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Working

Scanning Electron Microscopy imaging for Opaline Silica Single Cell Skeletons (Polycystines Radiolaria)

Version 3

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Recovering skeletons

- 1 After DNA extraction, recover skeleton from the eluted pellet under binoculars or inverted microscope.

Note: During DNA extraction: dilute waste from the extraction procedure (i.e. pellet debris, containing the skeleton) in milliQ water and store skeletons at -20°C.

Protocol adapted from Biard et al. (2015).

Biard, T., Pillet, L., Decelle, J., Poirier, C., Suzuki, N., Not, F., 2015. Towards an Integrative Morpho-molecular Classification of the Collodaria (Polycystinea, Radiolaria). *Protist* 166, 374–388. doi:10.1016/j.protis.2015.05.002

Rinsing

- 2 Rinse skeleton several times in milli-Q water to decrease the concentration of SDS and other lysis and DNA precipitation reagents.

Cleaning

- 3 Transfer skeleton into 1.5 ml Eppendorf tubes containing 50 µl of hydrogen peroxide (H₂O₂).

- 4 Heat at 70°C for 10 min to remove residual organic matter.

Diluting

- 5 Add 1 ml of milli-Q water.

Rinsing

- 6 Handpick skeleton under binoculars or inverted microscope and repeat several rinsing steps.

Preparing for imaging

- 7 Transfer skeleton in a glued SEM pin stub mount with the less water as possible.

Note: no polycarbonate membrane, place the skeleton directly in the glue of the pin stub.

Drying

8 Let dry 30 minutes / 1 hour.

Imaging

9 Skeleton ready to be imaged.



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