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5.2.1 The inverted-microscope method

Grethe Rytter Hasle

The inverted-microscope method, or Utermöhl method, was introduced in the early 1930s. Another, less correct, name is the sedimentation method. Utermöhl (1931, p. 594) characterized the inverted-microscope method as a combination of the Kolkwitz (1907) chamber method and the sedimentation method of Volk (1906), or in his own words: `Die hier mitgeteilte Untersuchungsweise stellt eine Erweiterung and zum Teil Abändderung des Kolkwitzschen Kammerverfahrens dar, zugleich aber auch eine Vereinfachung des Volkschen Sedimentierverfahrens.' Volk's sedimentation method involves stepwise concentration to successively smaller volumes of water by transfer from larger to smaller containers. (Somewhat modified, the method is now used with success to obtain non-quantitative material for morphologic, taxonomic and distributional studies of coccolithophorids, small dinoflagellates and diatoms; and other plankton organisms too small to be collected adequately by nets; semiquantitative results may also be expected: see Chapter 5.2.2.) The simplification of Volk's sedimentation method made by Utermöhl is the combined use of the same chamber for sedimentation and counting without any transfer and consequent loss of material.

The Kolkwitz chamber was designed for a standard compound microscope. Because of its height an objective with a long working distance and correspondingly low power must be used. By using an `inverted' microscope and chambers with a glass bottom of coverslip thickness, Utermöhl made it possible to use objectives with shorter working distance and higher power.

'COUNTING" CHAMBER'S

Utermohl (1931) designed an assortment of counting (=sedimentation) chambers or cylinders in order to provide chambers for specific sample characteristics. A sample with a dense phytoplankton population or a lot of detritus is most easily examined in a chamber in which the bottom (floor) area is large relative to the volume. A sample with a sparse population and less detritus is best examined in a chamber in which the bottom area is small relative to the volume, reducing the time wasted scanning the empty bottom. The chambers constructed by Utermöhl (1931) were too tall to be used with a condenser;

however, he later (1958) described a chamber with a detachable bottom plate which is now manufactured by optical firms. The principle is to remove the upper part of the chamber (the sedimentation cylinder) after sedimentation, leaving organisms in the bottom part, which has a height less than the working

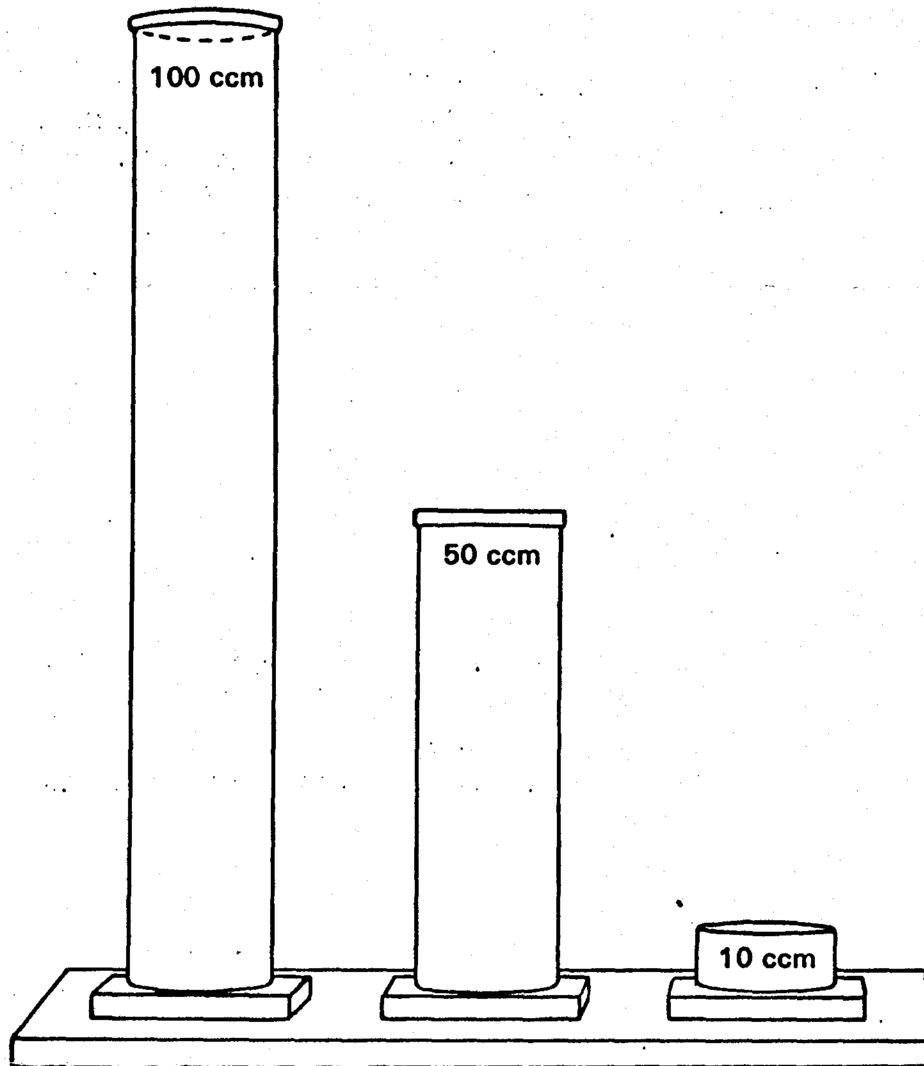

Figure 17

Top (sedimentation) cylinders of combined plate chambers (after Wild Ml, Catalogue 140d X11.69

distance of the condenser. The model now in common use is a combined plate chamber consisting of a top cylinder (sedimentation cylinder) of 10, 50 or 100 ml capacity (Fig. 17) (5 and 25 ml may also be available) and a bottom-plate chamber (Fig. 18). The parts of the plate chamber are a rectangular perspex plate (Fig. 18a); a ring (I 8b) and a circular bottom (base) plate of coverslip thickness. The plate fits into the mechanical stage of the inverted microscope of the type for which it is constructed. It has a small opening close to one end, and a circular central opening of 26 mm diameter, which is slightly larger than the

diameter of the sedimentation cylinders. The ring can be fastened to the underside of the rectangular plate by a key (Fig. 18c). The bottom plate is placed between the ring and the large **opening of the perspex plate and thus forms the floor of** the plate chamber (Fig. 19). The combined chamber is ready for use when the cylinder of the desired capacity is placed on top of the plate chamber. When the well-shaken preserved water sample has been poured into the combined chamber to overflow, a top plate is placed in position to eliminate dust and evaporation. Care should be taken to remove all water outside the chamber to keep, in particular, the thin glass bottom clean. After sedimentation the top cylinder is slowly pushed away from the plate chamber by using the square top plate of the plate chamber (Figs. 18-19). **Pushing stops when the cylinder reaches the** small opening near the end of the perspex plate of the plate chamber. As soon as the circular top plate of the sedimentation cylinder is removed, water is drained out of the cylinder through the small hole below.

In contrast to the assortment of counting chambers **he originally recommended,** Utermöhl (1958) ultimately found standardization of the bottom area **of the new chambers a convenience.** He **recommended examination of only a portion of the bottom area when high magnification is needed and when the population is dense** enough so that such a procedure will yield reliable results. This implies that the sediments generally. are randomly distributed on the chamber floor. When dealing with marine phytoplankton, non-random (aggregated) **distributions of the settled organisms are often encountered, particularly when chain-forming species are present.** Instead of examining a small part of the bottom of the commercial chambers; a one-piece chamber shallow enough to permit the use of a condenser, and with a small volume and a bottom area of reasonable size, can be constructed (see below).

Utermöhl (1958) warned against the use of tall cylinders because of attachment of organisms to the chamber wall. Paasche (1960) made comparisons which indicated that this might be the case with chain-forming species of the setae-bearing genus *Chaetoceros.* Counts of other phytoplankton forms may be unaffected by this source of error (Margalef, 1969c). Personal investigations of the spindle-shaped. marine dinoflagellate *Ceratium fusus* and the lens-shaped marine dinoflagellate. *Prorocentrum micans* showed no significant difference in cell densities estimated from a 2-ml (15-mm high) chamber and a 10-ml (40-mm high) chamber. Another objection to tall chambers was given by Nauwerck (1963, p. 16) **who observed that convection currents could not be avoided in chambers higher** than five **times the.chamber diameter. The problem of convection currents** in these chambers could apparently not be overcome by a longer settling time since it was emphasized that a considerable amount of plankton didnot settle at all. The height of the 100 ml commercial chamber greatly exceeds this relative size and

should therefore be used cautiously, since sedimentation of phytoplankton will be hindered by convection currents.

SEDIMENTATION TIME

Variations in preservation method and species composition probably account for the diversity of sedimentation time recommended in the literature. Utermöhl (1931) assumed that all organisms would have settled by the day after preparation of the sample, while in 1958 he wrote more explicitly that a settling time of at least 24 hours was needed. Lund et al. (1958) recommended 18 hours for 100-ml chambers, 3 hours for 10-ml and 1 hour for 1-ml, while Willén (1976) used about 8 hours for 10-ml and 48 hours for 50- and 100-ml chambers. In an investigation by Nauwerck (1963) a sedimentation time of 4 hours per centimetre chamber height resulted in a complete sedimentation of even the smallest organisms 'von µ-Grösse'. These apply to freshwater phytoplankton preserved - by an iodine solution. Steemann Nielsen (1933) recommended 24 hours as sedimentation time for marine phytoplankton preserved with formaldehyde, and Margalef (1969c) as a general formula suggested the sedimentation time in hours to be at least three times the height of the sedimentation chamber in centimetres.

Since iodine preservation apparently precludes a proper investigation of coccolithophorids (Chapter 4), neutralized formaldehyde is often used. Formaldehyde lacks the advantage of iodine, which increases the weight of the cells, thereby decreasing the settling time.

A test performed on formaldehyde-preserved marine phytoplankton stored for 12 to 13. years and counted in 2-ml (about 15-mm high) chambers showed that in particular cases a period of more than 24 hours was necessary to ensure sedimentation of the algae (Hasle, `1969, p. 19). The error due to incomplete sedimentation never exceeded a factor' of two, and it varied from one species to the other. In addition to colony formation, shape, size and silicification appeared to be decisive factors. No similar test was performed on other sizes of chambers but, based on experience with the 2-m1 chambers, a settling time of at least 40 hours was recommended for formaldehyde preserved marine phytoplankton independent of chamber size.

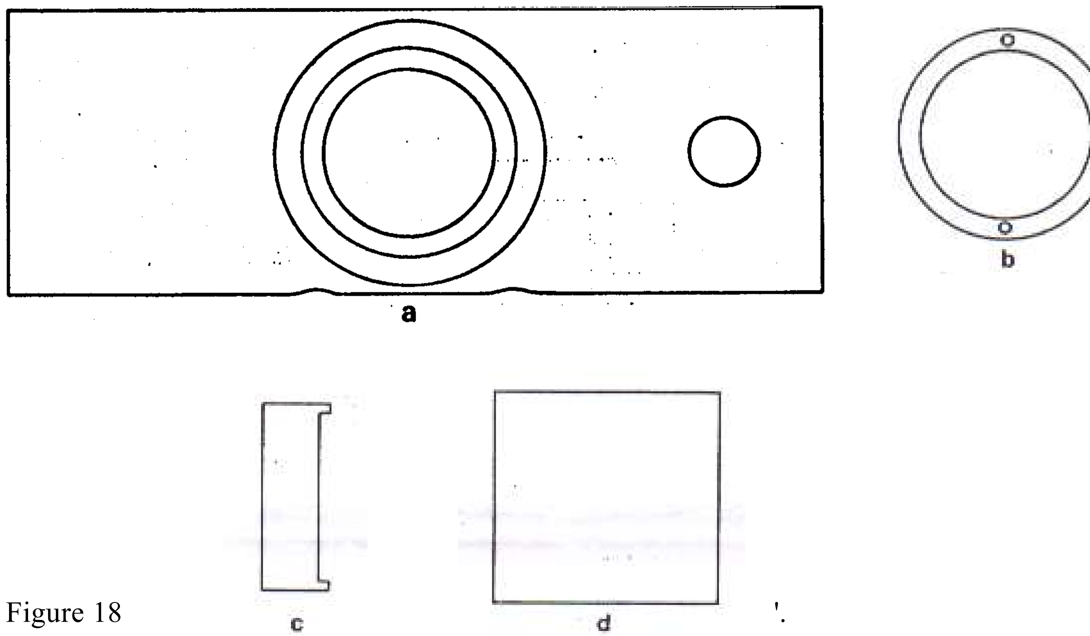

Figure 18

Bottom part of combined plate chamber: (a) perspex plate with larger opening for top cylinder and smaller drainage hole; (b) ring for support of the bottom (base) plate; (c) key to fasten ring to underside of perspex plate; (d) top plate of sedimentation chamber, also to be used to remove top cylinder after sedimentation.

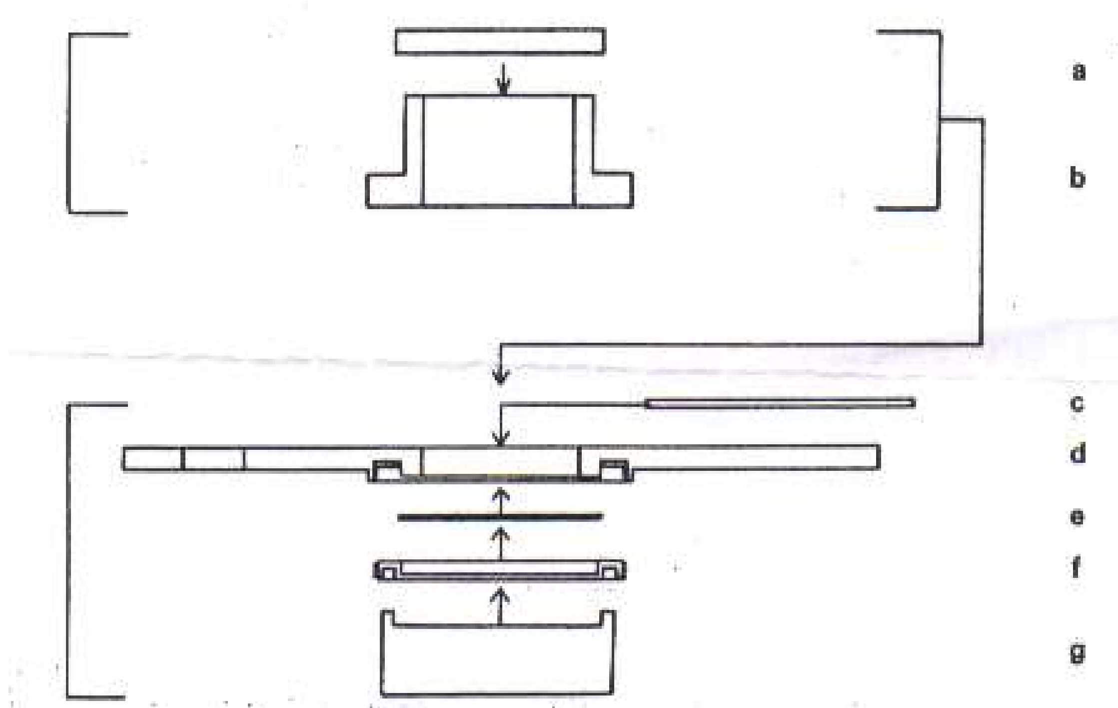

Figure 19

Vertical cross-section of combined plate chamber (partly after Wild Ml, Catalogue 140d XII.69): (a) top plate of sedimentation cylinder; (b) sedimentation cylinder; (c) top plate of bottom plate chamber; (d) perspex plate; (e) bottom (base) plate; (f) ring; (g) key.