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PREPARING WOODY TISSUES FOR  
MAKING MICROSCOPIC MOUNTS

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## PREPARING WOODY TISSUES FOR MAKING MICROSCOPIC MOUNTS

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In order to cut thin sections of wood for microscopic study or for photomicrography it is important to select and to treat the material to be sectioned with great care. Thick, imperfect sections may be prepared simply by wetting the surface of the wood to be cut and by using a sharp hand razor. Such sections, however, are usually not thin or uniform enough or do not present a large enough field to be photographed with good results and have only a temporary value. For exacting work permanent sections 5 to 25 micra thin, stained and mounted on a glass slide are desirable.

The wood may be collected at any time of the year, but it is preferable to cut the samples when the leaves and flowers or fruit are on the trees so as to have reinforced evidence as to the identity of the samples. Whenever possible specimens showing typical structure should be taken from green trees. If green material is not available, dry wood may be used, but in air-seasoned or kiln-dried material numerous fine checks may be found which often preclude good results. For most purposes it is better to take the sample from the stem of the tree or shrub, because the wood in the roots and branches usually shows somewhat different development from that of the stem. It is important that the material be labeled as soon as collected giving name of species, location and conditions governing growth, part of tree from which specimen is taken, date of collection and name of collector. It should then be wrapped in parafin paper, oil cloth, or dampened cloth till further treatment can be given it, which should be as soon as possible. If kept damp in warm weather, molding of the specimen can be prevented by thoroughly wetting the surfaces with a weak solution of corrosive sublimate. Killing and fixing are not necessary unless a study of the protoplasm is to be made.

Several blocks should be cut from some distance beneath the surface of the specimen. At least one annual ring or preferably several rings of average width should be included in the blocks for radial and transverse sections. The dimensions of the face from which the sections are to be cut may vary with the object in view and the hardness of the wood. From some wood sections 1/2 inch square may be readily cut. For ordinary study smaller sections will suffice and are easier to cut than the larger ones, especially in case of very hard woods. Three-eighths inch cubes are of convenient size for most work.

It is sometimes possible to cut good sections from the blocks of green wood without further treatment. In many cases, however, it is desirable to soften the wood somewhat before cutting sections. If the wood is to be softened it may be treated as follows: Immediately after the blocks are prepared they should be boiled in water for about two hours to remove the air so that they will sink when placed in acids or other liquids.

In many cases wood is too hard to cut immediately after boiling and further treatment is necessary. Hardness of wood is dependent on the density of the wood and the presence of mineral matter in the cell walls, or in the cell cavities in the form of crystals. The minerals most commonly met with in wood are calcium oxalate, calcium carbonate, and silicious compounds. Such mineral matter should be removed as far as possible in order to maintain a sharp edge on the knife.

Immersion in commercial 25 to 40 per cent or chemically pure hydrofluoric acid for a period of several days to two weeks or longer is the method most commonly used for removing some of the mineral matter. It, however, does not remove all the mineral matter in wood but enough to make the difference in cutting noticeable. It has been found that the use of 30 per cent acid for the softer woods and 40 per cent for the harder woods gives the best results. The use of hydrofluoric acid stronger than 40 per cent is not advisable except for a period of one or two days, since acid of 50 per cent strength distorts the elements and tends to macerate the wood. In using hydrofluoric acid, gutta-percha bottles are best for containers, but paraffin, lead, or glass bottles lined with paraffin may also be used. The volume of the acid should be about ten times that of the wood to be treated.<sup>1</sup>

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<sup>1</sup>If the wood is to be tested for chemical differences, it is not advisable to treat it with acid, as the acid may modify its chemical nature. For certain purposes even boiling of the wood may be objectionable.

After the treatment with acid the blocks must be boiled in at least six changes of water to remove the acid. (Glass beakers may be used for this; uncoated metal dishes will be attacked by the acid too rapidly.) In case of extremely hard woods like lignum-vitae, locust, and osage orange further softening may be effected by allowing the woods to remain in a solution of chloral hydrate (5 parts of chloral hydrate to 2 parts of water) for 10 to 20 days. In order to hasten the process the wood may be boiled in the solution for several hours using a condenser so as to keep the same strength of solution.

Another softening method recommended<sup>2</sup> is to place the blocks, after the air has been removed in pure acetone for 1 to 2 hours, and then in a solution of cellulose acetate (12 per cent cellulose acetate in acetone) for from 2 to 14 days. This not only softens the tissues but infiltrates them with a matrix which aids in sectioning. Before staining the sections should be washed in pure acetone for 1 or 2 minutes and then in alcohol for the same length of time.

For softening hard conifers it has been found helpful to boil the woods for one or two hours in a 2 to 4 per cent solution of potassium hydroxide in 95 per cent alcohol. This has the dual effect of softening the cell walls and dissolving the resin out of the wood without macerating it, though in stronger solutions the cell walls often swell, especially in the late wood. In this operation a condenser is also necessary. After the wood has been boiled in this solution it should be placed in 50 per cent alcohol for several hours and then boiled one hour in several changes of water to remove the potassium hydroxide. Hard pines so treated may be sectioned with very little difficulty.

Most woods, however, do not require the treatment with chloral hydrate or potassium hydroxide but may be softened sufficiently by placing them in a solution of glycerine 1 part, alcohol 2 parts, and water 3 parts for a week or indefinitely till ready to be sectioned. If the stopper to the container does not fit perfectly tight, the alcohol and water will evaporate leaving too large a percentage of glycerine, which may soften the material too much if it is allowed to remain in the solution for a year or more, depending on the nature and kind of wood. Boiling in glycerine is recommended

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<sup>2</sup>Williamson, H.S., A new method of preparing sections of hard vegetable structures: *Annals of Botany*, January, 1921.

# SOFTENING WOOD WITH STEAM<sup>1</sup>

(Abstract)

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Hard materials, such as locust wood and vegetable ivory, may be sectioned by allowing a regulated jet of steam from slowly boiling water to flow on the material while being sectioned. By allowing the steam to flow through a coil which is separately heated condensation within the tube with consequent dripping is avoided. Copper tubing causes particles of copper oxide to blow out, monel metal or aluminum tubing is preferable. This method permits immediate sectioning and avoids chemical changes due to HFl treatment.

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<sup>1</sup>Crowell, Ivan H. (Department of Botany, Miami University Oxford, Ohio) "Cutting microscopic sections of wood without previous treatment in hydrofluoric acid." *Stain Technology* 5:149-150, Oct., 1930.

See also

Kisser, Josef. *Der Heutige Stand Botanisch-Mikrotechnischer Schneidmethoden.* *Biologia Generalis*, 4, Lieferung 1/2, 151-3. Emil Haim and Company, Wien and Leipzig, 1928.

by some for softening woody tissues. Blocks of hard wood may also be softened by storing them in commercial formalin for a week or longer.<sup>3</sup>

Fairly good sections may be made from the blocks of some woods taken right out of the alcohol-glycerine mixture, or even immediately after the softening agents have been removed by boiling. Ash, maple, oak, gum, elm, orange wood and others afford excellent sections without further treatment. However, very soft woods have thin-walled cells, especially the epithelium of resin ducts, section poorly without infiltrating them with a matrix that offers backing to the cell walls. Paraffin cannot be used since it will not allow glycerine to soak into the cell walls to soften them after the hardening due to the dehydration with alcohol. Celloidin is much used for infiltrating and serves the purpose well.

The celloidin method with hard tissues has been described fully by Plovman in the Botanical Gazette, Vol. 37, pp. 456-461, and by Bailey in the same journal, Vol. 49, pp. 57-58. The method with some slight modification is given below.

Remove blocks from the alcohol-glycerine solution and boil in two changes of water to remove the glycerine. Dehydrate by placing in 70, 95, absolute alcohol, and ether-alcohol (1 part ether to 1 part absolute alcohol) successively, leaving the material 12 to 24 hours in each. It is absolutely essential that all water and glycerine be removed from the tissues. Transfer the blocks successively into 2, 4, 6, 8, 10, 12, 14, 16, and 18 per cent celloidin dissolved in equal parts of absolute alcohol and ether. The vials containing the material and celloidin after having the stopper clamped down tightly with wire or a stout cord are placed for 12 hours in an incubator or oven having a temperature of 50 to 60° C. When taken out the vials should be placed in cold water, but no water must be allowed to come in contact with the celloidin. When the strongest solution is reached it is allowed to evaporate gradually by removing the stopper for an hour or two each day, or chips of celloidin may be added till the solution is quite firm. The blocks with the bulk of celloidin trimmed off are placed for 24 hours in chloroform, and then into a solution of equal parts of 95 per cent alcohol and glycerine where they should remain a few days, or many remain indefinitely till ready to be sectioned.

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<sup>3</sup>Weinstein, A.I., "Methods for cutting and differential staining of microscopic sections of hardwood glue joints." Colloid Symposium, Vol. 4, 1926.

It has been found that this process may be shortened by placing a dozen or more vials or crystallization dishes containing the material to be infiltrated and the celloidin solution into a heavy glass jar fitted with a heavy, brass, air-tight lid having a stopcock and a pressure gage inserted into the top. The material is first put into a liberal quantity of a 10 per cent solution of celloidin, the lid fastened on and the apparatus put into an oven at about 50° C. The heat evaporates some of the solvents and produces a pressure. After the apparatus is thoroughly heated through, the air valve is opened allowing part of the gas (air, ether, and alcohol) to escape gradually while more evolves from the solution, thus thickening the celloidin. Next air is pumped into the chamber by means of a foot pump until a pressure of 30 pounds per square inch is reached and it is then set into a basin of cold water. When sufficiently cooled it is again placed in the oven and the whole process repeated. By this method wood may be thoroughly infiltrated in about 2 days.

After sectioning and before staining the celloidin may be removed by first washing out all water and glycerine with alcohol (95 per cent followed by absolute alcohol) and then placing the sections in ether for 15 minutes. Or if the material is fragmentary the celloidin may be left in the sections to hold them together. In which case absolute alcohol must not be used for dehydrating and clove oil must not be used for clearing, but the sections are passed quickly through 95 per cent alcohol and then into a mixture of equal parts of oil of bergamot, oil of cedar, and carbolic acid.

A great variety of stains may be used for woody tissues. For single staining 1 to 2 per cent alcoholic (80 per cent) solutions of Bismarck brown, cyanin, fuchsin, iodine green, or safranin may be used. For double staining the following methods are recommended: Place the sections for a few minutes in a 3 per cent aqueous solution of ammonia sulphate of iron, (iron alum) then wash in water for half an hour and place for about 10 hours in a one-half per cent solution of haematoxylin ripened in a bottle plugged with cotton for two months. Remove from the haematoxylin, rinse in water for 5 minutes, and place on a 1 per cent solution of the alum to reduce the too deep stain to the proper intensity and then wash one-half hour in several changes of water. Now place the sections for several hours in a 1 per cent solution of safranin in 50 per cent alcohol, after which they may be rinsed, dehydrated and cleared.

Cyanin and eosin are excellent double stains for coniferous woods. Place the sections after dehydration in a saturated solution of cyanin in clove oil for several hours. Drain off the oil and rinse out the surplus stain with clear clove oil and then place for one minute in a 2 per cent solution of eosin in clove oil. After rinsing once in clove oil and twice in cedar oil the sections are ready for mounting.

With hardwoods excellent results have been obtained and with conifers fairly good results by placing the sections for several hours or overnight (10-15 minutes only for conifers) in a 1 per cent solution of safranin made by dissolving one gram of safranin in 50 cc. of 95 per cent of alcohol and adding 50 cc. of water. After rinsing in 50 per cent alcohol cover the sections with 70 per cent alcohol containing one-half per cent acetic acid till they appear a pale pink. After rinsing in water the sections are placed for several hours or over night (the longer period is better especially for conifers) in a 2 per cent aqueous solution of methyl blue which has been filtered through an alundum filter. After removing the blue stain and rinsing with water till practically no more blue color is given off the sections are quickly passed through a one per cent solution of Bismarck brown, made by dissolving the stain in hot water and filtering through ordinary filter paper. The sections are then washed in water several times and then passed through 95 per cent alcohol and absolute alcohol into a clearing agent. This combination of stains colors the middle lamella red and the secondary walls olive green.

Another useful differential stain is produced by the usual treatment with alcoholic safranin (30 minutes) followed by a few seconds in a 1 per cent solution of light green in 95 per cent alcohol. This is especially serviceable for the rapid differentiation of so-called muscilagenous cells.

For clearing woody sections clove oil followed by cedar oil is found better than xylene because the latter has a hardening effect on the wood making the sections often too brittle to handle with a camel's hair brush. Canada balsam is the most satisfactory mounting medium. It should be of such consistency that it readily drops from a glass rod. The cover-glass should not be pressed down firmly until several days or a week after it has been placed on the slide, otherwise so little balsam will remain between the slide and cover-glass that when it dries out bubbles will form under the glass often ruining the mounts.



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