# An Improved Form of Sedimentation Apparatus for Use with an Inverted Microscope

By

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## Introduction

The preparation of material for the quantitative estimation of phytoplankton, including nanoplankton, by the sedimentation technique and inverted microscope is now well established — see for example UTERMÖHL (1931 a, b, 1936), LUND (1951, 1957), LUND, KIPLING and LE CREN (1958). In the final stages of sedimentation the excess liquid is usually siphoned off tending to disturb the settled organisms or leaving too large a volume of liquid to allow proper adjustment of the microscope condenser for the higher powers of magnification.

The apparatus to be described was devised to provide a simple and compact means of removing the excess liquid to leave quite a small volume. It reduces the errors, and at the same time facilitates the process of preparation prior to microscopical analysis and enables the slide to be examined by oil immersion lens if desired.

A prototype was demonstrated to the Plankton Committee at the 41st meeting of the International Council for the Exploration of the Sea in Copenhagen in 1953 (see BRAARUD, 1958; FRASER, 1954) and the present apparatus was exhibited to the Challenger Society at Aberdeen in 1958.

## Construction

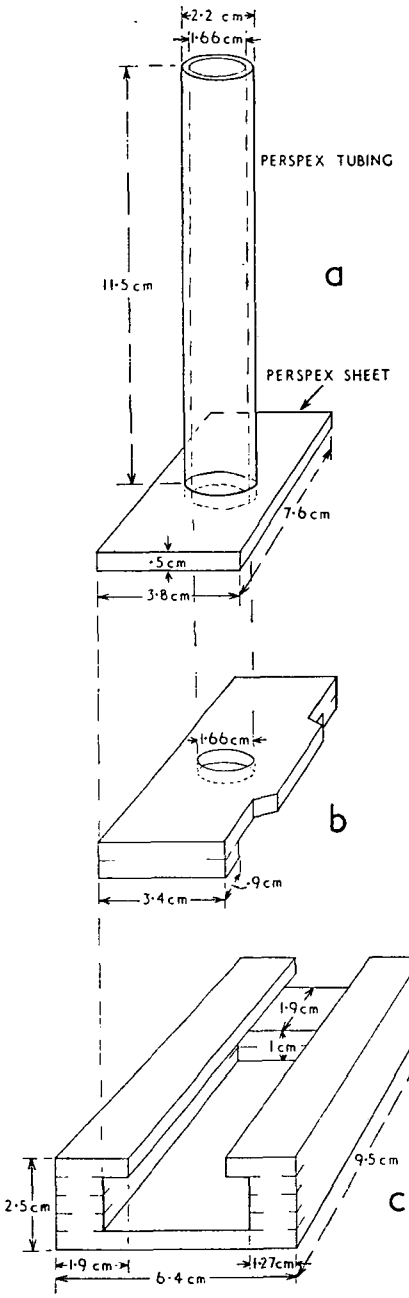
The apparatus is constructed from perspex sheet and tubing. The sizes given here are those used in Aberdeen but they can be varied to suit individual requirements.

The entire assembly (Fig. 1) consists of three components:—

(a) *A sedimentation tube* to contain 26 ml of sample material.

(b) *A sedimentation chamber* to receive the settled organisms, etc. and with a capacity of approximately 1 ml. Parts (a) and (b) form the *settling column* with a total capacity of 27 ml.

(c) *A holder* to align and contain the other two components.



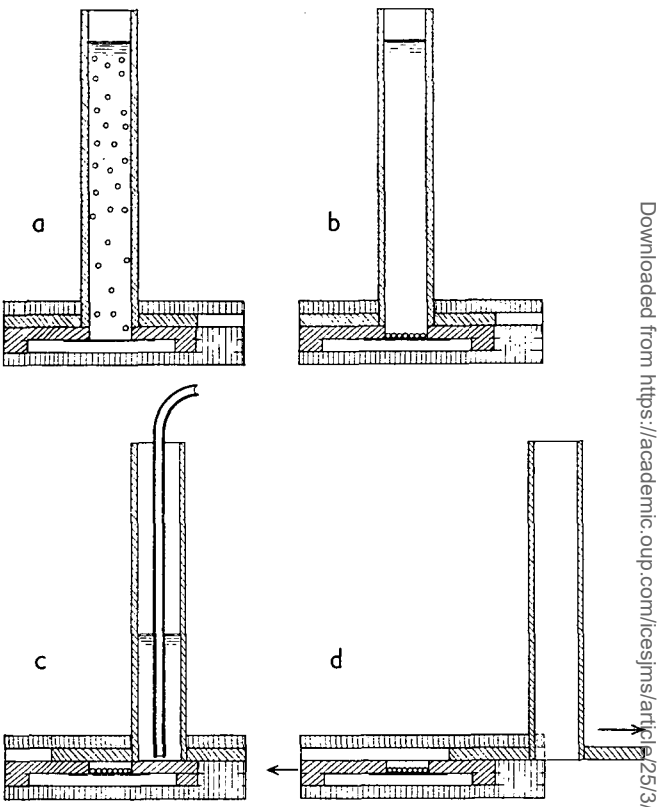




Figure 2. Stages in the operation of the apparatus.
(a) Settling of material in the sedimentation column.
(b) Settling of material completed.
(c) Removal of surplus liquid.
(d) Separation of components.

Figure 1. Component parts of sedimentation apparatus.
(a) Sedimentation Tube. (b) Sedimentation Chamber. (c) Holder.
The sizes given are those used in Aberdeen for convenience and availability of material.

The *sedimentation tube* as provided by the makers of the plastic has no claim to consistency in wall thickness or circularity of section and so it is drilled out to the required size. The bottom plate is drilled to take the outside diameter of the tube which is then inserted and cemented in, flush at its base.

The *sedimentation chamber* slide has the same dimensions as the tube plate but the central hole is the same diameter as the internal bore of the tube. It is shaped to fit into the contours of the mechanical stage of the microscope and has two runners on the undersurface to raise it slightly above the stage level. The chamber itself is formed by attaching a coverslip to the undersurface of the slide by an adhesive made from resin dissolved in chloroform, a waterproof cement devised by SCHLAUDER (1945). Because the slide is slightly raised by the runners it can be moved by the mechanical stage without risk of disturbing the coverslip. Examination of the contents of the water samples are made through this coverslip which can be No. 1 thickness and so allow the use of an oil immersion lens if desired.

The *holder* consists of a larger plate along three of whose sides are cemented layers of perspex strip cut from the same thickness of sheet to produce the required dimensions. Three layers of narrow strips are cemented lengthways and capped with a further wider strip to act as a rim retaining the other components. One end of the holder is blanked off with two layers of wide strip to provide an end stop for the other components. The internal dimensions of the holder should be slightly larger than those of the chamber slide and tube plate to allow for smooth movement of these parts.

## Operation

A smear of vaseline is spread over the upper surface of the chamber slide and on top of this is pressed the tube plate to make a good seal. The settling column is thus formed and is placed in the holder where final alignment takes place.

The sample to be examined is now placed into the column (Fig. 2 a) and allowed to stand and settle for three hours which should be sufficient for the larger forms but this time should be extended for smaller forms — see LUND (1958). All organisms and other matter present settle on the coverslip base (Fig. 2 b) and the unwanted liquid can then be removed. This is achieved simply by sliding the tube plate to one side, the chamber is retained in the holder by the end stop and the column is now broken without disturbing the settled material and the waste can then be siphoned off (Fig. 2 c). The Tube plate is then slid completely free of the chamber (Fig. 2 d) leaving the latter ready to be taken out for examination, with any required power of magnification, on the inverted microscope. As the cell is only 5 mm deep the condenser can be brought close enough for high power examination and the shallow depth facilitates the removal of a particular organism if required.

### Collection and analysis of water samples

Sea-water samples of 300 ml are taken with the water bottle and preserved with LUGOLS' solution in dark green bottles. (LUGOLS' solution consists of 10 g iodine, 20 g KI in 200 ml of distilled water to which is added 20 cc

glacial acetic acid.) A few drops of this solution is added to the water sample to produce a light tea or cognac colour which will preserve as long as the colour lasts but must be kept in the dark. The samples stand for some time aboard ship before return to the laboratory when a further period of at least a day should elapse before they are analysed. During this time settling has occurred and it is a safe and simple matter to siphon off the excess liquor to leave about 15–20 ml containing the material present in the original container. This is poured into the settling column, together with washings from the bottle making up the 27 ml capacity of the column where final settling occurs.

Numerical analysis is carried out at Aberdeen using a square ocular micrometer covering approximately $^1/_{1800}$ of the total floor area of the chamber when used with $^2/_3$ in objective and × 10 Wide Field eyepieces. If six such fields are examined then the total numbers observed are expressed directly as thousands per litre of sea water since the original sample volume was 300 ml. When only a small number of organisms are present the squared type of ocular micrometer is very useful for carrying out a traversing examination of the complete floor area of the chamber.

**Standard net phytoplankton analysis**

The sedimentation chamber with its capacity of 1 ml is also ideal for the examination of Stempel pipette sub-samples from net hauls. The net sample with formalin is made up to a volume of 100 ml and well mixed. Six Stempel pipette sub-samples of $^1/_5$ ml each are taken, placed in separate chambers and one field in each examined. The ocular micrometer provides a field of known size and relationship with the total area, from which suitable final values can be worked out.

**Ancillary equipment for the "Prior" inverted microscope**

The model of inverted microscope used in the Marine Laboratory is that marketed by W. R. Prior & Co. Ltd., London, but this basic model has been fitted with a binocular attachment and × 10 Wide Field eyepieces. It is used as a multipurpose instrument in the analysis of sedimentation samples and standard silk net samples, and for the general purposes of a high power microscope. Various pieces of apparatus, other than the sedimentation apparatus, have been developed to extend and improve its operation and as these may be useful to other workers they are briefly described here.

To bring the oculars to a comfortable working level the instrument is raised seven inches on a wooden staging fitted with drawers containing spare optical accessories, lens cleaning tissue, micrometers, pipettes, needles, etc. (Fig. 3). All equipment is thus readily at hand. The raised portion supports the microscope and it has attached to it the switch and the illuminant which is fitted to a freely rotating bar for easy adjustment.

The lower extension of the staging provides a more stable base and also acts as a rest for the elbow when operating the controls of the moveable stage. Fixed on its upper surface, under a protective transparent sheet, are the various conversion tables for the micrometers and other data to which reference is often required.

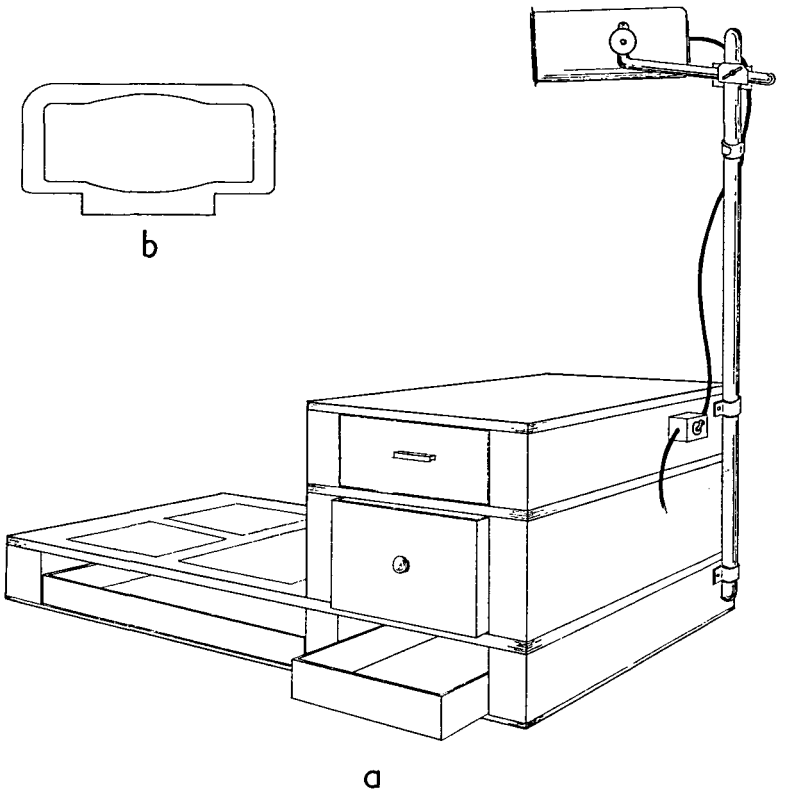



Figure 3 a. Stand for use with the inverted microscope. (For details see text).
b. Perspex distance piece.

When using the inverted microscope for general purposes the normal slide must be turned upside down so that the coverslip faces the objective. To prevent restriction of movement, caused by the coverslip catching the edges of the stage opening, the slide can be raised on a perspex distance piece (Fig. 3b), or the sedimentation chamber can be used for unmounted material. If, however, it is known that only low power examination is required the slide can be mounted the usual way up thus facilitating removal of organisms for more detailed examination.

## Summary

1. A description is given of a new form of sedimentation apparatus for use with an inverted microscope. It is easily constructed and is simple in operation with the minimum of interference to the settled material and no losses are possible when siphoning off excess liquid. In the form described it has a capacity of 27 ml but sizes may be adjusted to suit individual requirements. No modifications of a standard inverted microscope are necessary and all powers of magni-

fication may be used. The sedimentation chamber can also be used for the analysis of Stempel pipette sub-samples from net hauls.

2. Various pieces of ancillary equipment for use with the inverted microscope and developed in this laboratory are described as are the methods of analysis.

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