

Use of the Sykes-Moore Tissue-Culture Chamber and the Cambion Heating-Cooling Stage for Spore Germination Research

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ABSTRACT

A microscopic method is described for observing spore germination and subsequent mycelial growth of fungal organisms. The method consists of placing spores within a Sykes-Moore tissue-culture chamber mounted on a Cambion heating-cooling stage. The Cambion stage is placed on a mechanical microscope stage that provides full coaxial movement of the Sykes-Moore chamber. Under these conditions, the entire contents of the chamber may be observed at $\times 100$ to $\times 600$. The primary advantages of the method include contamination-free observation over long periods, temp control between -10 and $+40$ C, and light control. It is suggested that the Sykes-Moore tissue-culture chamber, in combination with the Cambion heating-cooling stage, could be adapted to physiological and fungicide research and provide a means for improving some existing microscopic techniques.

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Additional key word: techniques.

Research with spore germination and subsequent mycelial growth often requires direct, constant, or periodic observation to record the various events of germination and growth as they occur. Such studies necessitate placing the organism on slides, or in culturing containers, that can be placed on a microscope stage for indefinite periods. Several problems are inherent in these procedures, depending on the duration of the observations. Contamination is of primary importance where observations must be made over several hours; it may be necessary also to control temp and light. The method described herein provides a means by which such studies can be conducted with existing equipment and minimize the inherent problems of contamination, temp, and light. The method makes use of the Sykes-Moore tissue-culture chamber (11, 12), and the Cambion heating-cooling stage and temp indicator (3).

The Sykes-Moore tissue-culture chamber was developed for ease of assembly and sterilization and for maintenance of sterility for in vitro study of mammalian cells (11, 12). The Cambion heating-cooling stage provides constant temp from -10 to $+40$ C and can be continuously monitored with the temp indicator. The heating-cooling stage can be used on any conventional microscope.

Components of the Sykes-Moore chamber (Fig. 1) and equipment used for placing spores in the chamber

should be autoclaved. To further prevent contamination, spores should be introduced into the chamber within a positive-pressure transfer chamber. Spore suspensions may be placed in the chamber in two ways. They may be introduced into an assembled chamber as described by Sykes and Moore (11, 12), for mammalian cells, by inserting a hypodermic needle (No. 22 or 24) into one of 4 ports in the base of the chamber and through the silicone rubber "O" ring gasket (25 mm dia.) (Fig. 1). The needle serves as an air vent as the spore suspension is introduced with a syringe through a port on the opposite side of the chamber. Spores also may be introduced into the chamber by placing the lower cover glass (No. 1 or 2, 25 mm) and gasket in the base of the chamber and then placing the spore suspension directly on the lower cover glass with a syringe or pipet. The upper cover glass is then placed on the gasket, and the chamber top is screwed into place. Chamber volume is ca. 1.0 ml with the standard 2.5-mm thick gasket.

The Sykes-Moore chamber, with spore suspension, is placed on the Cambion heating-cooling stage with the bottom side up (Fig. 2). In this position, the top of the chamber fits within the open center of the heating-cooling stage, and the upper edge of the chamber bottom comes to rest on the stage surface. It may be necessary to scrape off a small amount of insulating material from the sidewall of the center opening of the stage to allow the chamber top to slide smoothly into the opening. That the top of the Sykes-Moore chamber fits into the center opening of

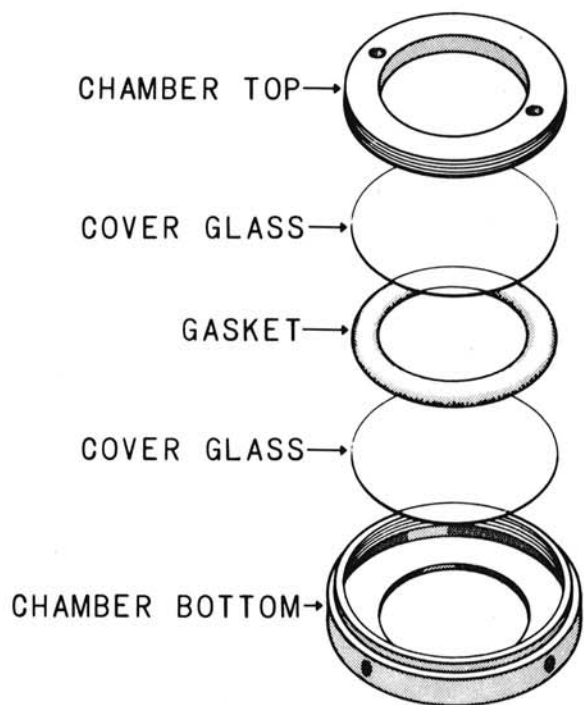


Fig. 1. Components of the Sykes-Moore tissue-culture chamber (11,12). Available from Bellco Glass, Inc., Vineland, N. J.

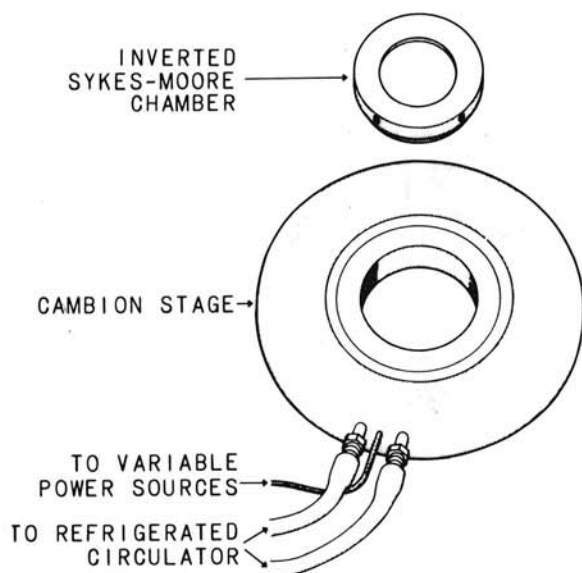


Fig. 2. Positioning of the inverted Sykes-Moore chamber on the Cambion heating-cooling microscope stage (3). The Model 806-3000-01 stage is shown. Available from Cambridge Thermionic Corp., Cambridge, Mass.

the Cambion stage is coincidental; the two items were designed and produced independently. Once the chamber is in place, the temp-indicator probe may be pasted onto the inverted bottom of the chamber for direct, continuous temp monitoring. Although the Cambion stage is provided with a rubber base that allows it to set securely in one position on a microscope stage, it is desirable to have the stage mobile. This is accomplished by locking the Cambion stage into the slide holder of a mechanical microscope stage; this allows full coaxial mobility of the heating-cooling stage, and the entire contents of the chamber can be examined in position on the microscope. It is probable, however, that slide holders of all mechanical stages will not be equally adaptable to this procedure.

The method described here for observing spore germination and subsequent mycelial development utilizes existing equipment and minimizes problems of contamination, temp, and light. Contamination can be prevented if care is taken to sterilize all equipment; when a nutrient medium is used, however, contamination is best avoided by placing the medium in an assembled chamber with a syringe. Temperature control via the Cambion stage is precise; chamber temp variation does not exceed ± 0.2 C. When studies are conducted at temp of 5 to 10 C, the cover glass nearest the objectives tends to fog and requires periodic wiping with lens paper. The duration and intensity of light can be readily controlled with substage illuminating units (Koehler types) found on most research microscopes.

The range of magnification for examining spores within the chamber is $\times 100$ to $\times 600$, utilizing various combinations of $\times 10$ and $\times 15$ eyepieces with $\times 10$, $\times 20$, and $\times 40$ objectives. The effective range of

magnification for scanning the contents of the chamber is $\times 100$ to $\times 300$; use of the $\times 40$ objective for magnifications of $\times 400$ to $\times 600$ is effective for detailed examination of spores and photography, but it is not effective for scanning the chamber. Conventional $\times 40$ objectives cannot be used because of inadequate working distance (ca. 0.54 mm). Long-working-distance $\times 40$ objectives (ca. 2.0 mm) can be used when the standard silicone rubber gasket (2.5-mm thick) is replaced by a thinner gasket (1.5-mm thick). When this is done, however, a thin gasket also should be placed between the chamber top and the cover glass to prevent the top from screwing all the way into the bottom; if this occurs, the chamber top will not provide a flange to set within the heating-cooling stage, and the chamber slides about uncontrollably. The thinner gasket also will reduce the chamber volume to ca. 0.6 ml.

Use of the Sykes-Moore tissue-culture chamber and Cambion heating-cooling stage for spore germination studies has been limited to conidia of *Curvularia* and *Helminthosporium* species in distilled water or liquid nutrient media. Germination of conidia of these organisms is excellent (7, 8) within the chamber, and it is probable that the method might be of considerable value in various physiological studies. The chamber might also provide a convenient closed container for studies with various hanging-drop (5) and hanging-block methods (4, 5). Sykes and Moore (12) also describe a tissue-culture method utilizing cellophane within the chamber; methods utilizing cellophane for cytological studies of fungi have been devised (5, 9, 10) and might be further developed with the Sykes-Moore chamber. The chamber might also be adapted to spore-germination methods for fungicide evaluation. Current methods (1, 2, 4) for evaluating spore germination in soluble fungicides on glass slides might be further improved in a closed chamber where temp and light were controlled.

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