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Determination of absolute coccolith abundances in deep-sea sediments by spiking with microbeads and spraying (SMS-method)

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Abstract

A quick new method is described for the quantification of absolute nannofossil proportions in deep-sea sediments. This method (SMS) is the combination of Spiking a sample with Microbeads and Spraying it on a cover slide. It is suitable for scanning electron microscope (SEM) analyses and for light microscope (LM) analyses. Repeated preparation and counting of the same sample (30 times) revealed a standard deviation of $\pm 10.5\%$. The application of tracer microbeads with different diameters and densities revealed no statistically significant differences between counts. The SMS-method yielded coccolith numbers that are statistically not significantly different from values obtained from the filtration-method. However, coccolith counts obtained by the random settling method are three times higher than the values obtained by the SMS- and the filtration-method. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: coccoliths; absolute abundance; microbeads; preparation technique

1. Introduction

There is an increasing need for absolute calcareous nannofossil counts in order to calculate coccolith fluxes from the photic zone into sediment traps or to estimate the varying coccolith carbonate accumulation rates in geological records. In the past, several different techniques were developed, although there are currently only two basic methods that are in use: (a) the random settling technique introduced by Beaufort (1991) and modified by different authors (Williams and Bralower, 1995; Su, 1996; Flores and

Sierro, 1998) and (b) a filtration technique described by Backman and Shackleton (1983) and modified by Andruleit (1996). The accuracy and reproducibility of both techniques depends on three factors: (1) the even distribution of the particles on a filter or on a cover slide, (2) the assumption that no size-dependent fractionation of coccoliths occurred during the preparation procedure, and (3) that there is no loss or bias due to splitting procedures, filter funnels or settling devices.

Here, we demonstrate the application of the combination of two techniques that have been already applied or suggested for nannofossil analyses: (a) a spraying technique used by McIntyre et al. (1967) and (b) the addition of microbeads as tracer parti-

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cles (suggested by Okada, 1992). Furthermore, we compare the results obtained by this new method with the counts obtained by the filtration- and the settling-method.

1.1. Background

McIntyre et al. (1967) used a spray gun in order to distribute coccoliths of various sizes homogeneously on a slide and Okada (1992) suggested adding a known weight of microbeads (Potter Ballotini Inc., type MB10) to a sediment sample in order to calculate absolute abundances of nannofossils. Okada (1992) assumed a constant number of microbeads per unit weight without knowing the real number of microbeads per weight unit. Therefore, his method enables only the counting versus a relative standard but not the calculation of absolute nannofossil content per gram sediment. However, Okada (1992) also suggested calibrating the number of microbeads per weight unit using a suitable standard if this standard were available.

Adding tracer particles or tracer chemicals is a common method, especially in chemistry, to calculate the unknown amount of a component. Particle tracers have been used by micropaleontologists since the 1960's (Benninghoff, 1962; Stockmarr, 1971). Benninghoff (1962) added a known number of *Ly-copodium* spores to a sediment sample in order to calculate the absolute abundance of pollen per gram sediment. However, until now it was not possible to apply this simple concept to coccolith counts because (a) no uniform tracer particles were available in the size range of coccoliths and (b) it was difficult to calculate the number of tracer particles that had to be added to a sample with an acceptable standard deviation.

2. Materials and methods

2.1. Microbeads

Microbeads of uniform diameter have recently become available commercially and thus allow a simple theoretical calculation of microbeads per solid weight (instead of enumerating complete aliquots, see Stockmarr, 1971) with a standard deviation of

$\leq 2\%$. These microbeads are made of polystyrene, latex or borosilicate and they are available in many different sizes, accuracy of diameter and physical and chemical properties. In order to use these microbeads for calcareous nannofossil counting they have to be stable in water suspensions with a pH of 8.5 or in alcohol. Furthermore, they have to be resistant to ultrasonic treatment and they should not form large aggregates during the preparation procedure.

We used two different kinds of microbeads that meet the above-mentioned requirements in our new method: (a) polystyrene microbeads (mean diameter $4.0 \mu\text{m} \pm 0.06 \mu\text{m}$ standard deviation; density = 1.09 g/cm^3) and (b) borosilicate microbeads (mean diameter $5.1 \mu\text{m} \pm 0.8 \mu\text{m}$, density = 2.5 g/cm^3 ; for details see Table 1).

The number of microbeads per solid weight (e.g. 1 g) can be calculated as follows:

$$M_{\text{TOT}} = \frac{1}{W_{\text{M}}} = \frac{1}{\rho_{\text{M}} \times V_{\text{M}}} = \frac{1}{\rho_{\text{M}} \times \frac{4}{3} \times \pi \times r^3} \quad (1)$$

where M_{TOT} = number of microbeads per gram; W_{M} = weight of one microbead; ρ_{M} = density of one microbead; V_{M} = volume of one microbead.

2.2. Sediment samples

In order to test the new method we used two different sediment samples: (a) the fine fraction ($<38 \mu\text{m}$) of a pure coccolith ooze from the mid-Pleistocene *Gephyrocapsa* dominance interval (Bollmann et al., 1998) of North Atlantic DSDP Hole 607 that is composed of 93% of *Gephyrocapsa* placoliths (607, 2–2, 29–32 cm), and (b) a hemipelagic Holocene sample (bulk) with low carbonate content (Blagnac sample: off Cape Verde; W $18^\circ 15.1$; N $21^\circ 19.76$; 2002 m water depth). This sample was already used for an international intercalibration experiment between thirteen nannofossil experts. Until now, the results of this experiment have not been published and therefore, we show only our own determinations (Table 2).

2.3. Spraying device

We used an Effa Spray Mounter and small glass capillaries (for details see Table 1). The distance between the target and the spray mounter was found

Table 1
Materials used for SMS preparation

Item	Ordering No.	Amount	Price	Supplier
Polystyrene Microbeads; diameter: $4.0 \pm 0.06 \mu\text{m}$; density: 1.0^9 g/cm^3	Product number: PS055N/001168; Inv. Nr. L970305E	1 g	\$ 270,-	Bangs Laboratories, Inc., 9025 Technology Drive, Fishers, IN 46038-2886 USA, e-mail: info@bangslabs.com
Borosilicate Microbeads; diameter: $5.1 \pm 0.8 \mu\text{m}$; density: 2.5 g/cm^3	Product number: 9005; LOT Nr. 19324	1 g	~\$ 150,-	Duke Scientific Corp., 2463 Faber Pl., Palo Alto, P.O. Box 50005, CA 94303, USA, e-mail: info@dukesci.com
Spray gun	11805 or 11800	1	\$ 95,-	Ernest Fullam., 900 Albany Shaker Rd. Latham, New York 12110, USA, e-mail: sales@fullam.com
Small glass capillary tubes	11802	100	\$ 13,-	Ernest Fullam., 900 Albany Shaker Rd. Latham, New York 12110, USA, e-mail sales@fullam.com
Triton X 100 detergent	X100	100 ml	\$ 28,-	Sigma Chemical Company, P.O. Box 14508, St. Louis, Missouri 63178-9916, USA
Lasert granulometer, GALAI CIS 1				L.O.T. – Oriel GmbH, D-64295 Darmstadt, Germany

to be optimal at ca. 20 cm with respect to particle density and distribution.

2.4. Sample preparation

2.4.1. Spraying (Figs. 1 and 2)

Step 1. Microbeads and dry sediment are weighed with a high-precision balance (e.g. 1.0 mg with a Mettler balance AE 260 with a precision of 10^{-6} g). We suggest adjusting the ratio between the weight of microbeads and the weight of sediment in order to avoid counting excessively large numbers of microbeads with low numbers of coccoliths, or vice versa. For pure coccolith oozes a weight ratio of microbeads (polystyrene) to coccoliths ($<38 \mu\text{m}$ fraction) of 2 to 1 is recommended and for samples with low carbonate content (e.g. 40%) we recommend to use a ratio of 1 to 4. If bulk samples are used, we suggest increasing the amount of sediment to a minimum of 10 mg in order to avoid inhomogeneous samples. However, if fine-fraction samples are used about 1 mg sediment is sufficient in order to guarantee homogeneous samples.

Step 2. Polystyrene microbeads and sediment are suspended in 2 to 4 ml of denatured alcohol. We used alcohol in order to provide a homogeneous mixing between polystyrene microbeads and sediment because of the comparatively low density of

these microbeads. Borosilicate microbeads and sediment are suspended in 2 to 4 ml water. We buffered the water with NH_3 to a pH of 8.5 in order to avoid dissolution or overgrowth of coccoliths. In addition, a very small amount of Triton X 100 detergent was added to avoid coagulation of the particles.

Step 3. The suspension is ultrasonicated for about 30 s at 35 kHz.

Step 4. The suspension is sprayed onto a cover slip (5 to 10 times) using small glass capillaries (Figs. 1 and 2). However, for routine work we recommend using a 5 ml syringe in combination with a glass capillary (Fig. 2). The syringe is filled with the suspension and the suspension is sprayed onto the target. This offers the advantages of (a) spraying a complete suspension onto a target without refilling capillaries and (b) a better control on how much material is sprayed onto the target.

Step 5. The dry cover slide is mounted on an aluminium stub for scanning electron microscope (SEM) analysis or on a microscope slide for light microscope (LM) analysis.

Detailed ordering information (Table 1) and a full description of the spraying setup is given on: http://www.geology.ethz.ch/mp/data/microbeads/beads_main.html

Table 2
Test for reproducibility and precision of the SMS-method

Sample	Method	Microbead type	Microbeads ($g \times 10^{-4}$)	Sediment ($g \times 10^{-4}$)	Microbead counts	Coccolith counts	Coccoliths ($\times 10^{10}/g$)	<i>E. huxleyi</i> ($\times 10^{10}/g$)	<i>Gephyrocapsa</i> spp. ($\times 10^{10}/g$)	<i>F. profunda</i> ($\times 10^{10}/g$)	Others ($\times 10^{10}/g$)	Error (%)
DSDP607-1	SMS	P	7.68	26.15	203	1228	4.87	–	4.53	0.20	0.13	8.01
DSDP607-2	SMS	P	5.44	15.03	180	937	5.16	–	4.89	0.14	0.13	8.54
DSDP607-3	SMS	P	5.44	15.03	201	1054	5.20	–	4.79	0.19	0.22	8.13
DSDP607-4	SMS	P	3.78	11.19	212	1045	4.56	–	4.20	0.21	0.14	7.97
DSDP607-5	SMS	P	8.14	25.72	201	1123	4.84	–	4.56	0.13	0.16	8.09
DSDP607-6	SMS	P	6.04	13.24	201	812	5.05	–	4.84	0.09	0.12	8.30
DSDP607-7	SMS	P	9.5	34.22	153	1025	5.09	–	4.73	0.20	0.20	9.05
DSDP607-8	SMS	P	4.68	14.90	203	1180	5.00	–	4.69	0.21	0.10	8.03
DSDP607-9	SMS	P	8.1	16.35	205	810	5.36	–	4.69	0.17	0.13	8.24
DSDP607-10	SMS	P	5.94	5.93	307	681	6.09	–	5.64	0.25	0.20	7.35
DSDP607-11	SMS	P	5.49	4.46	309	525	5.73	–	5.28	0.29	0.15	7.63
DSDP607-12	SMS	P	10.78	18.19	209	590	4.99	–	4.71	0.14	0.14	8.46
DSDP607-13	SMS	P	15.94	17.44	303	744	6.15	–	5.72	0.24	0.19	7.29
DSDP607-14	SMS	P	11.55	14.52	301	777	5.62	–	5.24	0.24	0.14	7.27
DSDP607-15	SMS	P	12.62	14.76	203	533	6.15	–	5.65	0.33	0.16	8.65
DSDP607-16	SMS	P	13.14	20.88	204	622	5.26	–	4.83	0.25	0.17	8.48
DSDP607-17	SMS	P	21.06	31.06	274	842	5.71	–	5.29	0.24	0.17	7.42
DSDP607-18	SMS	P	9.61	12.21	264	761	6.21	–	5.71	0.29	0.21	7.60
DSDP607-19	SMS	P	8.49	7.94	204	432	6.20	–	5.69	0.30	0.22	8.88
DSDP607-20	SMS	P	8.53	6.29	306	501	6.08	–	5.49	0.39	0.21	7.71
DSDP607-21	SMS	P	9.52	19.08	206	918	6.09	–	5.65	0.33	0.11	8.14
DSDP607-22	SMS	P	6.9	12.48	204	904	6.71	–	6.32	0.20	0.19	8.18
DSDP607-23	SMS	P	7.98	12.74	212	741	6.00	–	5.58	0.23	0.19	8.21
DSDP607-24	SMS	P	18.5	13.68	315	500	5.88	–	5.41	0.15	0.32	7.65
DSDP607-25	SMS	P	11.77	11.77	201	714	5.12	–	4.74	0.17	0.20	8.40
DSDP607-26	SMS	P	13.9	16.69	309	830	6.13	–	5.79	0.13	0.21	7.15
DSDP607-27	SMS	P	10.4	13.16	301	619	4.45	–	4.20	0.14	0.12	7.49
DSDP607-28	SMS	P	8.65	15.86	302	981	4.85	–	4.54	0.16	0.15	7.08
DSDP607-29	SMS	P	10.17	10.10	307	607	5.45	–	5.04	0.25	0.16	7.47
DSDP607-30	SMS	P	7.18	9.41	209	567	5.67	–	4.98	0.27	0.42	8.50
Average							5.52					7.98
Standard deviation							0.58					
% Standard deviation							10.53					
DSDP607-31	SMS	B	77.46	33.65	200	844	5.60	–	4.97	0.31	0.31	0.28
DSDP607-32	F + MB	B	59.68	25.68	283	1093	5.17	–	4.90	0.12	0.15	0.28
DSDP607-32	F	B	0	25.68		1093	4.50	–	4.27	0.10	0.13	
DSDP607-33	SE + MB	B	35.51	26.35	220	1133	4.00	–	3.87	0.05	0.08	0.28
DSDP607-33	SE	B	0	26.35		1133	12.34	–	11.95	0.15	0.24	
Blagnac-1	F	B	0	102.00		1276	0.27	0.083	0.08	0.04	0.06	
Blagnac-2	SMS	B	72.03	293.63	210	438	0.29	0.096	0.11	0.05	0.04	0.28

The results of the repeated preparations and counting of DSDP sample 607-2-2, 29–32 cm and the Blagnac sample are shown. In addition, the counts of the same samples obtained by the filtration and settling method are given. The abbreviations in column 'Method' are: SMS = estimates obtained by spiking with microbeads and spraying; F = estimates obtained by filtration based on particles per area; F + MB = estimates obtained by microbeads added to filtered sample (ratio microbeads/coccoliths); SE = estimates obtained by settling based on particles per area (after Williams and Bralower, 1995); SE + MB = estimates obtained by microbeads added to a settled sample (ratio microbeads/coccoliths). The type of microbead is indicated by P = polystyrene microbeads, and B = borosilicate microbeads. Note that the numbers of coccoliths per gram sediment have to be multiplied by 10^{10} . The error value is calculated from the number of microbeads and coccoliths counted and the variance in microbead sizes. See text for details.

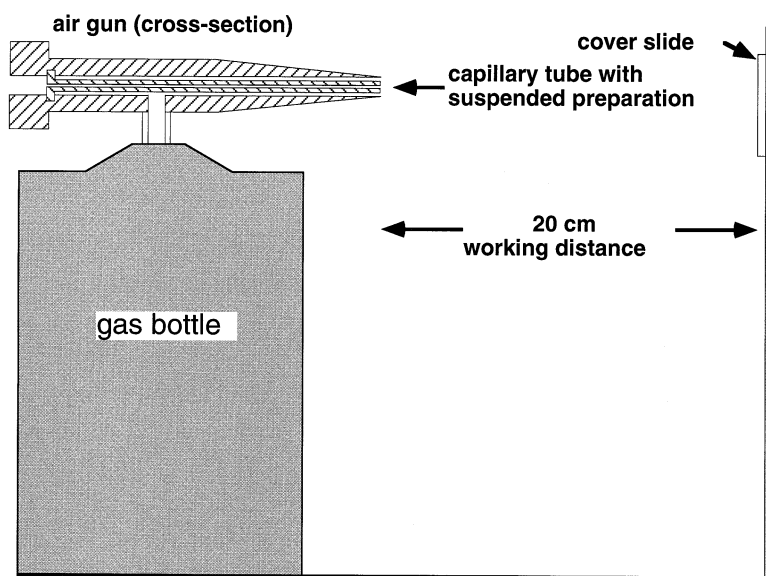


Fig. 1. Sketch of the spraying set-up. A distance of 20 cm between gun and target was found to be optimal with respect to particle distribution. Cover slides and SEM stubs can be mounted with a small strip of double-sided tape or a small strip of spray glue.

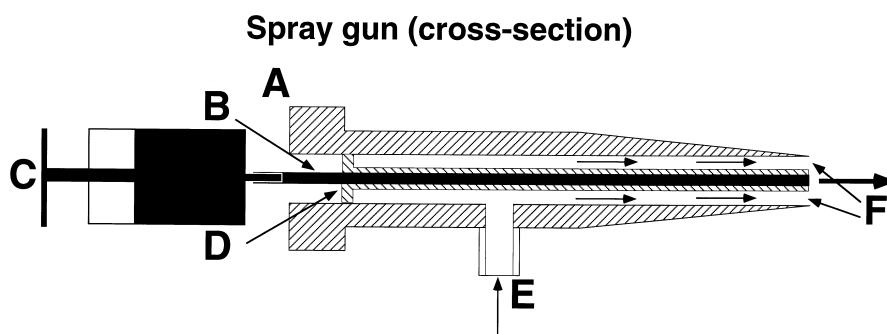


Fig. 2. Close-up of the spraying device: A = spray gun nozzle; B = capillary tube filled with sediment suspension; C = syringe with suspension; D = pipette tip; E = air pressure supply; F = underpressure. Note, for routine work we recommend using a syringe (C) in combination with glass capillaries (B). This offers the advantage (a) to spray a complete suspension onto a target without refilling capillaries and (b) of a better control on how much material is sprayed onto the target.

2.4.2. Filtration

Both samples (DSDP607 and the Blagnac sample) were prepared once using the filtration technique after Andrulleit (1996). In contrast to Andrulleit (1996), we split the sample manually (see step 3) and not with a rotary splitter. In order to obtain an independent measure of the accuracy of this method, we added a known amount of borosilicate microbeads to the DSDP sample.

Step 1 and 2 same as SMS-method.

Step 3: the suspension was manually split in

two by pouring the suspension repeatedly from one glass beaker to another until the same amount of suspension was in both beakers ($25 \text{ ml} \pm 1 \text{ ml}$). One of the beakers was filled to 50 ml with buffered water and split again as described. This procedure was repeated four to five times in order to obtain an appropriated amount of material to be filtered for optimal particle density on the filter membrane.

Step 4: the last split was filtered on a Nucleopore PC $0.8 \mu\text{m}$ membrane using a Gelman 1119 inline filter gasket.

2.4.3. Settling (after Williams and Bralower, 1995)

We used the same settling box as Geisen et al. (1999) and we placed the slides at water column height of 2 cm. In order to obtain an independent measure for the accuracy of this method we added also a known amount of borosilicate microbeads to the DSDP sample.

Step 1, 2 and 3 as SMS-method.

Step 4: the suspended sample was transferred into a volumetric flask and diluted to 1000 cm³ and homogenised by a magnetic stirrer for several minutes followed by four inversions of the flask.

Step 5: the suspension was poured into the settling device and left to settle for 24 h.

Step 6: the water was drained carefully using a drain valve at the bottom of the settling device (for details of the settling device see Geisen et al., 1999). Once the remaining water on the cover slide had air-dried the slide was mounted on a SEM stub (same as SMS-method).

2.5. Counting

All counts were done using scanning electron microscopes (HITACHI S2300 and PHILIPS XL30) at magnifications of 3000× and 6000×.

2.5.1. Spraying

All coccoliths and microbeads were counted in each field of view. The counting was terminated when at least 100 to 300 microbeads were counted (Table 2). From the ratio between counted and added microbeads the number of coccoliths per weight can be calculated as follows:

$$C_{\text{TOT}} = \frac{C_{\text{count}}}{M_{\text{count}}} \times \frac{M_{\text{TOT}}}{W_{\text{TOT}}} \quad (2)$$

where C_{TOT} = number of coccoliths in a sample; C_{count} = number of coccoliths counted; M_{TOT} = number of microbeads added; W_{TOT} = sample weight; M_{count} = number of microbeads counted.

2.5.2. Filtration

Counting on filter membranes was done according to Cortés (1998). Calculations are based on the number of particles per observed area and the number of coccoliths per solid weight (C_{TOT}) was calculated as

follows (from Andruleit, 1996):

$$C_{\text{TOT}} = \frac{F \times C \times S}{A \times W} \quad (3)$$

where C_{TOT} = number of coccoliths in a sample; F = filtration area; C = number of coccoliths counted; A = analysed filtration area; W = sample weight; S = split factor.

2.5.3. Settling

The analyses of the random settling sample were done according to Williams and Bralower (1995). Calculations are based on the number of particles per observed area and the number of coccolith per solid weight can be calculated as follows (from Williams and Bralower, 1995):

$$C_{\text{TOT}} = \frac{C \times V}{W \times F \times A \times H} \quad (4)$$

where C_{TOT} = number of coccoliths in a sample; C = number of coccoliths counted; V = volume of water used for dilution; W = sample weight; F = number of fields of view observed; A = area of one field of view; H = height of water column above slide.

2.6. Error estimates applying the SMS-method

The variance of the estimates of coccoliths/g sediment can be derived from information on the variance (a) of weighing microbeads, (b) of weighing sediment, (c) of calculating number microbeads per unit weight, (d) the number of coccoliths counted and (e) the number of microbeads counted. In order to calculate the variance of coccoliths per weight sediment, $\sigma^2(C_{\text{TOT}})$, we used the Gaussian law of error propagation (for details see Appendix A). The estimates of the standard deviation obtained for 100 microbeads and 100 coccoliths using the polystyrene microbeads are $\pm 14.4\%$. Additional counting up to 1000 microbeads and 1000 coccoliths reduces the standard deviation to $\pm 5.5\%$ (Fig. 3). However, additional counting cannot decrease the minimum standard deviation to less than $\pm 3\%$ for the polystyrene microbeads and to less than 27.7% for the borosilicate microbeads because of the error on the number of microbeads per weight unit that is given by the variance of the diameter of these microbeads ($\pm 0.0036 \mu\text{m}$ for polystyrene microbeads and $\pm 0.64 \mu\text{m}$ for borosilicate microbeads).

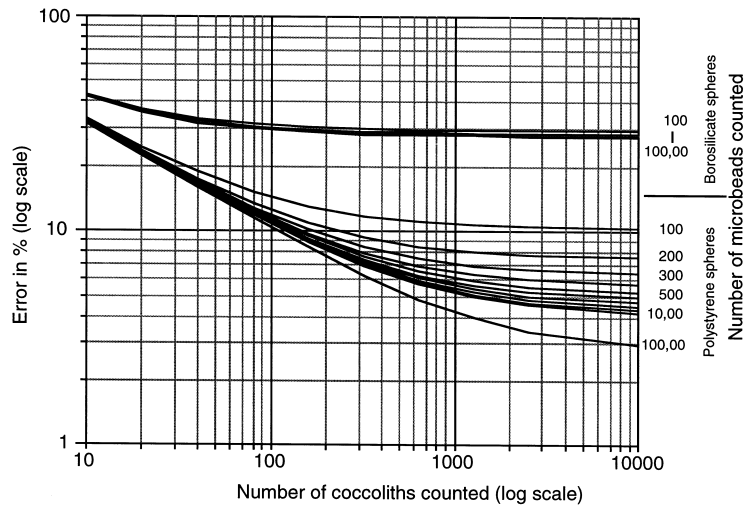


Fig. 3. Error estimates for the calculated number of coccoliths in a sample. The error is given in percent. The upper curves show the total error when 100 to 10,000 borosilicate microbeads were counted. The lower curves show the same estimates for the polystyrene microbeads with a smaller error on the calculated number of microbeads per weight unit.

3. Results

The SMS-technique was tested with 30 repeated preparations of the DSDP sample using polystyrene microbeads. The average number of coccoliths per gram fine-fraction sediment was 5.52×10^{10} with a standard deviation of $\pm 0.58 \times 10^{10}$ (Table 2). A single preparation of the same sample using borosilicate microbeads revealed 5.60×10^{10} coccoliths per gram fine fraction.

The filtration of the same DSDP sample (spiked with borosilicate microbeads as an independent measure for accuracy) revealed 4.5×10^{10} coccoliths per gram sediment and 5.02×10^{10} borosilicate microbeads (calculated from the number of particles per observed area, see Eq. 3). These measurements produced 13% fewer coccoliths than estimated from the coccoliths to microbeads ratio of the same sample applying Eq. 2 (5.17×10^{10} coccoliths/g, sample Nr.32 in Table 2) and 9% fewer than the average number of coccoliths calculated from all SMS-preparations. In addition, the number of microbeads is 13% smaller than the theoretical number of microbeads (see Eq. 1).

Application of the random settling method revealed 1.2×10^{11} coccoliths per gram fine fraction for the DSDP sample (spiked with borosilicate microbeads as an independent measure for accuracy)

and 1.78×10^{10} microbeads using the particle per area based Eq. 4. This is three times the number of coccoliths calculated from the coccoliths to microbeads ratio for the same sample using Eq. 2 (4.00×10^{10} coccoliths/g, in sample NR. 33; Table 2), 2.2 times the average number of coccoliths calculated from all SMS-preparations and three times the theoretical number of microbeads as calculated from Eq. 1.

The analyses of the hemipelagic Blagnac sample revealed 0.295×10^{10} coccoliths using borosilicate microbeads and 0.265×10^{10} coccoliths using the filtration method. This estimate is 11% smaller than the value obtained with the SMS-method (Table 2).

4. Discussion

Coccolith counts obtained by the random settling method applying the formula given by Williams and Bralower (1995) are three times higher than the values obtained by the SMS-method and the filtration method. These high values are apparently reproducible in repeated preparations (Geisen et al., 1999), suggesting the possibility of erroneous estimates of the number of borosilicate microbeads per gram spiking material. Therefore, we checked the number of borosilicate microbeads per gram us-

ing a lasergranulometer (GALAI CIS 1). The result of $5.86E \times 10^9 \pm 25\%$ microbeads confirmed the theoretical calculation of $5.762 \times 10^9 \pm 27.7\%$ microbeads per gram (Appendix B). We suspect that convection currents within the settling box might cause the apparent enrichment of particles on the elevated cover slide in the settling box. This is supported by an estimate of 4.94×10^{10} coccoliths per gram when the number of coccoliths is calculated assuming a total water column height of 5 cm instead of 2 cm in Eq. 4. This corresponds to a slide position on the bottom of the settling device instead of being elevated 3 cm.

The SMS-method, however, yields coccolith numbers that are not significantly different from values obtained from the filtration method. The application of microbeads with different densities also gave similar values. We conclude that both methods and both types of microbeads are suitable for coccolith analyses. Microbeads with small diameter variation are preferable because of the resulting reduction of the error estimates.

5. Conclusions

Microbeads can be used in order to calibrate absolute abundances in combination with any commonly used method, e.g. filtration or settling. However, the combination of microbeads with spraying (SMS-method) is a uniquely fast, accurate and reproducible technique for quantitative nannoplankton analyses in sediment samples. It is possible to prepare samples for SEM and LM analyses at the same time and the distribution of particles is homogeneous and size-independent. Additionally, potential loss of sediment and thus underestimation of coccoliths is of minor importance because there is no need for sample splitting. Furthermore, the potential dissolution of coccoliths is reduced because the contact between liquid and sediment is very short (a few minutes).

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Appendix A

In order to calculate the variance of coccoliths per gram sediment, $\sigma^2(C_{TOT})$, we used the Gaussian law of error propagation (Hartung et al., 1993, p. 326) and the variance calculations were done according to Hartung et al. (1993, p. 117). The variance of the estimates of coccoliths/g sediment can be derived from information on the variance of: (a) weighing microbeads, $\sigma^2(W_{M_{TOT}})$ and (b) sediment, $\sigma^2(W_{C_{TOT}})$. These errors are assumed to be negligible (<1%) because the accuracy of the Mettler balance is 10^{-6} g. (c) Calculating the number of microbeads per unit weight that has been added to a sample, $\sigma^2(M_{TOT})$, which is due to variance in the weight of a single microbead, $\sigma^2(W_M)$. $\sigma^2(W_M)$ is a function of the variance of the density of the microbeads and the variance of the volume of one microbead, $\sigma^2(V_M)$. The variance of the density is assumed to be zero, $\sigma^2(\rho_M) = 0$ and $\sigma^2(V_M)$ is a function of the variance of the radius, $\sigma^2(r)$, that is given by the manufacturer for different microbeads. The variance for the radius of the microbeads used is $\pm 0.0009 \mu\text{m}$ for the polystyrene microbeads and $\pm 0.16 \mu\text{m}$ for the borosilicate microbeads.

From this, the variance of the calculated number of microbeads per unit weight can be derived as follows:

$$\sigma^2(M_{TOT}) = \sigma^2\left(\frac{W_{M_{TOT}}}{W_M}\right) \cong \frac{1}{W_M} \times \left[\sigma^2(W_{M_{TOT}}) + \left(\frac{W_{M_{TOT}}}{W_M}\right)^2 \times \sigma^2(W_M) \right] \quad (5)$$

where

$$\sigma^2(W_M) \cong (\rho_M)^2 \times \sigma^2(V_M) \quad (5.1)$$

where:

$$\sigma^2(V_M) = \sigma^2\left(\frac{4}{3} \times \pi \times r^3\right) \cong \left(\frac{4}{3} \times \pi\right)^2 \times \sigma^2(r^3) \quad (5.2)$$

where:

$$\sigma^2(r^3) = \sigma^2(r \times r^2) \cong r^4 \times \sigma^2(r) + r^2 \times \sigma^2(r^2) = r^4 \times \sigma^2(r) + r^2 \times (2 \times r^2 \times \sigma^2(r)) = 3 \times r^4 \times \sigma^2(r) \quad (5.3)$$

(d) The variance related to the number of coccoliths counted, $\sigma^2(C_{\text{count}})$. It is assumed to follow a Poisson distribution with λ_C . (e) The variance related to the number of microbeads counted, $\sigma^2(M_{\text{count}})$. It is assumed to follow a Poisson distribution with λ_M .

From this the total variance of coccoliths per gram sediment, $\sigma^2(C_{\text{TOT}})$, can be calculated by:

$$\sigma^2(C_{\text{TOT}}) = \sigma^2 \left[\left(\frac{C_{\text{count}}}{M_{\text{count}}} \right) \times \left(\frac{M_{\text{TOT}}}{W_{C_{\text{TOT}}}} \right) \right] \cong \left(\frac{M_{\text{TOT}}}{W_{C_{\text{TOT}}}} \right)^2 \times \left[\sigma^2 \left(\frac{C_{\text{count}}}{M_{\text{count}}} \right) + \left(\frac{C_{\text{count}}}{M_{\text{count}}} \right)^2 \times \sigma^2 \left(\frac{M_{\text{TOT}}}{W_{C_{\text{TOT}}}} \right) \right] \quad (6)$$

where:

$$\sigma^2 \left(\frac{C_{\text{count}}}{M_{\text{count}}} \right) \cong \frac{1}{M_{\text{count}}^2} \times \left[\sigma^2(C_{\text{count}}) + \left(\frac{C_{\text{count}}}{M_{\text{count}}} \right)^2 \times \sigma^2(M_{\text{count}}) \right] = \frac{1}{\lambda_M^2} \times \left[\lambda_C + \left(\frac{\lambda_C}{\lambda_M} \right)^2 \times \lambda_M \right] \quad (6.1)$$

and:

$$\sigma^2 \left(\frac{M_{\text{TOT}}}{W_{C_{\text{TOT}}}} \right) \cong \frac{1}{W_{C_{\text{TOT}}}^2} \times \left[\sigma^2(M_{\text{TOT}}) \times \left(\frac{M_{\text{TOT}}}{W_{C_{\text{TOT}}}} \right)^2 \times \sigma^2(W_{C_{\text{TOT}}}) \right] \quad (6.2)$$

Appendix B

Number of borosilicate microbeads per gram solid weight estimated with a laser granulometer. Three samples of the borosilicate microbeads were measured several times.

Sample	Weight (g × 10 ⁻⁴)	Microbeads (×10 ⁵ /ml)	Estimated number of microbeads (×10 ⁹ /g)
1	5.54	8.20	4.44
1	5.54	8.70	4.71
1	5.54	8.10	4.39
1	5.54	8.10	4.39
1	5.54	8.10	4.39
1	5.54	8.10	4.39
1	5.54	11.00	5.96
1	5.54	8.60	4.66
1	5.54	9.70	5.25
1	5.54	8.10	4.39
1	5.54	14.00	7.58
1	5.54	9.70	5.25

Appendix B (continued)

Sample	Weight (g × 10 ⁻⁴)	Microbeads (×10 ⁵ /ml)	Estimated number of microbeads (×10 ⁹ /g)
3	3.74	6.50	5.21
3	3.74	9.40	7.54
3	3.74	7.80	6.26
3	3.74	6.70	5.37
3	3.74	5.90	4.73
3	3.74	10.00	8.02
3	3.74	10.00	8.02
3	3.74	13.00	10.43
3	3.74	9.60	7.70
4	4.00	8.90	6.68
4	4.00	7.50	5.63
4	4.00	8.00	6.00
4	4.00	7.10	5.33
4	4.00	8.20	6.15
4	4.00	7.50	5.63
4	4.00	8.70	6.53
4	4.00	6.70	5.03
Average			5.86
Standard Deviation			1.47
%			25

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