

A THIN CULTURE CHAMBER FOR TIME-LAPSE PHOTOMICROGRAPHY OF FUNGI AT HIGH MAGNIFICATIONS

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SUMMARY

The construction and use of a thin culture chamber for time-lapse photomicrography of fungi at high magnifications are described. A photomicrographic sequence of blastospore development illustrates the potential of the chamber.

In classifying Fungi Imperfecti, developmental characters are now considered more biologically significant than criteria based on conidium arrangement, conidium septation, and pigmentation (Hughes, 1953). For example, chains of conidia that look very similar at maturity may, in fact, have developed in several quite different ways—compare *Penicillium*, *Cladosporium*, and *Oidioidendron*. We wanted to investigate conidium ontogeny by means of time-lapse photomicrography, and needed a culture chamber that fulfilled the following requirements: (1) it would support growth and sporulation of the fungus over a period of several days without drying out or staling; (2) it could remain exposed on the stage of the microscope repeatedly and for long periods, without becoming contaminated; (3) it would be thin enough to permit time-lapse cinephotomicrography by phase contrast illumination under a 100 × oil immersion objective.

Several types of culture chambers were already available. The van Tieghem cell (Duggar, 1909, p. 57-61) and the Sykes-Moore tissue culture chamber (Sykes and Moore, 1959) fulfilled requirements (1) and (2). The method of slide culturing employed at the Commonwealth Mycological Institute, Kew (Herb. I.M.I. Handbook, 1960, p. 31-32), met condition (1). None, however, was thin enough to satisfy (3). It was apparent that the chamber could not be much thicker than an ordinary microscope slide if our third requirement was to be met.

After some experimentation, a satisfactory and inexpensive design was evolved which has subsequently proved itself in both 35 mm photomicrography and 16 mm cinephotomicrography at high magnifications.

The culture chamber is based on a simple glass cytology slide measuring $75 \times 50 \times 1$ mm. A hole, 18–20 mm in diam, is drilled through the slide toward one end, and a slot approximately 1 mm wide is cut from the hole to the opposite end of the slide (FIG. 1). If a diamond-tipped drill (cost, approximately \$15) is available, holes can be bored in three slides simultaneously. If the copper tube-carborundum technique must be used, only one slide can be treated at a time. The slot is cut with a diamond saw (a diamond-edged wheel) and several slides may be slotted at once.

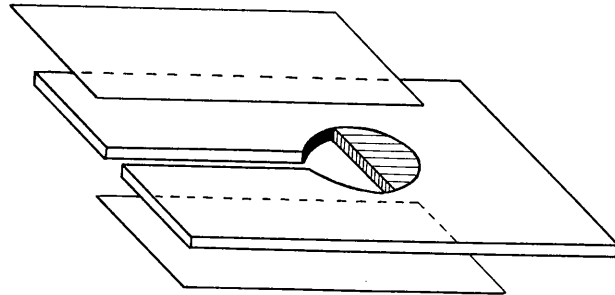


FIG. 1. An "exploded" view of the culture chamber, showing the drilled and slotted slide, the agar medium, and the two coverslips which seal the upper and lower surfaces.

The drilled and slotted slide is autoclaved, then a sterile 60×24 mm #1 coverslip is sealed to one side of the slide with nail varnish, covering the hole and the slot. As a further precaution against contamination, the open chamber is irradiated by a germicidal ultraviolet lamp for 15 min. Next, a slip of thin card the same width as the hole, and 5 mm high, is sterilized under the germicidal lamp or by autoclaving, and is placed across the middle of the shallow circular chamber, parallel to the short axis of the slide. Now, by means of a sterile micropipette, sterile molten nutritive agar is injected into the semicircular chamber on that side of the card away from the slot, and allowed to solidify. The card is then removed and the freshly exposed vertical surface of agar is inoculated with the fungus to be investigated. The chamber is now completed by sealing a second 60×24 mm coverslip over the top of the hole and slot (FIG. 1). A sterile petri dish is lined with filter paper soaked in 50% glycerol, to maintain high humidity, and a V-shaped glass rod laid horizontally on top of the wet paper supports the slide chamber. The whole is now incubated at 25–30 C.

Some fungi will grow and sporulate in the chamber for more than 2 weeks. The chamber must, of course, be removed from its petri dish when sporulation commences, and remain exposed on the open microscope stage for hours or even days while photomicrographic sequences are obtained. During these periods, desiccation may be prevented by covering the open end of the slot with a piece of paper tissue soaked in 50% glycerol.

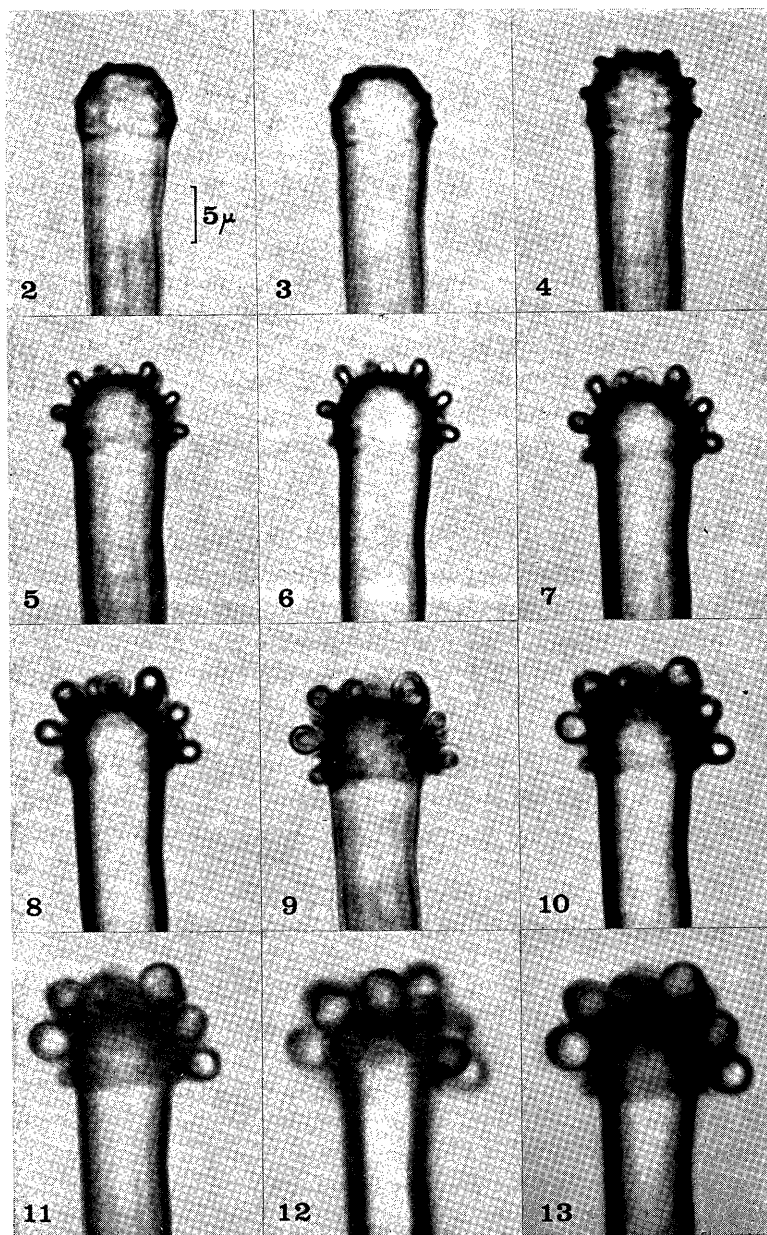
The chamber may be viewed from *either* side. In practice, we have found that only those conidiophores growing along the surface of one or other of the coverslips may be photographed at high magnifications. Fungi can be fixed and stained *in situ* at any stage of development desired, fixative being introduced through the slot by a hypodermic needle.

Certain of the dimensions of our chamber appear to be quite critical. The hole should not be made much less than 18 mm in diam as this could seriously reduce the amounts of both agar and air available to the fungus. The slot connecting the chamber with the outside air should be 35–45 mm in length. If it is shorter, desiccation sets in too soon and the risk of contamination is increased. If it is longer, gas exchange may be restricted, and serious condensation may occur on the coverslips, interfering with sporulation of the fungus and photomicrography.

We originally made our chambers from rectangles of $\frac{1}{16}$ inch thick perspex, because this material is so easy to drill and cut. We soon discovered that the thin perspex warped rather easily, and its lack of rigidity proved a fatal drawback under oil immersion, where very small vertical movements threw the subject completely out of focus, and where no horizontal instability could be tolerated. These problems were virtually eliminated when we adopted glass slides in place of the perspex.

Growth and sporulation of many fungi are inhibited if they are subjected to excessive amounts of heat and/or light. These factors were controlled by placing a heat filter in the light path below the stage of the microscope, and by decreasing the illumination to a low level between camera exposures. This last function, electrically synchronized with the automatic timing of exposures, is achieved by a time-lapse mechanism which has been developed in our laboratory and which will be described elsewhere.

This technique for studying spore development in fungi imperfecti became operational in April 1967, and we have so far successfully recorded the growth and sporulation of the following fungi: *Phialophora*



FIGS. 2-13. 35 mm time-lapse sequence of primary blastospore formation in *Gonatobotryum apiculatum* taken under the 100 \times oil immersion objective. FIGS. 2-13 at 0, 5, 10, 20, 25, 30, 40, 45, 50, 60, 70, 85 min, respectively.

richardsiae (Nannf.) Conant, *P. americana*, (Nannf.) Hughes, *P. lagerbergii* (Melin & Nannf.) Conant, *Oidiodendron truncatum* (Robak) Barron and *Gonatobotryum apiculatum* (Peck) Hughes. A cinemicrographic time-lapse study of the spore ontogeny of the last three named has been made on 16 mm Ektachrome EF color film (Cole and Kendrick, 1967). A sequence of time-lapse 35 mm photomicrographs obtained by using our slide chamber is reproduced here. (FIGS. 2-13). It was taken under oil immersion. Because the fungi will only sporulate normally in air, the definition attainable in this type of work is not strictly comparable with that obtainable in liquid mounts, but much new information concerning the fine detail and the timing of the process of sporulation may still be derived from our chamber. We suggest that this chamber would be useful not only for photomicrographic purposes but also to those who wish merely to observe fungi growing under the highest magnifications of the light microscope.

We are willing to supply sample drilled and slotted slides to interested mycologists on request.

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