

# A filtration technique for quantitative studies of coccoliths

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**ABSTRACT:** A quantitative preparation method is described in order to enable high precision coccoliths analysis with the scanning electron microscope (SEM). This technique is fast and easy to apply and very thorough in the counting procedure. An equivalent basis for the comparison of datasets from sediments and water column is accomplished.

## INTRODUCTION

There is a current demand to improve micropaleontological investigations in order to calculate absolute abundances of microfossils. A high precision counting is required for the comparison with other planktic groups as in synoptic studies (Samtleben et al. 1995) or for detailed understanding of coccoliths assemblages of Late Quaternary to Recent age (Andruleit 1995). In the past few years an increasing number of new preparation techniques for coccoliths counting have been published aiming for quantitative analysis (Baumann 1990; Beaufort 1991; Henriksson 1993; Okada 1992; Samtleben and Schröder 1992; Wei 1988). However, each method has its certain restrictions. For example, extensive processing may cause alterations of the assemblages, or studies using the light microscope may have taxonomic problems. Also, a corresponding method is needed for comparison of coccoliths fluxes obtained from sediment trap investigations with coccoliths accumulations in the sediments. The presented method combines techniques from different applications to improve quantitative coccoliths analysis and allows the comparison of coccoliths assemblages from differing datasets.

## PROCEDURE

The whole procedure is summarized in text-figure 1.

### Part A:

Step 1: Freeze dry the sediment sample

Step 2: Weigh about 0.05 to 0.5g of the freeze-dried sediment on a high precision balance (the amount depends on the coccoliths content).

Step 3: Dilute the sample with about 10ml of water (e.g. carbonate saturated tap-water or demineralized water buffered by ammonia with pH=9).

Step 4: Ultrasonicate the suspension for about 15 sec. If there are still small lumps visible repeat the ultrasonic treatment for another 5 to 10 sec.

Step 5: Fill the suspension up to about half a liter or more and homogenize by thorough shaking.

### Part B:

Step 6: Split the diluted suspension by using a rotary splitter at about 40 to 50 rotations per minute. To minimize splitting errors mix the opposite sample splits together after each splitting round. Optimal splits are mostly accomplished with the 1/256 fraction (i.e. split factor=256). Depending on the amount of sediment or coccoliths content other split fractions may be more suitable.

### Part C:

Step 7: Filter the split fraction through Millipore filter (0.45µm pore width) by means of a vacuum pump.

Step 8: Dry the filter for about six to twelve hours at 40°C.

Step 9: Cut a small piece (about one cm<sup>2</sup>) out of the filter and prepare it for the SEM

Step 10: Investigate species composition and abundances on measured transects.

The numbers of coccoliths per gram dry sediment can then be calculated as follows:

$$\text{Coccoliths [no.g}^{-1}] = \frac{F \cdot C \cdot S}{A \cdot W}$$

F = filter area [mm<sup>2</sup>]

C = number of counted coccoliths

A = investigated area [mm<sup>2</sup>]

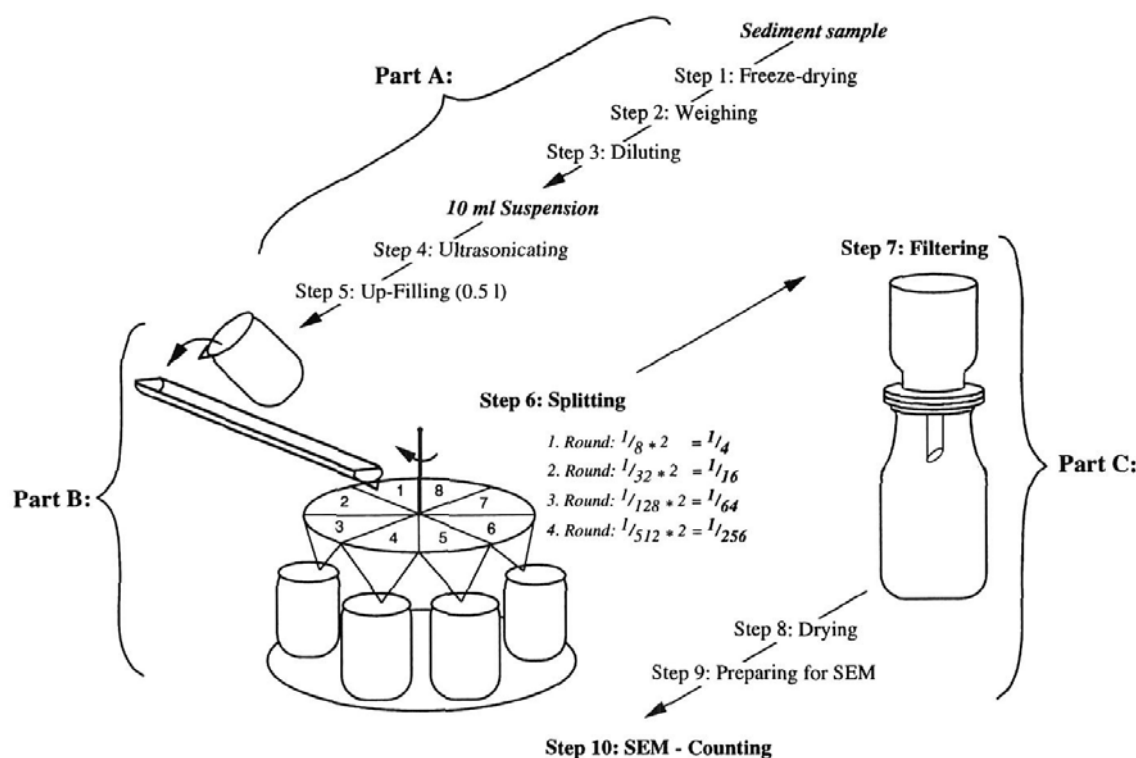
W = weight of sample [g]

S = split factor

### Reproducibility

The whole procedure was already applied to about 150 sediment samples of Holocene age from the northern North Atlantic (Andruleit 1995). The reproducibility of the counts mainly depends on 1) the homogeneity of the particle distribution on the filter and 2) the precision of the splitting.

1) Standard counts were performed on areas of only 0.05mm<sup>2</sup> in size with a magnification of 3000 to 5000. The homogeneity of the particle distribution is documented by the comparison of the counts of two different areas of the same examined filter



TEXT-FIGURE 1  
Graphical illustration of the preparation technique.

(text-fig. 2). Generally, the counts of at least two areas should be added together. On some occasions, greater differences between the two areas have been observed. In such cases the areas have to be enlarged until enough specimens (normally about 300) are identified. Counting can be done in two steps: a) first counting the common and ubiquitous species and b) repeated counting of the rare species or sporadically occurring intact coccospheres on enlarged areas, if necessary at lower magnification.

2) To test the overall reproducibility of this method a box core profile of early Holocene to Recent age from the Norwegian Sea was examined twice. From each depth two sediment samples were weighed and independently processed. Absolute abundances of the dominating species *Coccolithus pelagicus* and *Emiliania huxleyi* exhibit a high similarity (text-fig. 3). It is also obvious that the agreement of the relative abundances is very good and does not seem to be influenced by slight differences in absolute abundances (text-fig. 3).

## DISCUSSION

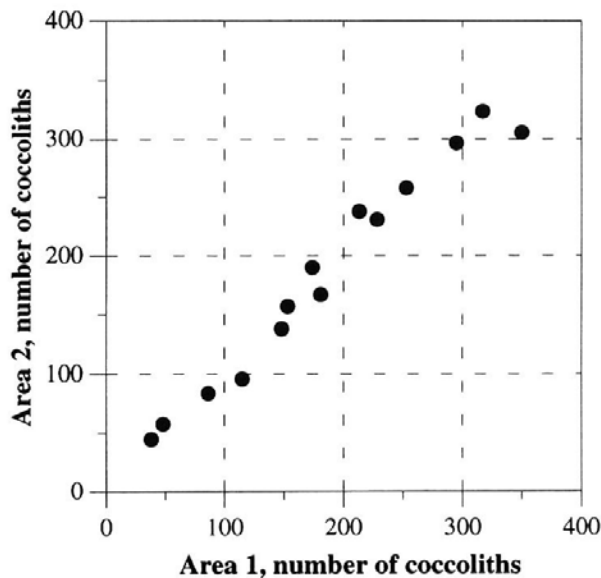
The dilution/filtering method was originally developed by McIntyre and is described in Backman and Shackleton (1983). But it has not been accepted as a standard method because smear-slide investigations are much faster and yield similar abundance variations despite of being semi-quantitative. However, this method has been adapted to current investigations comprising samples from water column and sediments considering the demand for the comparability of different datasets. Furthermore, a detailed analysis on species level is accomplished simultaneously.

The whole procedure consists of distinct parts for different purposes which are modified for quantitative coccoliths analysis (text-fig. 1). The filtering technique (part C) is commonly used for plankton investigations of living coccolithophore communities. Sea water is filtered through e.g. Millipore filters without any preceding processing, and counting can be done by light microscopy (e.g. Okada and Honjo 1973) or SEM (e.g. Samtleben and Schröder 1992). The splitting technique (part B) is applied for investigations of sediment trap samples for bulk parameter (e.g. v. Bodungen et al. 1991) or plankton groups (e.g. Bathmann et al. 1991, Samtleben et al. 1995). Part A is required to prepare sediment samples for processing in part B and C. Combining all three parts, a single technique is available for all purposes. This approach avoids methodological discrepancies and thus allows actuo-paleontological studies which compare data from plankton, sediment trap and sediment on the basis of coccosphere units (according to Samtleben and Schröder 1992) to calculate coccolithophore/coccoliths flux and accumulation.

## Comparison with other methods

All methods for quantitative coccoliths counting which were previously published can be divided into two main groups: A) light microscopic and B) SEM techniques.

A) Methods using the light microscope are known to be rather fast in processing and counting (e.g. Henriksson 1993, Okada 1992). However, the small size of many species (e.g. the group "small placoliths") or the high diversity of some genera (e.g. the *Syracosphaera* genus) may hamper a quantitative and taxonomic complete analysis. Also, fragments of coccoliths are difficult to recognize and thus have to be neglected in most cases.



TEXT-FIGURE 2

Comparison of coccoliths counts of two different areas of the same examined filter, performed at a box core profile from the Aegir-Ridge, Norwegian Sea (GIK 23411, 65°47.9'N / 03°30.6'E; 2849m water depth).

B) There are only few SEM methods for quantitative coccoliths counting published yet. Samtleben and Schröder (1992) applied a modified settling method according to Atterberg. The whole procedure takes about four to five weeks during which the coccoliths stay in contact with demineralized water. Despite of buffering with ammonia, etching is a common phenomenon indicating the influence of dissolution. Thus, alteration of the coccoliths assemblages cannot be ruled out.

Generally, the presented method is a combination of different techniques being fast and easy to apply and very thorough in the counting procedure. Due to the careful and short sample processing an alteration of the coccoliths assemblages by mechanical breakage or dissolution is negligible. However, ultrasonication should be done as briefly as possible to avoid fragmentation of delicate species.

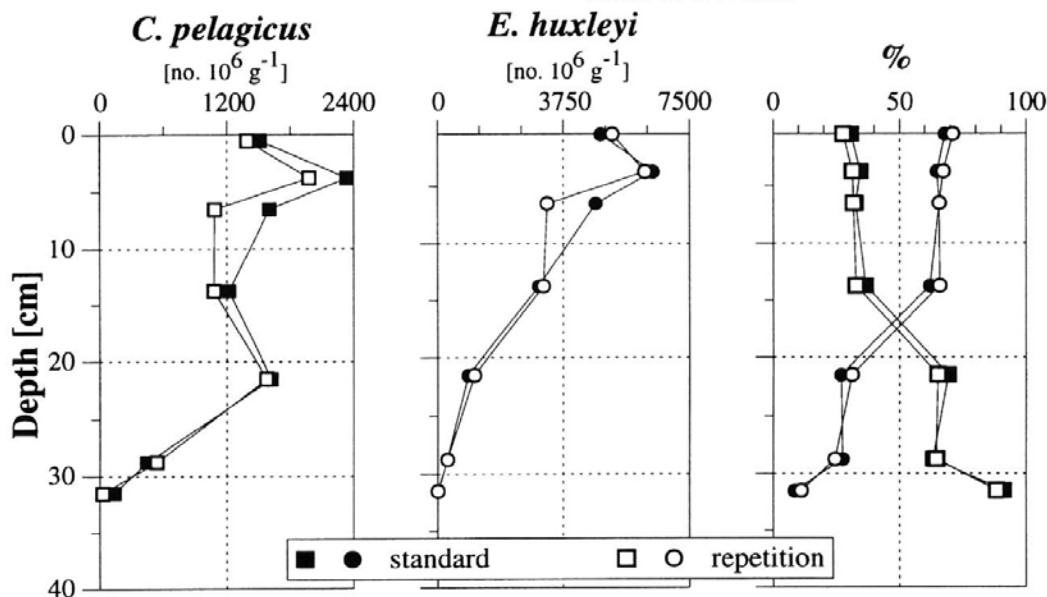
The difficulty to get coccoliths evenly distributed on slides as discussed by Beaufort (1991) is overcome due to the active filtering. All particles are randomly distributed on the filter surface without size fractionation that is common during smear slide preparation. Also, no disturbance by settling or evaporation processes occurs. Due to the small area size the counting under the SEM can be done rather fast and with very high precision.

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TEXT-FIGURE 3

Repeated counting of the dominant species *C. pelagicus* and *E. huxleyi* for absolute and relative abundances performed at a box core profile from the Aegir-Ridge, Norwegian Sea (GIK 23411, 65°47.9'N / 03°30.6'E; 2849m water depth), ranging from Early Holocene to recent age. Other species are very rare and are not considered for relative abundances.

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