

COMPARISON OF DIFFERENT PREPARATION TECHNIQUES FOR QUANTITATIVE NANNOFOSSIL STUDIES

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Abstract: Four different preparation techniques to calculate coccolith abundances, using the scanning electron microscope (SEM) as well as the light microscope (LM), are briefly described and compared to each other. Sample preparation techniques applied include smear-slides, a modified settling method for the LM, a modified settling method for the SEM, and a filtration technique for the SEM. All samples were prepared from the same site (ODP Site 643, Norwegian Sea). In addition, the reproducibility of the individual counts, and the homogeneity of the individual methods, were tested and the data of the individual counts have been checked with each other.

Introduction

In the past few decades, calcareous nannofossils have been increasingly studied for palaeoceanographic and palaeoecologic purposes. A great number of different methodologies are, therefore, available for both investigations using scanning electron microscope (SEM), as well as light microscope (LM), analysis. Generally, the introduction of the SEM has greatly improved calcareous nannofossil studies. This instrument has contributed to the identification of small forms (e.g. nannoliths, holococcolithophores, etc.), to the ultrastructure of most of the taxa and, thus, to the taxonomy of the coccolith species. However, although SEM studies provide a better resolution of coccolith ultrastructure, calcareous nannofossils are still most commonly studied with the LM. Studies of calcareous nannofossils have lead to advances in palaeoceanographic studies most probably due to more-detailed and precise data (e.g. Backman *et al.*, 1986; Gard, 1988; Williams & Bralower, 1995; Flores *et al.*, 1997; Andruseit & Baumann, in press). Stronger emphasis was put on techniques applicable to absolute counts of calcareous nannofossils. Several different preparation techniques for quantitative studies have been introduced (Backman & Shackleton, 1983; Wei, 1988; Beaufort, 1991; Okada, 1992; Henriksson, 1993; Andruseit, 1996). However, with the exception of Backman & Shackleton (1983), who compared results of smear-slide analysis with counts of absolute coccolith abundances obtained by a procedure developed by McIntyre for SEM examinations (see also Backman & Shackleton, 1983), tests of the reproducibility and accuracy of the methods have only been carried out on a few samples using the method described in the respective paper (Wei, 1988; Beaufort, 1991). Thus, a comparison of different methods is still an outstanding problem.

Consequently, we experimented with different preparation techniques and used SEM as well as LM for quantitative analysis. Techniques include a modified settling method for LM samples (Beaufort, 1991; Su, 1996), a settling method for SEM samples (Baumann, 1990; Baumann & Matthiessen, 1992), and a filtration technique for SEM (Andruseit, 1996). In addition, another technique to calculate absolute numbers of coccoliths, which we at least would like to enumerate, proposes to use a known

amount of microbeads to estimate the absolute abundance of nannofossils (Laws, 1983; Okada, 1992). This methodology is used by palynologists (Stockmarr, 1971) and is also a standard technique for the preparation of dinoflagellate cysts (see Baumann & Matthiessen, 1992). However, since we have not experimented with this method we cannot comment on it.

Samples were all prepared from the same site (ODP Site 643, Norwegian Sea) and combined with previously published data derived from smear-slide counts (Gard, 1988). Abundance data of calcareous nannofossils in the Late Pleistocene section of this site have previously been shown to be highly variable and only of low diversity (Gard, 1988; Henrich & Baumann, 1994). Taxonomic uncertainties may therefore be negligible, making this site appropriate for the purpose of our study. In addition, the reproducibility of the individual counts and the homogeneity of the individual methods was tested.

Brief descriptions of applied preparation techniques

Smear-slide analysis:

Analysis of smear-slides is still a commonly used technique for calcareous nannofossil studies (Gard & Backman, 1990; Flores *et al.*, 1997). This method is fast, easy and only a small amount of sediment is needed. Slightly different techniques to prepare the slides are used by different nanno-workers. In general, a tiny amount of sediment is mixed with a drop of water and spread evenly across a microscope cover glass (see also Roth, 1994). After the suspension has dried on a hot plate, the cover glass is mounted on a glass slide with a mounting medium. Different mounting media can be used (see van Heck, 1996).

Backman & Shackleton (1983) have introduced the use of smear-slides as a semi-quantitative method and used the data to refine the precision of some nannofossil datums for biostratigraphic purposes. The preparation is as usual, but the abundance data is expressed as units of slide area (number of specimens/mm²).

Modified random settling method:

In order to prepare a large number of samples for LM analysis, the method proposed by Beaufort (1991) has been slightly modified (Su, 1996) and is briefly explained in the following. In principle, this technique is adapted from a

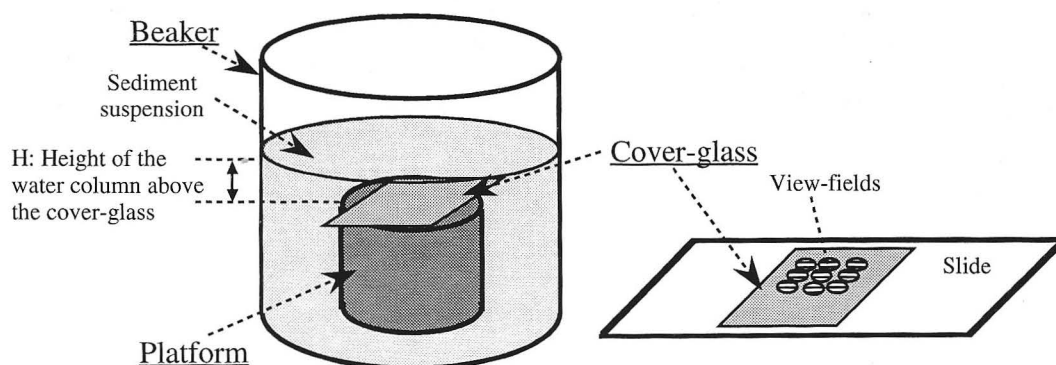


Figure 1: Schematic illustration of the parameters involved in the settling technique and the calculation of coccoliths per g of sediment (modified after Beaufort, 1991).

method described by Moore (1973), which is most commonly used for the study of radiolarians.

A certain amount of the dried sediment sample (about 0.005g) is weighed and diluted in a beaker with a given volume (200ml) of buffered water (pH of ~8.5). It is ultrasonicated for about 10 seconds and stirred/shaken thoroughly for several minutes, in order to get a homogenised sediment suspension. The suspension is carefully poured into a beaker in which a coverglass attached to a platform has been previously placed before (Figure 1). We have limited the height of the sediment suspension above the coverglass to a few millimetres. The beaker is put into an oven (40°C) where particles settle randomly on the coverglass while the water slowly evaporates. After the water level has dropped below the coverglass (usually after 2-5 days) it is mounted on a slide and counting analysis using LM can then be carried out. Usually more than 400 coccoliths on a slide were counted. The absolute number of a species can then be calculated as following:

$$\text{Coccoliths (No./g sed.)} = \frac{A \times V}{N \times S \times G \times H}$$

A = number of counted coccoliths

V = volume of the sediment suspension (ml)

N = number of view-fields investigated

S = surface area of view-fields (mm²)

G = weight of sample (g)

H = height of water column above coverglass (mm)

Modified SEM settling method:

In order to count coccoliths in clay-rich sediments by means of SEM, the sample is cleaned from the <2µm fraction by using a settling technique (Baumann & Matthiessen, 1992). Therefore, the sediment sample (about 200mg) has to be diluted in 200ml test-tubes with buffered water (0.01N NH₄). Every 24 hours the upper half of the suspension is sucked off, new solution added and homogenised with the sample. This process is repeated until the supernatant was nearly clear (usually 5-8 days with the clay-rich sediments at Site 643, but only few hours to 1-2 days for carbonate-rich sediments).

A drop of the suspension is placed on a round coverglass, pasted on a SEM stub when it has dried, and

then sputter-coated with gold-palladium. For quantitative analysis, micrographs of an arbitrarily selected part of the scanned sample are taken and all particles (usually >1500 particles!) are counted. Upto about 800 coccoliths are counted in the sample using x2000 magnification. The quantitative data are recorded as particle percent (= grain percent) for coccolith species.

Filtration technique for SEM:

Another quantitative method to enable high precision coccolith counts with the SEM uses a combined dilution/filtration technique (Andrulleit, 1996). A small amount of freeze-dried sediment is weighed (generally 0.05 to 0.1g) and put into suspension (about 100ml). After dilution with a rotary splitter (our optimal split is about 1/100), the suspension is filtered through a polycarbonate membrane filter (from Schleicher & Schuell™, 0.4µm pore-size) or a cellulose nitrate filter (from Satorius AG with 0.45µm pore-size). After drying the filter at 40°C, a section of the filter can be studied by means of SEM. For more details of the whole procedure see Andrulleit (1996). The number of coccoliths per gram dry sediment was calculated as follows:

$$\text{Coccoliths (No./g sed.)} = \frac{F \times C \times S}{A \times W}$$

F = filter area (mm²)

C = number of counted coccoliths

S = investigated area (mm²)

W = weight of sample (g)

S = split factor

In general, this approach allows one to directly compare data of the plankton and sediment traps with coccolith counts of the sediment.

Reproducibility and homogeneity of the individual methods

The internal reproducibility of different preparation methods (also those which have not been applied here) have already been discussed by Beaufort (1991). Nevertheless, reproducibility of the individual counts and the homogeneity of the individual methods presented have been checked.

The reliability of the abundance counts from the smear-slide procedure has already been tested on a sedi-

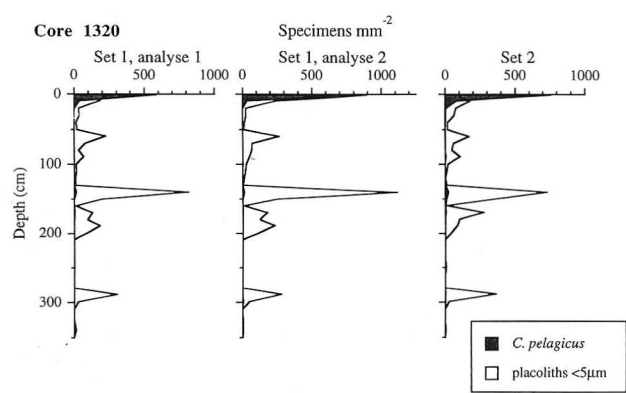


Figure 2: Comparison of abundance curve patterns which result from duplicate smear-slide preparation (modified after Gard, 1988).

ment core, which was prepared twice and counted three times for its content of coccoliths (Figure 2; Gard, 1988). The magnification was been x750 and x800, and the total area observed varied from between 1.1 and about 1.6mm². The abundance patterns were generated to specimens per mm² as described above. In general, Gard (1988) reported that the deviation in sediments with increased abundances of coccoliths (>20 specimens per mm²) is less than 40%, while variation is much higher in samples with fewer coccoliths. However, only minor differences occurred between the three abundance patterns generated and, obviously, reproducibility further increased with increasing coccolith number.

The comparison of the semiquantitative data of different samples, however, is heavily affected by the density and distribution of the grains on the glass slide. Even slight differences in the amount of sediment smeared on the slide, and/or an uneven distribution of the particles, may result in much higher differences than observed by Gard (1988). Our study reveals that the number of coccoliths can increase by upto a factor of three when preparing slightly thicker slides of a single sample (Table 1). Nevertheless, slides prepared by only one person could certainly be used for counting semi-absolute abundances in a fast and accurate way, as shown by Backman & Shackleton (1983) and Gard (1988). In addition, results obtained by this technique are highly comparable to SEM counts using a filtering technique which allowed calculation of coccoliths per gram of sediment (see Backman & Shackleton, 1983).

The reliability of the abundance counts using the modified settling technique for LM was tested by repeated counts of distinct species in separately produced slides (Table 2). Generally, slides produced with this technique show an even distribution of particles. This is demonstrated by the fact that repeatedly counted numbers within one slide deviate by less than ±4% of each other. In addition, comparison of duplicate slides gives a high reproducibility of about ±7%.

The high reproducibility of the settling technique has also previously been illustrated. Beaufort (1991) counted duplicate slides of different samples and got a correlation (r²) of 0.98, while Williams & Bralower (1995) demonstrated that the percentages of individual taxa in duplicate slides

Sample (Site 608)	Coccolithus pelagicus		Discoaster spp.	
	Slide 1 ("thicker")	Slide 2 ("thinner")	Slide 1 ("thicker")	Slide 2 ("thinner")
	No./mm²	No./mm²	No./mm²	No./mm²
10-3, 66-71	310	116	114	34
11-3, 70-74	312	148	100	68
12-6, 88-93	104	50	80	26
13-5, 67-71	332	220	274	152
14-2, 54-58	281	137	48	33
14-5, 91-96	205	83	81	21
16-3, 88-93	1329	657	191	54
17-3, 64-68	385	246	46	11

Table 1: Results of repeated counts on differently prepared smear-slides from samples at Site 608 (all counted at magnification x1000). Numbers of C. pelagicus and Discoaster spp. were calculated according to the procedure given by Backman & Shackleton (1983).

Sample (Site 608)	Sample weight	No. of Discoaster spp.		
		Count 1	Count 2	Count 3
17-3, 52-53	0.0037g - slide 1	308	309	305
	0.0037g - slide 2	322	313	319
	0.0037g - slide 3	301	290	299
	0.0013g - slide 4	114	111	110
17-2, 102-103	0.0010g	43	43	42
17-4, 2-3	0.0013g	71	69	73
17-CC, 18-19	0.0018g	110	110	108

Table 2: Results of repeated counts on slides using the modified settling technique (after Beaufort, 1991). Note that the data have not been calculated as nannofossils per g of sediment.

(and also in four separately produced slides) always were less than ±5% of the original slide. In addition, the latter authors showed that counts are highly comparable to each other, even if the number of counted individuals drastically varies.

To test the reproducibility of the settling technique for SEM analysis, counts were carried out on 10/12 different samples of Holocene age. Duplicate samples were prepared and one of the sample series was counted twice. Comparison of the two repeated counts of the same sample shows that the variation in the numbers of coccoliths was lower than 5%, while the duplicate samples show higher differences (Figure 3). The results vary in parallel, although differences of up to 25% were observed.

In general, this technique is simple and, in addition to that, technical as well as financial expense is minimal. The data can be compared to component analysis of the grain-fraction. Samples must not be separated into different grain size fractions as described by Samtleben & Schröder (1992). These samples are also suitable for other investigations, such as biometric studies, due to the mostly plane position of the coccoliths.

The quality of the filtration technique for SEM was tested on box-core sediments from the Norwegian Sea which were examined twice (Figure 4). From each depth, two samples were separately prepared and counted. The graphical comparison of the two datasets already underlined the high reproducibility of this method (Andrleit, 1996). The mean deviation between the repetitive counts for absolute abundances of coccoliths in samples with more than 100 counted specimens was

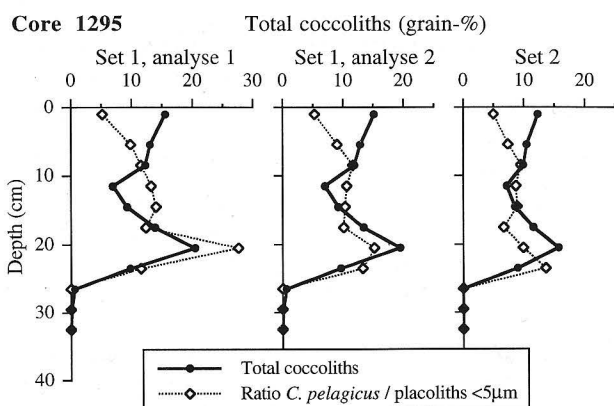


Figure 3: Comparison of abundance curve patterns of separately produced samples, using the settling method for SEM.

lower than 10%. Mean deviation of relative abundances of species was even better with a value of lower than 7%.

Generally, this method is relatively fast (less than 45 minutes), easy to apply and very thorough in the counting procedure. The careful and short sample-processing minimises alteration of coccoliths due to mechanical breakage or dissolution. All particles are evenly distributed on the filter surface without any fractionation due to the active filtering. One advantage of this method compared to all others is the range in the amount of sediment used. One can use very small amounts if the material is rare, but it is also appropriate to use a larger amount of sample material to minimise errors due to inhomogeneity of the sediment. Larger sample weights may increase the homogeneity of the sediment sample.

Comparison of the different preparation techniques

The patterns of absolute coccolith abundance variation generated by the four different methods applied are similar to each other (Figure 5). All major abundance features are present in each of the countings and the results generally vary in parallel, although there are differences in the absolute size of individual peaks. While most of the relative peak variations between the smear-slide estimates, the SEM settling method and the filtration technique for SEM (Figure 5a, b, d) are essentially identical, differences mainly occur in comparison to the settling technique for LM (Figure 5c). Nevertheless, we believe that there is a fairly high correspondence between the datasets. The peak in the dataset obtained by the settling technique for LM (Figure 5c) at 2.40m does not have a representative sample in any of the other datasets, while the peak at 7.65m nicely agrees with a corresponding data-point of the settling technique for SEM (Figure 5a). Except for this latter finding, the absolute number of coccoliths per gram of sediment in the uppermost 12m at Site 643 matches very well the settling technique for LM and the filtration technique for SEM (Figure 5c, d). Obviously, the absolute numbers vary by a factor of two to three below 12m in the coccolith-rich assemblages of isotope stages 11, 13 and 15. However, this is possibly due to the fact that a less dense dataset has been gained by the settling technique for LM. The general features of coccolith variation in this section are highly comparable between all preparation techniques.

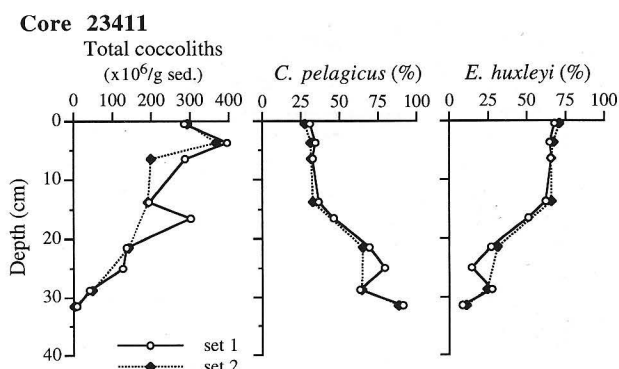


Figure 4: Repeated countings of the total coccolithophores and the relative abundance of *C. pelagicus* and *E. huxleyi* in core 23411 from the southernmost Norwegian Sea (modified after Andruseit, 1996).

Unfortunately, a direct comparison of the individual counts is not possible, since the data collection has been developed during the last few years. Many of the samples which have been prepared as smear-slides (Gard, 1988) and/or by the SEM settling method (Baumann, 1990) were not available for absolute quantification of coccoliths using the settling technique for LM as well as the filtration technique for SEM. Even the numbers of available samples for the applied techniques vary between 53 and 130 (see Figure 5). This fact has also influenced the apparently most conspicuous differences between the results of the settling technique for LM as well as filtration technique for SEM (Figure 5c, d).

Thus, our experiment seems to remain a little superficial, although countings performed after the described methods yielded relatively similar results even at species level (Figure 6). Therefore, at least trends in the development of the coccolithophore assemblages are regarded as being comparable between the different methods.

Nevertheless, each method has its particular restrictions, disadvantages and profits and, therefore, the technique which should be used mainly depends on the types of questions to be answered. Extensive and time-consuming processing via the settling methods in general may cause alterations of the assemblages. Standard smear-slides, while easy to prepare, can only provide semiquantitative data. Additional ideas for estimating absolute abundances of nannofossils in smear-slides (Henriksson, 1993), or at least for the calculation of nannofossil accumulation rates (Flores & Sierro, 1997), have been proposed. The modified settling technique and the filtration technique need certain technical equipment (*e.g.* oven, rotary splitter, *etc.*) but are the only two, of the four techniques applied, which allow calculation of absolute calcareous nannofossil abundances in terms of numbers of coccoliths per gram of sediment. In addition, the filtration technique also avoids methodological discrepancies between water-samples, sediment-trap material, and sediment samples, and allows direct comparisons between data from the plankton with coccolith counts of the sediment. The latter fact is the reason why we usually use the filtration method (Baumann, Andruseit), although we also use the settling technique for LM (Su).

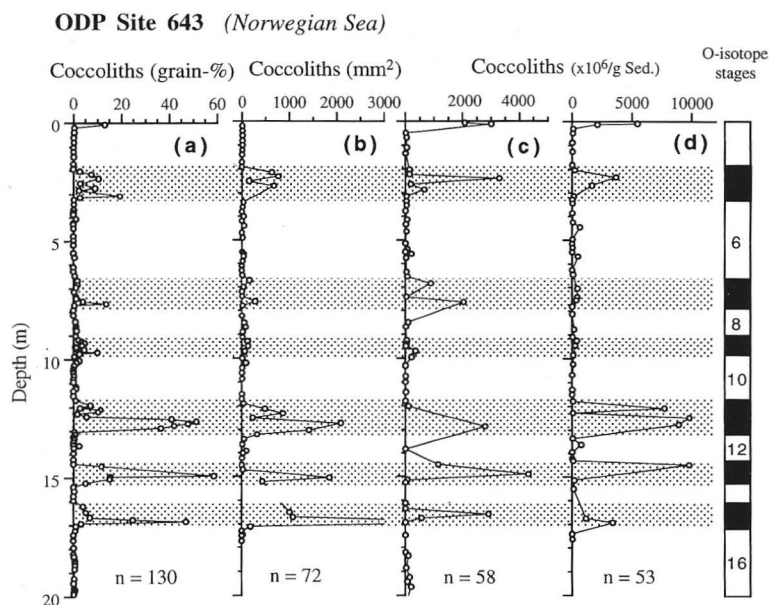


Figure 5: Comparison of abundance curve patterns to demonstrate the reproducibility of the applied techniques (a = settling technique for SEM, b = smear-slide data, c = settling technique for LM, d = filtration technique for SEM). Quite obviously, the patterns generated by the different methods vary in parallel. Differences may also be due to different sample availability, since the data has been collected over past years.

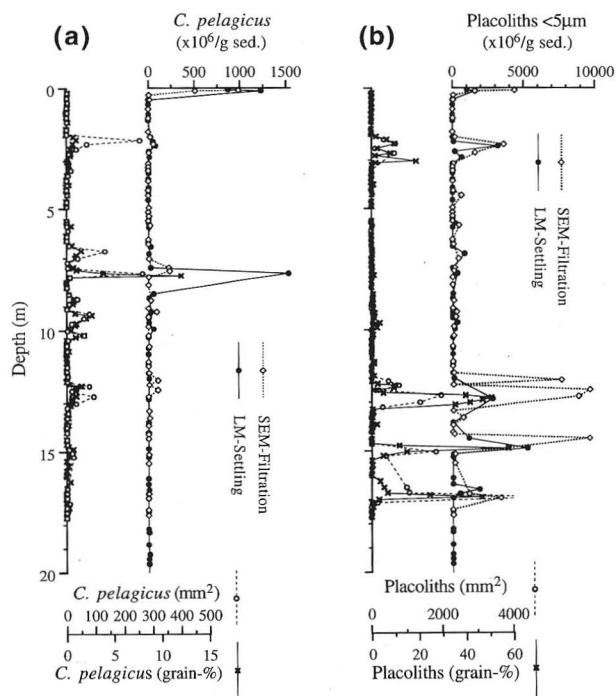


Figure 6: Variations in the numbers of the dominant species between the applied techniques: (a) *C. pelagicus*, (b) placoliths = *E. huxleyi* and *G. muelleriae*.

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