Using LR White for Electron Microscopy

When using 'LR White' embedding resin for dedicated electron microscopy, very few changes need to be made to the regime used for epoxy resin embedding. Every laboratory has its own individual embedding schedule but we have laid out here a 'typical' schedule for LR White as guidance for its use.

Fixation:
No change from normal fixation should be made if EM analysis only is required from the final blocks. If, however, good ultrastructure and a wide range of LM staining is required then we have found that the use of aqueous paraformaldehyde in a phosphate buffer pH 7.2 with 2.5% w/v sucrose is the best compromise. Glutaraldehyde alone and Karnovsky's glutaraldehyde-formaldehyde mixtures may lead to patchy LM staining and some stains not working or giving "false positives" (e.g. PAS) whereas normal formalin fixation yields unacceptable EM ultrastructure.
For dual LM/EM applications, Osmium tetroxide should be avoided due to its effect on many LM stains but 1% phospho tungstic acid (w/v) in the first absolute ethanol step of dehydration improves electron contrast without adversely affecting most LM stains. Osmium tetroxide may be used if the blocks are required for dedicated electron microscopy only.

Dehydration:
A graded ethanol series is the method of choice when embedding in 'LR White'. Acetone acts as a radical scavenger in the resin system and therefore traces of acetone left in the tissue at curing can interfere with this polymerisation. For this reason the use of graded acetone series and 2,2 - dimethoxypropane (which generates acetone) are best avoided. If the use of 2,2 - dimethoxypropane is considered vital we recommend either a protracted resin infiltration or washing the tissue with dry ethanol prior to infiltration in order to minimise the chance of acetone contamination of the final resin.

Infiltration:
The extremely low viscosity of 'LR White' may be exploited by allowing the use of short infiltration times or large specimens but not both! A 1mm cube of animal tissue will be adequately infiltrated in about 3 hours if 4-6 changes of 'LR White' at 60˚C are employed during this period.
An overnight infiltration at room temperature, followed by two short changes of resin will often be more convenient, however. The long shelf life and low
extraction rate of 'LR White' allows specimens (e.g. reserve tissue) to be stored safely in resin for many weeks at 4˚C if required. Larger blocks do require significantly longer infiltration times than small ones.

Polymerisation:
Samples stained with osmium tetroxide should not be 'cold-cured' with the accelerator. This process is strongly exothermic, and the dark colour of the tissue leads to a focal heat accumulation which can cause local problems in and around the tissue.
If the tissue is not fixed with osmium tetroxide then curing with 'LR White' accelerator may be employed. As with curing blocks for light microscopy we recommend cooling the moulds during polymerisation, but there is no need to exclude oxygen from the surface of the curing block.
Thermal curing should be used for osmicated specimens and may be used for all specimens. Here it is important to limit the contact of oxygen with the resin while polymerisation occurs. The most convenient way of achieving this with capsule-type embedding is to use gelatin capsules (available from Agar Scientific), fill up to the brim and slide the other half of the capsule on.

If flat embedding is required for cutting orientation then the surface of the resin must be covered to prevent contact with oxygen.
One convenient method is to utilise the JB-4-type moulds and chucks, useful for light microscopy, and after polymerisation the block may be sawn off the stub and mould re-used.
Polymerisation time and temperature are fundamental to the physical characteristics of the final block, to a much higher degree than with undercured epoxy systems.
We strongly recommend a temperature of 60˚C ±2 for a period of 20-24 hours. Some ovens are not capable of controlling polymerisation temperature so closely, and if faced with over brittle blocks, this is the first parameter to check.
LR White has extremely good powers of penetration and can penetrate and soften some low-density polyethylene capsules. This causes them to distort and collapse. Also polyethylene is not impermeable to oxygen and may allow enough contact with atmospheric oxygen to give the blocks an inhibited 'tacky' surface.
Both these problems may be overcome by the use of gelatin capsules (size 00 is similar to the popular polyethylene capsule size) and these are much cheaper and easier to seal during polymerisation.
Resin may be used straight from the refrigerator and has a very low toxicity both in monomeric and polymerised state, unlike epoxies. The cold cure accelerator does have some toxic risk and contact with the skin and eyes should be avoided.
For cold curing the accelerator should be used at one drop per 10ml of resin and this should cause polymerisation in between 10 and 20 minutes. If polymerisation occurs faster than this we recommend either more careful metering of the one drop of accelerator or a higher volume of resin per drop of accelerator.

Trimming and cutting:
Trimming the block may be done with jewellers saw, razor blade or with a glass knife on the ultramicrutome as with epoxy resin blocks. Cutting may be performed in the same way as for epoxy resin with glass or diamond knives. A typical cutting speed of about 1mm per second is suitable.

Section staining:
All the common section stains give good results on tissue embedded in 'LR White' resin. Stains made up in ethanol or methanol should be avoided as these solvents soften the resin and may remove sections from grids. As an alternative to uranyl acetate, 1% phosphotungstic acid has proved a good general-purpose stain, both as a block stain, as mentioned earlier, and as a section stain.

In the Electron Microscope:
An initial reduction in electron density may accompany the initial exposure of the resin to the beam. This is thought to represent a loss of water, imbibed from knife-boat or staining solutions. Thinning as such does not occur and specimens have been kept stationary under a 120kV electron beam for 3 hours with no obvious signs of damage.

London Resin - acrylic resins for microscopists

This product is just one of the London Resin's range of resins specifically formulated for the needs of the microscopist. All the resins are manufactured to the same rigorous standards from one of the world's largest suppliers of histological resins.

LR White

A convenient and economical premixed resin with very wide application. Being both hydrophilic and electron beam stable it is equally suitable for light and electron microscopy, and with appropriate fixation the same specimen may be used for both techniques. Published work shows that immunocytochemical methods may be used through LR White sections without etching or any pre- treatment.

Histocryl

A conventional multi-component acrylic system offering a direct alternative for other commercial HEMA systems, but at a fraction of the cost of most.
Economical enough to allow the histologist who recognises the high quality resin histology can bring to his work to use resin more widely and cost effectively than ever before.

LR Gold

A special acrylic resin for very specific purposes. Its infiltration and polymerisation at low temperatures down to -20°C means that unfixed tissue may be embedded in LR Gold. This enables enzyme histochemistry and immunocytochemistry of many fixation sensitive enzymes and epitomes to be performed on 1 – 2µm resin sections. Bringing the quality of resin histology to an area where only cryostat sections were previously available. LR Gold is a real step forward in histochemical technique. This resin has the ability to be cured by blue light thus making expensive ultra-violet sources unnecessary.

All these acrylic resins combine low viscosity, low toxicity and case of use, reflecting the safety-conscious standards of London Resin products.

London Resin - manufactured and supplied by:
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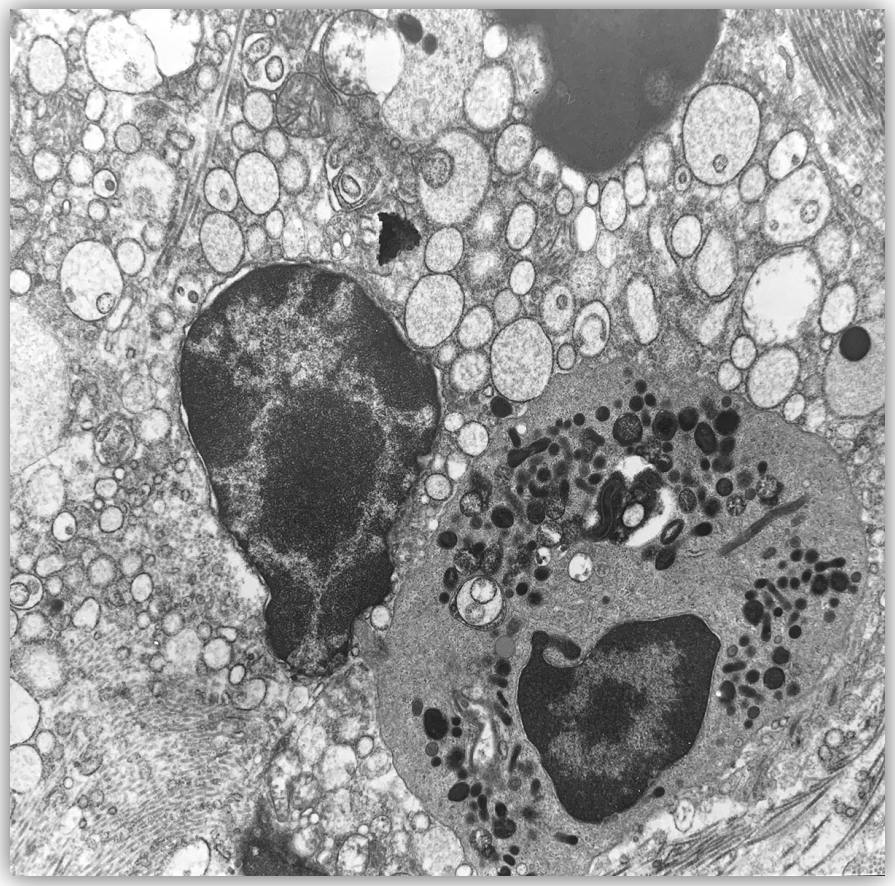

PMN Phagocytosis of Spirochaetes in connective tissue in Juvenile Periodontitis.