

## A Device for Continuous Microscopic Observations of Aquatic Microorganisms

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The device consists of a sealed microscopic mount provided with an inlet for liquid medium and an outlet, permitting continuous flow of the liquid. It permits observation of development of marine microorganisms such as thraustochytrids for 3 d.

Identification of the marine protists, the thraustochytrids, requires a study of various developmental stages for 1 to 2 d. Many<sup>1-3</sup> have used paraffin-oil chambers or continuous flow chambers<sup>4,5</sup> for microscopic observation of species of this group. Basically, these devices allow gentle and continuous inflow of seawater or nutrient medium from a reservoir through a shallow chamber placed under a microscope and exit through an outlet.

However, following problems are encountered in these chambers: The cells may not float or grow on the floating substrates, but may tend to sink to the bottom of the chamber. This makes microscopy difficult, since the chamber may be about 1 mm deep. This happens during development of species such as *Corallochytrium limacisporum*<sup>6</sup>. Secondly, the conventional chambers are tedious to prepare. Hence, a modified device for this purpose is described in this paper.

A microscope slide is cut into 4 pieces of  $2.3 \times 1.1$  cm and one central piece of  $3 \times 2.3$  cm (Fig. 1A). The 4 smaller pieces are joined with glue (Araldite) overnight, 2 on each side of the larger piece, leaving a gap of at least 1.5 mm in the middle (Fig. 1B). A coverslip of  $24 \text{ mm}^2$  is fixed overnight, one on either side of the slide on the same face, so that the grooves are closed on one side and the coverslip extends slightly beyond the joints as support (Fig. 1C). A dispensable hypodermic needle (Steriware;  $0.8 \times 38$  mm) is embedded overnight in glue in one of the grooves. Care must be taken to insert a fine wire through the nozzle of the needle and application of glue in the groove and over the syringe should stop about 3 mm away from the nozzle to prevent glue blocking the nozzle. Embedding should be proper and the syringe should not rotate in the groove. This is assured by fixing a narrow strip of coverglass over the syringe at the edge of the slide (Fig. 1C). In the other groove, a fine Teflon tube (ca. 1 mm inner diam.) is similarly fixed. Excess dried glue above the

surface of the slide is scraped. This unit and few coverslips ( $25 \times 50$  mm) are sterilized in an oven at  $100^\circ\text{C}$  for 2 to 3 h.

The unit consisting of a glass tube (b), a larger rubber tube inserted over it (c), along with a screw-type pinchcock over it and another smaller tube (d) fitted into (c) is assembled (Fig. 1E). The small rubber tube (d) should be of a size which can later be closely fitted into the hypodermic needle. This unit is sterilized by autoclaving. A suitable liquid medium, such as seawater for thraustochytrids, is filled in a flask along with a plastic tube (a) and is also autoclaved. The plastic tube 'a' should be of a size which can later be fitted onto the glass tube 'b'.

After sterilization, the flow chamber is assembled in an inoculation chamber. Prior to inoculation, the wire protecting the orifice of the needle is gently pulled out. A small drop of inoculum is placed in the centre of the slide. The long coverslip is placed on a firm base and silicon vacuum grease is applied on all the edges, using a syringe. The coverslip is lowered on the slide and gently pressed at the edges to make the grease spread thinly and evenly to seal the inoculum drop in the middle (Fig. 1D). As additional protection against water leakage during subsequent operation, nail varnish may be applied over coverslip to seal it and allowed to dry for 15 min. Then unit b, c, d is attached to the slide by inserting the tube into the hypodermic needle. The pinchcock over 'c' should be fully closed. With the water level in the flask higher than the slide, water is drawn out through the tube 'a' using a syringe and when the tube is full, it is inserted into the tube b. At this stage, the closed pinchcock over tube 'c' will prevent water entering into the slide. The setup is now transferred to the microscope, the flask resting on a jack adjacent to the microscope and the slide itself placed on the microscope stage. The height of the water level is adjusted to only a couple of millimetres above the slide on the microscope and the pinchcock over tube

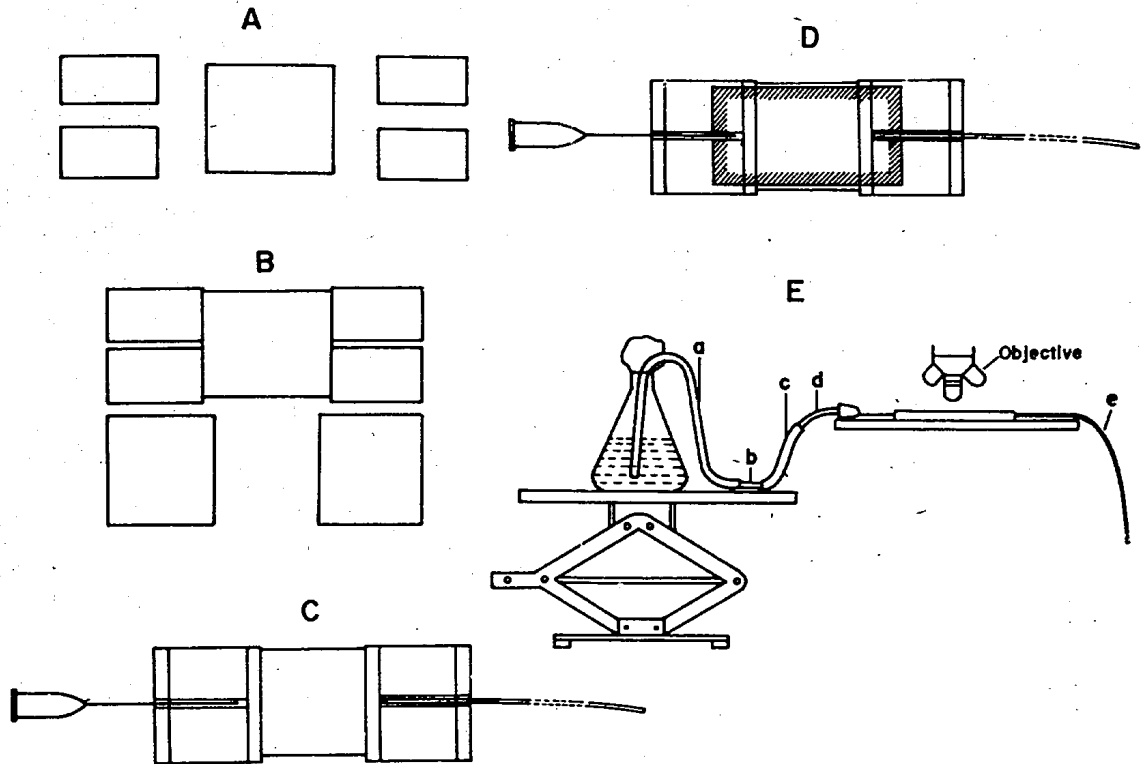


Fig. 1 — Continuous flow chamber assembly for microscopic observations (see text for details)

'c' is removed. Water now starts entering the chamber. The flow should be very gentle, otherwise the inoculum is washed off. The flow is regulated by adjusting the height of the jack. Caution should be particularly exercised when the medium starts flowing down through the 1 mm outlet tube 'e', since the flow now becomes faster; and once again the flow rate should be constantly adjusted till the medium starts dripping through the outlet of 'e'. One drop per minute is sufficient. The tube 'e' should be of sufficient length so that the final level of the medium in the flask is lower than the microscope stage, the orifice level of 'e' itself being slightly lower than the medium level in the flask. This is important since it ensures that any leak that develops in the chamber does not cause the water to spill on the microscope, but makes it flow back into the flask.

This device is routinely being used for observing the development of thraustochytrids in pure culture and also in natural microcosms. In the latter case, several groups of organisms, including diatoms, pro-

tozoans and thraustochytrids emerge out if bacteria are kept in check with the help of antibiotics.

Besides identification of thraustochytrids, the continuous flow chamber could also be used with advantage to study the development of aquatic fungi, protozoans and organisms of any aquatic microcosm in general under a microscope. The present device with some modifications can also be used to study larger organisms of about 500  $\mu\text{m}$  size. In this case, the surface of the chamber instead of being flat may be raised to a height of 1 mm by fixing strips of microscope glass slides on 4 sides. The long coverslip will rest on four sides on these strips.

#### References

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