An improved fungal mounting technique for Nomarski microscopy

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A new permanent mounting technique for Nomarski differential interference contrast and bright field microscopy of fungal tissue.

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Abstract

A new mounting technique for the production of permanent fungal slides for Nomarski and bright field microscopy is described. This technique produces a completely clear background free from stress induced interference patterns in Nomarski microscopy and permanent stained slides for bright field microscopy.

The "sellotape technique" of Butler and Mann (1959) with lactophenol cotton blue stain is widely used in these laboratories for the production of non permanent fungal mounts for class examination using bright field microscopy. The technique allows very little disturbance of fungal structure for identification and taxonomic work. The major drawbacks of this technique for permanent mounts are the fact that the tape itself is not inert and the adhesive reacts with the lactophenol forming droplets which cover the field of view (Onions et al., 1981). The stressed nature of the tape also gives rise to problems due to interference patterns during Nomarski microscopy. The increasing use of differential interference contrast (Nomarski) microscopy in mycological studies prompted a re-evaluation of techniques used in the preparation of fungal mounts (Fairclough, 1984). Other types of non-stressed tapes were found but these proved not to be sticky enough to pick up fungal structures from agar media cleanly and without undue damage. The technique described below uses a silicone based adhesive in conjunction with the slightly modified polyvinyl alcohol mountant of Salmon (1954), updated by Omar et al., (1978).

Reagents

(1) Clean glass coverslips are coated on one side with an inert synthetic adhesive (Dow Corning 280A Pressure Sensitive Adhesive, obtainable from Hopkin and Williams, Romford, RM1 1HA, Essex). The adhesive is diluted to 1% (w/v) in xylene and one drop (about 0.1 ml) is spread over the surface of the coverslip and allowed to dry, adhesive side up, in a dust-free atmosphere. Once prepared the coverslips have been stored for up to four months without any deterioration in adhesive properties.

(2) The permanent mounting medium first described by Salmon (1954) and used by Omar et al., (1978) was modified slightly by using hot water soluble polyvinyl alcohol (P 1763 Type III Low Molecular Weight PVA, obtainable from Sigma Chemical Company Ltd., Poole, Dorset, BH17 7NH). The hot water soluble PVA was found to produce a more viscous solution and to fully harden in a much shorter time than cold water soluble PVA. The formulation is given below:

Hot water soluble PVA (Sigma)	1.66 g
Distilled Water	10.0 ml
Lactic Acid (ANALAR)	10.0 ml
Glycerol	1.0 ml

The solution is gently warmed to 45 - 50 °C during preparation and then allowed to mature at room temperature for 24 hours. No filtration was found to be necessary.

Procedure and Modifications

For Nomarski microscopy the coated coverslip is held by forceps and gently pressed, adhesive side down, onto the edge of a fungal colony and then placed, adhesive side down, onto one drop of mountant on a clean glass slide. We have found that prewarming the slide with mountant to 40 °C assists greatly in the removal of air bubbles from the specimen , e.g. in sporing heads of Penicillium spp. (Figure 1). Excessive heating causes discolouration and hardening of the clear mountant. The differences between this technique and the sellotape technique in Nomarski microscopy can be illustrated by reference to Figures 2 and 3, taken under identical conditions and settings on our Olympus AH Vanox Universal Microscope. Both techniques allow minimal disruption of fungal structure but interference patterns in the sellotape, together with trapped air bubbles obliterate much true detail. Delicate structures such as the branched spore chains in Cladosporium spp. can also be faithfully preserved using this technique (Figure 4).

For bright field microscopy 0.01% (w/v) aniline blue can be incorporated into the mountant after maturation. The stain is taken up preferentially by the fungal tissue over a period of several days and a clear, light blue background remains (Figure 5). Higher concentrations of the stain give an overstained specimen.

Permanent mounts of both types of slide can be produced by heating the prepared slides at 40 °C for 48 hours.

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