Imaging Degraded Wood by Confocal Microscopy

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The application of confocal laser scanning microscopy (CLSM) in the studies of biological materials is rapidly expanding because of the opportunity to produce sharp, high resolution images through optical sectioning and computer assisted 3-D reconstruction. At our institute CLSM is being used in a wide range of forestry and wood science studies.

Recently we investigated the potential usefulness of CLSM in characterizing biologically degraded wood. The following are images produced from an archaeological wood which has been buried in a wet environment (rice field) for nearly 2,000 years in South Korea and is apparently degraded by bacteria. In an attempt to develop suitable techniques which can be used for routine examination of fragile degraded wood with CLSM, we have compared two different embedding methods for their suitability in preserving the integrity of cells. The embedding media are paraffin wax and LR White resin.

The illustrations (Figures 1, 2, and 3) provide a comparison of the images of degraded wood tissues embedded in paraffin wax and deparaffinized with xylene, embedded in paraffin wax but not deparaffinized, and embedded in LR White resin, respectively. Specimens fixed with glutaraldehyde and paraformaldehyde (1:1, v/v) were examined in a Leica TCS/NT CLSM using wavelengths of 488 and 568 nm for excitation and 530 and 590 nm for imaging with a 63X oil immersion lens. Specimens were observed directly after sectioning (15 µm thick for wax-embedded and 4 µm thick for resin-embedded samples) without treatment with any fluorescent dyes, using the autofluorescence of lignin present in wood cell walls. All images are sharp with a high contrast between the degraded and undegraded parts of wood cell walls, and this has made it possible for us to make critical comments on the advantages and disadvantages of the methods used. It is apparent that in Figure 1 few cells are intact, whereas in Figure 3 all cells retain their integrity, with the heavily degraded parts of wood cell walls being also well preserved as wall residues. The quality of preservation of the integrity of wood cells in Figure 2 is intermediate between the two.

Clearly paraffin wax embedding cannot be a method of choice for confocal microscopy of degraded archaeological wood (This may also apply to other degraded woods which are fragile), although paraffin embedded biological materials have been widely examined in confocal studies, particularly in medical sciences. We therefore suggest that anyone interested in the imaging of degraded wood tissues should use resin and not paraffin wax as an embedding material. However, if it is absolutely necessary to use paraffin to save time in processing samples or if there is no access to an ultramicrotome, paraffin embedded sections should not be deparaffinized. ■

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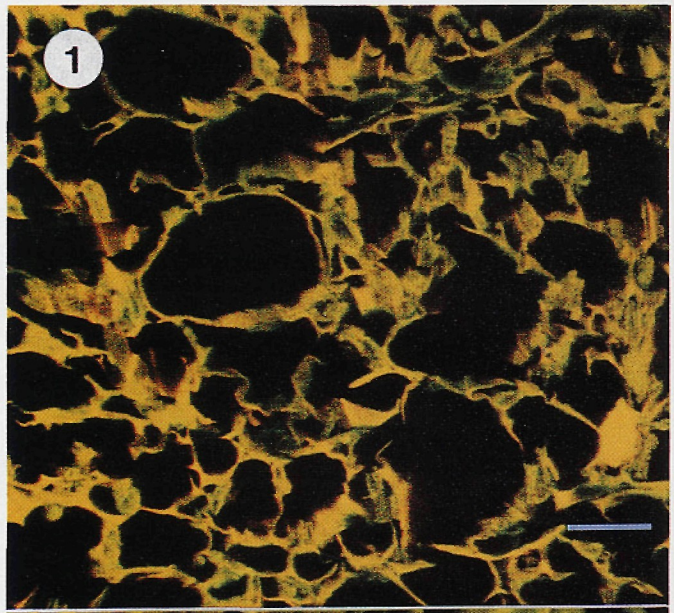

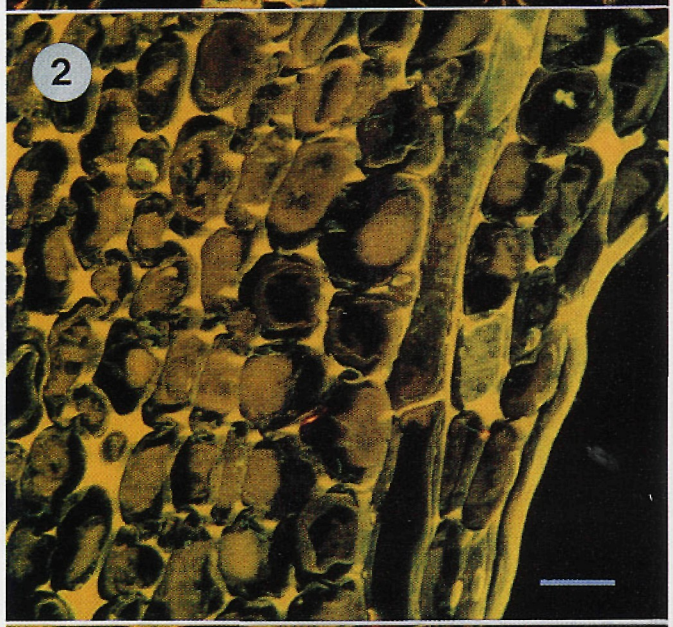

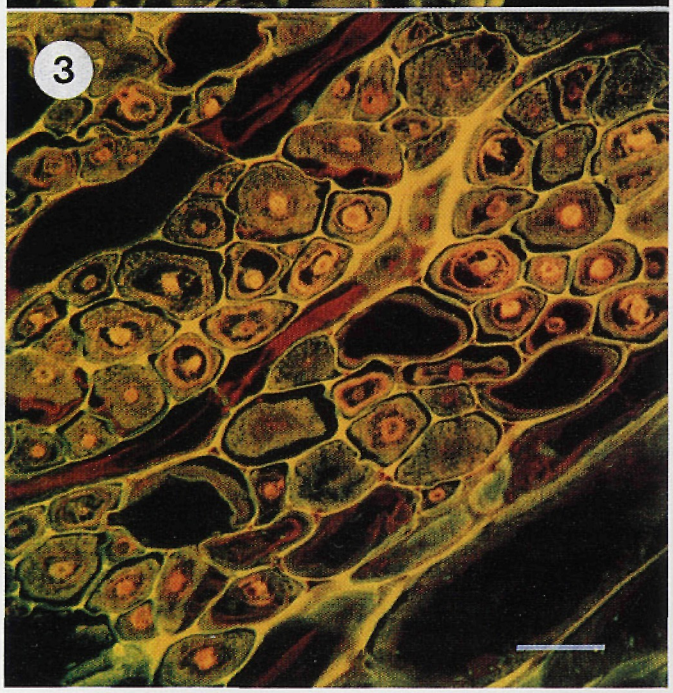

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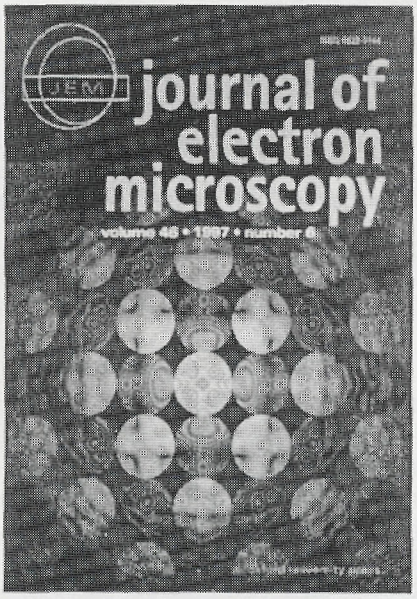
Figures 1 - 3. Images of waterlogged archaeological wood (oak; *Quercus* spp.) depending upon the different embedding methods. 1) Sample embedded with paraffin and deparaffinized with xylene. 2) Same sample embedded with paraffin but not deparaffinized. 3). Image of same sample embedded with LR White. Bar = 16 µm.



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