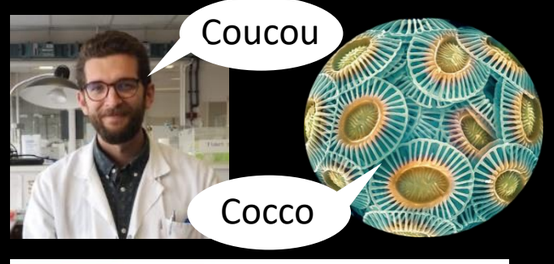

Hard X-ray nanoprobe imaging to follow calcification in coccolithophore microalgae

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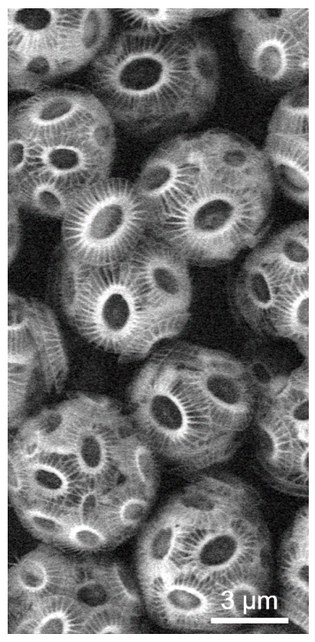
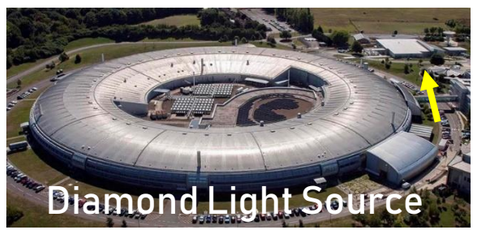
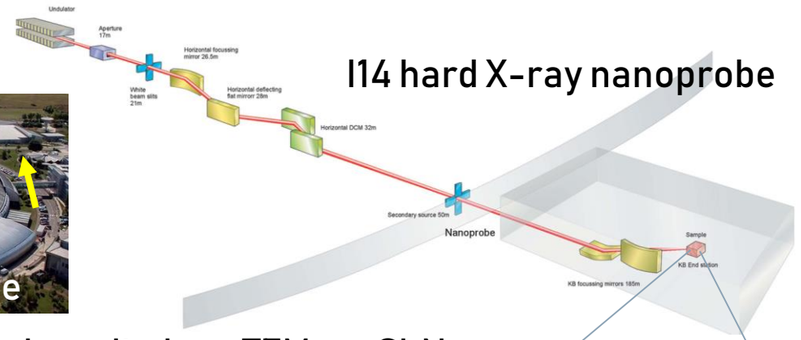
Introduction

Coccolithophore microalgae are phytoplankton that intracellularly biomineralize microscopic calcitic plates (coccoliths), which are extruded to the surface of the cell. They produce almost half of oceanic carbonates and thus significantly contribute to the carbon cycle (photosynthesis, calcification, coccolith sedimentation).[1] Despite this impact, the calcification process (e.g., the role of Ca-bearing bodies and delivery of substrates to coccolith vesicle) is still left to be unveiled. This work presents how hard X-ray nanoprobe techniques and sample environments can bring us closer to capturing the dynamics of coccolith formation.

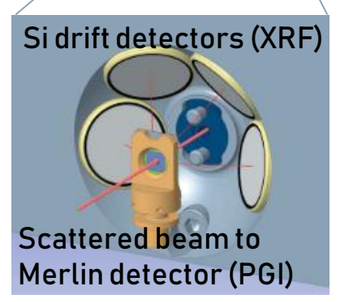


Experimental





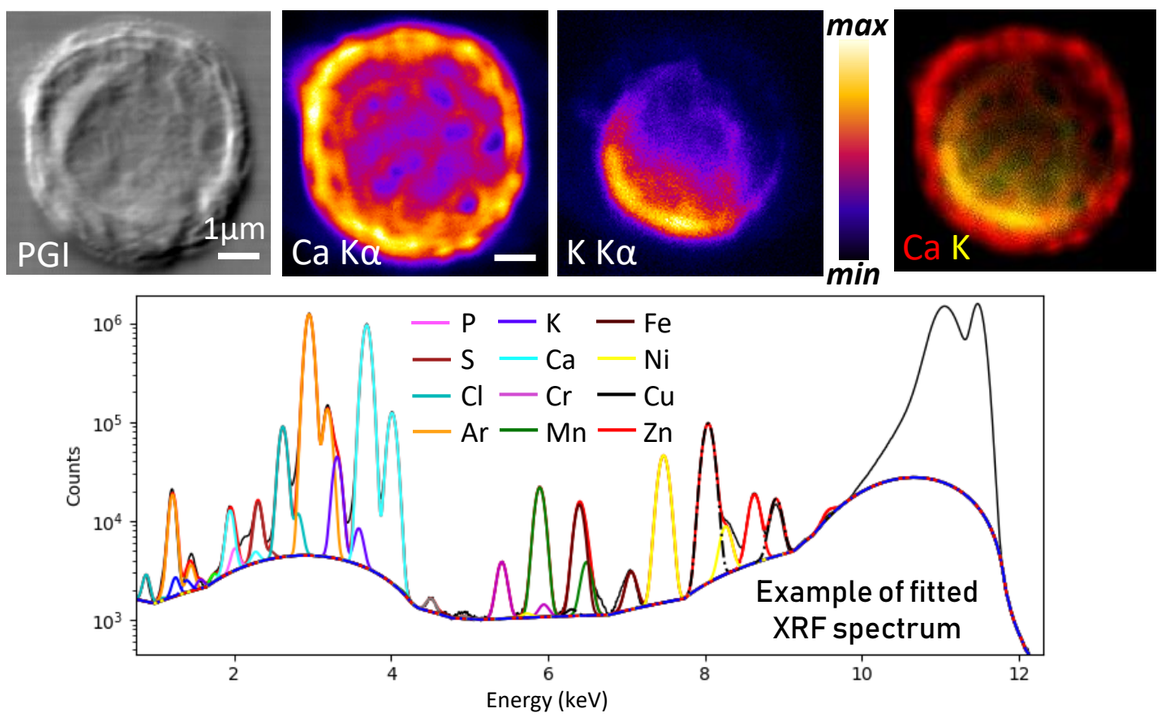
Coccolithophores were deposited on TEM or Si_3N_4 substrates for dry measurements. For wet measurements, coccolithophores were deposited between Si_3N_4 substrates and sealed in a custom sample holder. Scanning X-ray fluorescence (XRF) and phase-gradient imaging (PGI) were collected simultaneously using step size 50-100 nm with 20-50 ms dwell time. Incident X-ray energy 8, 11.5 or 16.5 keV. Beam size 50-70 nm (energy dependent).



Ref. 2

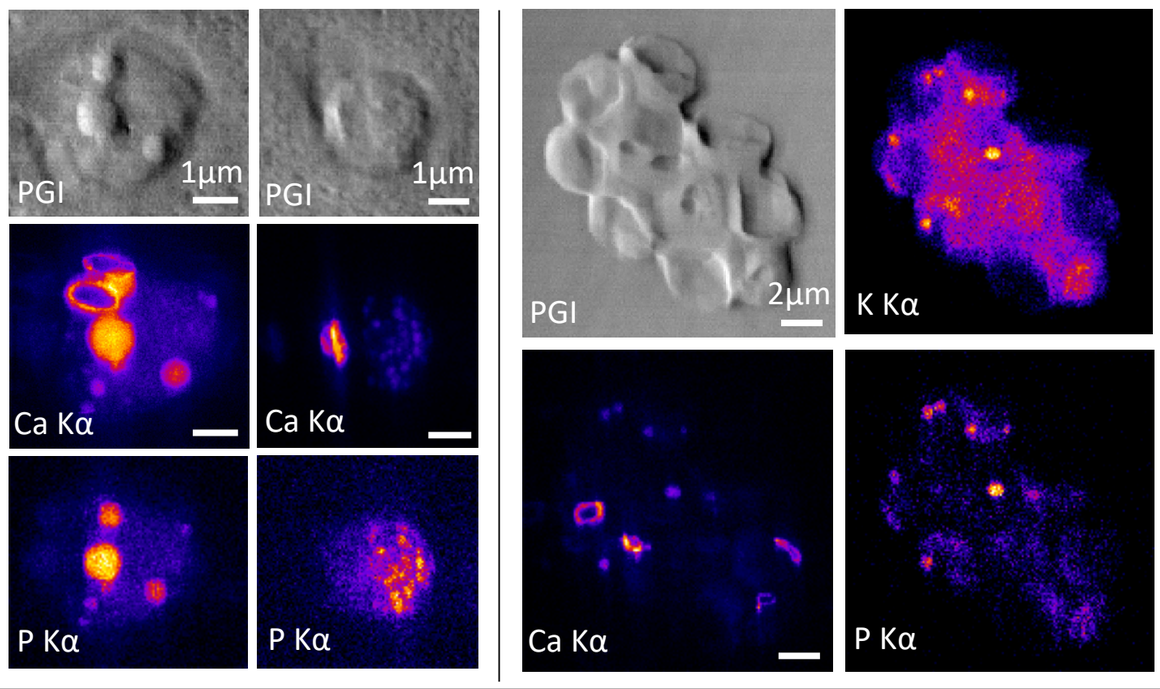
Phase gradient and X-ray fluorescence imaging

- *E. huxleyi* cells with complete coccosphere (shell of coccoliths)
- PGI and XRF signals represent structural and elemental composition



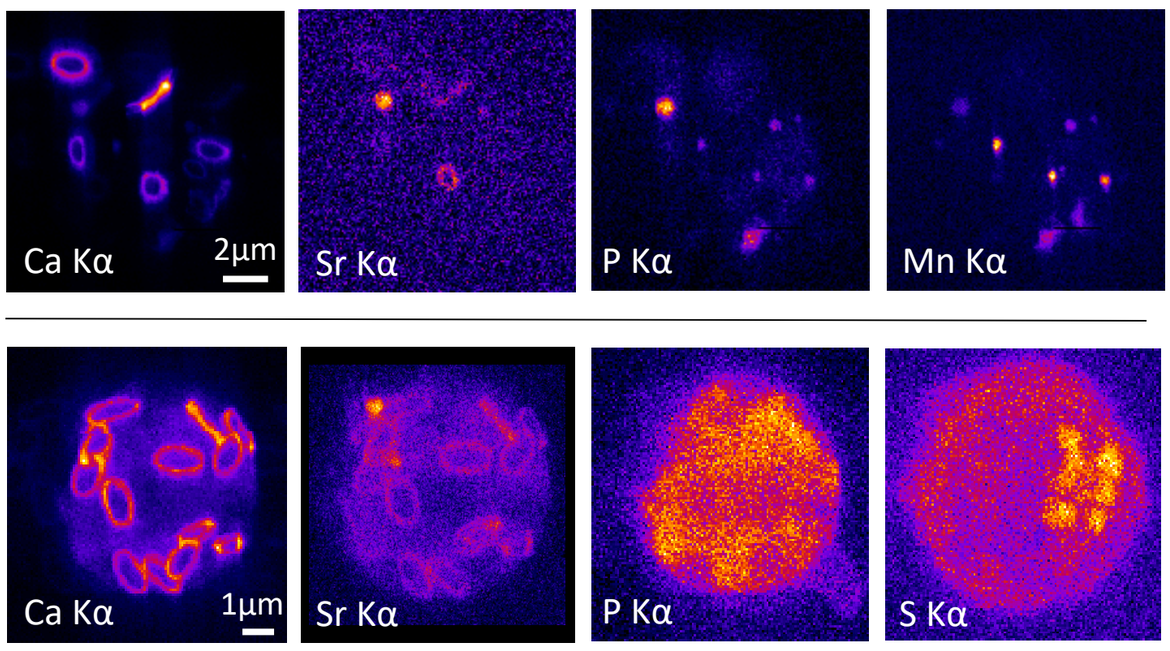
Coccolith formation

- *C. carterae* (left) and *E. huxleyi* (right) extracted during calcification
- Dense intracellular bodies and dilute concentrations of macronutrients and metals detected



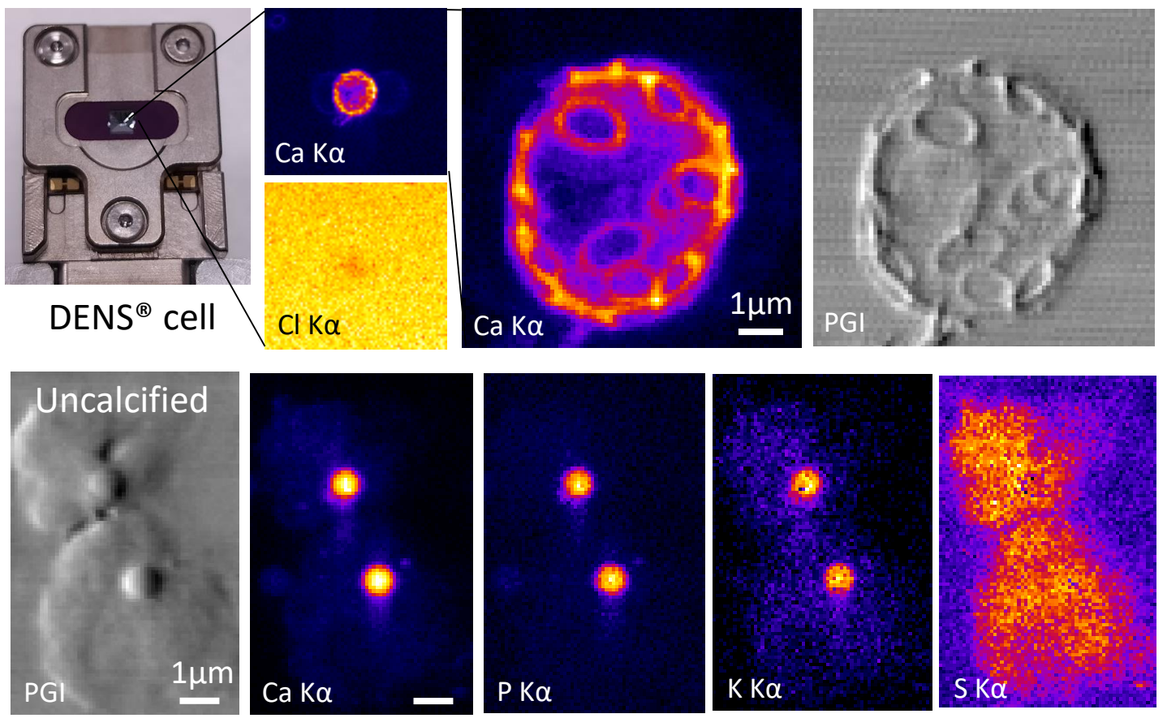
Dopants, trace- and oligo-elements

- *E. huxleyi* (top) and *C. carterae* (bottom) with added Sr^{2+} in media during coccolith formation
- Incorporation into coccoliths and dense intracellular bodies



Liquid cell measurements

- *E. huxleyi* introduced between Si_3N_4 membranes (8 µm spacer)
- Coccoliths imaged with XRF and PGI in sea water media



Summary

- Hard X-ray nanoprobe techniques offer subcellular structural and elemental information of coccolithophores
- Liquid sample environments for hydrated cell measurements are promising to image in near native-state
- Improvements on sample preservation (dry) and X-ray dose management (wet) will be invaluable to capture the chemical composition of coccolithophores during calcification

Acknowledgements

Beamtime proposals: MG23693, MG23602, MG28868
Special thanks to the I14 team at Diamond Light Source
References and image sources:
[1]Balch, W.M. *Annu. Rev. Mar. Sci.* 2018, *10*, 71-98
[2]Quinn, P. *et al. J. Synchro. Rad.* 2021, *28*, 1006-1013

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