Observing fungal growth with an inexpensive, easy-to-make microchamber for microscopy

HANS HALBWACHS
Danziger Str. 20, D - 63916 Amorbach
halbwachs@mykologie-bayern.de

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K e y w o r d s: cellular level, live cells, mycology, in vivo observation.

A b s t r a c t: Microchambers are a tool for peering into microbial lifestyles and the behaviour of organisms at the cellular level. The microchamber presented here – in contrast to previous designs – is a low-cost, robust and simple design that allows all kinds of experiments with live cells observed under the microscope. It can easily be mounted using readily-available material.

Practical application in the field of spore germination over three years has demonstrated its suitability for mycological research. The device is of particular interest for both professional and amateur mycologists and researchers in various other disciplines.

Z u s a m m e n f a s s u n g: Mikrokammern sind ein Instrument, um mikrobiologische Lebensweisen und das Verhalten von Organismen auf Zellebene zu untersuchen. Im Gegensatz zu früheren Konstruktionen ist die hier vorgestellte Mikrokammer preisgünstig, robust und einfach aufgebaut. Sie erlaubt verschiedenste Experimente mit lebenden Zellen unter mikroskopischer Beobachtung. Die Herstellung aus überall erhältlichen Komponenten ist einfach.

Die praktische Anwendung bei der Keimung von Sporen während drei Jahren hat die Eignung für die mykologische Forschung gezeigt. Das Gerät ist vor allem für Berufsmykologen und Amateure, aber auch für Wissenschaftler verschiedener anderer Disziplinen interessant.

Introduction

At times when molecular techniques govern biological research, live investigations of developmental aspects or organismic interactions at the cellular level of fungi seem to be outdated, looking at relevant publications during recent years. Appraising, e. g. morphological changes during life cycles of fungi under different conditions are underrepresented. One contributing reason could be that such endeavours are viewed as ambitious in terms of cost and sophistication.

Microchambers for microscopic observations offer a means for such challenges. They have been used for many years in biological and medical research, but the designs have had various drawbacks in terms of cost, sophistication, durability, handling and flexibility (Bartnicki-garcia & Lippman 1966, Federlin et al. 1971, Feder 1981, Fantini et al. 1987, Hill 1995, Focht 1996, Friedman et al. 2002, Hausen & Riebesell 2002)

In mycological research, experiments sometimes involve continuous observation of cells subjected to various regimes. Typical examples include investigations of hyphal growth, spore germination or living conditions of protozoans. In general, microchambers need to exhibit the following features:

- volume < 500 µl
- defined chamber thickness (0.4–1 mm)
- sterilisable
- easy to load (under sterile conditions)
- loadable with fluid, gaseous and (small) solid agents
- suitable for microscopic magnification up to 1,000× (oil immersion)

In addition, it is desirable to have a device that is easy to build, and that is affordable.

Design and assembly

The new microchamber basically consists of a conventional microscope slide, a cover glass and a silicone seal (Fig. 1).

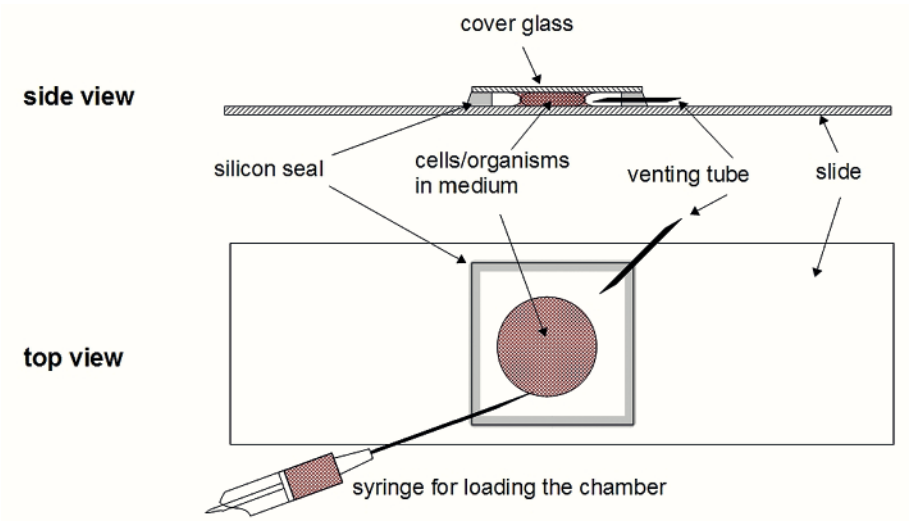

Fig. 1: Design of the microchamber Drawing: H. Halbwachs

Hardware needed (Fig. 2):

Parts for the chamber

- standard microscope slide, 76 × 26 mm
- cover glass, e.g. 18 × 24 mm
- venting tube: 10 mm piece of a hypodermic needle, e.g. Gauge 23 (0.6 mm) for
- a chamber of 1 mm thickness, cut with a needle file or the like

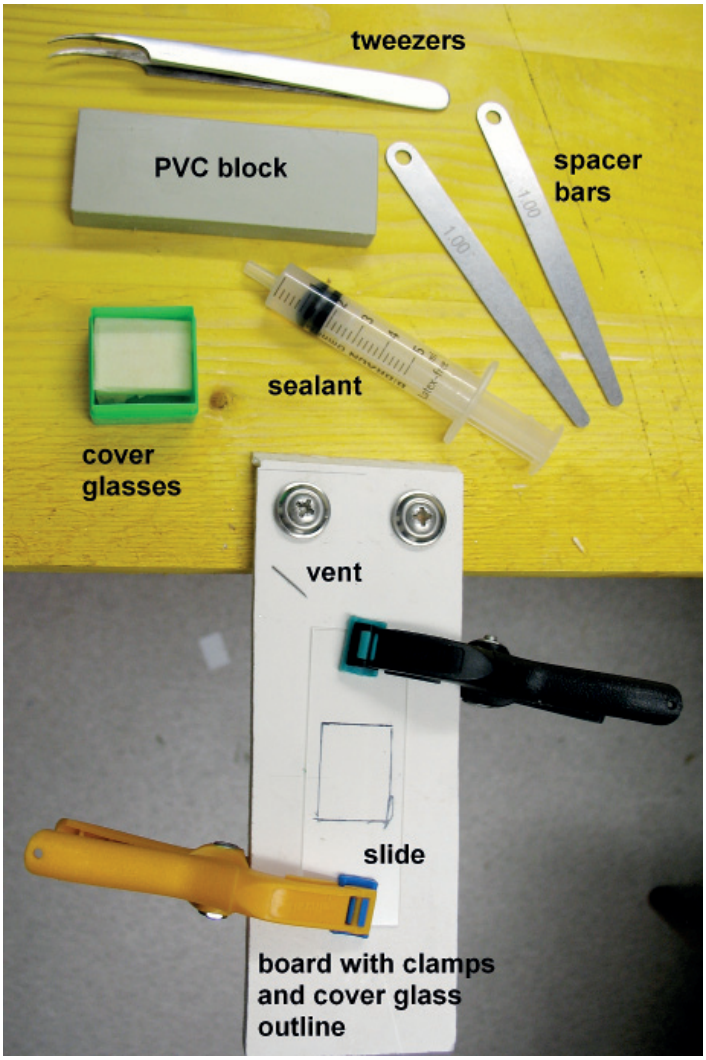

Fig. 2: Hardware for mounting the microchamber Photo: H. Halbwachs

Materials and tools for assembling the chamber

- acetone for degreasing slide and cover glass
- transparent silicone sealant (acetic cure) filled in a 5 ml plastic syringe (Luer slip)
- a small mounting board (ca. 150 × 50 × 10 mm) showing an outline of the cover glass that for instance is fixed at the edge of a table
- 2 clamps
- 2 spacer bars removed from feeler gauges (see Appendix); the thickness of the spacer bars determines the thickness of the chamber, e.g. 1 mm
- 1 block of rigid PVC (ca. 10 × 30 × 85 mm)

The assembly comprises the following steps:

1. degrease slide and glass cover (if needed, covers are mostly greaseless)
2. place slide on board and fix with clamps (ref to fig. 2)
3. apply sealant in a ca. 2 mm thin line along the interior side of the cover glass outline (ref to fig. 3)
4. place the venting tube with fine tweezers into one of the corners and push it down
5. place the cover glass on the sealant, adjust the fit on the sealant line
6. remove clamps and place spacer bars left and right on the slide (ref to fig. 4)
7. press PVC-block on the arrangement (ref to fig. 5) and remove
8. let the assembly set overnight and sterilise at 150 °C for 1 hour, which expels residuals of the curing agent (acetic acid) as well.

The assembly of one microchamber takes less than two minutes, the materials and parts add up to less than 0,5 Euro (incl. cost share for the tools).

Loading takes place with sterile disposable syringes of 0,5 or 1 ml fitted with hypodermic needles with a maximum diameter of 60% of the chamber thickness. For a 1 mm chamber a 0,4 mm needle (Gauge 27) is a save choice (ref. to fig. 6).

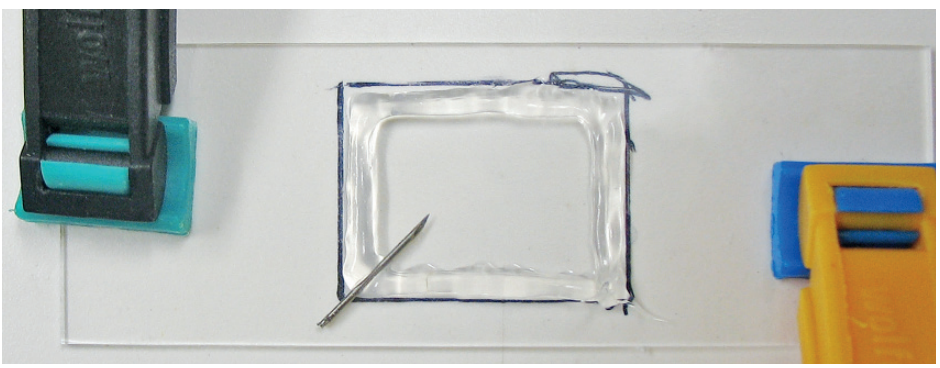

Fig. 3: sealant line and vent Photo: H. Halbwachs

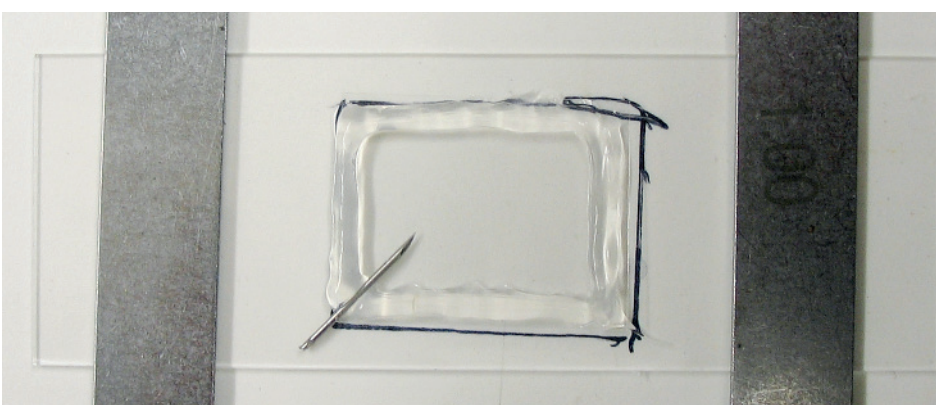

Fig. 4: spacers Photo: H. Halbwachs

Results and Discussion

1 mm microchambers have been used in approx. 50 basidiospore germination experiments during three years, using various liquid or semi-liquid media. Handling and durability proved to be reliable. Only the cover slip broke twice during this time while somewhat hastily piercing the silicone seal with a 0,6 mm (Gauge 23) hypodermic needle in course of inserting a fragment of a hair root (ref. to fig. 7). Breakage never occurred with the recommended 0.4 mm (Gauge 27) needles.

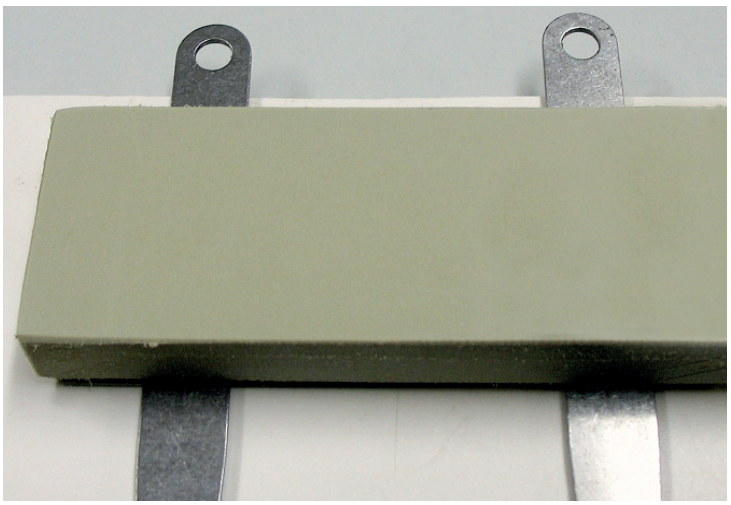

Fig. 5: PVC-block
Photo: H. HALBWACHS

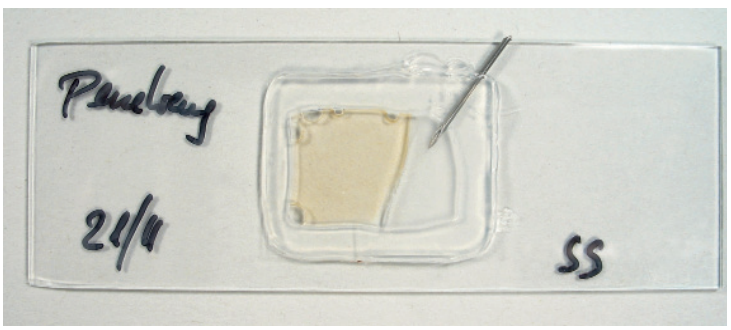

Fig. 6: chamber loaded with a spore suspension
Photo: H. HALBWACHS

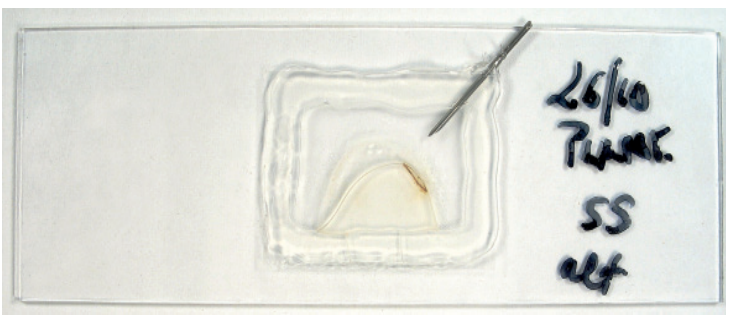

Fig. 7: hair root inserted into chamber
Photo: H. HALBWACHS

Bright field microscopical investigations were mostly carried out at a magnification of 400 (ref. to fig. 8). For closer inspection an oil immersion objective (1000X) was used, in most cases with good results (ref. to fig. 9 and 10). Objects situated at the bottom of the chamber appear more hazy due to the medium and particles above, if the aperture is too small. Though not tested, the chambers are expected to be well suitable for microscopes with heating stages.

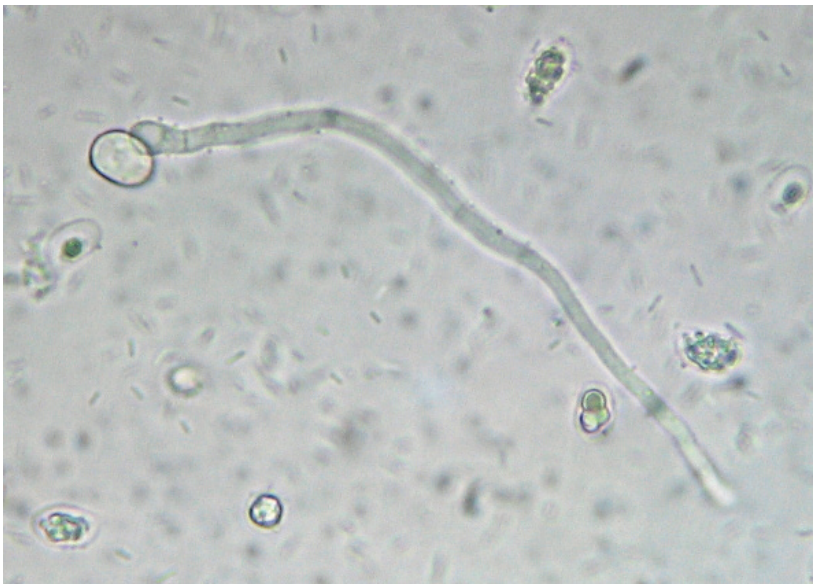

Fig. 8: germinating spore of *Armillaria borealis* (400x) Photo: H. Halbwachs

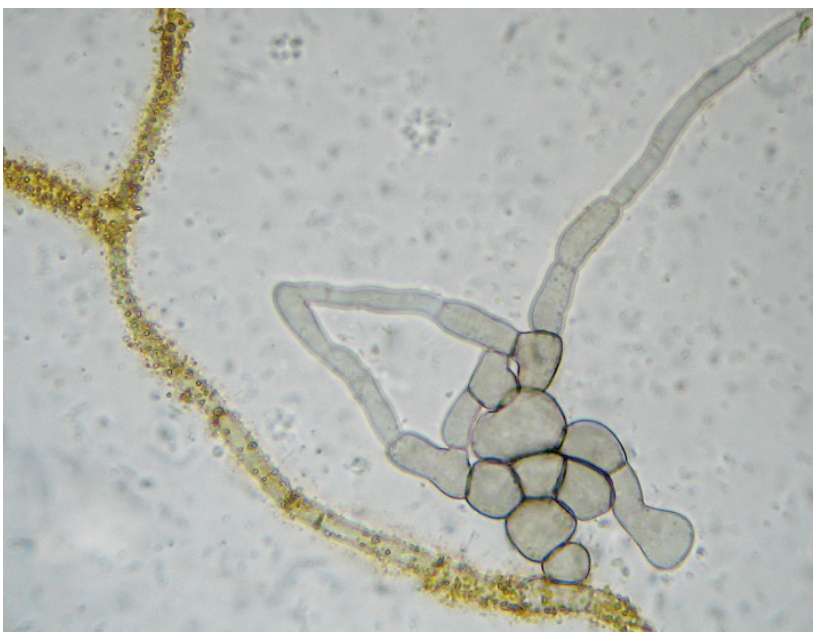

Fig. 9: sclerotia of a dark septate endophyte (DSE) (1000x) Photo: H. Halbwachs

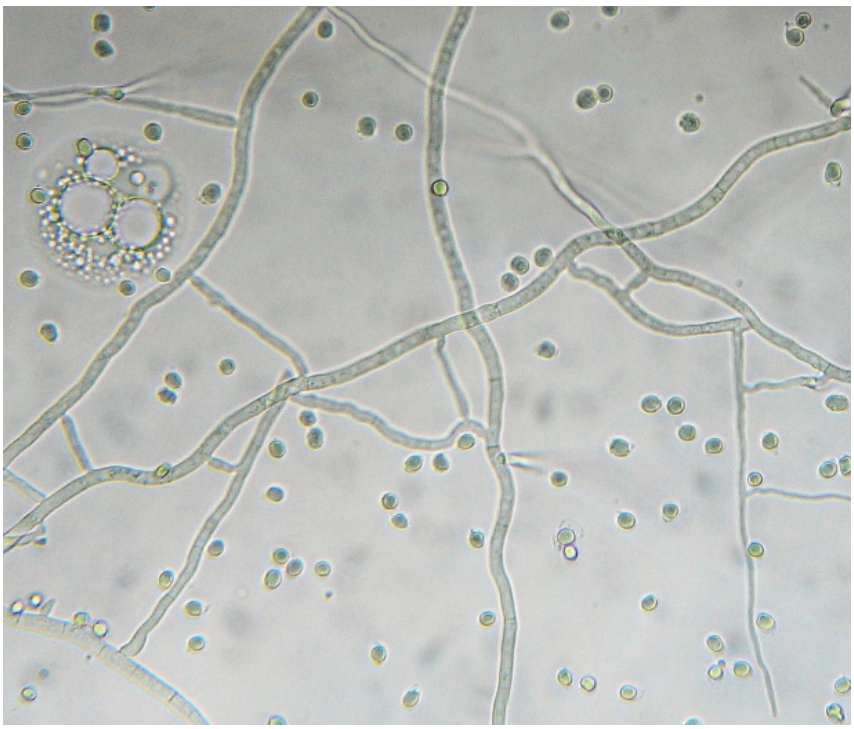

Fig. 10: ascomycetes appearing in a basidiospore suspension (400x)
Photo: H. HALBWACHS

Theoretically the chambers can be reused after flushing with cleaning agents and distilled water, followed by sterilisation. But since the production of the chambers takes only little effort and time, re-utilisation is not worthwhile.

For the mycologist the microchamber is of particular interest. It is an easy-to-handle tool for investigating germination behaviour and mycelial differenciation under differing conditions. Even explant experiments are possible.

The chamber may also be used for demonstration and teaching purposes, particularly with microscopical projection techniques. Simplicity and price makes the device attractive to amateur mycologists, as well.

In conclusion, the microchamber presented fulfils all properties required. Its design is flexible and may be adapted to various experimental designs that e. g. use electric pulses via electrodes, continuous gas flow or periodic adding of agents.

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Appendix

The material and parts mentioned can be acquired from regular stores, in most cases even online:

Item	**Supply**	**Remarks**
microscopic slides, cover slips, disposable syringes, hypodermic needles	lab stores, medical supply, pharmacies	
silicone sealant	sold as glue for styrofoam in small quantities by art and craft shops	make sure that the sealant is acetic cured
spacer bars: feeler gauge as used e.g. for spark plugs	DIY-stores, motor car accessories	www.reluctantmechanic.com/using-tools/thickness_gauge.php
PVC-block	DIY-stores, online auction portals	
clamps	sold for press sizing of wooden pieces by DIY-stores and online auction portals	

A compilation of microchambers available on the market is provided by OLYMPUS: http://www.olympusfluoview.com/resources/specimenchambers.html