MICROSCOPICAL TECHNIQUES

staining with Congo Red

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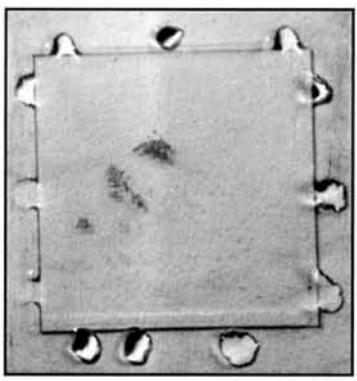
Congo Red is a particularly effective stain for staining the walls of hyphae. Surprisingly it gets hardly any mention in any of the books on microscopical staining techniques, perhaps because these are mainly concerned with bacteriology and histology of mammals. It is hard to find mention of it in general field guides and popular books. However, a glance in fungal monographs at techniques used will reveal the popularity of the stain among serious workers, especially with larger Basidiomycetes.

It is effective in alkaline solution and best in strong ammonia solution. The actual strength is not very important. If the concentration of ammonia falls, causing the dye to precipitate out, the problem is easily reversed by adding a little strong ammonia. The ammonia solution obtainable from pharmacies is suitable and is described as 10%. I use .880 ammonia* diluted to about 20% with water. Heinz Clémençon, who is one of the world's leading mycological microscopists, uses aqueous congo red with a surfactant (a substance like washing up liquid, which reduces surface tension and aids the wetting of the section) added. He uses 1% sodium dodecyl sulphate which is not a readily available substance.



Closeup of a stained section of tissue in Congo Red. Drops of glycerol can be seen around the outer edge, as described in the text, preventing the slide from drying out.

I have tried various surfactants, but with no satisfactory results using readily available compounds. Precipitation is a problem. For most of us ammonia will do the job but beware of corrosion effects, easily overcome by washing as described below.

The technique is to place a small section of the required tissue, the edge of a lamella for example, on a slide and carefully cast a drop of the stain onto it, or place a section in a drop already on the slide. The dye is taken up very quickly with thin-fleshed species, rather more slowly with polypores. After a minute or so, lower a cover slip onto the section and carefully squash with gentle pressure from forceps or a rubber-topped pencil.

Have small squares of blotting paper handy to remove excess stain. Wash the preparation with a little potassium hydroxide solution (KOH) or else just water. To do this, place a drop of the KOH solution at one edge of the cover slip and "pull" the liquid through the preparation by applying a square of blotting paper to the far side of the cover slip. If preparations still in ammoniacal solutions are left in close proximity to microscope objectives, etching of the metal parts can occur. It is wise to wash the excess ammonia away first, after staining. The result will be a cleaner section to study with less tendency to dry out. For prolonged study of a section, e.g. during drawing or photography the following technique was suggested to me by R A Maas Geesteranus, and I have found it to be very effective.

*Ammonia is a gas at room temperature but is very soluble in water. A saturated solution contains a large amount of ammonia and has a specific gravity of 0.880 which is why it is known as eight eighty ammonia. This is dangerous material and even the more dilute solutions available should be treated with great respect, being strongly alkaline. Eye protection is recommended when preparing the dye solution.

Using a drawn out glass rod or a seeker, place a small drop of glycerol (glycerine from the pharmacy is the same thing), at each edge of the cover slip (Fig 1). Carefully help the small drop to spread along the edge, taking care not to move the cover slip if possible. Leave for a few minutes and the glycerol will diffuse in the outer layer of the solution within the mount. The section will not then dry out for hours. If you wish to preserve the slide for a short period, carefully blot off excess glycerol from around the cover slip and seal with nail varnish. I have prepared slides in this way and they have remained the same for six months. This technique will only be possible with 18mm cover slips or smaller; large cover slips do not leave space for sealing so easily.