Cryosectioning as new tool for studying marine snow composition and colonization/degradation dynamics

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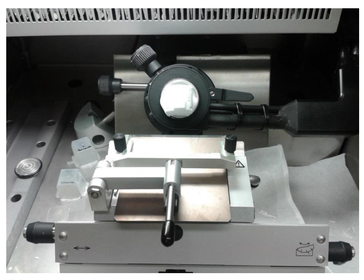
Because of their high organic matter (OM) content, marine snow particles are hotspots for microbial activity. Due to the heterogeneous composition and intricate three-dimensional structure of marine snow, microbial dynamics and microbe-substrate interactions are hard to examine using standard filtration and microscopy. To resolve this, we have developed thin-sectioning of frozen embedded marine snow as new tool for high-resolution visualization of individual aggregates. Cryosectioning lends itself to a wide array of applications: selectively staining the aggregate matrix and specific clades of bacteria makes it possible to analyse and correlate their distribution within an aggregate. Moreover, 3D-reconstruction of entire aggregates enables a direct assessment of their porosity, which is of significance for colonization, nutrient release, oxygen supply, and sinking velocities and mass fluxes.

Method

1. Pick aggregate from roller tank or Marine Snow Catcher
2. Fix in 3% formaldehyde
3. Wash in filtered seawater
4. Staining (e.g. Alcian Blue)*
5. Embed in cryogel (Tissue-Tek®)
6. Cryosectioning at OT -25°C/CT -30°C
7. Staining (e.g. DAPI, CARD-FISH)*

* time point depending on intended use



Block with embedded sample mounted in cryotome ready for sectioning.

FAQs

What is the optimum slicing thickness?

Depends on the features you want to see. Thin (10-20µm) is good for 3D reconstruction and looking at bacteria. Thick (~30-60µm) is better for viewing larger organism (diatoms, flagellates, coccolithophores ...)

When to stain?

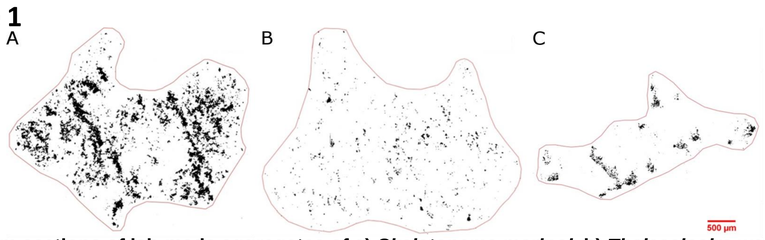
Depends on stain and purpose of slicing. To keep matrix optimally intact, staining prior to embedding is recommended.

To wash or not to wash?

Washing of the cryogel after slicing eliminates the weak fluorescent background signal caused by the gel and prevents interference with CARD-FISH. However, it cannot be ruled out that some of the sample gets washed off or smeared.

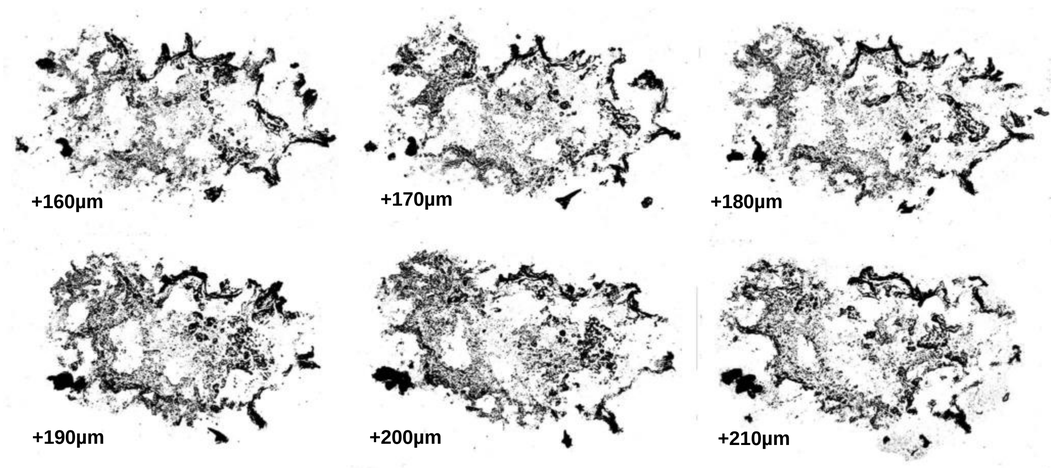
Porosity

Example 1



60µm sections of lab-made aggregates of a) *Skeletonema marinoi*, b) *Thalassiosira weissflogii* and c) Mix of *Skeletonema* and *Thalassiosira* stained with Alcian Blue.

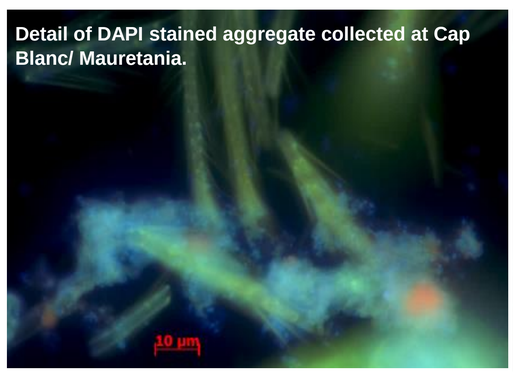
Example 2



Consecutive 10µm sections of aggregate collected at the Porcupine Abyssal Plain Observatory stained with Alcian Blue. Length of whole particle: 500µm.

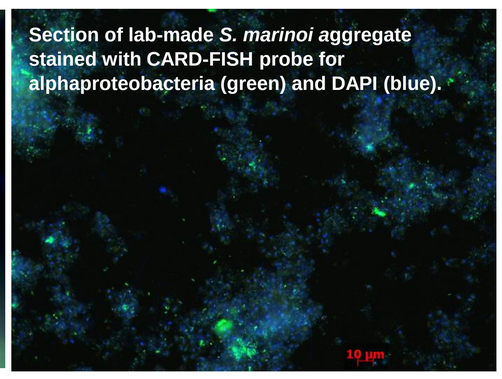
Colonization patterns and identification of bacteria

Example 1



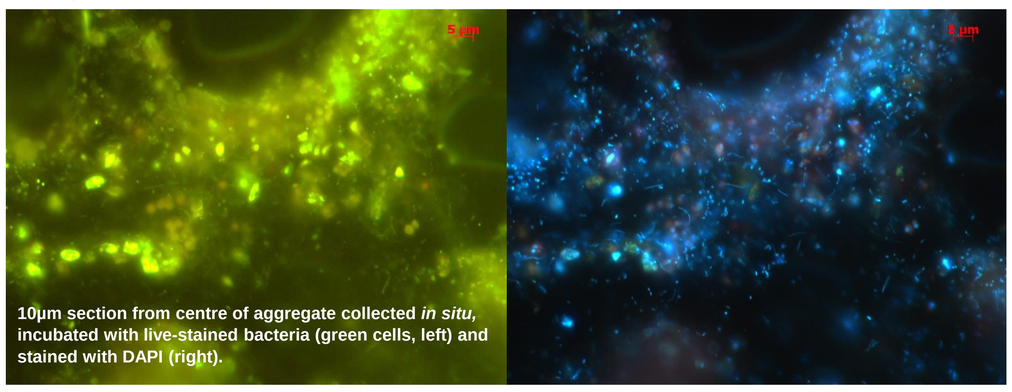
Detail of DAPI stained aggregate collected at Cap Blanc/ Mauretania.

Example 2



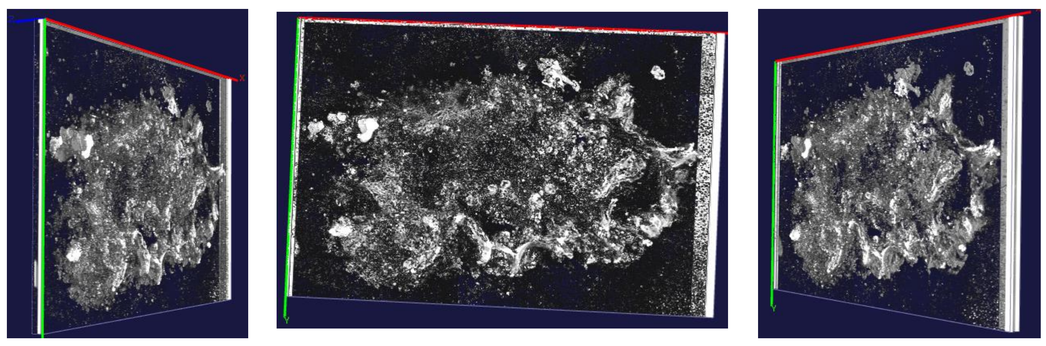
Section of lab-made *S. marinoi* aggregate stained with CARD-FISH probe for alphaproteobacteria (green) and DAPI (blue).

Example 3

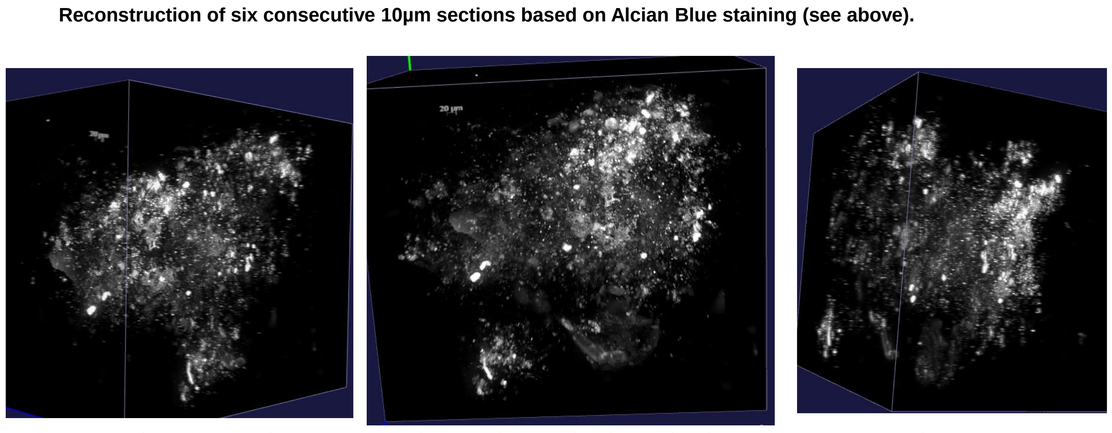


10µm section from centre of aggregate collected *in situ*, incubated with live-stained bacteria (green cells, left) and stained with DAPI (right).

3D reconstruction and volume rendering



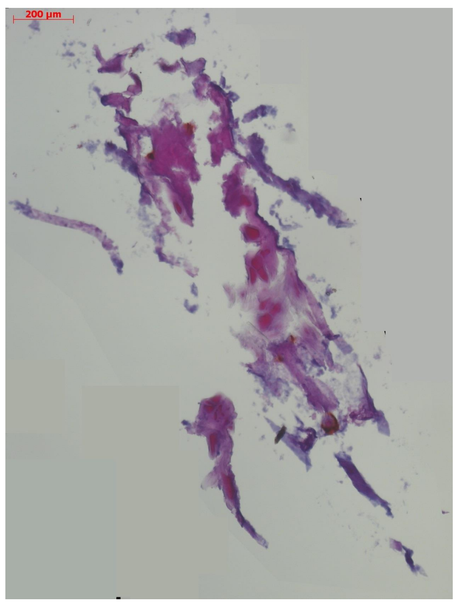
Reconstruction of six consecutive 10µm sections based on Alcian Blue staining (see above).

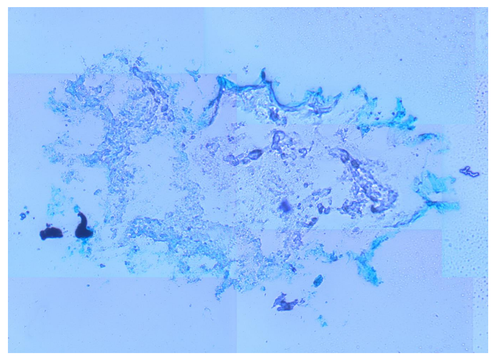


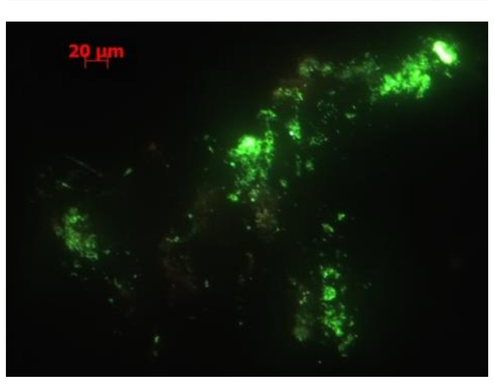
57 consecutive 10µm sections of aggregate collected *in situ*. Reconstruction based on green autofluorescence.

Substrate Identification

Below: Periodic acid-Schiff Stain (for polysaccharides and mucosubstances)
Top right: Alcian Blue stain (for acidic polysaccharides)
Bottom right: Autofluorescence (unknown origin)







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Acknowledgements

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