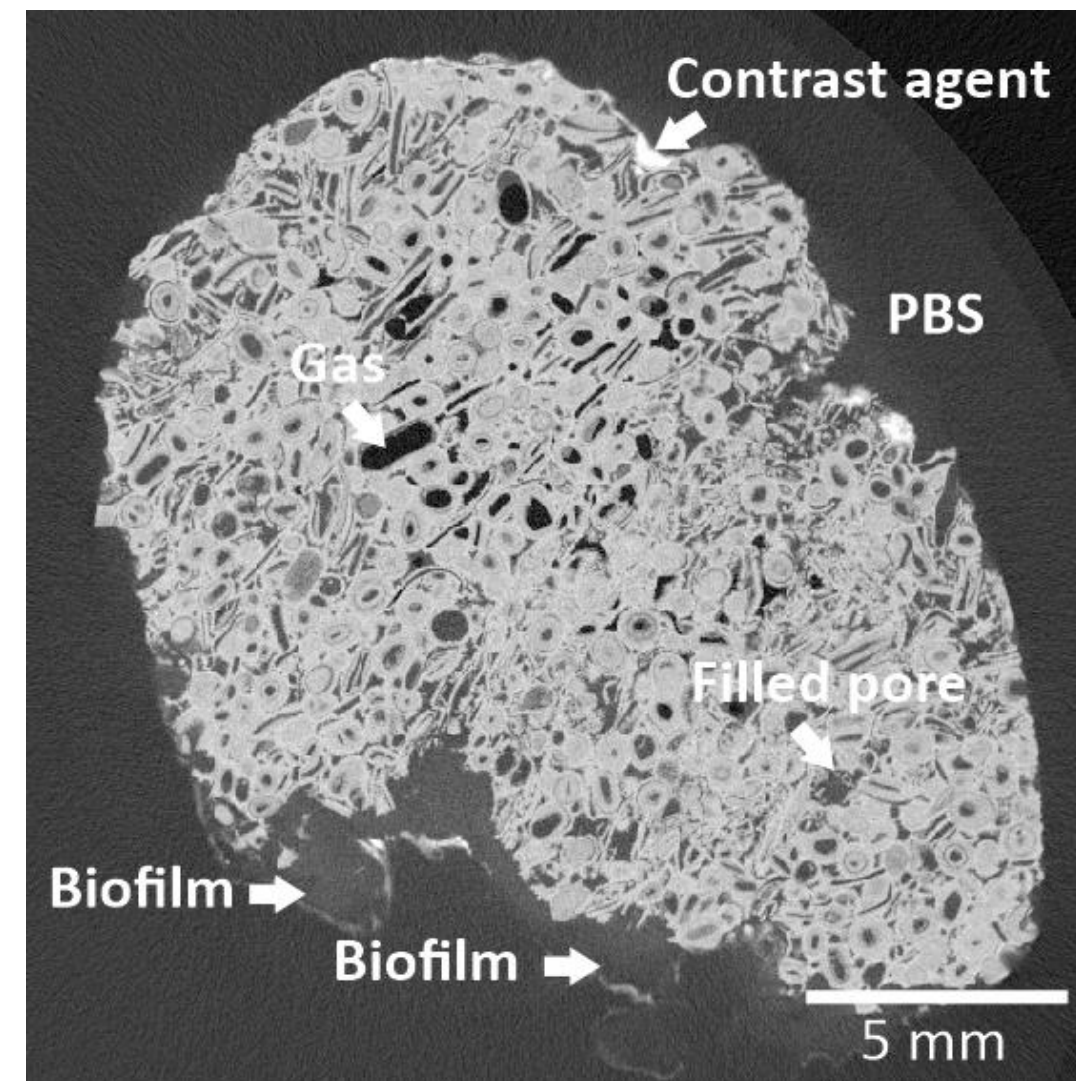
1. Cultivate the biofilm
2. Add the contrast agent
3. Remove non-attached contrast agent with PBS
4. μ CT scan

NUMEROUS CONTRAST AGENT WERE TESTED AND MONO-WD POM GAVE PROMISING RESULTS

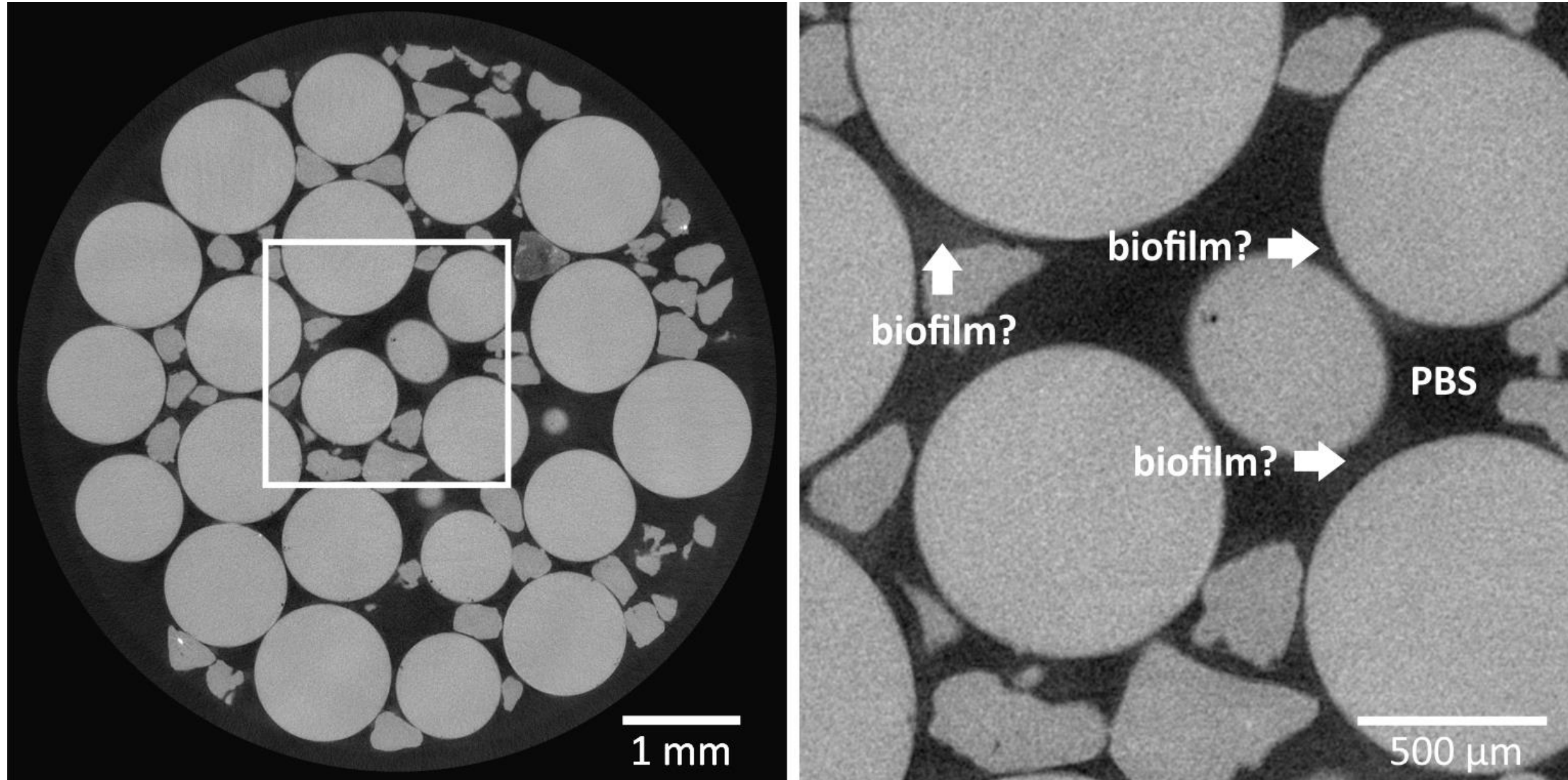
Mono-WD POM: Dripflow experiment

Tested, but less promising

- KI
- KBr
- Iron-oxidation
- Ba-absorption
- PTA



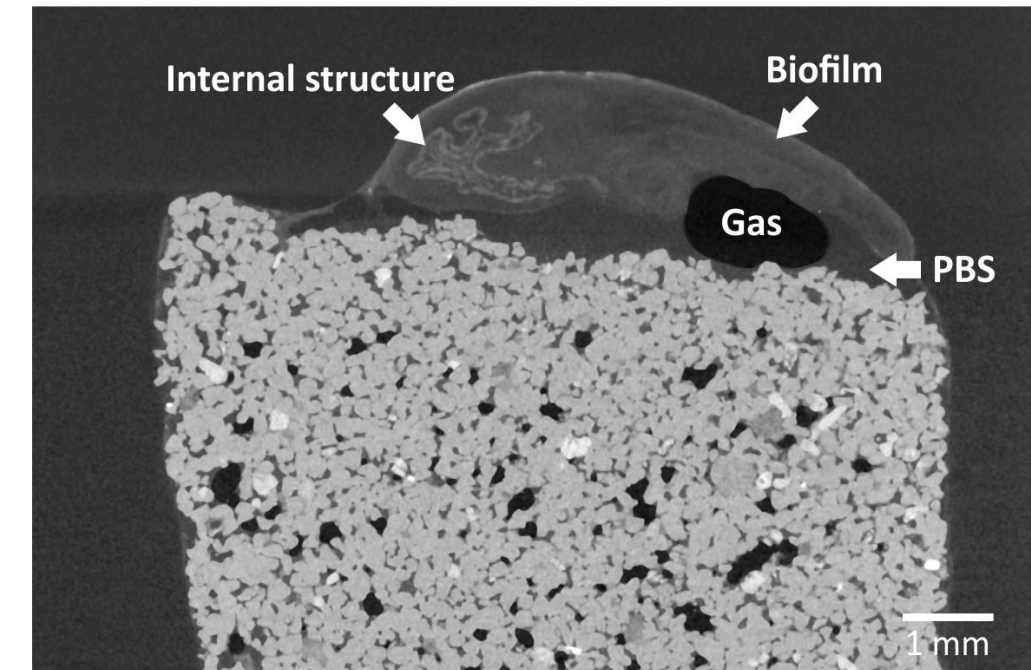
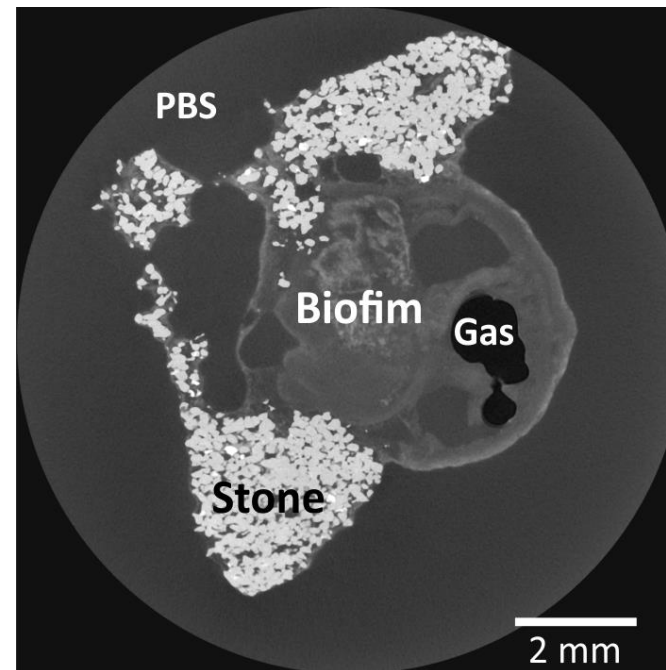
VISUALIZING BIOFILMS INSIDE THE FLOW CELL REMAINS CHALLENGING



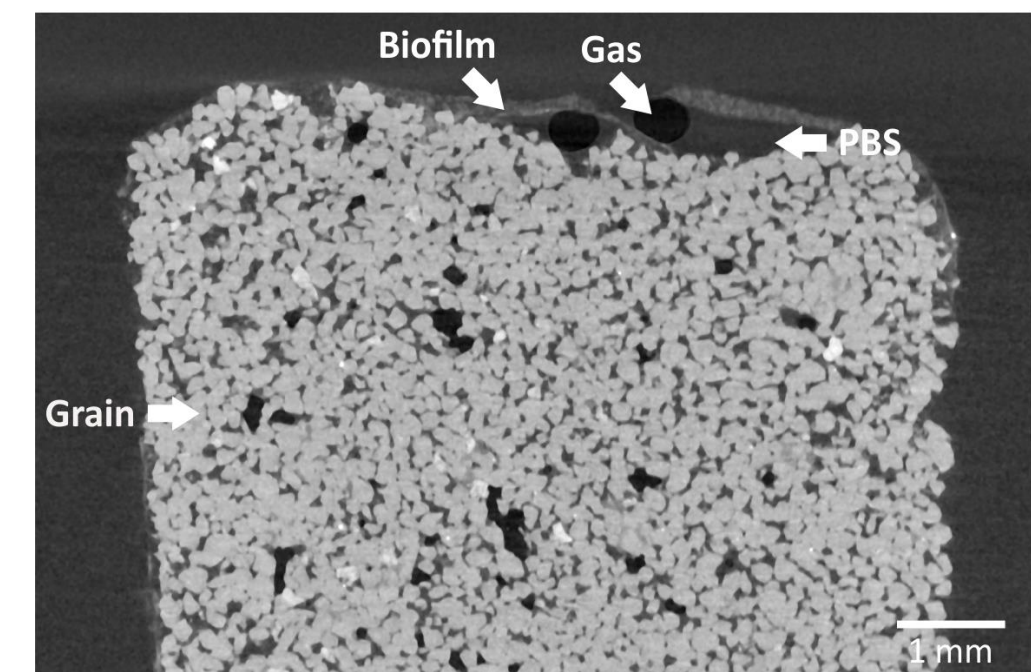
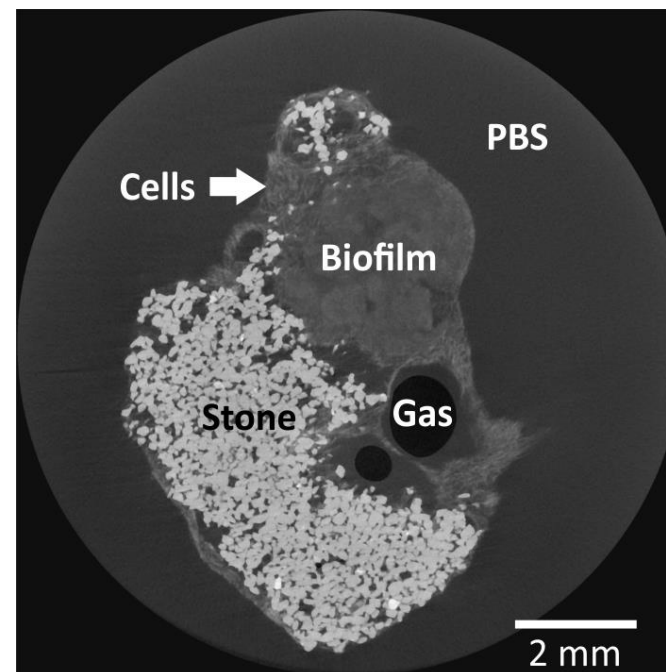
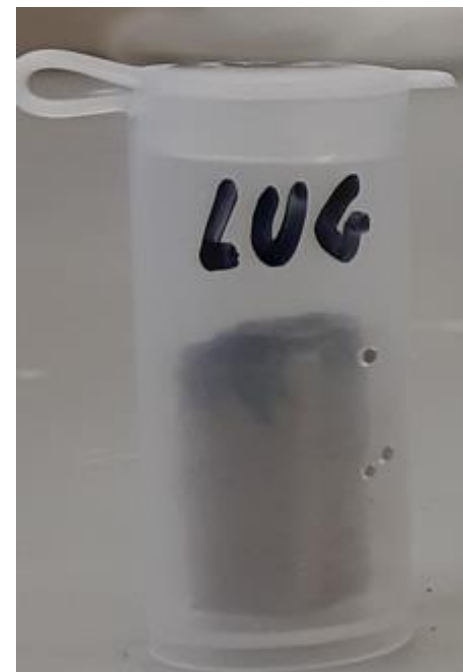
FIVE CONTRAST AGENTS WERE COMPARED

ISOTONIC LUGOL AND HF-WD POM WERE PROMISING

Hf-WD POM

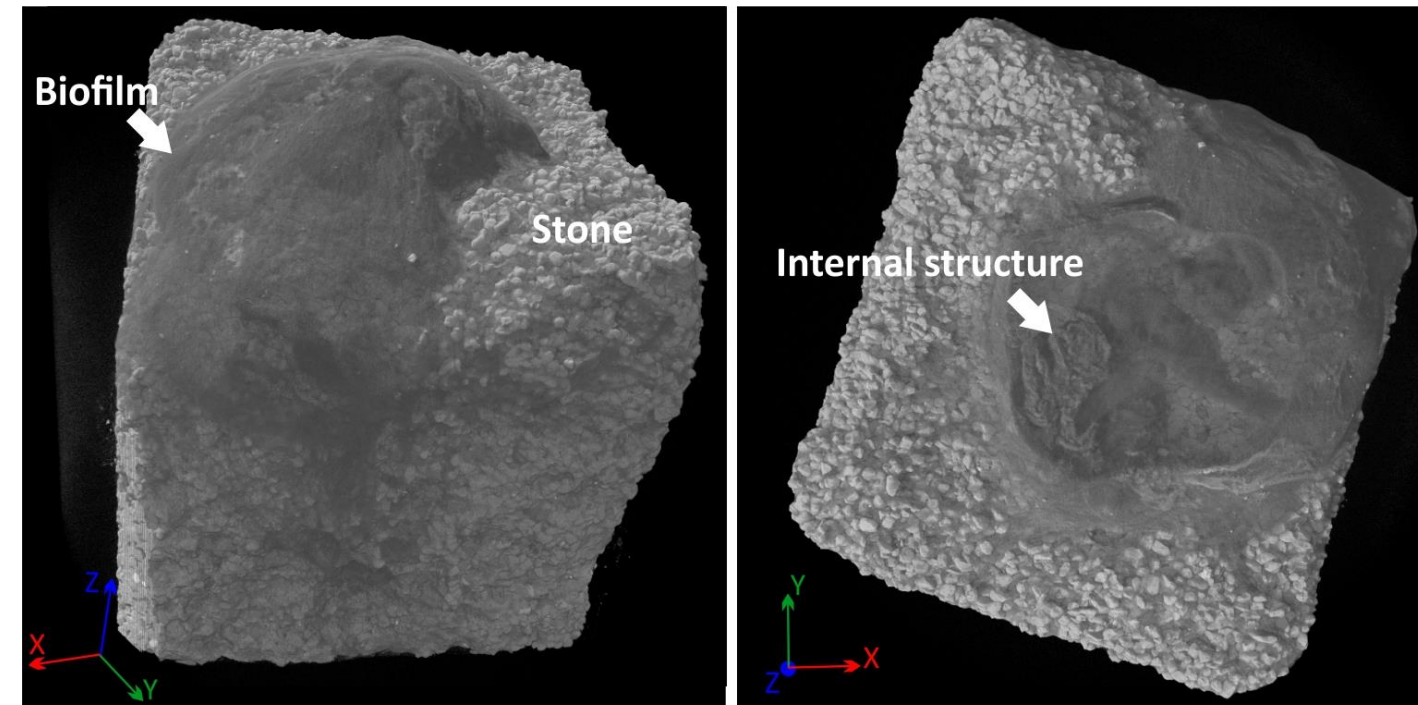


Isotonic lugol

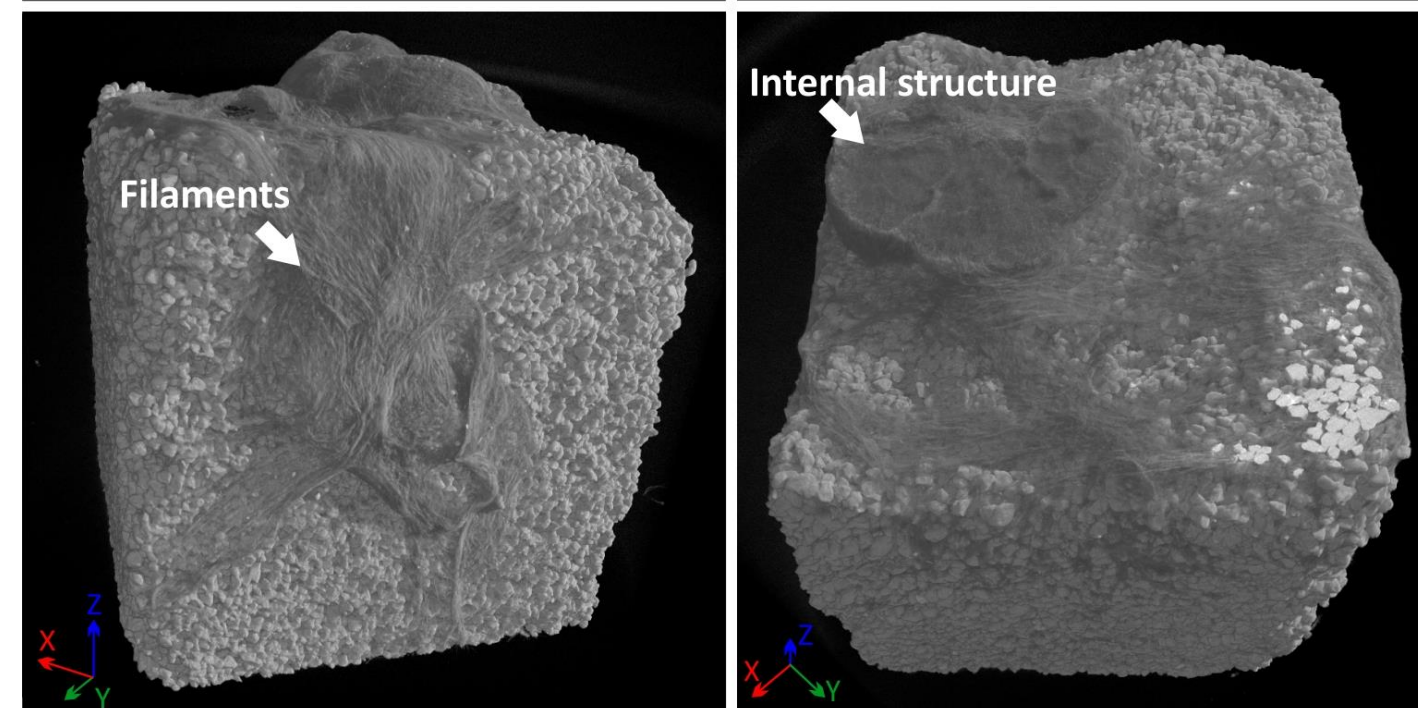


3D VISUALIZATION OF THE BIOFILM STRUCTURE

Hf-WD POM

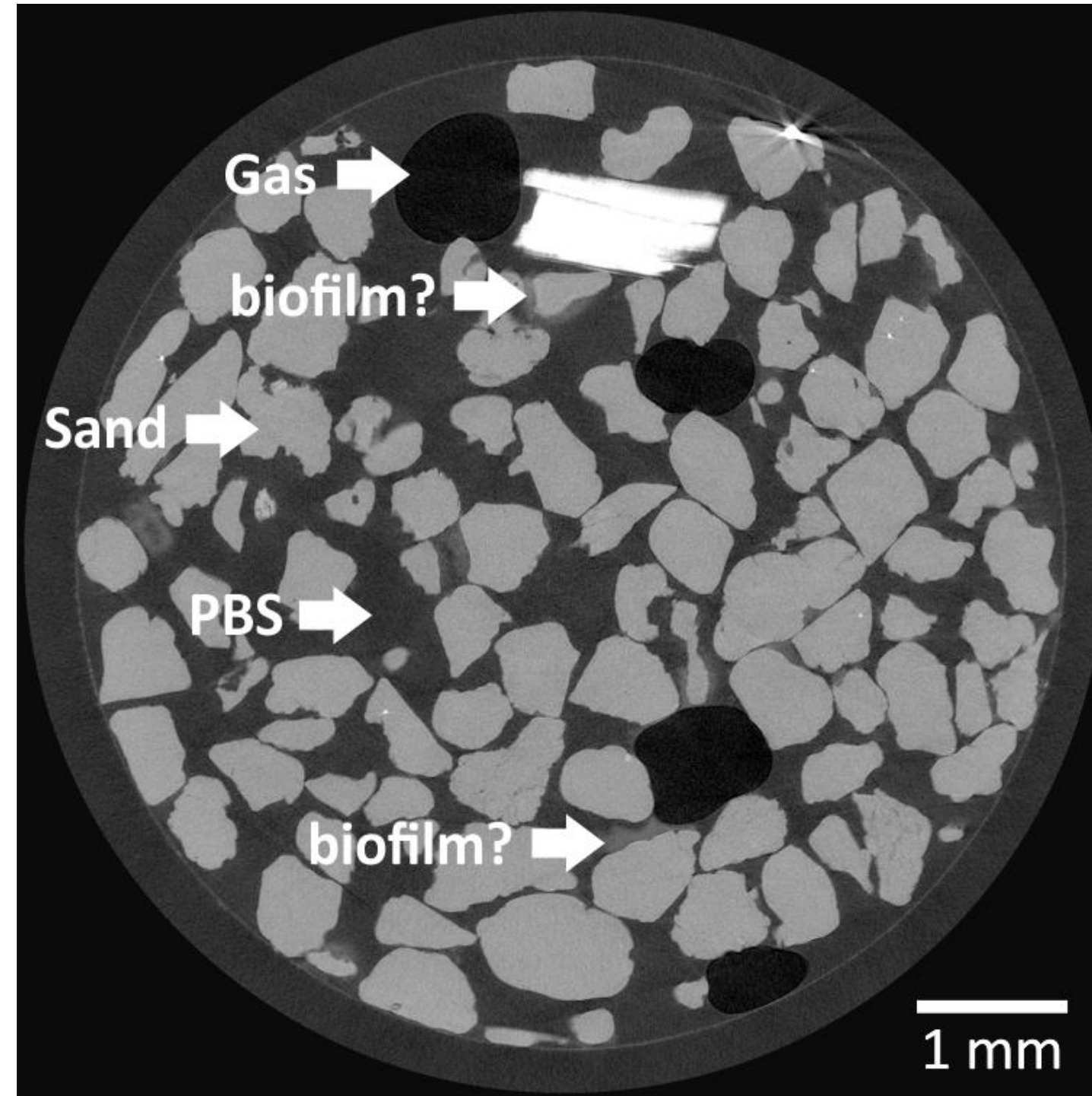
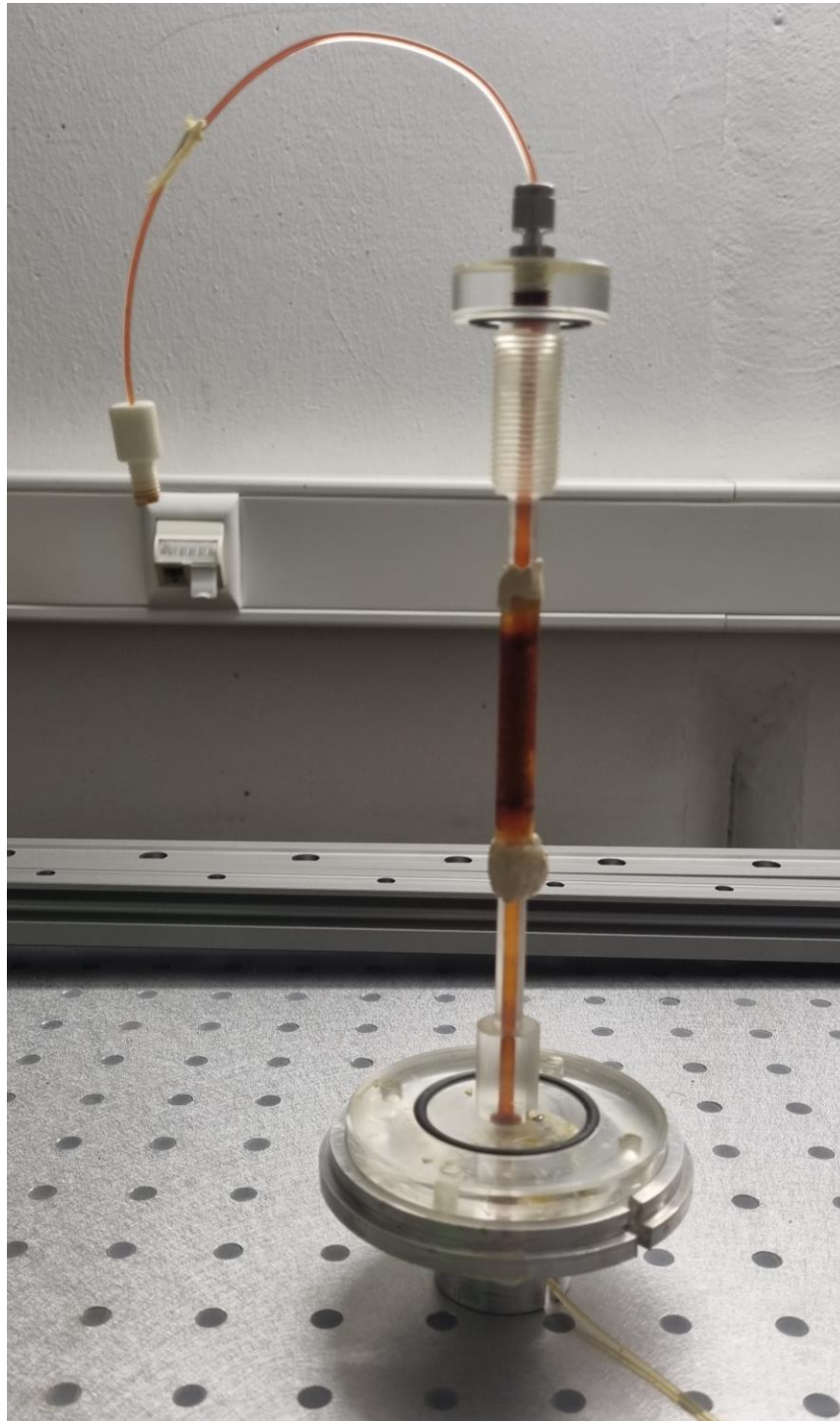


Isotonic lugol



Both contrast agents can be used complementary

ISOTONIC LUGOL VISUALIZES POTENTIAL BIOFILMS INSIDE POROUS MEDIA BUT SEGMENTATION REMAINS DIFFICULT



CONCLUSIONS AND FUTURE RESEARCH

- Negative contrast agents are able to stain biofilms allowing visualization with μ CT
- Hf-WD POM, Mono-WD POM and isotonic lugol are the most promising contrast agents
- Visualization of biofilms inside porous media remains challenging
- Future research should focus on how these contrast agents interact with the biofilm and how we can enhance absorption



15 Institutions

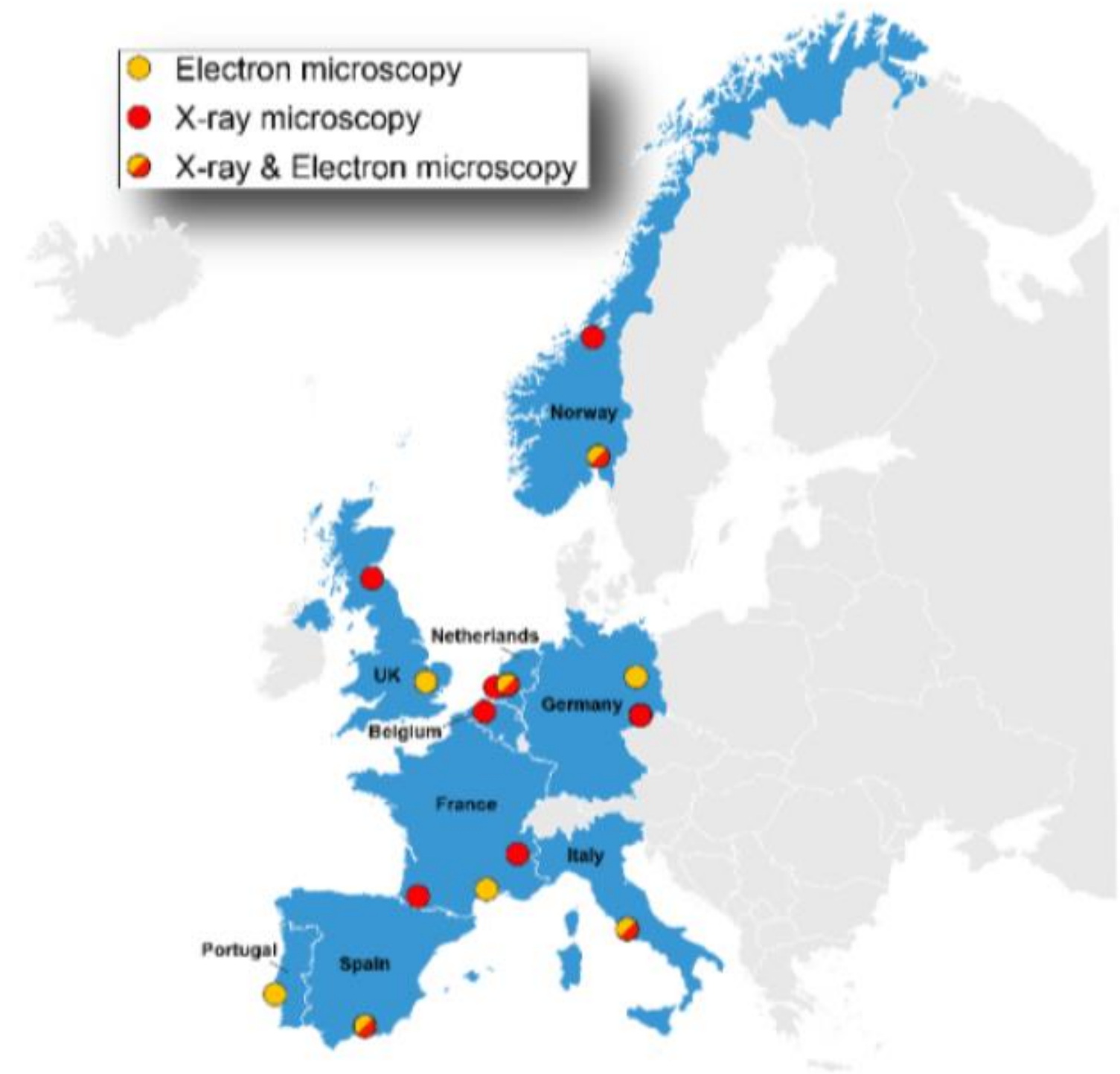
9 Countries

Facilities:

- 11 Electron microscopy
- 14 X-ray Microscopy

Transnational access, joint research activities, industry events, workshops, etc.

<https://excite-network.eu/>



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