

## A new method for the quantitative separation of diatom frustules from lake sediments

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### Abstract

Achieving a purified fraction of diatom frustules from soft sediment samples is an essential requirement for using their biogenic silica as a carrier of paleolimnological and paleoenvironmental information. Because diatom frustules behave hydrodynamically unlike most mineral grains, these types of particles can theoretically be separated in liquids. Based on this principle, a new method has been developed, which employs split-flow thin fractionation (SPLITT) as a tool for separating diatom frustules from other sedimentary particles. The SPLITT channel selected for this study had a length of 20 cm, a breadth of 4 cm, and a height of 371  $\mu\text{m}$ . For optimum results, samples were pretreated, e.g., suspended and wet-sieved prior to any processing with the SPLITT cell. The sample concentrations used in the fractionation process were below 0.1% (w/v) to minimize particle-particle interaction, which affects the quality of the separation. The advantages of SPLITT fractionation over other tested methods are good reproducibility, high throughput by continuous flow, minimum losses of frustules, and minimum contamination of the diatom fraction by minerals or sponge spicules. Separation results from representative sediment samples of different origin, age, and compaction as well as various organic (0.5% to 10% total organic carbon) and biogenic opal contents (1% to 20% biogenic silica) are presented and discussed. They demonstrate that the developed separation technique yields highly purified and, within the processing tolerances, quantitative samples of diatom frustules from bulk lacustrine sediments.

Diatoms contribute significantly to the total amount of biogenic silica in marine and lacustrine sediments. Their valves are preserved in sediments and remain nearly unchanged over long time periods. Because the species composition of these algae depend on environmental conditions, frustules are frequently used in ecological and related earth science studies (Stoermer and Smol 1999). For example, different communities of species determined from sediment cores can enable the reconstruction of environmental conditions prevailing during the time of their life (e.g., Pienitz and Smol 1993). Several attempts have been made to use diatom silica

for oxygen isotope paleothermometry (Juillet 1980; Juillet-Leclerc and Labeyrie 1987; Shemesh et al. 1992; Brandriss et al. 1998; Leng et al. 2001; Schmidt et al. 2001). However, success was limited due to the high preparatory and technical requirements necessary for reliable oxygen isotope analyses. Taking into account the still growing importance of stable isotope techniques in the study of global change, biogenic silica nevertheless remains an alternative for isotope geochemists in cases where carbonaceous materials are not available, e.g., in annually laminated maar lake sediments (Negendank et al. 1990).

The basic prerequisite for geochemical investigations on diatom silica is the capability to extract a highly purified diatom fraction from bulk sediment. This is especially compelling in the field of isotope geochemistry. Even modest remains of mineral grains, sponge spicules, or chrysophycean cysts alter the oxygen isotope composition of the diatom fraction. Alteration of the diatom species composition of the sample might lead to deviating isotope signals due to species dependent vital effects. Sample material is always restricted and, thus, for high resolution palaeolimnological reconstructions the yield of frustules should be maximized.

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It was the aim of this study to develop a standardized and quantitative method for the separation of diatom frustules from bulk lacustrine sediments. Because an independent measure for the quantitative recovery of diatom frustules obviously does not exist—current methods only quantify biogenic silica as a whole—we define the term “quantitative” based on image analysis as follows: The gained sample of diatom frustules should fulfill two criteria: (a) a maximum grade of purification, especially with respect to sponge spicules and mineral grains (<5% contaminants) and (b) minimal losses of frustules referred to the original sample or to the fraction of sample used for separation.

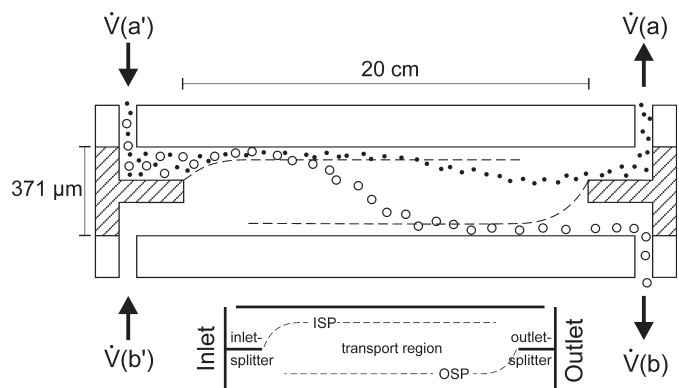
Initially different separation methods were tested, such as differential settling in a beaker (Schrader 1973; Juillet-Leclerc 1984; Shemesh et al. 1988; Ellwood and Hunter 1999), separation in an inhomogeneous magnetic field (Janér and Jungner 1982), and the use of heavy liquids, i.e., aqueous solution of sodium polytungstate, in centrifugation experiments (Skipp and Brownfield 1993), but they were found to be inadequate for lacustrine samples. All tests were performed with samples pretreated with hydrogen peroxide ( $H_2O_2$ ) to destroy the organic matter.

Tests with differential settling in a beaker resulted in partial loss of diatom frustules and contaminated samples. Because the sample suspension fills the whole liquid column in the beaker while particles separate only in the upper part of the column, diatom frustules will also settle together with mineral grains and be lost. Repeated settling of a sample increases the yield of diatom frustules, but considerable losses of diatom frustules still cannot be avoided.

The type of magnetic separation that has been tested is typically used for separating diamagnetic quartz grains with a size > 200  $\mu m$  from paramagnetic minerals for thermoluminescence dating. A suspended sediment sieve fraction < 80  $\mu m$  exposed to a magnetic field showed no separation of diatoms from minerals, most probably because magnetic separation operates with a concentrated and highly viscous aqueous solution of a manganese salt. The magnetic force of the used separator was not sufficient to move the particles within the suspension and small particles (e.g., < 10  $\mu m$ ) started to cluster inhibiting any separation.

Separation by heavy liquid was tested with an aqueous solution of sodium polytungstate and a sample sieve fraction < 20  $\mu m$ . A density gradient was chosen for the heavy liquid with a maximum at 2.5  $g/cm^3$ . After repeated centrifugation no separation of diatom frustules from minerals was achieved. Similar to the magnetic separation, small particles started to cluster and inhibited separation.

The method that was finally selected uses the different sinking velocities of particles in a liquid that is gently pumped through a narrow channel. This technique is called SPLITT fractionation (split-flow lateral-transport thin separation cells) and was first introduced by Giddings (1985). SPLITT fractionation is applied in a wide variety of fields (Keil et al. 1994;



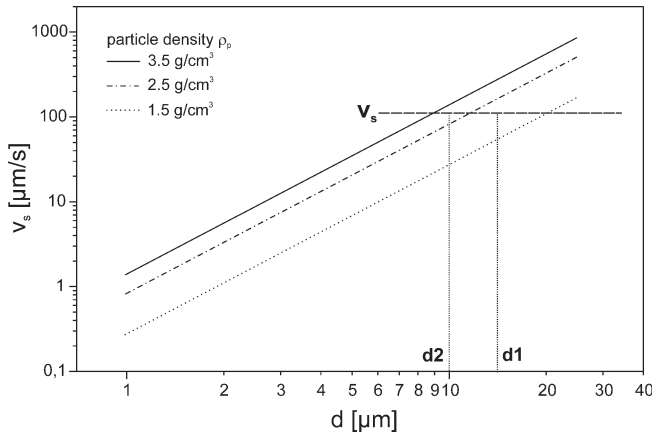
**Fig. 1.** Cross-section of a SPLITT-cell (not to scale). ISP = inlet splitter plane; OSP = outlet splitter plane. The height and length, given as the distance between inlet and outlet splitter, refer to the cell used.

Zhang et al. 1994; Fuh and Giddings 1995; Bergamaschi et al. 1997; Contado et al. 1997; Fuh and Giddings 1997; Dondi et al. 1998; Fuh and Chen 1998; Fuh 2000; Gustafsson et al. 2000), but its usefulness for separating diatoms from sediments has not yet been tested.

Here we describe a new approach for the separation of diatom frustules from lacustrine sediment samples using SPLITT fractionation and demonstrate the quality of the developed technique. The sedimentary materials discussed are chosen out of a larger set processed with the developed SPLITT technique and represent typical examples from the field of paleolimnological research. Other areas of application for this purification technique can be trace element geochemistry, the enrichment of diatom frustules from highly diluted samples for the determination of species frequencies, as well as separation of other materials such as pollen, chitin shells (head capsules), chrysophycean cysts, and sponge spicules.

*Theoretical background*—The basic principle of SPLITT fractionation is the gradual separation of particles of varying properties (size, density, etc.) in a laminar flow with a field of force, i.e., force of gravity applied perpendicular to the direction of flow (Giddings 1985; Springston et al. 1987). A homogeneous suspension of the particles is continuously introduced through the upper inlet  $a'$  of a SPLITT cell, while a carrier liquid (deionized water) is introduced through its lower inlet  $b'$  (Fig. 1). Within the cell the two flows merge smoothly, developing into a laminar current. Particles migrate according to their density, shape, and size toward either of the two outlets. As a result, particles will be split into two fractions,  $a$  and  $b$ . The cutoff diameter  $d_c$ , which determines through which outlet ( $a$  or  $b$ ) a particle leaves the cell, is controlled by the flow rates and the field strength.

The sinking velocity  $v_s$  of a particle with density  $\rho_p$  and diameter  $d$  is given by Stokes' law:  $v_s = F_g/f = m_{eff} g/3\pi\eta d(f/f_0)$  where  $F_g$  = force of gravity;  $f$  = friction coefficient ( $f = f_0$  for spherical particles);  $m_{eff}$  = effective mass of particles in the liquid allowing for their buoyancy ( $m_{eff} = [\rho_p - \rho_l] \cdot V$ );  $V$  = vol-



**Fig. 2.** Dependency of the sedimentation velocity  $v_s$  on particle size ( $d$ ) for different particle densities (double log scale). The density of the liquid is chosen as  $\rho_l = 0.998 \text{ g/cm}^3$ . Note that, for instance, a light spherical particle ( $\rho_{p1} = 2 \text{ g/cm}^3$ ) of  $d_1 = 14 \text{ } \mu\text{m}$  diameter has the same sedimentation velocity as a denser particle ( $\rho_{p2} = 3 \text{ g/cm}^3$ ) of  $d_2 = 9.9 \text{ } \mu\text{m}$  diameter.

ume given by  $(1/6) \cdot \pi \cdot d^3$ ;  $g$  = acceleration due to gravity;  $\rho_l$  = liquid density;  $\eta$  = liquid viscosity. The frictional ratio ( $f/f_0$ ) allows for nonspherical particles (Springston et al. 1987). From this, it follows that

$$v_s = g \cdot \frac{d^2}{18 \cdot \eta \cdot (f/f_0)} \cdot (\rho_p - \rho_l). \quad (1)$$

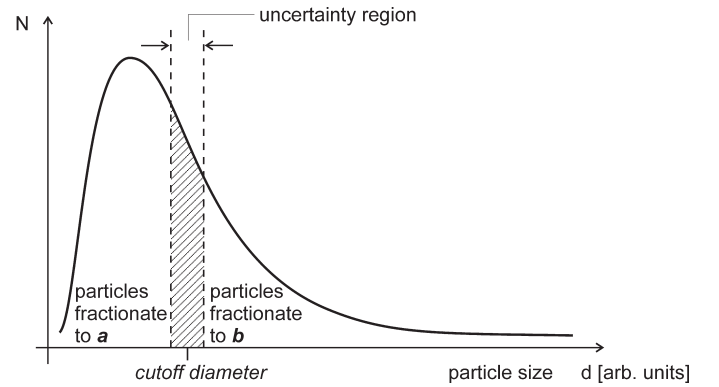
Thus, the particle properties ( $\rho_p$ ,  $d$ ,  $f/f_0$ ) and the fluid properties ( $\rho_l$ ,  $\eta$ ) determine the velocity  $v_s$ .

Assuming for simplicity, that we are dealing with spherical particles, Eq. 1 contains two independent quantities of a particle ( $d$  and  $\rho_p$ ). Thus,  $v_s$  is not unequivocally characterizing one class of particles (Fig. 2). Particles of different sizes may have the same sinking velocity  $v_s$ , if the densities are chosen accordingly. Therefore, particles of so-called “corresponding densities” or “corresponding sizes,” respectively, cannot be separated from each other. They show identical sinking velocities ( $v_1 = v_2$ ).

$$\frac{\rho_{c1}}{\rho_{c2}} = \left( \frac{d_2}{d_1} \right)^2 \quad (2)$$

with  $\rho_c = \rho_p - \rho_l$ .

Because diatom frustules are less dense than most mineral grains (specific gravity [ $\text{g/cm}^3$ ]: opal 1.95, kaolinite 2.6, quartz 2.65, calcite 2.72 [Klein and Hurlbut 1985]), a separation should be possible in most cases. The threshold of separation, i.e., the smallest mineral grains that can be separated from diatom frustules, is theoretically determined by the corresponding density or size. Thus, the number of mineral grains that cannot be separated from diatom frustules by SPLITT fractionation (or differential settling) depends on the grain-size distribution of the sediment. The distribution of grain sizes can be narrowed by sieving.



**Fig. 3.** Quality of fractionation, i.e., resolution of the SPLITT technique. Distribution of equally shaped particles with different diameters in a suspension before fractionation. If for all particles equal density is considered, they fractionate to  $a$  or  $b$  depending on their size and the used flow rates. The uncertainty region indicates the size interval in which particles fractionate to  $a$  as well as to  $b$ . The so-called cutoff diameter gives the particle size at which fractionation leads to a 50/50 distribution, i.e., equal distribution to  $a$  and  $b$ .

An important aspect of SPLITT fractionation is its resolution, defined as the capability to separate a single size distribution of grains with equal shape and density into two, nonoverlapping grain-size distributions. In most cases, there is no ideal separation or cutoff (Fuh et al. 1995; Jiang et al. 1997). Under normal conditions an overlapping grain-size interval exists (Fig. 3) out of which particles can appear in both fractions, i.e.,  $a$  and  $b$  (Giddings 1992). The width of this grain size interval determines the resolution and quality of the SPLITT technique and is governed by flow rates, inlet splitter geometry, channel geometry, and particle interactions (Gupta et al. 1997). Resolution will also deteriorate by particle interactions within a dense suspension. Improvement of resolution can be reached by an inclination downstream of the SPLITT cell. This extends the pathway of sedimentation and allows for lower flow rates, thus, reducing turbulences occurring at higher flow rates (Jiang et al. 1997).

## Material and procedures

### Material

Sample characteristics: The selected samples comprise lacustrine materials of different origin and composition (Table 1): (A) is a compacted soft sediment typical for Holocene varved sediment material with medium organic content; (B) is a typical uncompacted surface sediment, often used to establish regional diatom calibration sets; (C) comprises settling matter of a eutrophic lake with high contents of organic matter and biogenic silica; (D) is an example of a consolidated material that is 40,000 years of age; and (E) is a typical sediment containing a diatom valve concentration that is too low for representative diatom determinations. These materials were chosen as typical examples of various types of sediments from lacustrine systems.

**Table 1.** Characteristics of materials used to demonstrate the quality of separation for diatom frustules from a particle mixture with the SPLITT technique\*

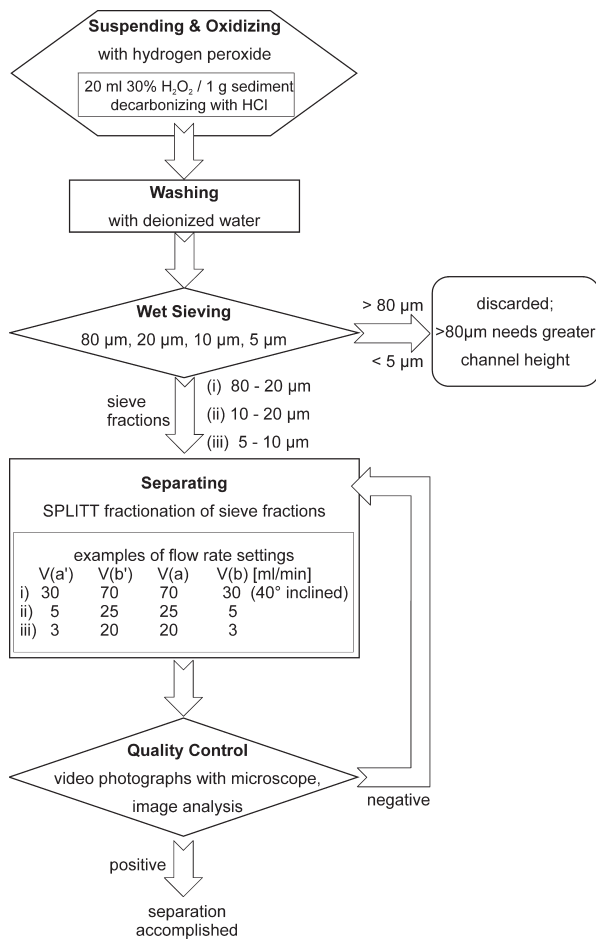
| Sample | Sediment type          | Composition             | Depth and age        | Location   |
|--------|------------------------|-------------------------|----------------------|--|
| A      | Sediment core          | 5% TOC†                 | ca. 3.5 m depth      | Lake Holzmaar,<br>Germany (Eifel)<br>50°7'N 6°53'E |
|        | Compacted material     | 10% BSiO <sub>2</sub> ‡ | ca. 2000 y BP§       |  |
| B      | Surface sediment       | 8% TOC                  | upper 2 cm           | Lake Holzmaar,<br>Germany (Eifel)                  |
|        | Uncompacted            | 12% BSiO <sub>2</sub>   | recent               |  |
| C      | Sediment trap material | 10% TOC                 | fresh organic matter | Lake Holzmaar,<br>Germany (Eifel)                  |
|        | (7-m water depth)      | 20% BSiO <sub>2</sub>   | 2 weeks (1995)       |  |
| D      | Sediment core          | 7% TOC                  | ca. 29-m depth       | Lake Huguang,<br>Maar, China<br>21°9'N 110°17'E    |
|        | Consolidated material  | 8% BSiO <sub>2</sub>    | 40,000 y BP          |  |
| E      | Surface sediment       | no data available       | upper 2 cm           | Lake Bachsee,<br>Switzerland<br>47°N 8°E           |
|        | Diatom-poor material   |                         | recent               |  |

\*The described materials represent typical lacustrine sediments important in palaeolimnological research.

†TOC, total organic carbon content.

‡BSiO<sub>2</sub>, biogenic silica content.

§BP, before present (before 1950 A.D.)



**Fig. 4.** Flow chart describing the developed standard procedure for separating diatom frustules from lacustrine sediments with SPLITT fractionation. This method accounts for different material properties ranging from fresh algal material to consolidated sediments.

*Experimental design*—The SPLITT cell (Fig. 1) used was of the type SF1000 (FFFractionation LLC) with channel dimensions of 4 cm × 20 cm × 371 μm. The splitter edge was slightly rounded, which ensures easy removal of air bubbles that may form in the liquid and improves flow conditions in the channel. The inflow of the sample suspension into the SPLITT cell was controlled by a peristaltic pump (BVP CA8, Ismatec), and the carrier liquid was transported by a cogwheel pump (Reglo Z, Ismatec). Sieve fractions > 20 μm were processed with a bubble trap at the sample inlet. The inlet for the carrier liquid was always equipped with a bubble trap. At the inlets, pumping rates were measured with a measuring pipette and at the outlets with a measuring flask. Flow rates at the outlets were controlled by narrow-bore tubings.

#### Methods

General procedure: A flow chart describing the developed standard processing procedure is presented in Fig. 4. Freeze-dried material is suspended homogeneously in deionized water by a mild process that doesn't use strong oxidizing acids (HClO<sub>4</sub>) to preserve diatom frustules intact; organic matter and carbonates are removed with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>; 30%) and hydrochloric acid (100 μL of HCl 32%/20 mL sample suspension). The material is subsequently rinsed in deionized water for 24 h and wet-sieved to obtain sediment fractions of > 80 μm, 20 to 80 μm, and < 20 μm. The sieve fraction > 80 μm is discarded because particles are too big for separation by the type of SPLITT cell that is being used. This is unproblematic because diatom frustules (freshwater) are rare in this fraction and, if necessary, sieve fractions > 80 μm can be processed using a SPLITT cell with different channel geometry.

The sieve fractions are set to an appropriate sample concentration (< 0.1% [w/v]). To prevent particles from sticking

**Table 2.** Experimental settings for SPLITT fractionation steps: Flow rates at the inlets and outlets for materials (A) to (E)\*

|       | Sieve fraction | Run nr | Flow rate (mL/min) |      |       |      | Sample volume (mL) | Operation time (rounded) |
|-------|----------------|--------|--------------------|------|-------|------|--------------------|--------------------------|
|       |                |        | a'                 | b'   | a     | b    |                    |                          |
| A.i†  | 20 to 80 µm    | 1      | 31.2               | 68.2 | 80.0  | 20.7 | 100                | ~10 min                  |
| A.ii  | 10 to 20 µm    | 1      | 5.2                | 26.4 | 25.0  | 7.1  | 20                 | ~10 min                  |
| A.iii | 5 to 10 µm     | 1      | 3.2                | 20.3 | 18.6  | 4.9  | 20                 | ~10 min                  |
| B.i‡  | 20 to 80 µm    | 1      | 56.6               | 77.0 | 100.0 | 32.9 | 200                | ~10 min                  |
| B.ii  | < 20 µm        | 1      | 9.8                | 30.0 | 30.3  | 9.9  | 400                | ~1 h                     |
|       | < 20 µm        | 2      | 5.0                | 20.3 | 19.7  | 6.0  | 400                | ~1.5 h                   |
|       | < 20 µm        | 3      | 2.5                | 9.8  | 9.8   | 2.6  | 400                | ~3 h                     |
|       | < 20 µm        | 4      | 1.0                | 5.4  | 5.0   | 1.3  | 400                | ~7 h                     |
| C.i§  | 20 to 80 µm    | 1      | 58.8               | 77.9 | 103.8 | 33.8 | 200                | ~10 min                  |
| C.ii  | < 20 µm        | 1      | 5.1                | 20.0 | 18.8  | 6.3  | 600                | ~2 h                     |
|       | < 20 µm        | 2      | 0.8                | 2.7  | 2.4   | 0.9  | 500                | ~11 h                    |
| D     | < 20 µm        | 1      | 5.1                | 20.3 | 19.5  | 6.3  | 200                | ~45 min                  |
|       | < 20 µm        | 2      | 0.8                | 2.7  | 2.5   | 1.3  | 200                | ~4.5 h                   |
| E¶    | < 20 µm        | 1      | 4.9                | 20.3 | 19.6  | 6.3  | 400                | ~1.5 h                   |
|       | < 20 µm        | 2      | 0.8                | 2.7  | 2.5   | 0.8  | 300                | ~6.5 h                   |

\*The sum of flow rates at the inlet may differ somewhat from the outlet due to minor measuring inaccuracies. The given dry weight is the initial mass before sieving. Sample volume gives the volume used for SPLITT fractionation. Operation time gives an estimate for a single step SPLITT fractionation.

†A = sediment core, Lake Holzmaar, ~300 mg; Ai = channel 40° inclination (for details, see text)

‡B = recent sediment, Lake Holzmaar (Germany), ~1 g

§C = sediment trap, Lake Holzmaar, 346 mg

||D = sediment core, Lake Huguang Maar (China), ~1 g

¶E = recent sediment, Lake Bachsee (Switzerland), some tenths of a milligram

onto the splitter plate or channel walls, 0.05 vol.% tenside (AGEPON, Agfa) and 1 vol.% ethanol are added to the sample suspension and carrier liquid (deionized water). Before processing a sample, air bubbles are removed from the flooded channel either by high pumping rates or by pumping of several milliliters of pure ethanol. After each run, fractions *a* and *b* are concentrated by centrifugation for 10 min at 3260g.

**Sample processing**—Depending on specific material properties, each sample had to be treated in a slightly different way. Flow rates and respective processing times can be taken as typical for each material (Table 2). Details of the processing for samples (A) to (E) are described below:

(A) Sample amount used was 300 mg of dry matter. Microsieving with mesh widths of 5 µm and 10 µm was performed to further investigate the performance of the technique by avoiding clay particles. The fraction < 5 µm contained almost no diatom frustules and was discarded.

(B) Sample amount was ca. 1 g of dry matter. The material was treated four times with SPLITT fractionation to determine in detail the separation characteristics of the mineral grains and diatom frustules. The flow rates were successively reduced and for each consecutive run the previous fraction *a* was used.

(C) Approximately 350 mg of dry matter was used. This material was characterized by a very high content of organic

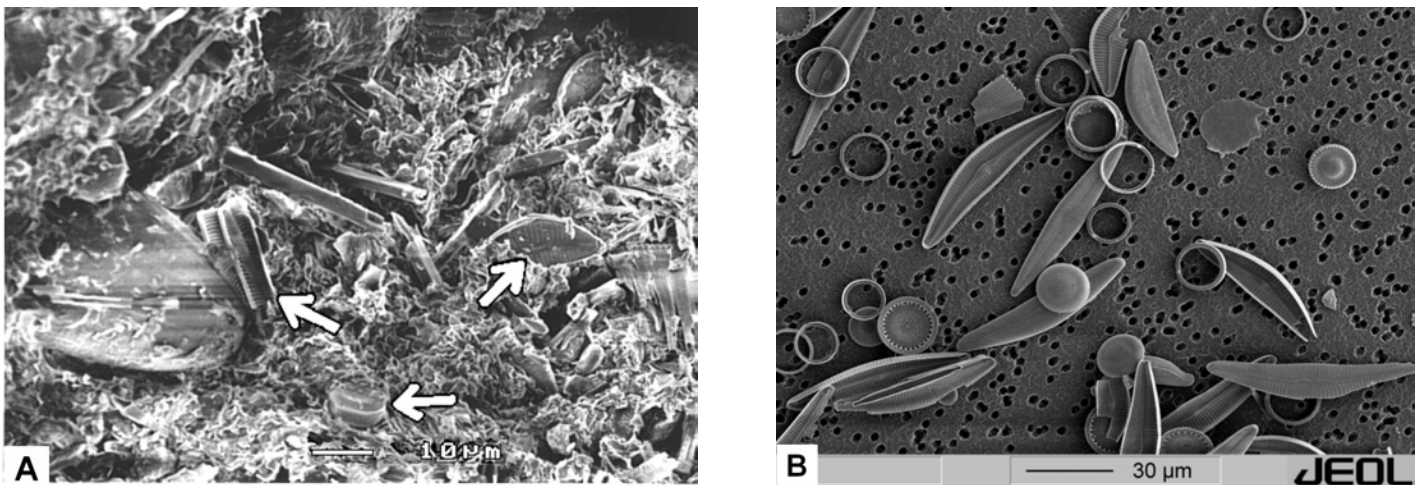
matter (30% to 40%). Therefore, the oxidation process had to be repeated three to four times prior to SPLITT fractionation.

(D) Sample amount was ca. 1.0 g of air-dried matter. The material was processed by SPLITT fractionation in two steps: first, removing coarse minerals (silt) and, second, removing fine minerals (clay).

(E) Some tenths of a milligram of this material were provided in suspension and, thus, did not have to be treated with H<sub>2</sub>O<sub>2</sub>. The material did not contain large organic particles and was solely wet-sieved. As described for (D), a two-step SPLITT fractionation was carried out.

**Quality control and quantification**—The quality of the cleaning and separation procedure was checked by light microscopy and scanning electron microscopy (SEM). A typical SEM example of a processed sample is given in Fig. 5. The highly purified *a*-fraction is uncontaminated and specifically no clay particles adhere to the frustules.

To determine the number and size of grains and diatom frustules, sub-samples were taken, diluted, and dried on 11 mm<sup>2</sup>-fields of an epoxy-coated slide (Diagnostika slides, Roth). Twenty-one video photographs (716 × 572 pixel, pixel scale = 0.761 µm) were taken of each sub-sample. The photographs were evaluated with image-processing software (KS 300, Zeiss) (Russ 1999). Mineral grains were automatically counted and



**Fig. 5.** SEM photographs. (Photo A) A lacustrine sediment (material [A], air-dried) with diatom frustules embedded in a mineral matrix. The picture shows a typical sample material before any treatment. (Photo B) Purified diatom material after SPLITT fractionation. The SEM photograph was taken from the  $\alpha$ -fraction after a one-step fractionation of material (A), sieve fraction 5 to 10  $\mu\text{m}$ . The background comprises a porous filter. Circular structures are cingulae. Broken parts of frustules are also identifiable. The frustules are completely free from adhering clay minerals.

measured in a range from 2 to 15  $\mu\text{m}$ . Grains  $> 15 \mu\text{m}$  were measured interactively by hand. Interactive measurement means that the maximum diameter of the particles had to be marked on the digital image using the computer mouse. Diatom frustules were also measured interactively and classified according to three shape classes: “round,” “long,” and “other.” The standard error over all size classes for three repetitive counts was 4% for diatom frustules (1000 counts) and 2% for mineral grains (50,000 counts).

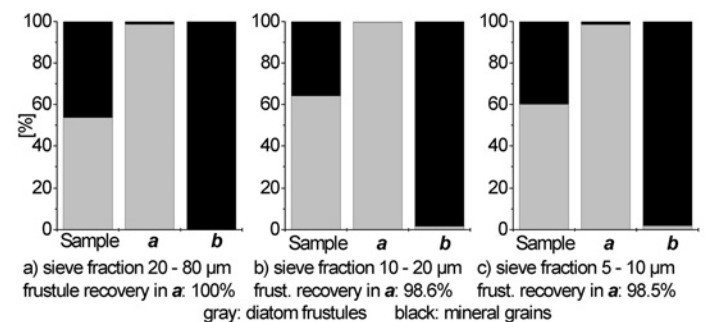
### Assessment and discussion

Initial tests with several separation methods as discussed in the Introduction quickly revealed the advantages of SPLITT fractionation. While diatoms  $> 80 \mu\text{m}$  could also be separated with other techniques, processing sieve fractions  $< 80 \mu\text{m}$  gave no clean diatom samples and high diatom losses were always unavoidable. Because the vast majority of diatoms in our materials, which represent typical lacustrine sediments, belong to the sieve size class  $< 80 \mu\text{m}$ , SPLITT fractionation was chosen as the adequate method to be optimized.

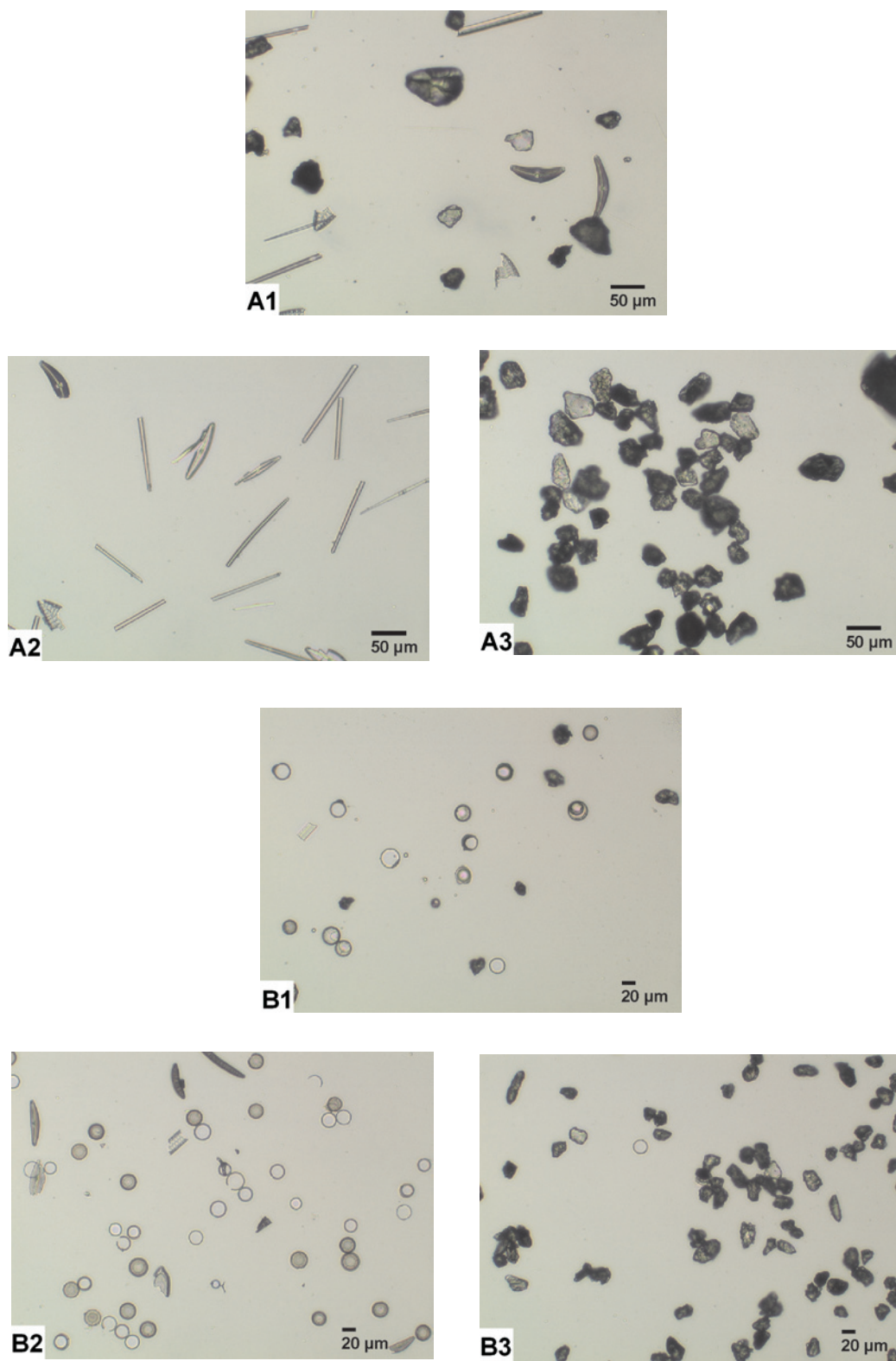
To increase resolution, all materials were sieved in the previously described manner (three sieve size classes) and, thus, the amount of diatom frustules and mineral grains with corresponding sizes was minimized. An appropriate particle concentration in the sample suspension was also found to be important for good separation results. Jiang et al. (1997) stated as a rule of thumb, “smaller particles or lower-density particles usually require preparation and separation at a lower concentration.” For sediments with a high amount of silt and clay, the concentration should be well below 0.1% (w/v).

Separation of diatom frustules was mostly achieved by one or two steps of SPLITT fractionation. Repetitive fractionations with identical flow rates showed that the separation results

can be reproduced reliably. Because of the relatively low amount of mineral grains found in the 20 to 80  $\mu\text{m}$  sieve fractions, separation of diatom frustules in these cases was accomplished after one separation within 15 to 20 min processing time. The generally obtained diatom fractions often included trace amounts of pollen, chitin shells, and chrysophycean cysts, which possess particle characteristics closely related to those of frustules (Duff et al. 1995; Wilkinson et al. 2001), whereas sponge spicules were separated with the mineral fraction. Pollen and chitin relicts can be combusted prior to oxygen isotope investigations. Hereafter further methodological details are discussed with regard to the different materials.



**Fig. 6.** Result of a quantitative extraction of diatom frustules from the compacted sediment (A) with SPLITT fractionation. Diagrams display the content of diatom frustules in the respective suspensions (sample, fraction  $a$  and  $b$ ) for the different sieve classes 20 to 80  $\mu\text{m}$ , 10 to 20  $\mu\text{m}$ , and 5 to 10  $\mu\text{m}$ . Because the whole sample suspension is processed, all particles have to show up in fraction  $a$  and  $b$ . If no frustules appear in fraction  $b$  the recovery in fraction  $a$  will be 100%. In sieve fraction 20 to 80  $\mu\text{m}$ , a recovery of 100% and a purity of 98.6% is achieved, whereas for class 10 to 20  $\mu\text{m}$ , there is 98.8% recovery and 99.7% purity, and for class 5 to 10  $\mu\text{m}$ , 99.3% recovery and 98.5% purity can be stated (compare Table 3).



**Fig. 7.** Microscope photographs taken from dried preparations. Upper plate: Sieve fraction 20 to 80  $\mu\text{m}$  of material (A) before (photo A1) and after SPLITT fractionation (photo A2, fraction *a*; photo A3, fraction *b*). Diatom frustules are separated after a single step of SPLITT fractionation. Lower plate: Sieve fraction 10 to 20  $\mu\text{m}$  of material (A) before (photo B1) and after SPLITT fractionation (photo B2, fraction *a*; photo B3, fraction *b*). Diatom frustules are separated after a single step of SPLITT fractionation. Circular structures in the lower left picture are cingulae of diatom frustules.

**Table 3.** Separation characteristics for diatom frustules as given by the number of counted particles before and after SPLITT fractionation of material (A)\*

|                                       | Counts†          |                |
|---------------------------------------|------------------|----------------|
|                                       | Diatom frustules | Mineral grains |
| Sieve fraction 20 to 80 $\mu\text{m}$ | 153              | 132            |
| SPLITT fraction <i>a</i>              | 71               | 1              |
| SPLITT fraction <i>b</i>              | 0                | 275            |
| Sieve fraction 10 to 20 $\mu\text{m}$ | 70               | 39             |
| SPLITT fraction <i>a</i>              | 331              | 1              |
| SPLITT fraction <i>b</i>              | 4                | 234            |
| Sieve fraction 5 to 10 $\mu\text{m}$  | 347              | 230            |
| SPLITT fraction <i>a</i>              | 911              | 14             |
| SPLITT fraction <i>b</i>              | 6                | 315            |

\*Initially each sieve fraction contains a high amount of mineral grains. After SPLITT fractionation, mineral grains are largely absent from the *a* fractions, thus showing highly purified diatom frustules, whereas the *b* fractions contain almost no frustules, indicating minimal losses. Therefore, diatom frustules are highly purified and quantitatively accumulated within fraction *a*. The numbers presented in the table are not absolute numbers and, therefore, sieve fractions cannot be balanced against respective SPLITT fractions.

†Image analysis of material (A).

*Compacted sediment core (A)*—Diatom frustules of every sieve fraction were quantitatively separated from mineral grains after one step of SPLITT fractionation using routine pretreatment and sample concentrations of < 0.02% (w/v) (Fig. 6; Fig. 7). Even frustule components and small broken parts were found in the cleaned diatom fractions. In this respect, pre-micro-sieving appeared as a major improvement, even if chrysophycean cysts still could not be completely removed. To avoid turbulences occurring at the high flow rates necessary to process the 20 to 80  $\mu\text{m}$  fraction, the SPLITT channel was inclined by 40°, and resolution was increased without raising the flow rates. Thus, an inclination can extend the application of the SPLITT technique to even larger particle sizes without changing the channel height.

The numbers of particles in the sample and in the separated fractions were determined by image analysis before and after SPLITT fractionation (Table 3). Initially each sieve fraction contains a high number of mineral grains. After SPLITT fractionation, mineral grains are largely absent from the *a*-fractions, thus, showing a highly purified diatom sample, whereas the *b*-fractions contain almost no frustules, indicating minimal losses. Because the whole sample suspension is processed, all particles have to show up in fraction *a* and *b*, distributed according to the quality of the SPLITT process. Apparently, if no frustules appear in fraction *b*, the recovery in fraction *a* is 100%. The grade of purity is given by the mineral content in *a*. Therefore it is evident that in sieve fraction 20 to 80  $\mu\text{m}$ , the recovery is 100% and the purity 98.6%, whereas for 10 to 20  $\mu\text{m}$ , 98.8% recovery and 99.7% purity, and for 5 to 10  $\mu\text{m}$ , 99.3% recovery and 98.5% purity is achieved (Table 3; Fig. 6).

The yield of diatom material achieved is given from the dry weights of the different *a*-fractions as 1.7 mg (20 to 80  $\mu\text{m}$ ), 1.1 mg (10 to 20  $\mu\text{m}$ ), and 1.6 mg (5 to 10  $\mu\text{m}$ ), respectively. The recovered diatom amounts in all fractions (4.4 mg) represent only 15% of the biogenic silica content of the original sample (Table 1). This bias can easily be explained by the principal difference between the biogenic silica content and the diatom frustule fraction. In the original sample inspection by light microscopy revealed large amounts of sponge spicules. These large, massive, and highly silicified biominerals are necessarily included in the biogenic silica content but excluded from the diatom frustule fractions separated with the SPLITT technique.

*Surface sediment (B)*—Because the fraction of this sample (20 to 80  $\mu\text{m}$ ) was quantitatively separated after only one step of SPLITT fractionation, our discussion concentrates on the smaller sediment fraction. The sieve fraction < 20  $\mu\text{m}$  consisted mainly of mineral grains < 5  $\mu\text{m}$  (silt and clay) and diatom frustules > 5  $\mu\text{m}$ . Because grains of this size class show similar sinking velocities as diatom frustules, separation problems were expected. Indeed, during SPLITT fractionation, the mineral grains were largely distributed into both fractions *a* and *b*. Despite the fact that most grains in fraction *b* should exceed the cutoff diameter, smaller particles (< 3  $\mu\text{m}$ ) appeared to “contaminate” each fraction of coarser grains. Presumably, this results from a high concentration of fine particles in the sample suspension, causing particle interactions and the formation of temporary aggregates. Particle interactions can, however, be avoided through further dilution of the sample suspension.

In fractionation step one and two, mostly mineral grains were removed through outlet *b*. In step three and four, a number of diatom frustules were also found in fraction *b*. The diatom frustules showed a rather complex behavior during fractionation. After two steps of SPLITT fractionation, frustules with wide forms, such as *Cymbella* or *Navicula*, were found together with larger centric forms, like *Cyclotella* in the first-step *b*-fraction. The smallest centric forms and spicular, i.e., pennate forms such as *Fragilaria crotonensis*, tended to stay in fraction *a* of each fractionation step. Additionally, small parts of broken frustules also remained in fraction *a*. Thus, the diatom frustule distribution between fractions *a* and *b* depends mainly on the shape of the frustules, together with size and silicification of the valves. This suggests a possibility of separating diatoms according to family or genus in the future.

An evaluation of the fractions by image analysis showed that more than 90% of the diatom frustules accumulated in the respective fraction *a* of every run. Thus, fraction *a* of the fourth run contained about 80% of the original amount of frustules. Taking into account the uncertainties of automatic particle analysis and counting, these results are completely satisfactory. The fractions *b* would have to be reprocessed to obtain an even better yield, which is, however, a matter of time and necessity.

*Sediment trap material (C)*—The specific problem with this material was its very high organic content (30% to 40%), typical for fresh materials. Independent of particle-size fractions,

remains of thread-like algae and other organic materials spoiled the SPLITT fractionation completely. Especially, the particles of the fraction  $> 20 \mu\text{m}$  formed aggregates, which partly clogged the SPLITT channel and prevented fractionation. This indicated that the sample had to be oxidized more intensively than in the routine pretreatment procedure. After three to four repetitions of the  $\text{H}_2\text{O}_2$  treatment, diatom frustules could be separated from the 20 to 80  $\mu\text{m}$  fraction. In the fraction  $< 20 \mu\text{m}$ , diatoms could not completely be separated from minerals because of clay minerals (compare material [B]). As a consequence, highly organic material must be repeatedly treated with  $\text{H}_2\text{O}_2$ .

*Consolidated sediment core (D)*—The fraction 20 to 80  $\mu\text{m}$  contained almost no diatom valves, and experiments concentrated on the smaller fractions where two effects were observed. The suspended particles were distributed as expected by theory, but the sample suspension contained aggregates that withstood the suspending process. These aggregates consisted mainly of clay and silt minerals and were found in both fractions *a* and *b*. During SPLITT processing, these aggregates “broke apart” and constantly released small particles that were found in either SPLITT fraction and, therefore, prevented a clean separation. Thus,  $\text{H}_2\text{O}_2$  treatment is not necessarily adequate to always gain a complete suspension of the sample and in special cases other treatments may have to be used, e.g., strong oxidizing acids (Juillet-Leclerc 1984). Once we had overcome the problem of aggregation by repeated  $\text{H}_2\text{O}_2$  and ultrasonic treatment, SPLITT fractionation resulted in a high-quality separation even for this consolidated sediment type.

*Diatom-poor sediment (E)*—The fraction 20 to 80  $\mu\text{m}$  was not processed, because diatom valves mainly were in the fraction  $< 20 \mu\text{m}$ . After one fractionation step almost all diatom frustules were found in fraction *a*, together with fine mineral grains. A second fractionation further improved the separation result. According to image analysis, the new fraction *aa* contained about 90% of the frustules. Although the amount of clay particles in fraction *aa* was still high, our task of diatom valve enrichment was successful. Thus, SPLITT fractionation is well suited for the enrichment of diatoms valves for the determination of species distributions in sediments with few frustules. Nevertheless, further studies are needed to prove that species composition is not altered by the SPLITT process.

### Comments and recommendations

The developed SPLITT fractionation technique can be used for the separation of diatom frustules from a variety of sedimentary materials and for the enrichment of diatom-poor samples for species determinations. Its outstanding characteristic is the ability to generate a quantitative sample of diatom frustules from lacustrine sediments with practically negligible contaminations by mineral grains and sponge spicules. The method is characterized by its high reproducibility, easy handling, and relatively short processing times (e.g., processing time [1-g dry matter]: sieve fraction  $> 20 \mu\text{m}$ ,  $\sim 30$  min;

sieve fraction  $< 20 \mu\text{m}$ ,  $\sim 90$  min). With the geometry of the SPLITT cell used (channel height: 371  $\mu\text{m}$ ), the sieve fractions from which diatom frustules can be separated cover the range of 5 to 80  $\mu\text{m}$ . For particles larger than 80  $\mu\text{m}$ , the cell used is only partially suitable. An inclination of the SPLITT channel can extend the particle range, but a channel with greater height is recommended for particles  $> 80 \mu\text{m}$ . Even frustules less than 5  $\mu\text{m}$  (smallest mesh width used for sieving) can be enriched but not completely cleaned. For this size, discrimination by another method, e.g., cross flow filtration (Gustafsson et al. 2000), should be applied.

An important step that must carefully be considered for a successful high quality separation with SPLITT is an appropriate pretreatment of the sample. Critical points are the destruction of organic matter in organic-rich samples and the possible formation of aggregates in the sample solution. With respect to the small mineral fraction, micro-sieving is an excellent means to separate undersized particles ( $< 5 \mu\text{m}$ ) in advance, leading to reduced processing times and to increased quality of the SPLITT separation.

The SPLITT method is independent of sample size and operates in a continuous flow mode. Therefore, small samples of some tenths of a milligram can be processed, as well as samples of grams of sediment. However, particle concentration in the suspension should be below 0.1% (w/v) to avoid disturbing particle interactions in the channel. The separation process can easily be controlled by the flow rates, therewith giving a high reproducibility. Because the flow rates determine the cut-off diameter, i.e., the resolution, and govern the formation of turbulences, they have to be determined carefully. Separation yield with respect to the diatom content is always greater than 90% after reunification of all *a*-fractions and reaches up to 100%, whereas the amount of contaminants is always smaller than 5% and can even be less than 2%. Because different families or species of diatoms have different sizes, forms and grades of silicification, frustules can to a certain degree be separated or enriched according to families or species. In the future, separation of other low density particles generally contained in lacustrine sediments (e.g., pollen, cysts, chitin head capsules, etc.) should be possible.

### References

- Bergamaschi, B. A., E. Tsamakis, R. G. Kiel, T. I. Eglinton, D. B. Montlucon, and J. I. Hedges. 1997. The effect of grain size and surface area on organic matter, lignin and carbohydrate concentration, and molecular compositions in Peru margin sediments. *Geochim. Cosmochim. Acta* 61/6:1247-1260.
- Brandriss, M. E., J. R. O'Neil, M. B. Edlund, and E. F. Stoermer. 1998. Oxygen isotope fractionation between diatomaceous silica and water. *Geochim. Cosmochim. Acta* 62:1119-1125.
- Contado, C., F. Dondi, R. Beckett, and J. C. Giddings. 1997. Separation of particulate environmental samples by SPLITT fractionation using different operating modes. *Anal. Chim. Acta* 345:99-110.

- Dondi, F., C. Contado, G. Blo, and G. S. Martin. 1998. SPLITT cell separation of polydisperse suspended particles of environmental interest. *Chromatographia* 48(9/10):643-654.
- Duff, K., B. Zeeb, and J. P. Smol. 1995. *Atlas of chrysophycean cysts*. Kluwer.
- Ellwood, M. J., and K. A. Hunter. 1999. Determination of the Zn/Si ratio in diatom opal: a method for the separation, cleaning and dissolution of diatoms. *Mar. Chem.* 66(3-4):149-160.
- Fuh, C. B., and J. C. Giddings. 1995. Isolation of human blood cells, platelets, and plasma proteins by centrifugal SPLITT fractionation. *Biotechnol. Prog.* 11(1):14-20.
- Fuh, C. B., E. M. Trujillo, and J. C. Giddings. 1995. Hydrodynamic characterization of SPLITT fractionation cells. *Sep. Sci. Technol.* 30/20:3861-3876.
- Fuh, C. B., and J. C. Giddings. 1997. Isoelectric split-flow thin (SPLITT) fractionation of proteins. *Sep. Sci. Technol.* 32(18):2945-2967.
- Fuh, C. B., and S. Y. Chen. 1998. Magnetic split-flow thin fractionation: new technique for separation of magnetically susceptible particles. *J. Chromatogr. A* 813:313-324.
- Fuh, C. B. 2000. Split-flow thin fractionation. SPLITT is a versatile family of techniques for separating macromolecules, colloids, and particles. *Anal. Chem.* 72(7):266A-271A.
- Giddings, J. C. 1985. A system based on split-flow lateral-transport thin (SPLITT) separation cells for rapid and continuous particle fractionation. *Sep. Sci. Technol.* 20(9/10):749-768.
- Giddings, J. C. 1992. Optimization of transport-driven continuous SPLITT fractionation. *Sep. Sci. Technol.* 27(11):1489-1504.
- Gustafsson, Ö., A. Düker, J. Larsson, P. Andersson, and J. Ingrid. 2000. Functional separation of colloids and gravitoids in surface waters based on differential settling velocity: Coupled cross-flow filtration-split flow thin-cell fractionation (CFF-SPLITT). *Limnol. Oceanogr.* 45(8):1731-1742.
- Gupta, S., P. M. Ligrani, M. Phillip, and J. C. Giddings. 1997. Investigations of performance characteristics including limitations due to flow instabilities in continuous SPLITT fractionation. *Sep. Sci. Technol.* 32(10):1629-1655.
- Janér, J. H., and H. Jungner. 1982. A simple method for separation of quartz in TL dating. *Pact. J.* 6:214-215
- Jiang, Y., A. Kummerow, and M. Hansen. 1997. Preparative particle separation by continuous SPLITT fractionation. *J. Microcol. Sep.* 9(4):261-273.
- Juillet, A. 1980. Structure de la silice biogénique: nouvelles données apportées par l'analyse isotopique de l'oxygène. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences, Serie D: Sciences Naturelles* 290(19):1237-1240.
- Juillet-Leclerc, A. 1984. Cleaning process for diatomaceous samples. Eighth International Diatom-Symposium, Paris, France, 27 Aug.–1 Sept. 1984. 8:733-736.
- Juillet-Leclerc, A., and L. Labeyrie. 1987. Temperature dependence of the oxygen isotopic fractionation between diatom silica and water. *Earth Planet. Sci. Lett.* 84:69-74.
- Keil, R. G., E. Tsamakakis, C. B. Fuh, J. C. Giddings, and J. I. Hedges. 1994. Mineralogical and textural controls on the organic composition of coastal marine sediments; hydrodynamic separation using SPLITT-fractionation. *Geochim. Cosmochim. Acta* 58(2):879-893.
- Klein, C., and C. S. Hurlbut Jr. 1985. *Manual of mineralogy* (after James D. Dana). 20th edition. Wiley.
- Leng, M., P. Barker, P. Greenwood, N. Roberts, and J. Reed. 2001. Oxygen isotope analysis of diatom silica and authigenic calcite from Lake Pinarbasi, Turkey. *J. Paleolimnol.* 25:343-349.
- Negendank, J. F. W., A. Brauer, and B. Zolitschka. 1990. Die Eifelmaare als erdgeschichtliche Fallen und Quellen zur Rekonstruktion des Paläoenvironments. *Mainzer geowiss. Mitteilungen* 19:235-262.
- Pienitz, R., and J. P. Smol. 1993. Diatom assemblages and their relationship to environmental variables in lakes from the boreal forest-tundra ecotone near Yellowknife, Northwest Territories, Canada. *Hydrobiologia* 269/270:391-404.
- Russ, J. C. 1999. *The image processing handbook*. CRC Press and Springer.
- Schmidt, M., R. Botz, D. Rickert, G. Bohrmann, S. R. Hall, and S. Mann. 2001. Oxygen isotopes of marine diatoms and relations to opal-A maturation. *Geochim. Cosmochim. Acta* 65(2):201-211.
- Schrader, H. J. 1973. Proposal for a standardized method of cleaning diatom-bearing deep-sea and land-exposed marine sediment. *Nova Hedwigia/Beihefte. Lehre* 45:400-409.
- Shemesh, A., R. A. Mortlock, R. J. Smith, and P. N. Froehlich. 1988. Determination of Ge/Si in marine siliceous microfossils: separation, cleaning and dissolution of diatoms and radiolaria. *Mar. Chem.* 25:305-323.
- Shemesh, A., C. D. Charles, and R. G. Fairbanks. 1992. Oxygen isotopes in biogenic silica: global changes in ocean temperature and isotopic composition. *Science* 256:1434-1436.
- Springston, S. R., M. N. Myers, and J. C. Giddings. 1987. Continuous particle fractionation based on gravitational sedimentation in split-flow thin cells. *Anal. Chem.* 59(2):344-350.
- Skipp, G. L., and I. K. Brownfield. 1993. Improved density gradient techniques using Sodium Polytungstate and a comparison to the use of other heavy liquids. U.S. Department of the Interior; U.S. Geological Survey. OF 92-0386.
- Stoermer, E.F., and J. P. Smol (eds). 1999. *The diatoms: applications for the environmental and earth sciences*. Cambridge.
- Wilkinson, A. N., B. Zeeb, and J. P. Smol. 2001. *Atlas of chrysophycean cysts, Volume II*. Kluwer.
- Zhang, J., P. S. Williams, M. N. Myers, and J. C. Giddings. 1994. Separation of cells and cell-sized particles by continuous SPLITT fractionation using hydrodynamic lift forces. *Sep. Sci. Technol.* 29(18):2493-2522.

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