Imaging liquids in pores - beyond X-ray tomography

Lipid Fixation in porous samples

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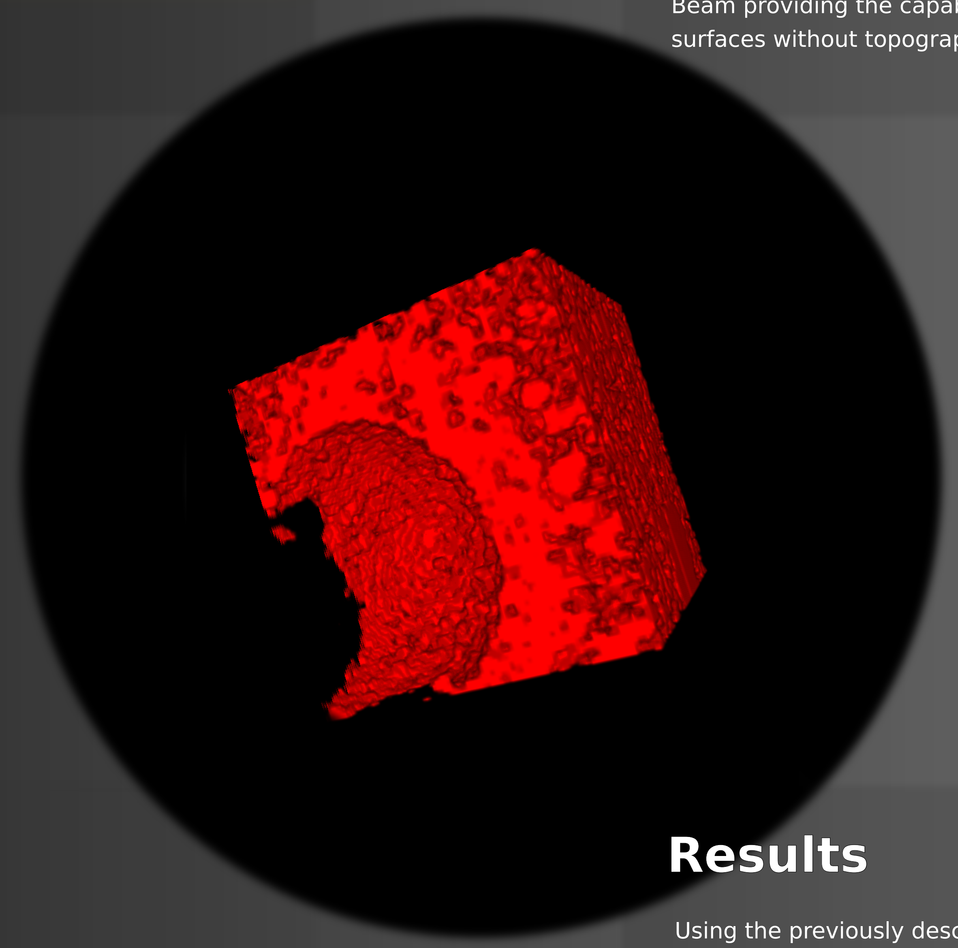


Why FIB/SEM?

Often tomography is used to acquire **3D images** of porous materials (see central image). Drawbacks of this technology however are the limited number of imaging modes and the expensive and complex image acquisition.
This work presents a **new sample preparation method** for imaging lipids in porous systems using **FIB/SEM**, overcoming the limitations of tomography and tackling some challenges of the classical SEM approach.

FIB/SEM offers:

- Fast image acquisition (can be done at all FIB/SEM setups)
- High spatial resolution
- Multiple imaging modes (BSE, SE, EDX)

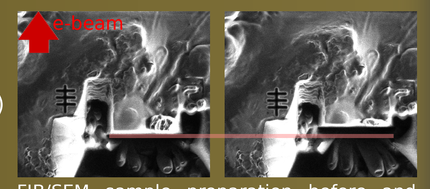
However FIB/SEM is a destructive method limiting the data collection to a one-time possibility.
The **direct observation** of the pore space, made possible by FIB/SEM, and its content eliminates the need for image reconstruction and offers a direct view on the area of interest.

Focused Ion Beam - Scanning Electron Microscopy (FIB/SEM)

FIB/SEM is combining the imaging capabilities of the Scanning Electron Microscope with the sample manipulation opportunities of the Focused Ion Beam.

Imaging steps:

1) Cutting a flat surface of the sample (FIB)
2) Take image of flat surface (SEM)
3) Remove thin slice (FIB)
5) Continue with step 2



FIB/SEM sample preparation before and after removing slices using the Focused Ion Beam - the direction of the imaging electron beam is indicated by the arrow

Why not cryo?

Immobilizing liquids by freezing them is a common approach to image liquids in the **Scanning Electron Microscope (SEM)**. However combining it with the ion milling process of the FIB/SEM is, especially considering porous rock samples, a challenging task.

The most popular approach for cryo-SEM imaging of rocks is a **fracture process** including fracturing the sample inside the SEM by the use of a blade (B. Lubelli et al., 2013).

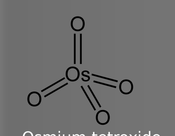
Fracture technique:

- No 3D information
- Topographic contrast due to the not sufficiently flat surface after fracturing

Fixating fluids **without the need of freezing** them (using the technique illustrated in this work) enables the use of the Focused Ion Beam providing the capabilities of 3D information as well as flat surfaces without topographic contrast.

OsO_4 fixation

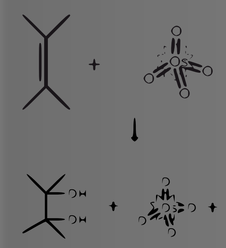
Osmium tetroxide (OsO_4) fixation is widely used in Life Sciences to immobilize lipids by reacting with unsaturated bonds in fatty acids to form diols.



Osmium tetroxide (OsO4)

Reaction steps:

- Opening up double bonds
- Cycloaddition to form osmate ester
- Rapid hydrolysis leading to vicinol diol



Oxidation of OsO4 leading to the fixation of unsaturated lipids (after J.C. Riemersma, 1962)

Sample preparation

To take advantage of the principle of Osmium Tetroxide Fixation the sample has to be flushed with a lipid containing a sufficient number of unsaturated fatty acids. **Sunflower oil** is a suitable choice.

- Emerge sample in Sunflower Oil
- Heat up to around 80°C for an hour
- Take the sample out
- Put sample in Osmium Tetroxide gaseous environment (~ 2 days)

After following this procedure the lipid inside the pores is fixed and can be treated as a solid.

The sample

Basic knowledge about the investigated chalk sample (Aalborg outcrop) is gathered by data analysis on tomographic images (see central image).

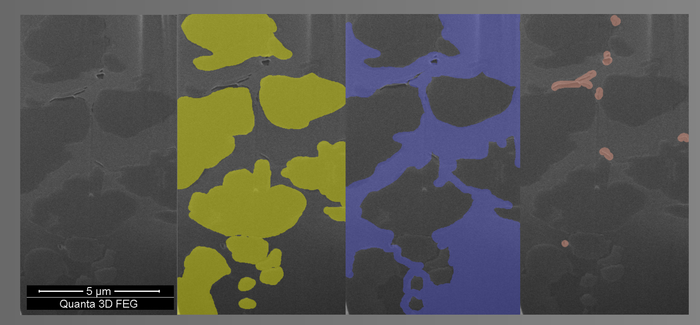
- Porosity: **~ 50.9%**
- Specific Surface Area: **~ 0.621 m^2/g**
- Permeability: **~ 32.24 mD**

The permeability is calculated using the Kozeny-Carman equation, i.e. based on the porosity and suface area.

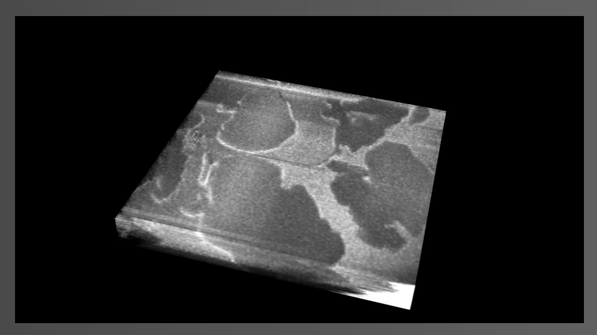
Results

Using the previously described preparation method images were taken showing three different phases:

- Liquid phase (lipid)
- Bulk phase (chalk)
- Air bubbles



Original image | Bulk coloured | Pores coloured | Air bubbles coloured



3D image data set with fixed liquid inside of pores

Verifying the observations:

- Air bubbles indicate the observation of a fixed liquid
- Elemental contrast due to different density of lipid and bulk

Information can be gathered from **2D images as well as from 3D image stacks** acquired by FIB/SEM.

Future possibilities:
Direct observation of fluid distributions for different saturations as well as the possibility of elemental mapping.

References

B. Lubelli, D.A.M. de Winter, J.A. Post, R.P.J. van Hees, M.R. Drury, Cryo-Fib-SEM and MIP study of porosity and pore size distribution of bentonite and kaolin at different moisture contents, Applied Clay Science, 2013, 80-81, pp. 358-365

J.C. Riemersma, Osmium Tetroxide Fixation of Lipids: Nature of the Reaction Products, J Histochem Cytochem 1963 11: 436

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