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TECHNICAL NOTE

An easy low-budget method to produce thin-sections of heavily decayed archaeological wood

Niels Bleicher*

Dendrochronological Laboratory, Department of the City of Zurich/Office for Urbanism, Urban Planning and Building, Switzerland

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Abstract

This article presents an easy, effective and cheap method to obtain good thin-sections of 20–30 μm thickness from decayed, soft archaeological wood using PEG-stabilization and bee's honey.

Keywords: Microscopy; Water-logged wood; Decayed wood; PEG

Introduction

When working with archaeological waterlogged wood (especially of deciduous species), it is often difficult to produce thin sections for detailed anatomical analysis or photographic and publication purposes. Anaerobic decay has destroyed most of the secondary cell walls in the wood-structure (Schweingruber, 1990, p. 198). Thus, razor blades and even freshly sharpened microtome-blades tend to tear the cells apart rather than rendering thin sections. Consequently, the wood-samples have to be stabilized prior to sectioning. A series of different methods and substances have been tested which yield good results and are neither time-consuming nor expensive. Among the substances used were melamine–resin, different acrylates, paraffin and polyethylene glycol (PEG) of different molecule sizes.

Methods and results

Melamine–resin, acrylates and silicone did not yield good results for different reasons.

Embedding in paraffin is an old technique in microscopy. It is time-consuming to prepare the samples since all water in the wood has to be removed which requires frequent changing of alcohol-baths (depending on the method used, since there are many methods of paraffin-embedding). The paraffin-embedded sample blocks have great sectioning properties. The problem is that the sections tend to roll and it is very difficult to unroll them without destroying the delicate thin section. A method to prevent this is to apply a strip of adhesive tape to the surface of the sample before sectioning. The next problem is to remove the paraffin from the section since it fills all cell-lumina. To do so, xylene is used, which conveniently also removes the adhesive tape. Often however, the xylene will render the thin-section extremely brittle. Thus, the chances are high that one will destroy the precious slice when staining and preparing the permanent slide. Fig. 4 shows on the left a 5300-year-old sample of beech cut with the paraffin-method. Mosaic-like splinters drifted apart when the

*Tel.: +49 177 234 90 74.
E-mail addresses: niels.bleicher@zuerich.ch, bleicher@zuerich.ch (N. Bleicher).

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cover glass was applied. Another problem with xylene is that it is considered by many people as very toxic. In German security recommendations, however, it is thought to be toxic only in larger doses although it would be advisable always to use it in a fume cupboard.

Embedding in PEG is a method that has been very popular among botanists for many years (Türler, 1972). In contrast to paraffin, it is water-soluble but has similar sectioning properties. Thus, no further chemicals are needed. It comes in several molecule sizes, which differ in terms of melting-point and hardness. It is possible to mix different kinds of PEG thus producing an individual optimal set of properties adapted to the species and degree of decay of the sample. Especially when working with the types of higher molecule sizes, it can be useful also to add a bit of glycerine to the mixture. The following mixture has yielded good results (and many others will): 87% PEG 4000, 10% PEG 400 (liquid), 3% glycerine. Pure PEG 1500 worked just as well.

The process is very easy: first small basins are required for the embedding. An easy way is to cut a small block of wood about 1 cm longer and some millimetres wider than the sample. Then ordinary silicone is applied on the outside. After it has hardened, one can remove the wood and use the silicon-skin as embedding basin. Then the size of the sample radius is reduced to 5 mm–1 cm thickness and put into the embedding basin. Cover it with as much PEG 1500 as possible and add some drops of water, which will facilitate the entry of the PEG into the wood. Then the basin must be put in a warm place – about 50–55 °C is needed – and stay in there for 2 or 3 days. The PEG will melt soon and the added water as well as the water from the sample will leave the wood while the PEG enters the cells. It is advantageous to check from time to time whether the sample is still covered with PEG and to refill if necessary. Then the basin should be removed and put onto a plate with about 1 cm cold water on it. One should be careful that no water gets into the PEG.

When the PEG has completely hardened (after approximately an hour) the block is ready for sectioning on the microtome (Fig. 1). One will also encounter the problem of rolling sections. When using adhesive tape to prevent this, one will again have to remove the tape with xylene and the section will become brittle again. It proved equally effective to use another method: a piece of paper the size of the sample, covered with a very thin layer of ordinary bee's honey, will work effectively as water-soluble adhesive tape. This honey-paper is pressed onto the surface of the block prior to cutting. The section will stick to the paper and cannot roll.

Now there are two options:

(1) Put the paper with the section onto the microscope-slide and add some ethanol. Ethanol will wash away both the honey and the PEG and one can easily remove the paper. There is no need to do this step with water (although it is possible), since the wood has already lost most of its water content and thus it is easier when preparing permanent slides not to add water and later have to remove it again. Now one can proceed with mounting as usual, as described, e.g. by Schweingruber (1990, p. 209). One general problem when using this method is that it sometimes happens that some PEG will stay in the pores which is unwanted, for instance when the aim is to do digital image analysis.

(2) Put the paper with the section onto the razor foil from an electric razor or a similar perforated foil and then for some minutes into a small basin with ethanol (Fig. 2). If the razor foil is placed on two matches to ensure there is some space between the bottom of the basin and the foil, the openings in the

Fig. 1. Sample embedded in PEG ready for sectioning.

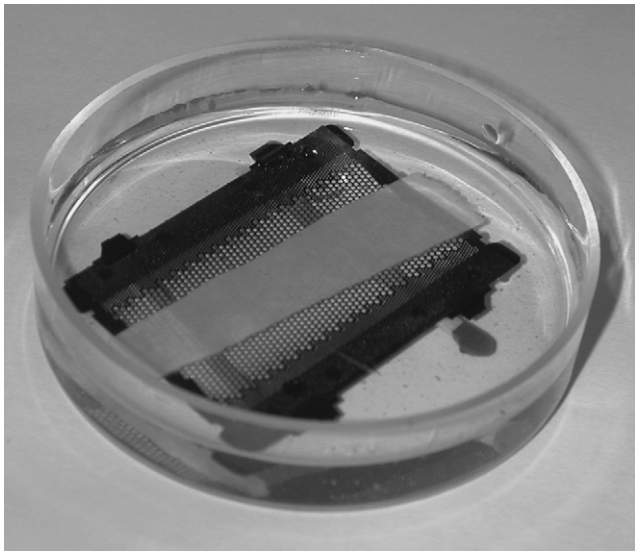

Fig. 2. Section applied to paper in an alcohol-bath on a razor foil.

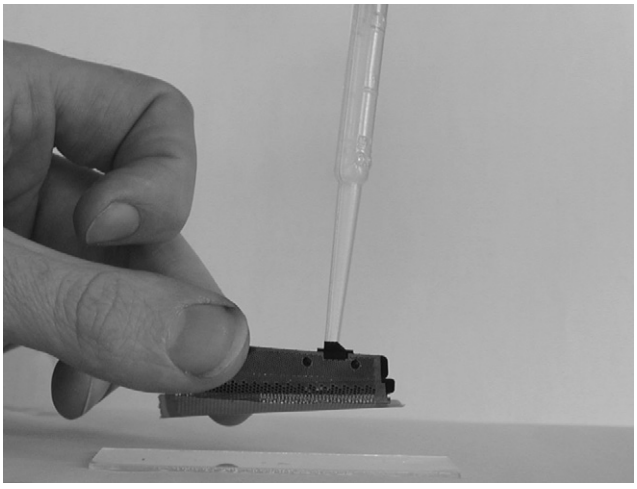

Fig. 3. Applying alcohol from the back of the foil to wash the section onto the microscope-slide.

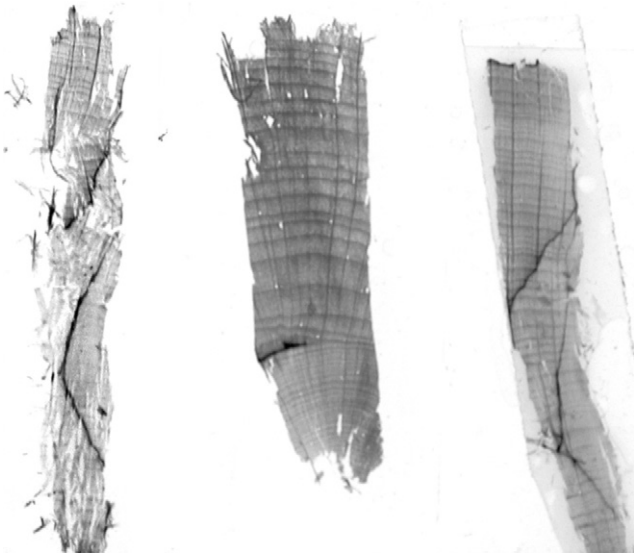

Fig. 4. Left: A 5300-year-old beech-sample cut with the paraffin-method. The xylene left the sample brittle. Right: The same sample left on the adhesive tape. Centre: A 5000-year-old beech-sample cut with the PEG-method. All samples waterlogged and cut to 30 µm. PEG-sample is 5.5 cm long.

razor foil will allow the ethanol to pass through and facilitate the complete removal of the PEG. It can easily be removed from the basin without the section drifting away as it would do on a glass-surface. Later the sample can be washed with absolute alcohol on the foil. Transferring the section onto a microscope-slide is easy since one can bend the foil and add some drops of alcohol from the back. The thin and flexible foil does not tend to tear the section when pressed against the slide (Fig. 3). Once transferred, proceed to prepare permanent slides as usual.

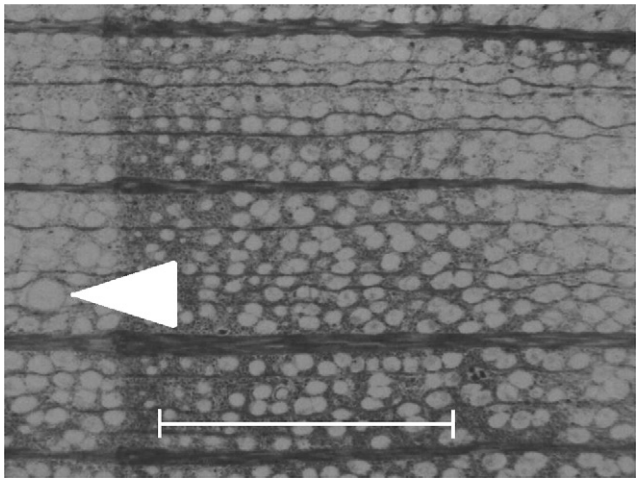

Fig. 5. Thin section of a 5000-year-old beech-sample. The bar is 1 mm wide. The arrow points to the rhizome of a cane grown through the decayed wood.

The whole process after embedding rarely takes more than 15 min per sample. Honey, paper, PEG and razor foil are cheap and the results are good while the process saves a lot of time compared to paraffin-based methods. Fig. 4 shows some results of the described methods, while Fig. 5 gives an impression of the achieved images.

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