



Test BioREE Lanthanoid Staining Kit

Sample description and imaging conditions

Imaging of a non conductive imprint of tongue on plastic.

Imaging goal: Imaging bacteria in well preserved cell structure

Problem: Analysis time → long time from shipping till analyzing, due to transport
Can the ultrastructure be preserved?

Imaging conditions:

GeminiSEM300 → VPBSD Detector

Operation Mode: VP Mode

EHT: 15 kV

Chamber pressure : 20 Pa

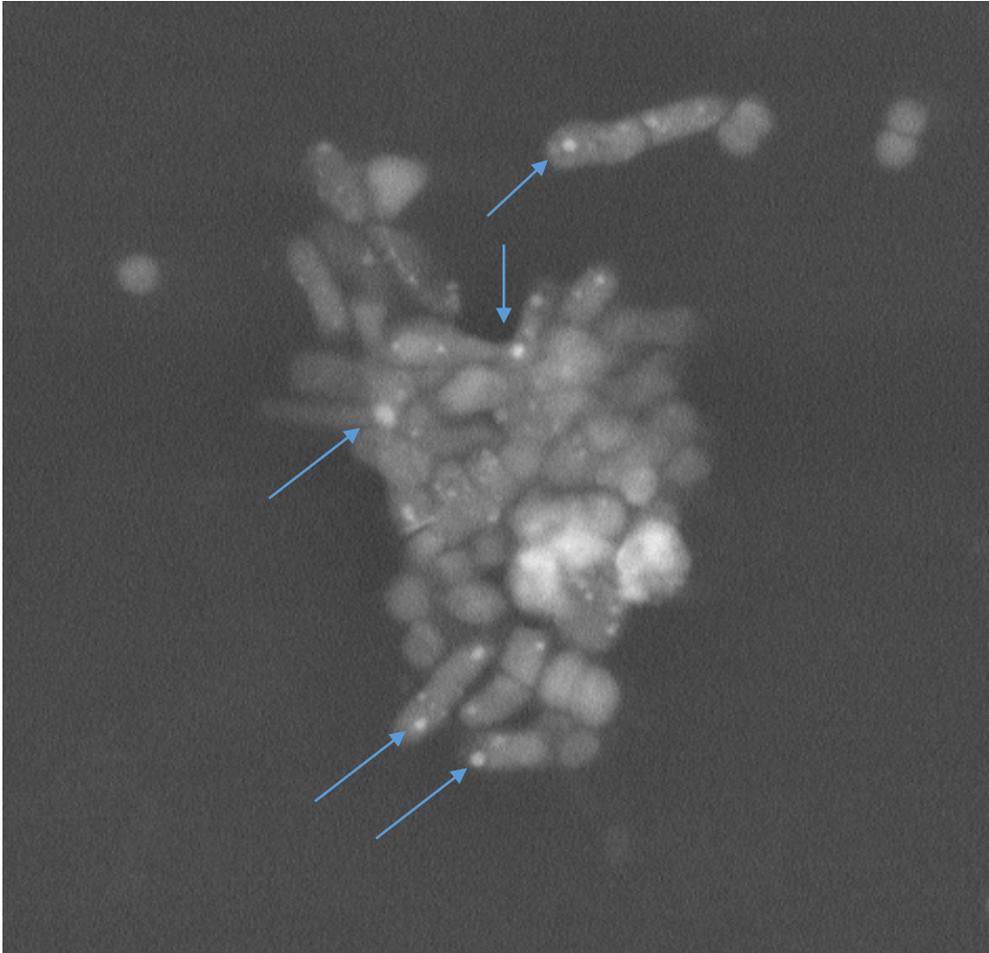
Aperture: 30 μm

Scanspeed 4-6

Noise reduction: Line average

The sample was stored in the fridge till analysis. No additional coatings were used.

Bacteria on a plastic sample



Some bacterial colonies could be identified

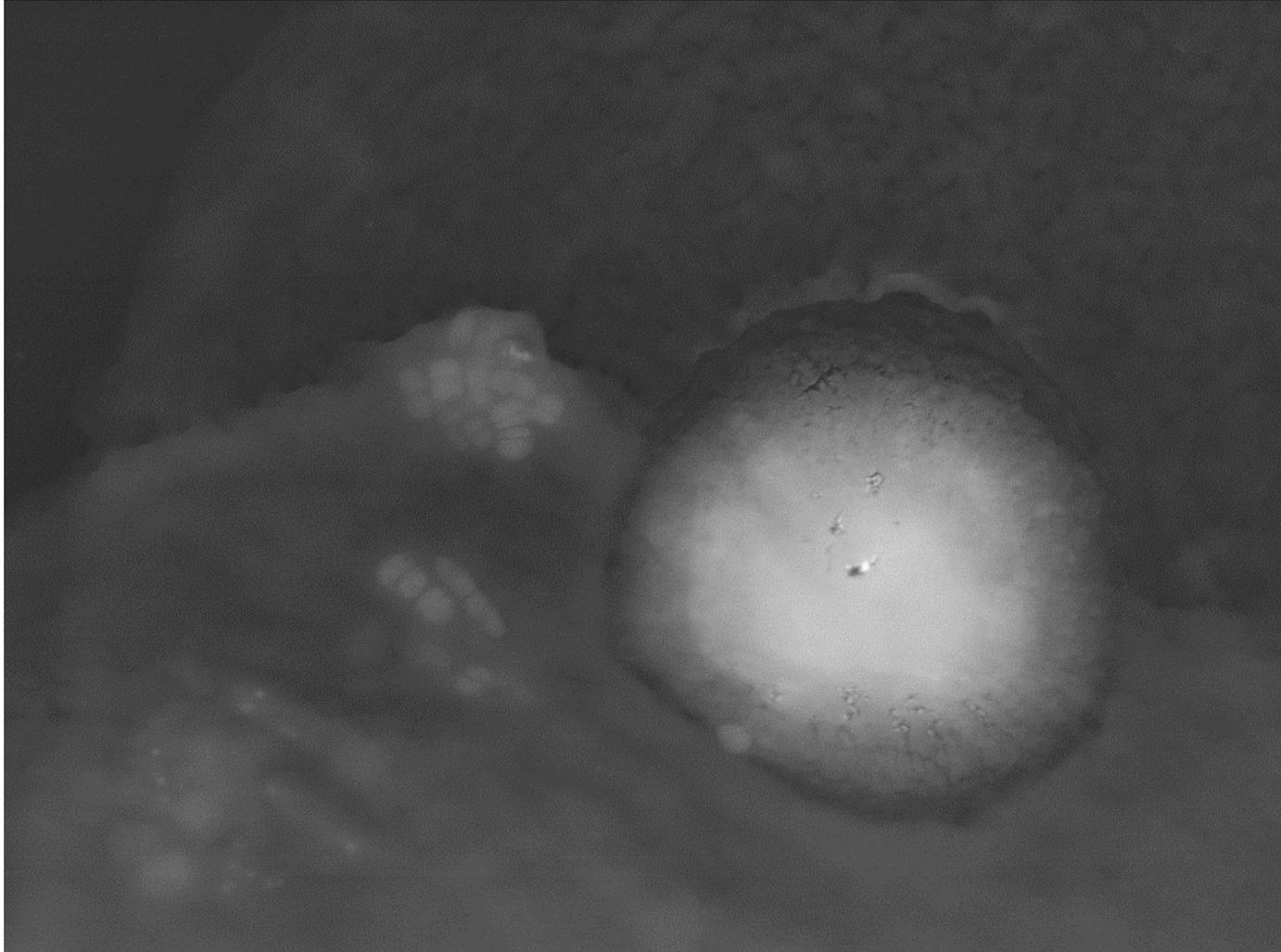
They were mostly grouped.

I couldn't get a signal with VPSE detector or with the VPBSD detector in topographic mode.

The bacterial colonies could be identified immediately. Inside the cells, labelled spots could be observed (see arrows).

The cell shape didn't look topographic but was clearly visible.

Probably caused by loss of water during transport and storage??



In this picture, the bacterial colonies also look flat.

They were clearly distinguishable from the surrounding areas.

Size of the single cell is $\sim 1\mu\text{m}$



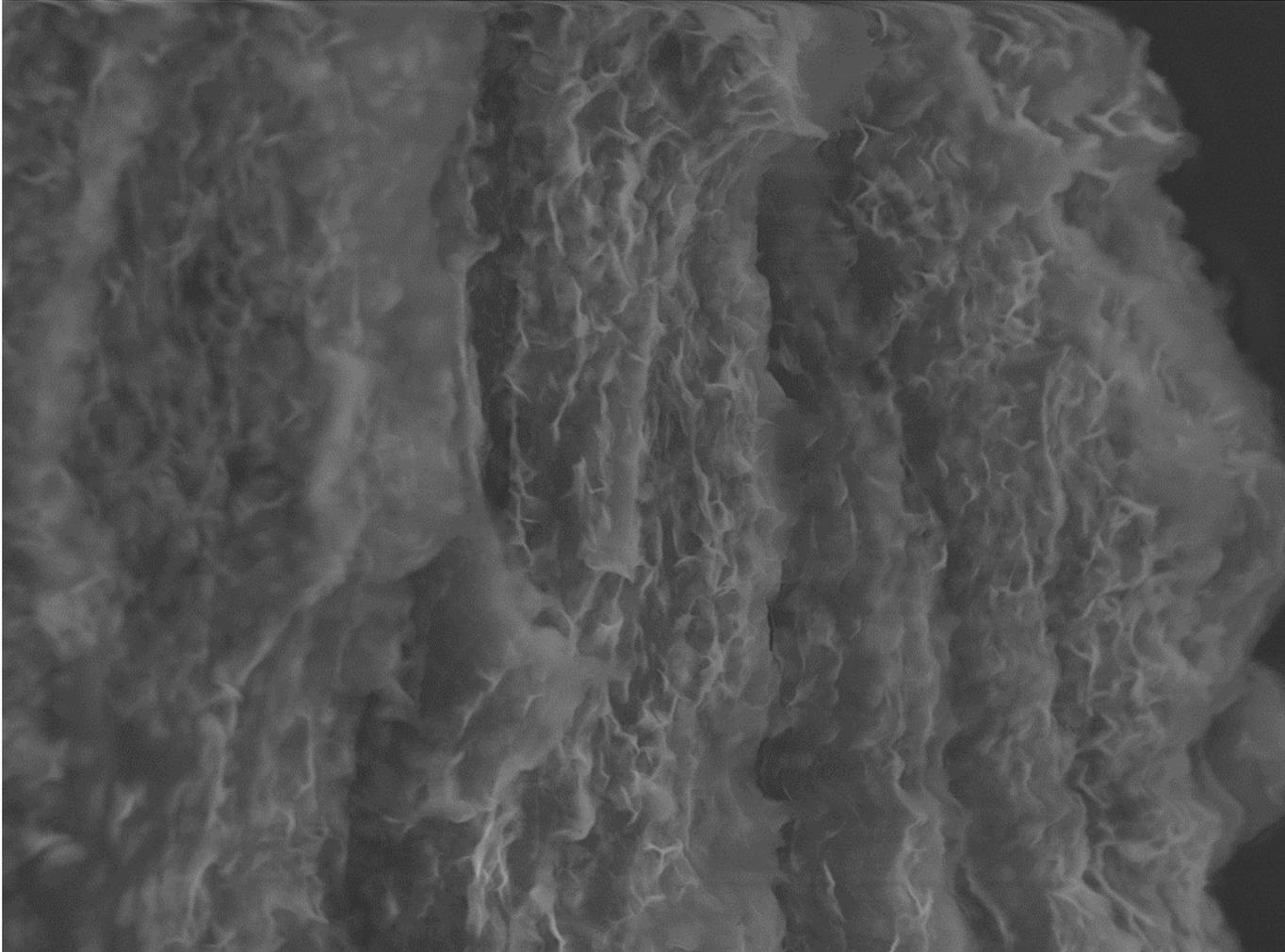
Some single bacteria with well preserved ultrastucture were found.

They show a more topographic structure and seem to be preserved better.

The spots observed in the more flat like colonies could not be observed here

The staining is much brighter compared to the cells described before

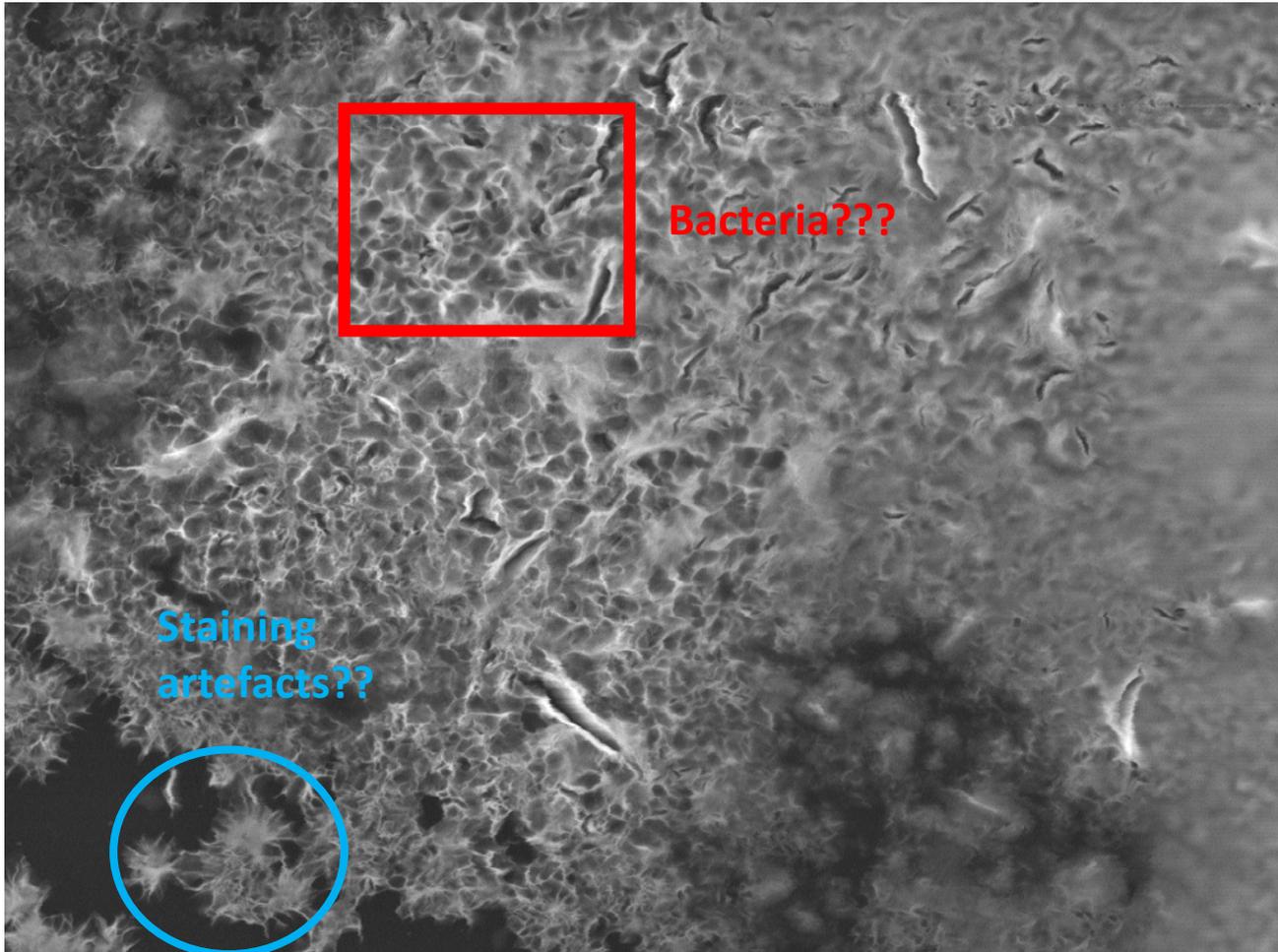
Other structures on the sample observed



Some other structures were also found on the sample.

Here I am not sure if there could have been some bacterial colonies on top and if structure is not preserved well.

Other structures on the sample observed – Staining artefacts???



Some other structures were also found on the sample.

On the first sight it looks like a big bacterial colonie.

(red square)

The contrast differs to those observed before. Also no internal structures were observed.

The blue circle looks like cristalline artefacts

→ Could it be that they result from the staining?

Pro:

- Staining seems to be easy in handling
- No toxic staining methods necessary
- Even after storage of some days → good ultrastructural preservation (just partly)
- Internal structures visible
- Good BSE contrast
- Imaging in FESEM possible

Contra:

- No SE signal detectable
- Kit lifetime is short → storing of a high number of kits not possible for longer periods
- VP Mode necessary → unfortunately VPSE Signal was too weak for detection (BSE Signal limited)
- Possibility of crystalline staining artefacts

Further suggestion and final words

The analysis of prestained cells was possible in the FESEM without additional conduction or coating of the sample. The VP Mode was necessary to analyze this sample (→ the plastic sample was also non conductive)

Even after storage of some days, bacteria with well preserved ultrastructure could be observed.

It would be great to see a comparison between commonly fixed cells (critical point drying) and cells stained with bioREE

All in all the staining seems to work, is non toxic and easy to handle.

It would be great to get a application collection to see where it is already used in the field.