The Hunt for Coccospheres: A New Method for Isolating and Analysing Coccospheres from Sediment

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1. Background

Coccolithophores, single-celled phytoplankton, produce a calcium carbonate exoskeleton, a 'coccosphere', comprised of plates (coccoliths) that interlock to surround the cell[1]. Fossil coccospheres in the sedimentary record provide cellular level information that can be compared to the living coccosphere, such as cell size[2]. However, coccospheres are highly delicate and therefore poorly preserved in sediments[2].

2. Project Summary

We introduce imaging flow cytometry (IFC) as a novel technique for rapid, high-throughput analyses to visually isolate coccospheres from marine sediments. Using the ImageStream Mk II (ISX; Luminex Corp. Seattle, US), we present the first application of IFC combined with cross-polarised light (ISX^{+PL}) to detect coccospheres based on their distinctive birefringent and morphological properties. High throughput imaging overcomes the constraints of laborious manual microscopy and allows the generation of coccosphere statistics despite low coccosphere concentrations in sediments.

3. Reconstructing Cell Size via ISX^{+PL}

Coccosphere and cell (coccoliths removed by acid addition) diameters of three cultured species were measured. Data was combined with individually measured coccolithophores[3-6] to establish a relationship between coccosphere and cell diameter (figure 1) and confirm coccosphere diameter obtained via ISX^{+PL} can be used as a proxy for coccolithophore cell diameter.

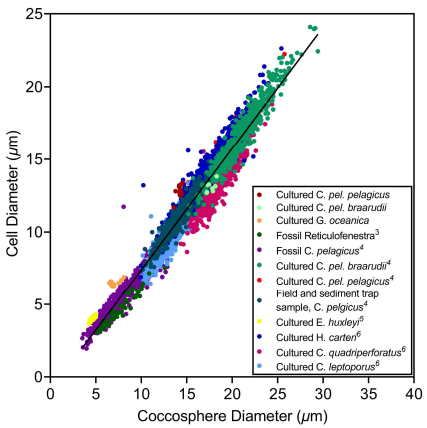

Figure 1: *Relationship between coccosphere and cell diameter of cultured C. pel. pelagicus, C. pel. braarudii and G. oceanica, combined with published data[3-6] Linear regression line for all data in black.*

4. ISX^{+PL} Method

1. Exploit the birefringence of coccospheres to filter out a considerable proportion of marine sediment
2. Utilise the morphological properties of coccospheres to distinguish them from remaining birefringent materials
   - 2a. Use circularity and diameter values to sort coccospheres from non-coccolith material
   - 2b. Use 'bright spot' count to sort coccospheres from coccoliths

1. The ISX fitted with polarising filters selectively detects birefringent particles. This was confirmed via employing the side scatter laser which detects all objects in the sample. A rate of ~1500 objects/sec was measured with the laser employed, compared to ~100 objects/sec without the laser.

5. Application to field sample

Appling the protocol and template (figure 2) to a North Atlantic core, 35 coccospheres were identified from 75,000 images of birefringent particles (0.047%) (figure 3). Based on the established relationship between coccosphere diameter and cell diameter (figure 1), diameter values obtained via ISX^{+PL} can be used to reconstruct coccolithophore cell size.

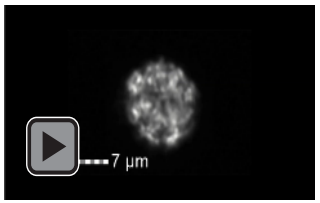

Figure 3: *Video of cross-polarised light images of sorted coccosphere population from North Atlantic core EN539-16MC.*

2. The morphological features of coccospheres allows coccospheres to be sorted from remaining birefringent material. A positive coccolithophore control and a negative control of marine sediment with $CaCO_3$ removed were investigated to classify morphological properties and to construct a template to which field samples could be applied.

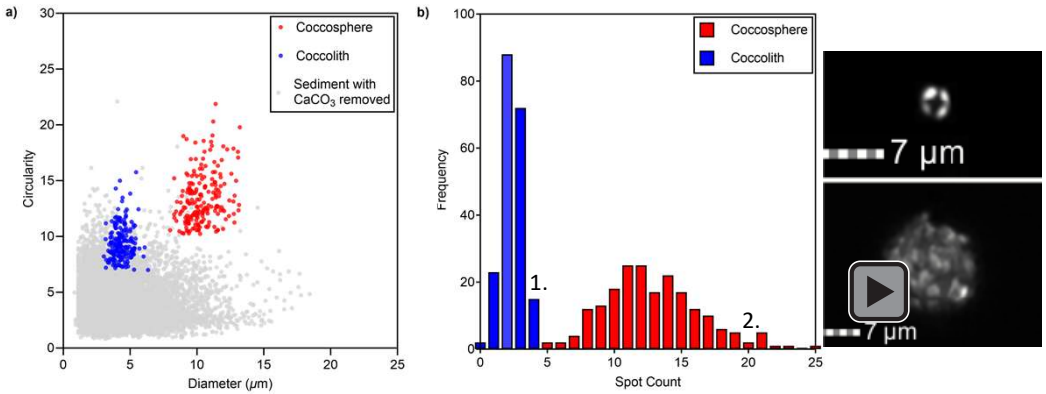

Figure 2: ***(a)*** *Diameter and circulatory distribution analysis of 150 coccospheres (red) and 200 coccoliths (blue) from G. oceanica sample and 10,000 marine sediment particles with $CaCO_3$ removed (grey).* ***(b)*** *Frequency distribution of spot count values for 150 coccospheres (red) and 200 coccoliths (blue) from G. oceanica sample. Video demonstrating spot count of a coccolith (top) and coccosphere (bottom).*

2a. Circularity and diameter provide a basis for visual separation of coccospheres with only 0.07% of marine sediment with $CaCO_3$ removed plotting in the identified coccosphere region.

2b. Where multiple species exist, and coccospheres and coccoliths fall within the same diameter range, 'bright spot' count allows coccospheres to be distinguished from coccoliths. Coccoliths consist of sub-radial and sub-vertical calcite orientations that appear light and dark in cross-polarised light[7]. Coccospheres exhibit a greater number of 'bright spots'.

6. Conclusion

- High-throughput imaging enables the analysis of sediments containing low coccosphere concentrations in which it would not be feasible to manually 'hunt' for via traditional microscopy.
- Application of this method to coccosphere rich sediments enables large datasets and statistically significant results to be generated.
- Objective analysis is achieved in the IFC software, that generates and archives datasets and images for reanalysis.
- Using ISX^{+PL}, we can move closer to a direct measurement of coccolithophore cell size.
- Caution should be exercised when interpreting changes in coccosphere and cell geometry through time due to biases in preservation.

[1] Bramlette, M. N. Significance of coccolithophorids in calcium-carbonate deposition. *Bull. Geol. Soc. Am.* **69**, 121–126 (1954). **[2]** Bown, P. R., Gibbs, S. J., Sheward, R., Sarah, O. & Higgins, D. Searching for cells: the potential of fossil coccospheres in coccolithophore research. *J. Nannoplankt. Res.* **34**, 5–21 (2014). **[3]** Henderiks, J. & Pagani, M. (Table S2) Mean coccosphere diameter, cell diameter and coccolith size of fossilized reticulofenestrid coccospheres from DSDP Site 72-516. *PANGEA* (2007). **[4]** Bown, P. Biometric data illustrating species-specific growth response of coccolithophores to Palaeocene-Eocene environmental change (NERC Grant NE_H017291_1) - data. (2018). **[5]** Schlüter, L., Lohbeck, K. T., Magdalena A. Gutowska, J. P. G., Riebesell, U. & Reusch, T. B. H. Adaptation of a globally important coccolithophore to ocean warming and acidification. *Nat. Clim. Chang.* **4**, 1024–1030 (2014). **[6]** Sheward, R. M., Poulton, A. J., Gibbs, S. J., Daniels, C. J. & Bown, P. R. Coccosphere geometry measurements from culture experiments on the coccolithophore species Calcidiscus leptoporus, Calcidiscus quadriperforatus and Helicosphaera carteri. *PANGEA* (2016). **[7]** Young, J. R. Biomineralization Within Vesicles: The Calcite of Coccoliths. *Rev. Mineral. Geochemistry* (2003).