

Anatomical techniques

The anatomy of the tree ring is, among other things, an expression of the physiological processes in the tree. The form and dimensions of the cell walls, the number and size of the cells and the proportions of the different cell types all provide information about the environmental conditions that are experienced by the tree.

Microscopic techniques have been used in the field of wood research mainly for differential anatomy and in

tree-ring biology, particularly in the investigation of cambial activity. Until recently, however, the microscope has been little used for work in dendrochronology. Today microscopic techniques are being increasingly applied to electronic tissue analysis, where the efficient production of long sample series is required. In radiodensitometry, microscopic preparations provide the basis for the understanding of density curves.

Preparation methods

The tissue structures can be made visible by three basic methods: polishing the sample surface, cutting a plane surface or cutting a section. Polished and plane surfaces are particularly important in the analysis of tree rings.

Microsections

The sledge microtome has proved invaluable in the production of microsections. Using this equipment it is possible to obtain 10 to 20 micrometer-thick sections of superb quality provided that the following conditions are met:

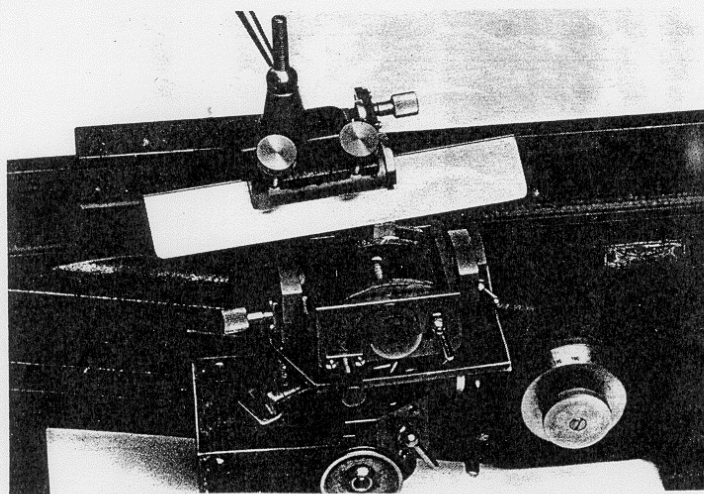
- the wood should be embedded in carbowax (polyethylene glycol)
- a top-quality blade must be used

Sections

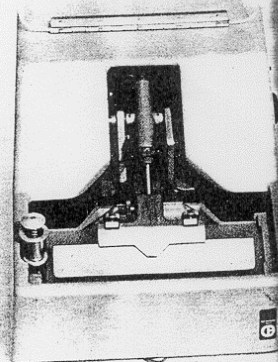
Sections can profitably be used where there is a need to produce photomicrographs of high quality. They are also used in the examination of cell walls and in the determination of the vitality of cells.

- the blade must be optimally sharp: sharpened with a special knife-sharpener, for example
- the sample must be positioned in the holding device with great care
- skilled handling of the blade is required.

In addition to these requirements a considerable amount of experience is desirable.



Sledge microtome with the blade in position.



Knife-sharpener: the friction of the blade against the fine-grained whetstone produces an optimal cutting edge.

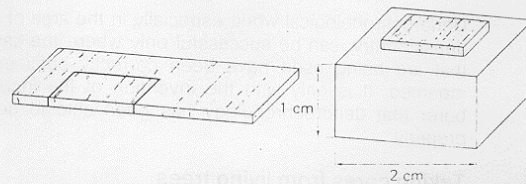
Cutting

The best sections are obtained from fresh wood. Dry wood must be softened in boiling water before cutting. Conifer pieces can be embedded in carbowax before cutting.

Where a sample which has been radiodensitometrically examined is also to be anatomically investigated, a part is removed after radiography and glued to a small wooden block. This allows fairly large transverse sections to be cut without difficulty.

a sample cut of a lath as a preliminary to taking a microsection

sample glued to a wooden block



A piece from a radiodensitometric lath is mounted on a wooden block prior to being clamped in the microtome.

Staining the sections

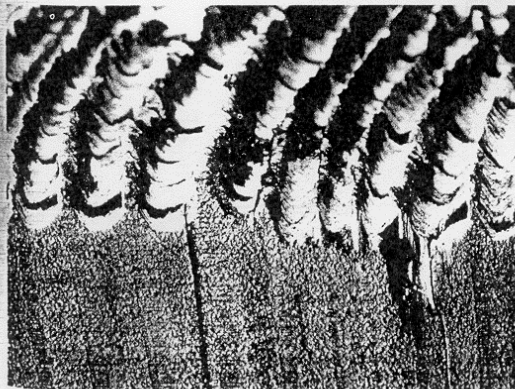
In dendroclimatology, the dimensions of the cell walls must be accurately measured. There are several ways of staining sections so that such measurements can be carried out.

The first step is to free the contents with Eau de Javelle (KOCI or NaOCI). After this the cell is stained red with safranin, which enables the cell walls to be clearly seen.

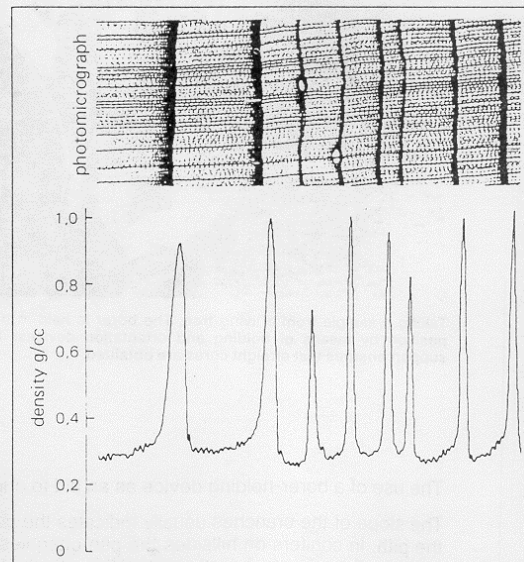
The procedure is different for biological work on tree rings. Here the cells themselves, their contents and the degree of lignification are of interest. Planeze IIIB stain can be used. The lignified cellwalls will then become green, the non-lignified ones colourless and the cell contents red.

The microstructure in relation to wood density

Density analyses reproduce anatomical features in a more general form. Density patterns, whether exposed naturally by weathering or artificially using radiodensitometry, can be understood only in the light of the cellular structure of the wood.



Here the natural density structure of the wood is the result of weathering. The soft earlywood has been eroded away and the denser latewood stands proud.



Photomicrograph and density diagram: the natural density structure can be made clear either by measuring the cell walls or by using light or X-ray techniques.

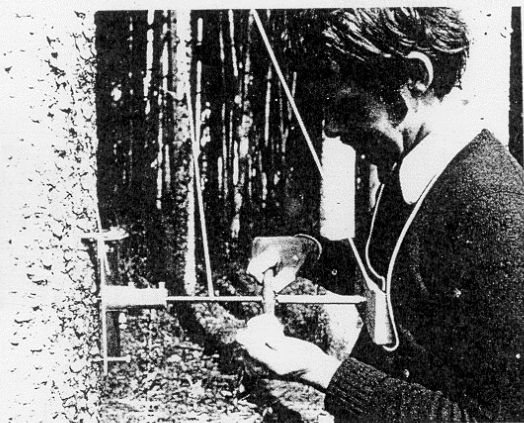
Taking samples

Dendrochronological work, especially in the area of radiodensitometry, can be successful only where the samples that are being used have been correctly obtained and mounted. It is only with the invention of the increment borer that dendrochronology has been able to develop properly.

Taking cores from living trees

Coring these days is done almost exclusively with Swedish increment borers. These are available from forestry suppliers. The 5 mm diameter borers are the most suitable for radiodensitometric work. The double-helix screw-thread enables the tree trunk to be easily penetrated. The wood outside the borer is pushed aside and only the non-compressed core passes into the drill tube, which is slightly wider towards the rear. When the desired depth of penetration is reached, an extractor spoon is pushed from the back of the borer between the core and the inner wall until it is tightly wedged between the core and the top of the borer. The core is broken off within the tree with an anti-clockwise movement and then pulled out.

orientation borer bore—support device



Taking a sample from a living tree. The borer is held in the correct position by means of holding and orientation devices. The bore-support ensures that straight cores are obtained.

The use of a borer-holding device as an aid to orientation

The slope of the branches usually indicates the position of the pith. In conifers on hillsides the pith often lies towards the hill. For boring, it is the orientation to the tree-axis rather than the radial orientation which is of vital importance. Where the sampling is carried out by two people, one of them should operate the borer while the other should indicate the perpendicular to the axis of the tree

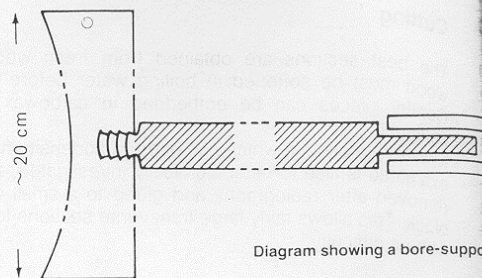
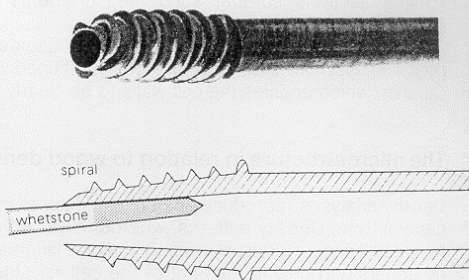


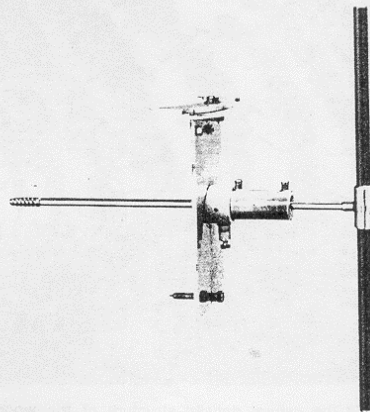
Diagram showing a bore-support

trunk from a distance of between two and three meters. Where the sampling has to be done by only one person a holding device is used. (See photo.) This can be adjusted in the axial direction by a peg and screw, and in the radial direction by a joint.

If, after some time, the borer gets blunt or notched, it can quite easily be sharpened parallel to the inner surface with a whetstone. Since it is the outermost annual rings which are decisive for dating, a bore support is used to ensure that the borer penetrates the tree trunk in a straight line.



An increment borer. The inner wall is conical and widens towards the rear produced by And. Mattsons, Mechaniska AB, Box 9, S-752 11 Mora, Sweden.

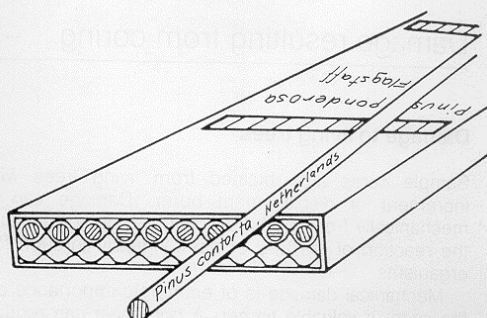


A borer holding device, showing the borer, the joint and the screw from above and from the side.

The labelling and transport of the cores

The cores should be labelled with a soft pencil while still moist. Ball-point and felt-tip pens have proved unsuitable for this. The cores can then be placed in specially-made holders or in drinking straws.

When the cores have dried out or are beginning to dry out, they can be taken out of the holder with a needle. The labelling should then be checked against the entries in the field-book, and the samples tightly bundled together and wrapped in newspaper.



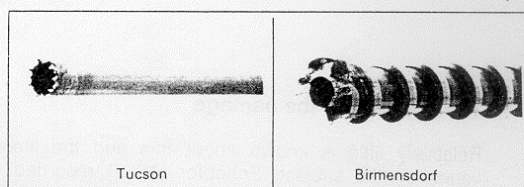
A core holder made out of corrugated cardboard. On top a core inside a drinking straw.

Taking samples from buildings and from fossil wood

It is important to obtain permission to take samples. Local residents can often be of help in acquiring the necessary permission. If it is impossible to cut a whole disc from a trunk in a building, the dry wood must be cored with a cutting borer. An American make (Tucson) is available.

In regions with relatively high air and wood humidity the only types of borers which are suitable are power-driven ones, e.g. Birmensdorf.

Discs are generally cut from fossil wood using a power-saw.

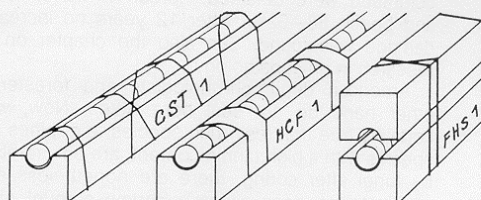


Borer for dry wood.

Mounting the cores

In order to cut the cores into strips or to polish their surfaces they are first glued into a groove in a wooden block using a water-soluble glue. They are then held together until they have fully dried out.

The cores are glued vertically into the groove when the annual ring surfaces are to be directly examined, and horizontally when they are to be radiographically investigated.

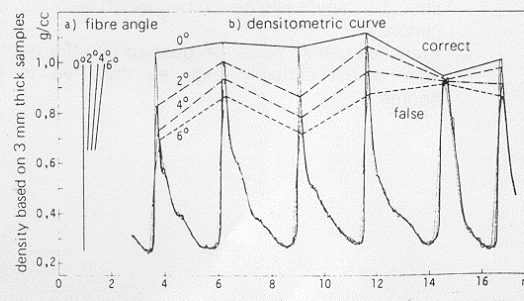


The cores mounted on supports:

- with cotton wool wound around the support;
- held in position with sellotape;
- held against another support with rubber bands.

The importance of proper coring for radiodensitometry

For radiodensitometric work in dendroclimatology the coring technique is vitally important. The accuracy of the density values later obtained depends largely on this.



The influence of fibre angle on the densitogram. Where the cores have been taken obliquely to the tree axis misleading values for the maximum density result.

Damage resulting from coring

Damage to living trees

Sample cores are obtained from living trees with an increment or displacement borer. Damage can result mechanically from the borer itself, physiologically through the reaction of the tree or pathologically through foreign organisms.

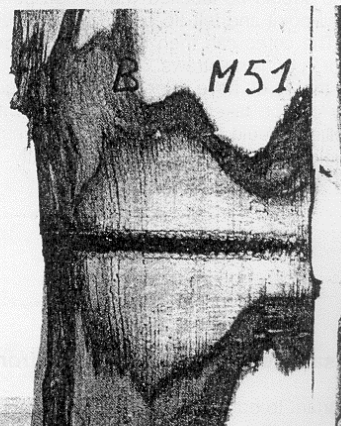
Mechanical damage is of economic importance only in the case of valuable timber. A bore hole can reduce the value of a whole stack of planks. The secretion of resin or anomalous heartwood transformation following access to oxygen results in discolouration. Many kinds of fungi cause the wood to become discoloured or to rot. Callusing around the bore hole reduces the value of timber.

The intensity of the damage

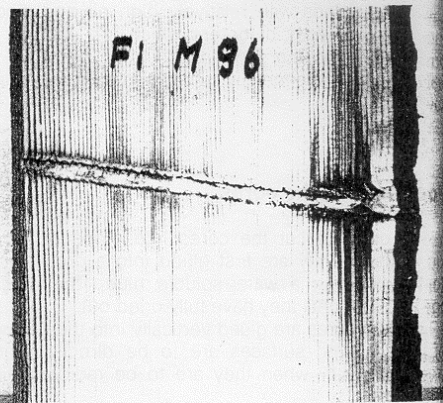
Relatively little is known about this and the literature varies on the subject. Schöpfer (1962) recorded 88% discolouration in spruce resulting from boring where the bore hole was not subsequently treated. Lenz and Oswald filled bore holes that they had made with grafting wax (1971). After six years the following percentage of discolouration were obtained: spruce 7–12%, fir 29–57% and beech 68–92%. After 12 years no increase in the damage was found. See also the chapter on tree ring research in phytopathology.

American dendrochronologists and foresters on the other hand do not seal bore holes. Now, while it is certainly true that resin-rich species, e.g. pines and some species with a high tannin content, are only rarely attacked by fungi after coring, there are nonetheless species in which coring does cause a certain amount of damage. In Switzerland an attempt is made to prevent damage resulting from coring by taking the following steps:

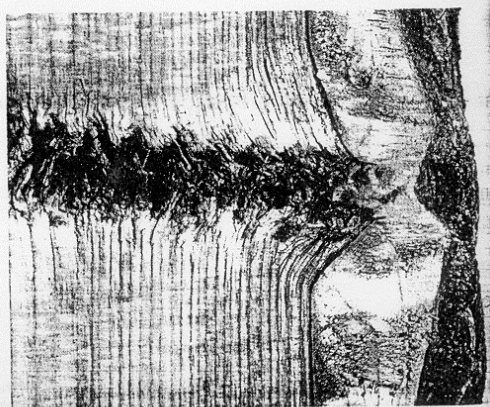
- The bore holes are sealed with grafting wax (which is available from garden centers and forestry suppliers). This very simple procedure should always be followed.
- In valuable timber stands or when samples are being taken from broadleaves with no coloured heartwood, e.g. beech, coring should be kept to the minimum. It should in any case never be undertaken out of idle curiosity.
- Branches should never be pushed into the bore hole. This almost certainly exposes the tree to fungal spores.



Beech: uneven discoloration resulting from anomalous heartwood formation and subsequent decomposition by fungi around the bore hole (Lenz, unpublished).



Spruce: discolouration by resins (Lenz, unpublished).

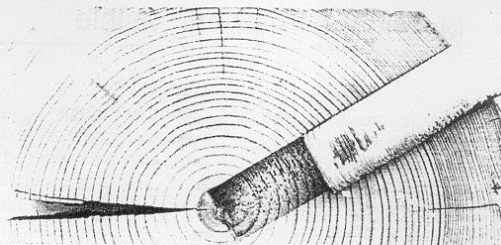


Spruce: callousing around the bore hole (Lenz, unpublished).

Damage to wood in buildings

Any damage caused to historical wood by coring is generally slight. As the wood is normally under cover, fungal decomposition is unlikely to occur.

Where the ends of beams have been sawn off the effects can be hidden by repainting. Bore holes should be plugged with a suitable dowel. The load-bearing capacity of beams is affected only in the case of narrow beams.



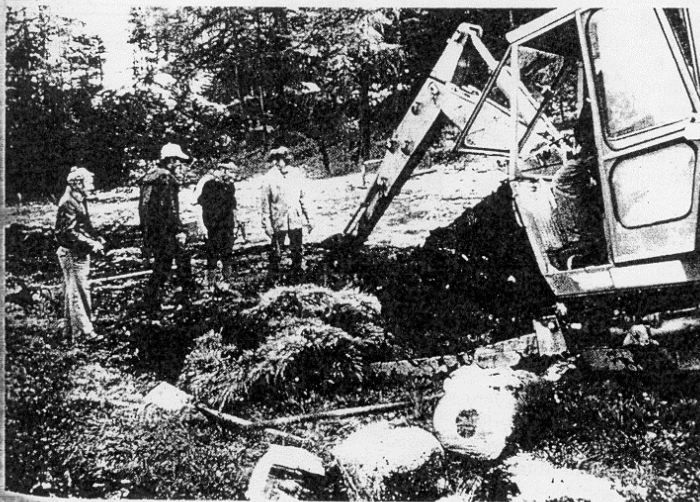
Repairing a bore hole in a beam with a plug.

Damage to the landscape through the excavation of fossil trunks

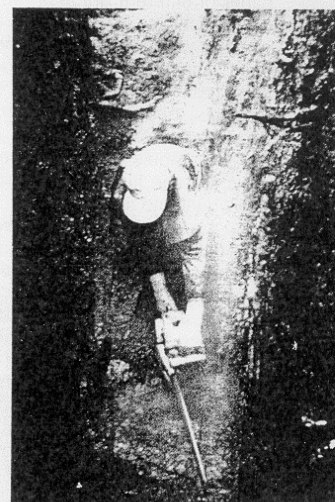
Where fossil trunks are to be examined whole discs are generally sawn off. If the trunks are buried in peat bogs or moraines they very often have to be freed using machines. Sampling must be kept to a minimum where the trunks are in deposits that are in protected areas.

Today the remains of trunks in arid areas are increasingly endangered. One of the reasons for this is that the use of 4-wheel-drive vehicles has led to the opening up of a number of desert regions to tourism. This has meant

that in the process of clearing up the areas the wood that was scattered about has been burnt. This wood is often centuries old. In the legendary Bristlecone Forests and in the Petrified forests it has become fashionable for tourists to take a piece of wood home with them as a souvenir. This practice is banned in many of the national parks. In some cases permission must be obtained from the authorities in the locality when it is desired to collect wood for dendrochronological purposes.



Excavating a peat-bog containing fossil trunks. The damage caused is considerable. In this particular case it is not important, however, as the land is shortly to be drained and built on (Bircher, unpublished).



Taking discs from fossil trunks found in a peat-bog near Saas Fee, Valais, Switzerland (Renner, unpublished).

Making the tree rings visible

The surfaces of samples, whether these have been obtained by sawing or by coring, are generally extremely rough. The structure of the rings can be rendered visible by making the surface smoother. There are various ways of doing this, depending on the condition of the material.

Material in good condition

Cutting a plane surface: Transverse radial strips are cut along discs or cores using a sharp blade, e.g. a multiple use snap-off blade knife, a carpet cutter or industrial razor-blades. Where the tree-ring boundaries are difficult to see, as is the case in diffuse-porous broadleaves, these boundaries can best be made visible by making the cut at an angle of between 20 and 40 degrees to the fibres. Here a satisfactory cut can be obtained only by drawing the blade cleanly along the surface (saw-cut), and this takes practice. Cut sections often expose the important latewood cells. Sections can prove to be an excellent method of making the structure visible, provided that the lighting is good.

Polishing: This process both smooths the surface and pushes planing dust into the lumen. This means that the cell wall, which is generally dark, can easily be distinguished from the lighter, filled, cell-cavity area. Sandpaper of different grades (100, 200, 320, 400) is used for the polishing. Power planers are generally necessary where a whole disc is to be polished. Cores, on the other hand, can as a rule be smoothed by hand. Particularly good results can be obtained by cutting a section which is then polished using fine sandpaper wrapped around a rubber eraser.

For samples from oak the contrast between the cell walls and the lumen can often be heightened by rubbing chalk dust into the pores.

In the case of materials where dating is problematic, whole discs should be examined wherever possible. This facilitates the location of 'missing' rings or the identification of false tree rings.

Microsections: Samples displaying little contrast between the tree-ring boundaries, e.g., poplar or rosaceae, should be mounted between two blocks and cut to about $\frac{1}{2}$ mm. They can then be examined in transmitted light.

Staining: So far this method has not proved to be very successful. Isolated cut samples stained with paper-dye have exposed the tree ring structure in diffuse-porous broad-leaves. This can be of particular use where trees in cities are being studied. (Cartasol, red and blue, K-2B; Sandoz, Basel, Switzerland)

Radiography: See page 64.

Soft, decomposed wood

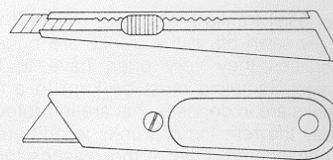
Very sharp blades must be used to make cuts in the surface of soft or damp wood, e.g. posts from lake dwellings or trunks from peat-bogs or from alluvial deposits.

Charcoal

Transverse fractures produce the best results. Where necessary brittle pieces can be wrapped up as they are collected, or stabilised by pouring hot paraffin over them. In large fractures the tree rings can then easily be seen and measured.

Examining the surfaces

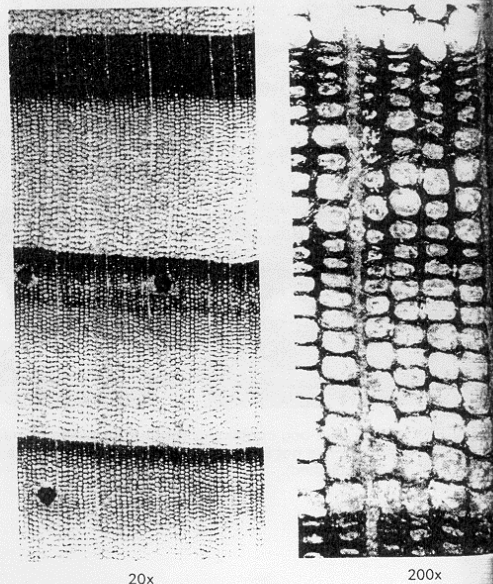
The surfaces can best be examined using a stereomicroscope with a spotlight or a hand lens. Small details, e.g. single rows of cells, can be seen only in very good light. On field trips the author has often used sun-light for this purpose.



Implements for cutting the cores: snap-off blade knife, carpet cutter, heavy industrial razor-blade.



Preparing cores which are to be examined in transmitted light.



Photograph of a polished transverse section of conifer wood (*Pinus ponderosa*) at different magnifications. The differing thicknesses of the cell walls can be seen at a magnification of 200x. The structure of the cell walls cannot, however, be seen.

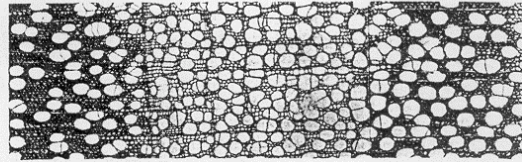
Counting tree rings

Dendrochronology is based on the counting of tree rings. This process provides important information and enables the age of the wood to be determined. In forestry, for example, all yield tables are based on tree age. Vegetation experts and geologists often need to know how old a tree is, i.e. when its first ring was formed, in order to estimate the age of a moraine or river-bed.

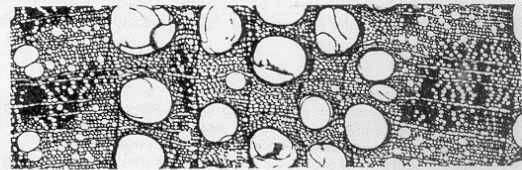
For trees in temperate zones the enumeration of the annual rings is generally straightforward. This is because the clearly defined early and latewood tissue make it easy to see the rings.

In other cases enumeration is more difficult: e.g. in species which tend to form less well-defined ring boundaries, or where the rings are very narrow, as is the case in ring-porous woods.

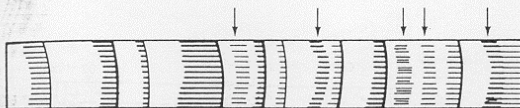
Enumeration becomes extremely problematic where false annual rings occur, or where the rings peter out or are missing entirely. It is impossible where the tree displays zones of irregular growth. In many cases the exact age of the tree can be determined only by applying the dendrochronological technique of cross-dating.



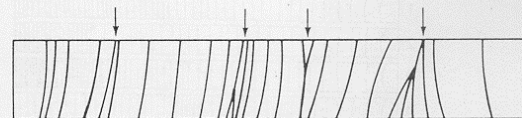
Poplar: tree-ring sequence with narrow rings and unclear boundaries between the rings.



Oak: tree-ring sequence with narrow rings. The earlywood vessels are almost touching each other and the latewood is barely developed.



Tree-ring sequence displaying a lot of width variations and 'false' rings. Enumeration here is problematic.

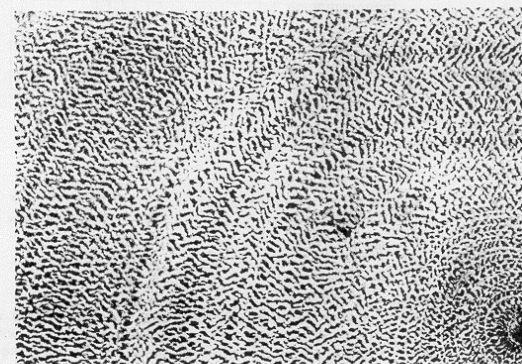


Tree-ring sequence where a number of the rings are missing and others wedging out. Enumeration here is problematic.

Trees and shrubs in temperate and boreal regions generally form one ring a year. The occurrence of clearly marked seasons in these regions means that very little variation in growth occurs within any given year.

There can, by contrast, be enormous variations in growth in trees and shrubs in arid and semi-arid regions. This is a result of the unevenly distributed precipitation. A ring may be formed every year, or there may be no growth whatsoever, or the tree may even appear to form two or three rings a year. For samples in these regions age can be determined only by cross-dating.

Enumeration is generally extremely difficult for samples in tropical regions. Only very few species are able to react morphologically to the very slight differences between the seasons and so produce an annual ring boundary as an expression of a break in growth. Very few species in these regions produce annual rings. Most produce either no ring at all or just a suggestion of a growth zone. (See pages 109, 237).



Cross-section through a piece of tropical wood. This species (Cassia) displays growth zones but no annual rings.



Tree-ring sequence of a tropical broadleaf (mahogany). No rings are discernible. Enumeration here is impossible.

Recording the changes of growth patterns

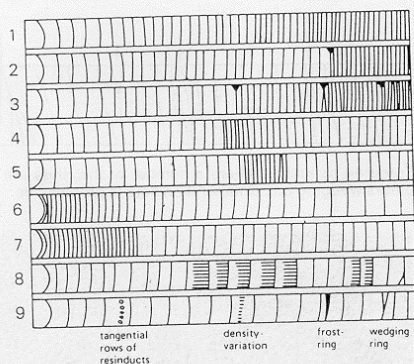
Every change in environmental conditions is reflected in some way in the annual ring structure. Relatively rapid changes sustained over a number of years occur as a result of the following:

- changes in the tree's position
- changes in the light, water or temperature conditions
- damage to the tree's crown, root or trunk system
- chemical changes in the environment.

The changes affect the number and size of the cells and the thickness of the cell wall. Such variations can often be

identified and dated with the naked eye or with the help of a stereo-microscope. For any given disc or core, both the point at which the variations began to occur, and the duration can be identified. The results of the analysis of individual samples can then be brought together in a single diagram. It is important to determine both the onset and the duration of these changes or events. The summary diagrams enable the identification of individual, local and regional changes. This is proving to be of particular value in our age of pollution.

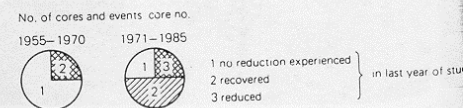
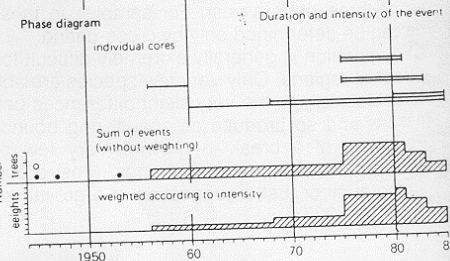
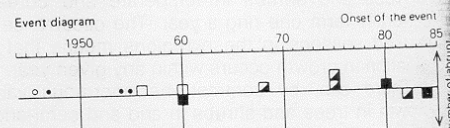
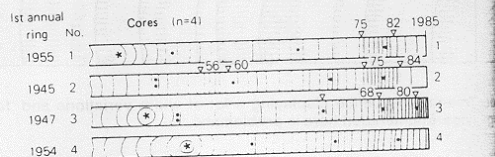
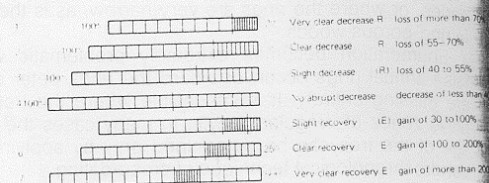
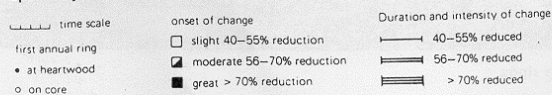
Examples of changes in tree ring width. Reductions in growth of less than 40% and recoveries of less than 150% following periods of reduced growth cannot be seen with the naked eye. The intensity of the change can be quantified by measuring the annual rings and comparing the number of those showing an increase or decrease in growth with the same number of previous rings.



- 1 age trend; the rings become continuously smaller
- 2 abrupt growth decrease
- 3 stepwise abrupt growth decrease
- 4 abrupt growth decrease and continuous release
- 5 abrupt growth decrease and abrupt growth release
- 6 continuous growth release
- 7 abrupt growth release
- 8 compression wood
- 9 special features

The most common patterns of change in the annual ring width. The abrupt changes — cores 2 to 5 and 7 — can be seen with the naked eye. Such changes can often be dated by counting the rings or by using pointer years.

Presentation of the beginning and duration of growth changes. Diagram showing 4 cores with abrupt growth reductions. The results are summarized in the event diagram, phase diagram and the circles to present the number of cores with reductions in a specific year or period.



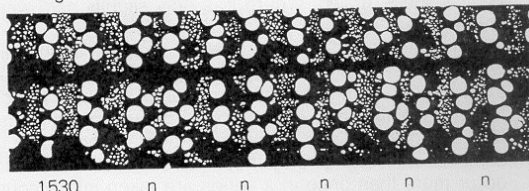
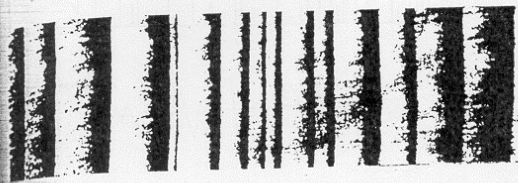
Pointer years and the associated characteristics of tree rings

The dating of a tree-ring series is based on the identification of rings with characteristic features. Dendrochronology is primarily concerned with those rings which are particularly narrow. Where these occur singly the years to which they relate are termed pointer years. Where they occur in a characteristic grouping they are referred to as signatures. Where there are a number of pointer years close together the tree-ring series may be referred to as *Sensitive*; where they occur in a loose sequence, it is

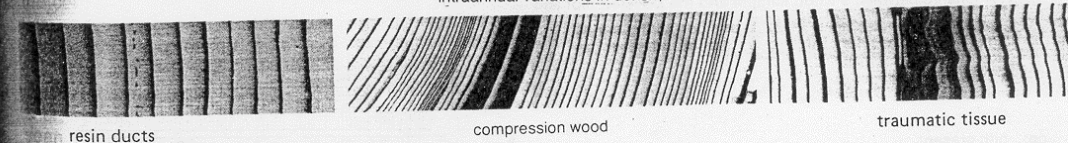
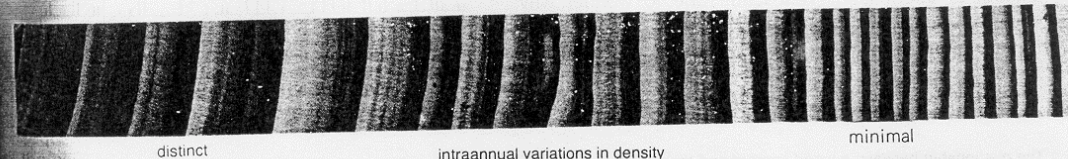
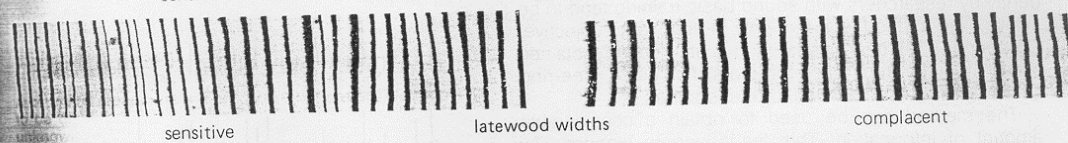
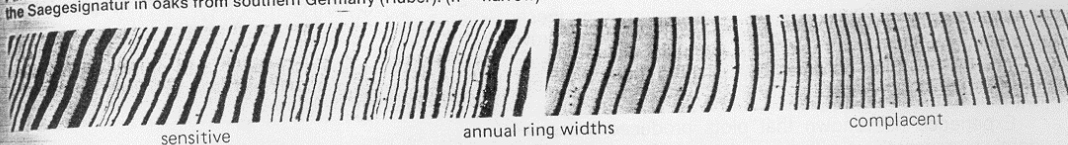
considered to be *complacent*.

The term pointer years has been variously defined. In the skeleton-plot method it is used to mean those narrow rings which occur fairly often. In the context of measured ring series and chronologies, it may refer either to those tree rings which are on average narrow or to those intervals in a chronology which tend to rise or fall.

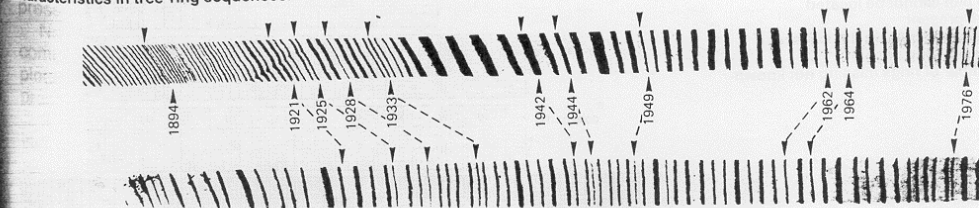
The width of the tree rings is one of a number of features displayed by the ring which may be of help in dating.



Famous signatures in tree-ring sequences. On the left the Pueblo signature in Conifers of the American southwest (Douglass). On the right the Saegesignatur in oaks from southern Germany (Huber). (n = narrow)



Characteristics in tree-ring sequences.



Visual matching of two tree ring sequences using pointer years. That for 1976 is narrow and displays very little latewood. Those for 1962 and 1964 are of normal width but have very little latewood. The samples were taken in the dry Rhone valley area, the Valais, Switzerland (Kienast *et al.*, 1981).

Cross-dating using the skeleton-plot method

American dendrochronologists have used this method for almost 80 years dating conifers growing under all types of climatic conditions. Before any actual measurements are made, an outline or skeleton of the tree-ring sequence is plotted. Those rings which are characteristically narrow are recorded subjectively, as they occur, on strips of paper with 2 mm divisions. The width of each ring is compared with that of the rings on either side of it. Where a ring is considerably narrower than its neighbours a long vertical line is drawn. Where it is only slightly narrower a shorter line is drawn.

Since age-trend and long-term changes in the ring width are not recorded using this method, it can be used to synchronize ring series of the same age but with different absolute ring widths. In this way samples from a fairly narrowly defined site can be aggregated to form a composite. Insofar as only those variations in width which occur more than once in a series are taken into account, these local series serve to highlight only those narrow rings which are characteristic for a locality.

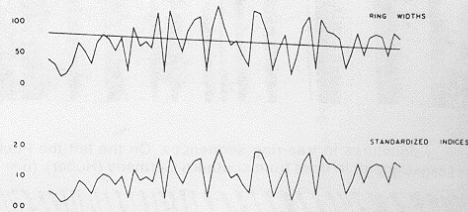
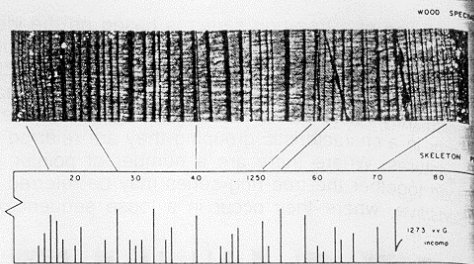
Master chronologies can be constructed from these local series. Such chronologies show only those narrow rings which characterize a region or a particular altitude or site. Samples can then be dated by matching these plots with recent tree-ring series.

Experience has shown that plots produced independently by researchers with sound basic training tend to be similar. The method is in fact only at first sight subjective. Cropper (1979) has illustrated this in that he obtained similar results from skeleton-plots for given tree-ring series and from calculations for these series.

The method can be used to obtain a considerable amount of information. This leads one to wonder why European dendrochronologists do not make use of it. The significance of skeleton-plots as a means of identifying ecological changes remains largely unrecognized.

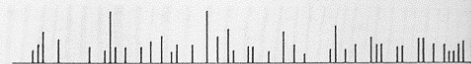
The relationship between a wood sample and the skeleton-plot and the tree-ring width curve for this sample. The skeleton-plot highlights the very narrow rings (Dean, 1978). In addition to the annual ring parameters other important information can be noted on the skeleton-plot:

- pp: pith present
- np: near pith
- ±: the pith cannot be located
- B: bark present
- G: beetle galleries on the surface
- r: terminal ring present
- vv: number of rings missing not known

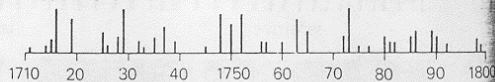


The skeleton-plots of individual radii have been aggregated to form a single plot. This can then be related to the master chronology (Dean, 1978).

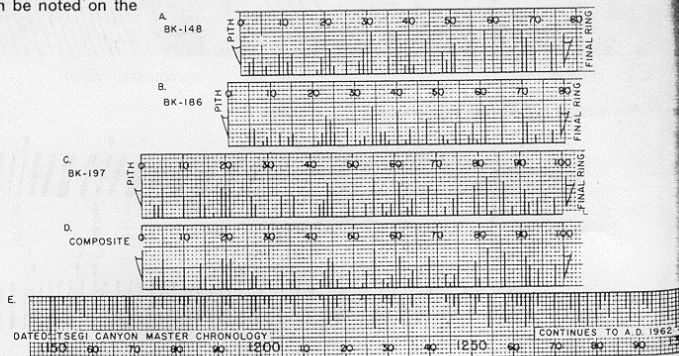
Plot DW 1969



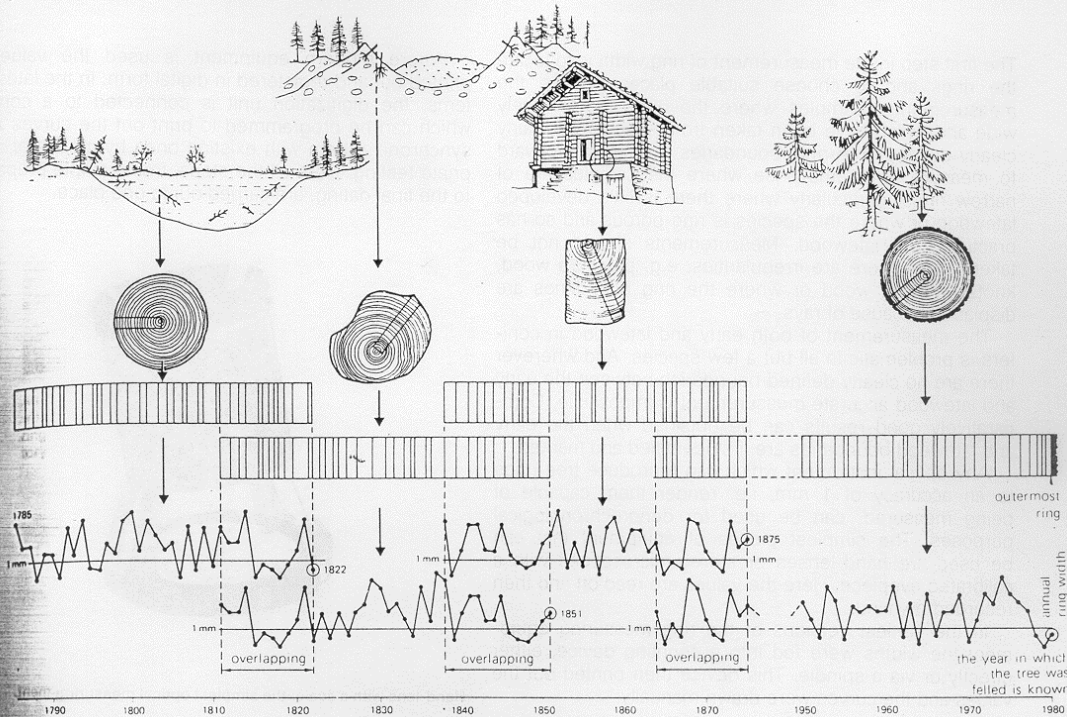
Plot BB 1973



Skeleton-plots of the same ring sequence drawn by two different people at different times. The agreement between the plots can clearly be seen (Courtesy of the University of Arizona).



Cross-dating using graphs



A schematic representation of the use of the bridging technique. The irregular occurrence of wide and narrow tree rings enables the samples to be dated. Matching the inner layers of living trees with the outer layers of beams in a building means that the samples of known and unknown age can be arranged in chronological order.

The measurement data are presented in the form of curves which are then optically aligned. This procedure is known as cross-dating.

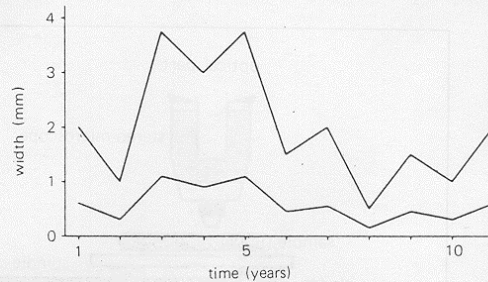
Ring width may vary considerably within any given sequence. The ring may, for example, be 4 mm wide in the center and 0.25 mm wide when the tree is a hundred years old. The ring width values tend, especially in Europe, to be expressed semi-logarithmically: The time-axis (abscissa) is linear while the axis showing the width values (ordinate) is logarithmic. This ensures that even in short series all the characteristically narrow rings can be seen clearly.

Because density varies only minimally it can be expressed on a linear scale.

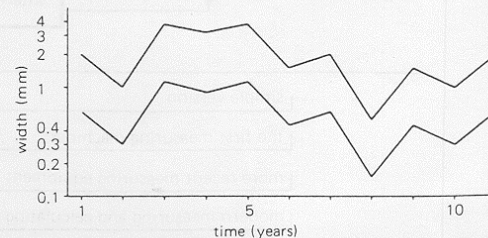
Nowadays, the curves are reproduced in the form of computer plots. But it is certainly worth emphasizing that plotting by hand is still a perfectly acceptable way of presenting the data.

It is essential that the coverage of the chronology be indicated, either in the form of a histogram or as a table of values on the diagram itself.

Where the material is to be expressed in summary form it is often confined to the number of tree rings analysed for a single tree or chronology. Bar charts are used to express these values.



Arithmetic presentation of a tree-ring sequence. The differences in width appear equally great for both narrow and wide rings.



Semi-logarithmic presentation of the same sequence. Differences in width emerge more strongly for the narrow rings than for the wider ones. (Aniol 1983)

The measurement of tree-ring width

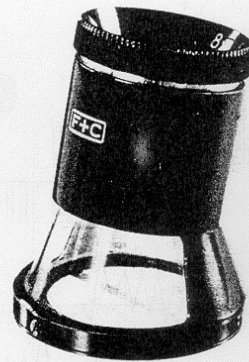
The first step in the measurement of ring width is to locate the rings and to choose suitable places to take the measurements. Samples where the rings are relatively wide and which have been taken from species with fairly clearly-defined tree-ring boundaries are straightforward to measure. Problems arise where the samples are of narrow rings, particularly where there is little developed latewood or where the species is ring-porous and so has practically no latewood. Measurements should not be taken where there are irregularities, e.g. pressure wood, knots or hazel wood or where the ring boundaries are displaced because of rays.

The measurement of both early and latewood in conifers is problematic in all but a few species. And wherever there are no clearly defined boundaries between the early and latewood accurate measurement is impossible. Comparatively good results can be obtained when the early and latewood boundaries are first identified and marked.

Any optical instrument which can reproduce tree rings to an accuracy of 1 mm, i.e. render them capable of being measured, can be used for dendrochronological purposes. The simplest pieces of equipment that can be used are hand-lenses or stereo-microscopes with a calibrated eyepiece. Here the values are read off and then recorded by hand.

In the earliest versions of the ring measuring equipment the widths were fed into a counting device, either directly or via a spindle. This device then printed out the values and the curves were drawn manually.

Where modern equipment is used the values are printed out and registered in digital form. In the latest systems, the digitization unit is connected to a computer which can be programmed to print out the curves and synchronize them with existing ones by means of appropriate testing. Here all the steps, from sample preparation to the final dating, are carried out in one place.



Hand-lens with a scale: the simplest optical measuring instrument.

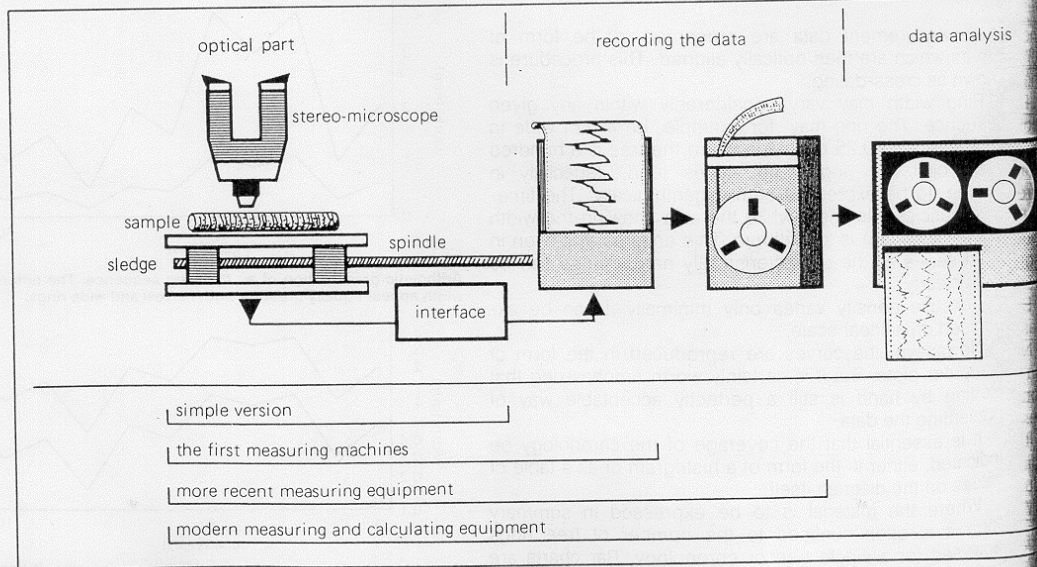
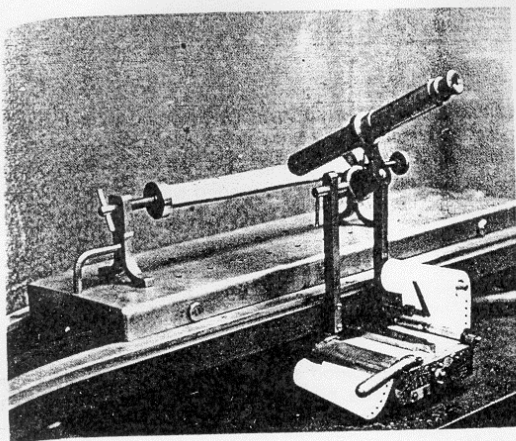
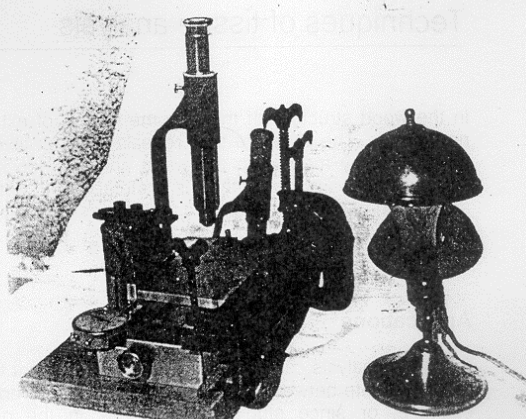


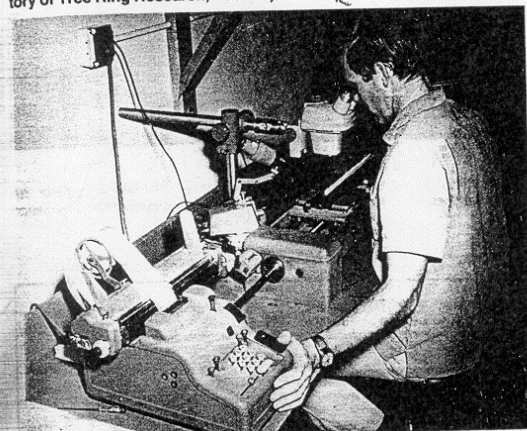
Diagram illustrating the stages in the development of tree ring measuring equipment.



An early version of a tree-ring measuring machine. This model, which was made in the 1930s, measured and recorded the annual ring sequences using a mobile telescope (Courtesy of the Laboratory of Tree Ring Research, Tucson).



An early version of a tree-ring measuring machine. The samples were placed on a stage and moved around under a monocular microscope (Courtesy of the Laboratory of Tree Ring Research, Tucson).



Machine-made tree-ring measuring equipment with a counting device. For twenty years this Eckland machine was the most widely-used annual ring measuring equipment (Courtesy of the Laboratory of Tree Ring Research, Tucson).

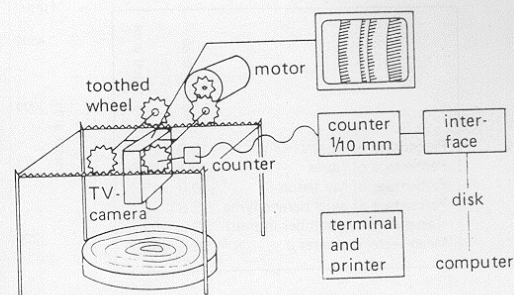
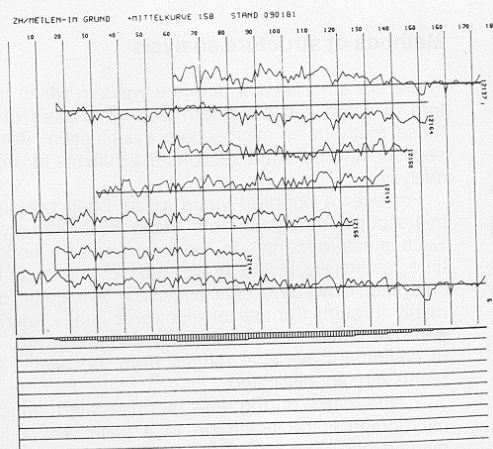


Diagram illustrating the tree-ring measuring system in the Centre for Forestry Research, Laurentide, Quebec. The camera can be moved about above the trunk disc. Observations are made using a color television screen.



A modern tree-ring measuring system with integral recording and calculating facilities. The dendrochronological laboratory of the City of Zurich.



The tree-ring curves are printed out synchronously. Statistical information about the reliability of the synchronization is thus obtained (Schweingruber and Ruoff, 1979).

Techniques of tissue analysis

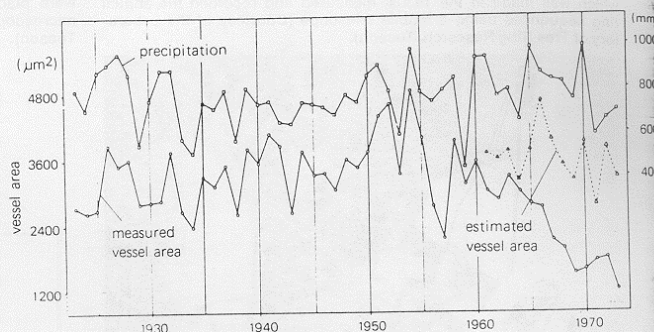
In the wood structure of the tree the effects of external and internal conditions are manifested in the form of a tree ring picture. The structure thus revealed can be investigated ecologically in one of two different ways: using structure analysis or by measuring the width and the density of the tree ring. Since more elements can be measured with structure analysis this method is preferable, but until recently the amount of time needed was prohibitive. Today, the application of modern electronic techniques enables structure analysis to be used efficiently.

Applications

Structure analysis can be applied to many different fields: to differentiate between various types of cells in wood, for example, or since studies of changes in the growth patterns of wood enable a picture of environmental conditions to be built up, in growth, in dendroecology and dendroclimatology.

	Normal area	Damaged area
Percentage of vessels	15.2	22.7
Percentage of fibres	70.1	54.9
Percentage of ray tissue	10.9	15.7
Percentage of axial parenchyma	3.8	6.7
Vessel density (number in mm)	53	82
Mean vessel diameter μ	56.6	65.7

Vessel area in the analysed stem dependent on the age of the cambium (Eckstein *et al.*, 1977).



Structure analysis of tree rings before and after damage. *Acer saccharinum* from the center of Hamburg affected by the drainage water from road-salt-ing (Eckstein *et al.*, 1974).

Methods of structure analysis

There are a number of different ways in which the structure of wood can be determined. The basis of most of these methods is microscopic examination. Both micro-sections and polished sections are suitable for this sort of analysis.

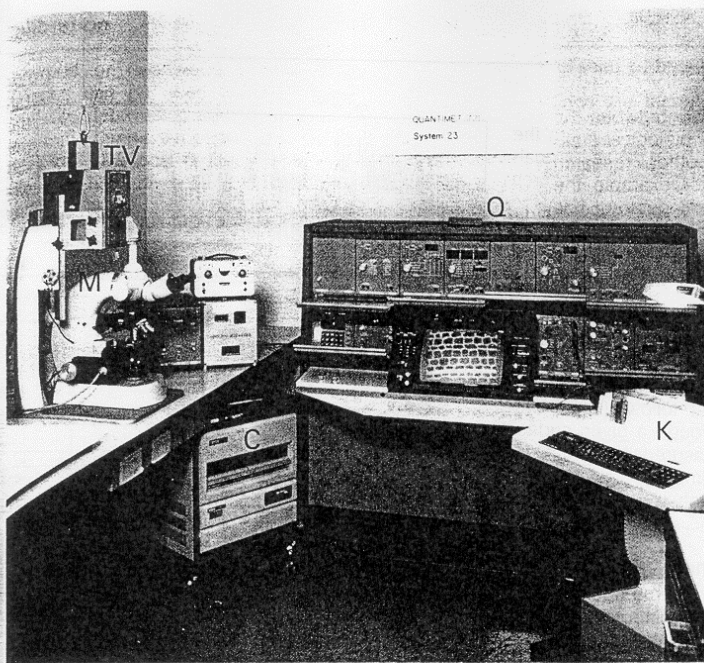
For over a hundred years it has been possible to do microscopic measurements using calibrated eyepieces. Such measurements do, however, require considerable time, especially in the preparation of the material to be analysed. Nowadays data acquisition is accomplished primarily through the use of electronic image analysis. The principle behind such analysis is straightforward. The type of equipment available ranges from the relatively simple to the very sophisticated.

In the case of the less sophisticated equipment a micrograph is projected on to a plate with a fine conducting grid. The structures can then be traced using a marking pen. This causes impulses to be triggered off by the grid which enable the x/y coordinates of the outlined structure to be identified. The area and the dimensions of

this structure can then be calculated. The more sophisticated models carry out image analysis proper.

It is clear from the work that has been done in this area up to now that structure analysis and radiodensitometry produce comparable results. The basic steps of structure analysis are presented below.

Material	Optical Examination	Digitalisation	Computer Plotter
Microsection	light transmittance microscope	T.V. monitor or plate with conducting grid	Grey-level analysis
Polished surface	surface illumination microscope	(identification of co-ordinates and enumeration)	→ Evaluation



An image analysis system: Q: analyzer 'Quantimet' (with monitor); C: computer with console K; M: microscope with mounted television camera (Sell, 1978).

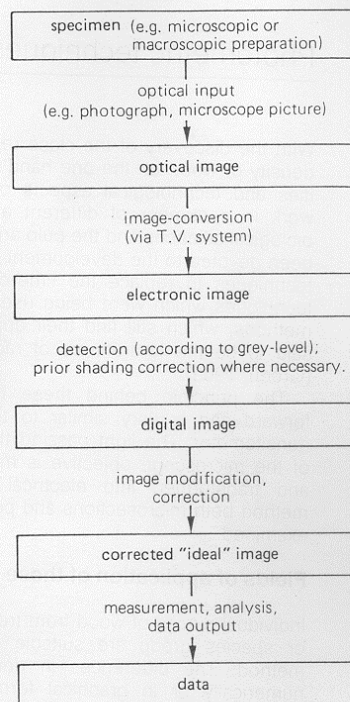
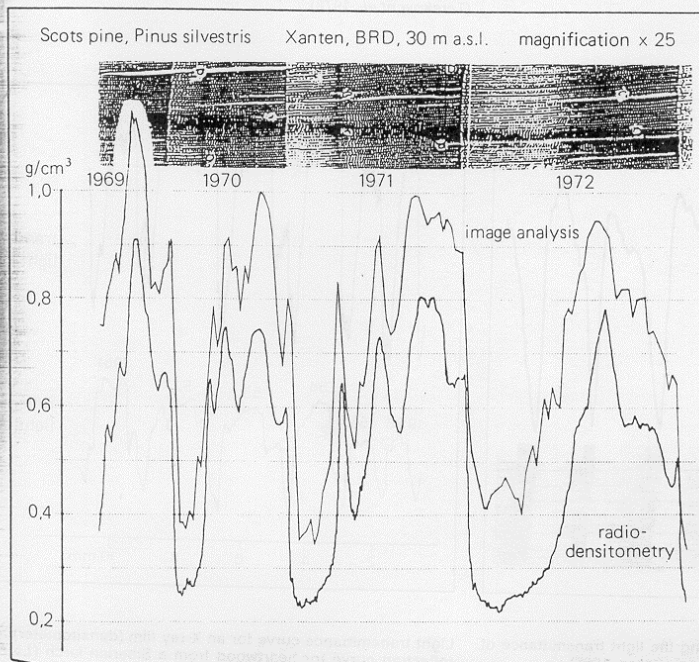


Diagram illustrating the stages in the process of electronic image analysis (Sell, 1978).



Comparison of the results of the determination of wood-density using:

- (a) image analysis — where the percentage area of the strips of tissue are multiplied by the value obtained for the density of the cell-wall 1.5 g/ccm.
- (b) radiodensitometry — where a value is obtained from the optical density of the X-ray film.

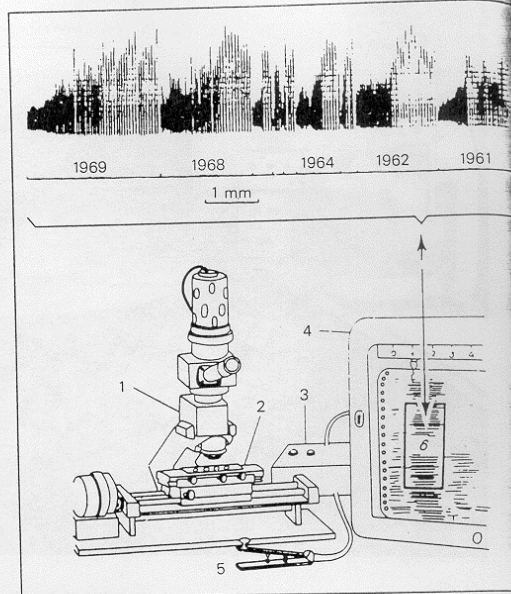
Photometric techniques

With the discovery of the close relationship between the density of wood on the one hand and its biological qualities and technological aspects on the other, research work in a number of different areas, for example the biological sciences and the pulp and paper industries, has been devoted to the development of efficient photometric techniques to replace the time-consuming microscopic techniques which were being used earlier. The resulting methods, which still find their application today, can be said to be the forerunners of radiodensitometry proper (Green, 1965).

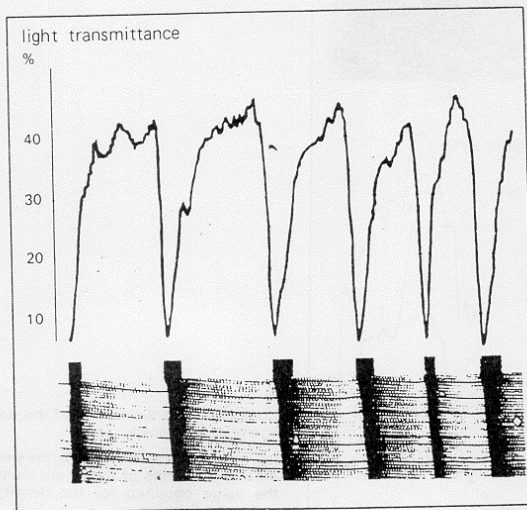
The principle behind these techniques is straightforward and is very similar to that followed in microdensitometry. The light passing through a given surface of the microscope objective is measured by a light cell and transformed into electrical impulses. Using this method both microsections and polished surfaces can be examined.

Fields of application of these methods

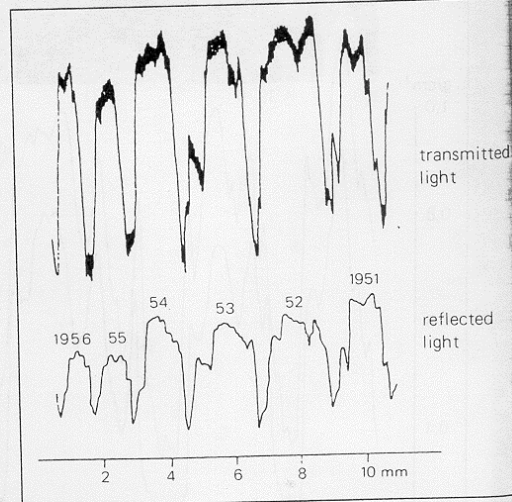
Individual pieces of wood from trees of the same species or species group are suitable for analysis using this method. The differences in density can be recorded numerically or in graphical form and then compared. Density profiles which are of as high a standard as those produced radiodensitometrically can be obtained using photometric techniques, provided that the equipment used is good.



Photometric measuring equipment for the analysis of wood sample under surface illumination — 1: microscope; 2: specimen table; 3: interface; 4: plotter; 5: impulse trigger; 6: plot (tracheogram) (Terskow *et al.*, 1978).



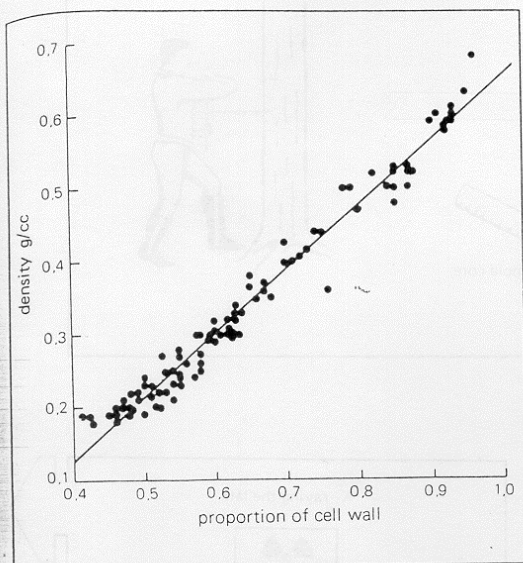
Photometrically produced curves showing the light transmittance of a microsection of red spruce (*Picea rubens*) (Green, 1965).



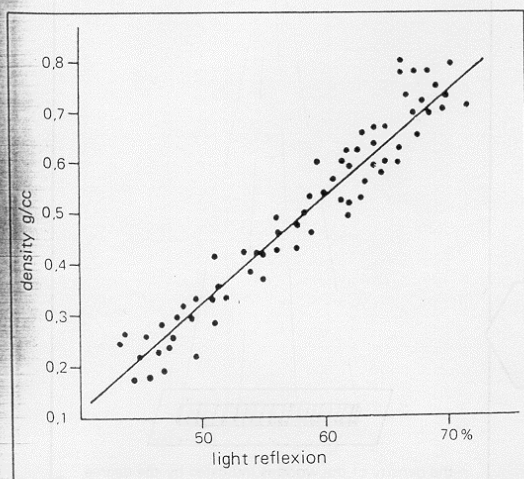
Light transmittance curve for an X-ray film (densitometer) and a reflection curve for heartwood from a Siberian larch (*Larix sibirica*) (Vaganov and Terskow, 1977).

Calibration

Sapwood or heartwood of microsections or polished surfaces can be used for calibration. Volumetric-gravimetrically determined densities agree with photometrically determined ones. An overall calibration, however, such as the X-ray analytical process provides, seems unfeasible with polished surfaces and light transmission through sections.



Relationship between wood density (ordinate) and photometrically determined proportion of cell walls in stained microsections of *Picea engelmannii*, *Pseudotsuga douglasii*, *Tsuga* sp., *Picea sitchensis* and *Abies balsamea* (Green, 1965).



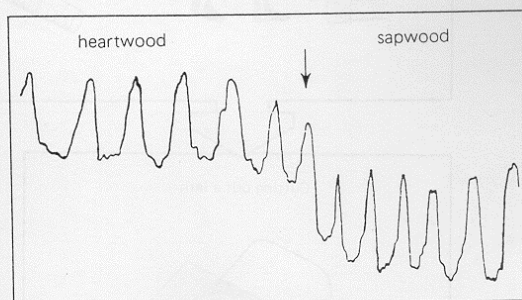
Relationship between volumetric-gravimetrically determined wood density (ordinate) and light reflection from a surface of Siberian larch. (*Larix sibirica*) (Vaganow and Terskow, 1977).

Polished surfaces

Since the technique is based on the reflection of light, colour differences within a trunk are reproduced and differences between sapwood and heartwood and irregularities in colour falsify the density values.

Microsections

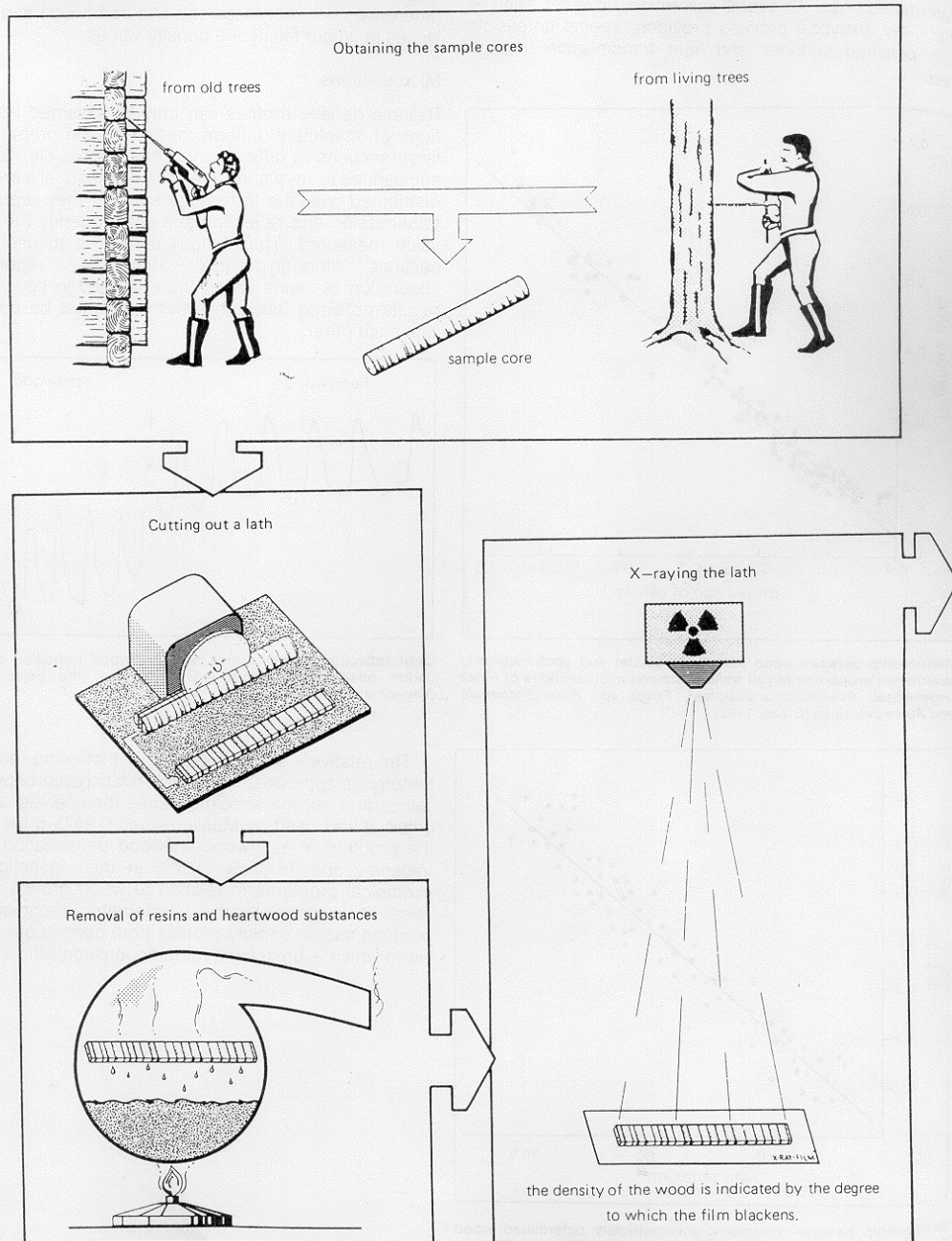
Reliable density profiles can only be obtained from sections of absolutely uniform thickness. The preparation of large sections is difficult and often impossible. Coloured substances in rays and other cell elements are irregularly distributed over the transverse surface; they reduce light transmission and reflection and consequently the density value measured. The obvious answer is to use stained sections, although Mueller—Stoll, 1947, reports that absorption of stains varies from species to species. The results obtained using this method cannot be compared with each other.

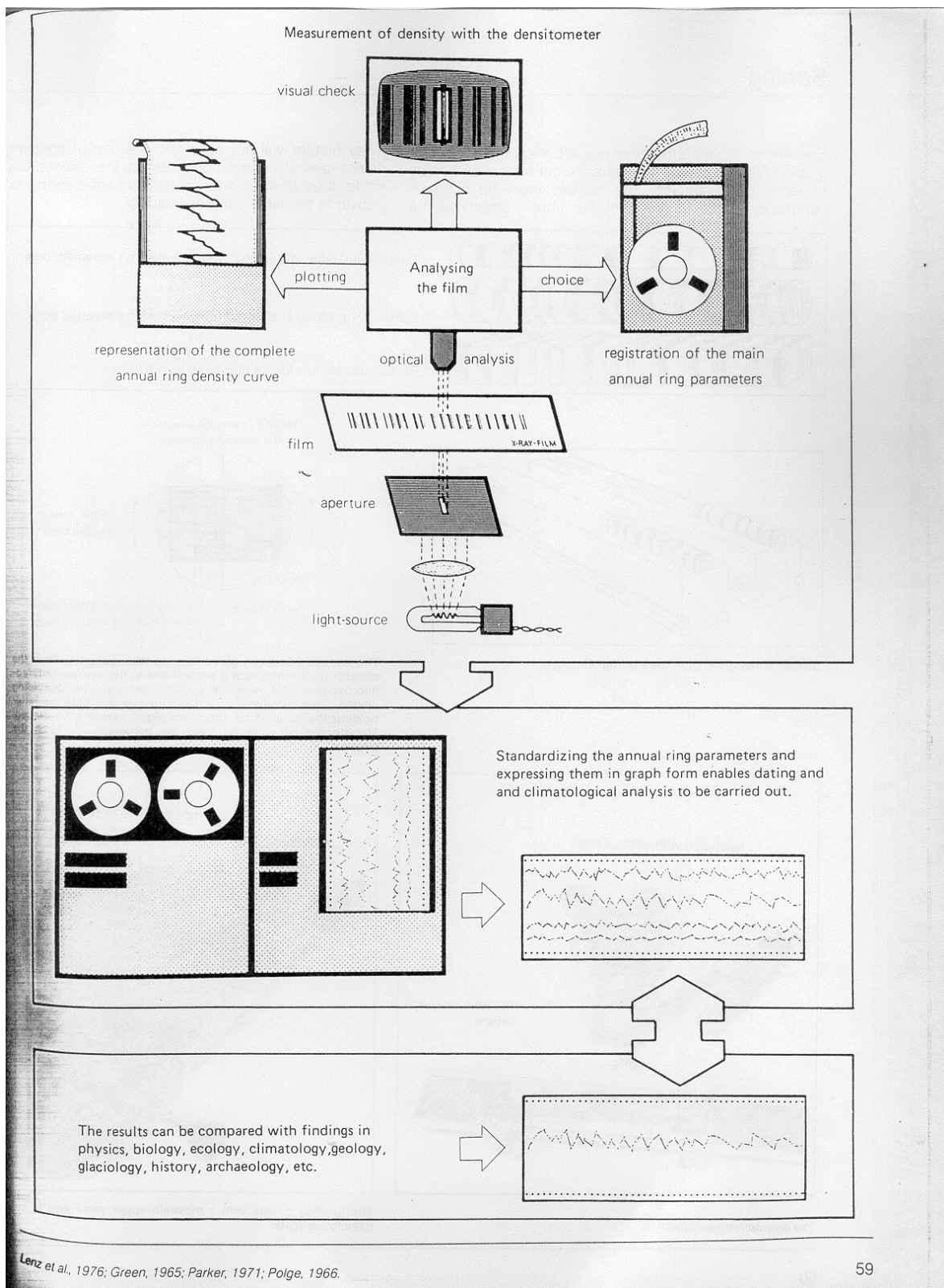


Light reflection curve from a sap-heartwood transition zone. The darker heartwood reflects less light than the paler sapwood (Vaganow and Terskow, 1977).

The relatively simple method i.e. that using radiodensitometry, is appropriate when the relationship between the values and not the absolute values themselves is investigated. It was used by Mueller—Stoll (1947) in his work on the problem of earlywood-latewood demarcation, and by Vaganow and Terskow (1979) in their investigation of ecological problems in relation to wood growth and tree rings. It is possible that photometric techniques can produce usable density profiles from decomposed conifer wood which is unsuitable for radiodensitometric analysis.

Basic steps in radiodensitometry

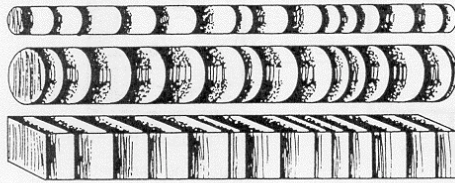




Sawing

Specimens of uniform thickness are required for radiography. For this reason the laths are cut from round cores or rectangular samples. The sample must be cut perpendicular to the direction of the fibres, otherwise the

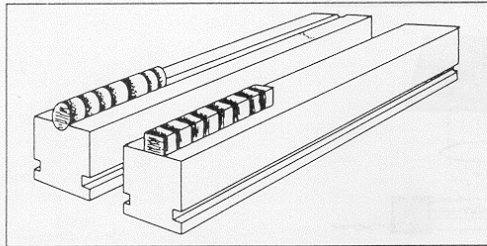
X-ray picture will not be clear. The use of an orientation device permits some correction to the orientation to be made, thus allowing such faults as oblique coring or spiral growth to be partly compensated for.



Sample core, 5mm in diameter, obtained using an increment borer.

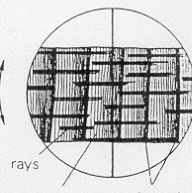
Sample core, 10mm in diameter, obtained using a drywood borer.

Rectangular sample from a disc taken from a trunk.



Sample cores glued onto the wooden supports.

vertical line in the eyepiece of the stereo-microscope



sample on the movable plate

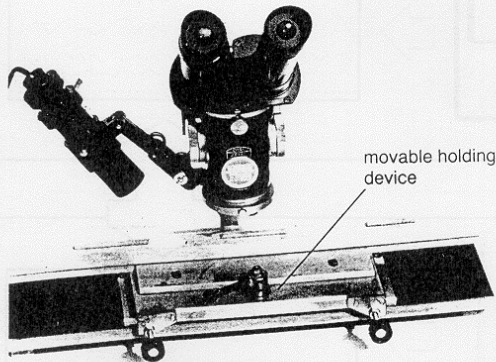
rays

wood fibres

here the annual ring boundaries deviate from the perpendicular by about 50°

The sample has been glued to a wooden support. The fibers in the sample are aligned with a vertical line in the eye-piece of the stereo microscope. This is made possible through the use of a holding device. This consists of a fixed and a movable plate, the latter holding the support on which the wood samples have been placed. The degree of variation from the perpendicular is noted on the support.

eyepiece with vertical line



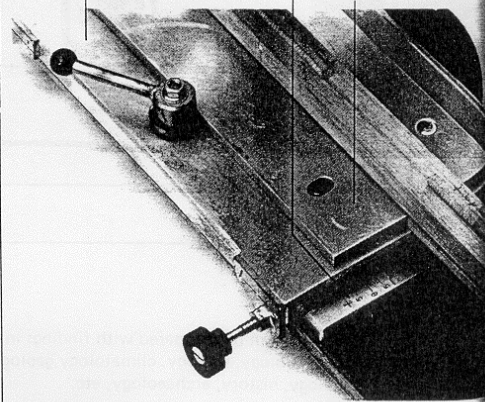
movable holding device

The orientation equipment.

scale on the fixed plate

movable plate

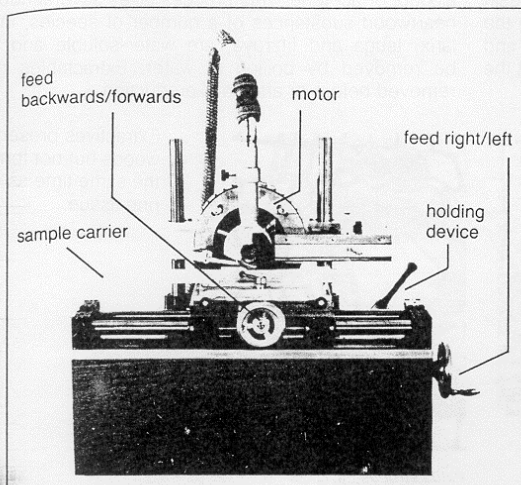
positioning device



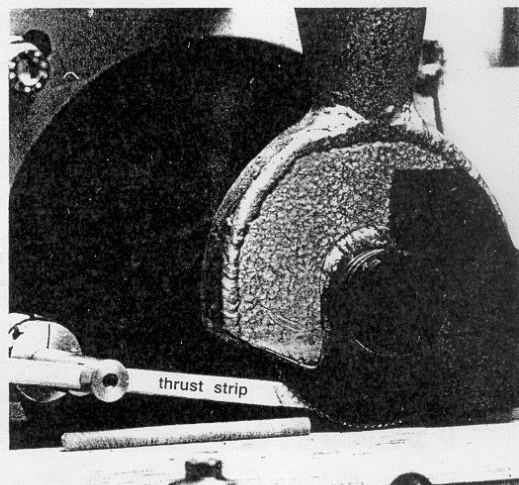
The holding device with a movable upper plate and a scale on a fixed lower plate.

With the sample still in position the holding device is placed underneath a twin-bladed circular saw and a lath, usually 1.25 mm thick, is cut out. The saw blades, which are of the sort normally used only for metalwork, cut with great precision (1–2% variation in width). In the case of well-bored samples only a single cut is necessary while for badly-bored ones two or three overlapping cuts may be needed. After sawing, the unwanted wood at the side of the lath is broken away with a rigid plastic strip. Incisions are made with a sharp blade along the base of the

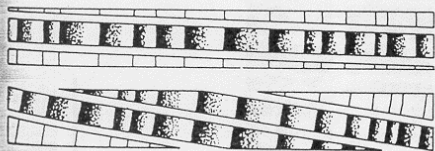
lath, which is in turn carefully detached from the support. The samples are labelled with India ink: this does not show up on the film. The thickness of the sample is then measured with a micrometer, this measurement being necessary for the subsequent density calculations. The accuracy of the coring and the cutting determines the clarity of the X-ray picture: for good samples the tissue structure is clearly visible, and for poorly-prepared ones it is blurred.



Sawing equipment for the production of laths for radiography.



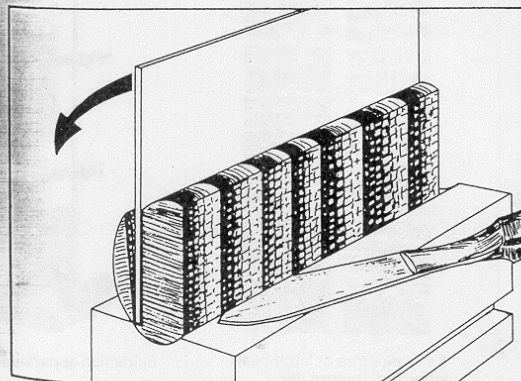
Detail: twin-bladed circular saw, with thrust strip in position, in operation.



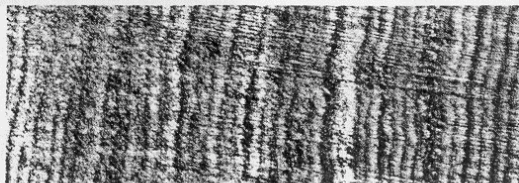
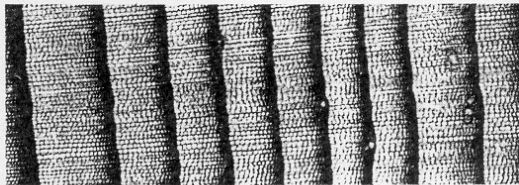
Core orientation in relation to the fibre angle

Well cored sample

Bad cored sample. Corrections have to be made by overlapping saw cuts.



Detaching the cut lath from the support.



The quality of the X-ray picture.

Top: Good — here the structure is clearly visible.
Bottom: Poor — here the structure is blurred.

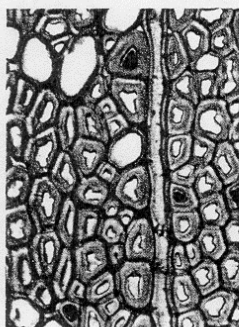
Removal of extractives

Certain parts of wood contain substances which do not directly belong to the tree ring structure itself. Where radiodensitometric work is to be done on the wood these substances must be removed because:

- such substances generally enter the tissue after the ring has been formed. Resins, for instance, are produced continually by the secretion cells of the resin ducts; other substances appear in the cells during the transformation of the sapwood to heartwood, and water is distributed more or less locally throughout the tree-trunk.

- they vary enormously in the rate at which they absorb X-rays: resins absorb relatively little compared cellulose: water absorbs slightly more; and certain other cell substances display considerably higher X-ray absorption.

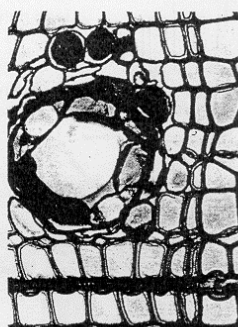
Resins should be extracted in a Soxhlet extractor, alcohol or acetone. This process takes several hours. The heartwood substances of a number of species, including larch, tsuga and fitzroya, are water-soluble and so can be removed by boiling in water. Extractables may be removed before or after lath-production.



Quercus sp. 400:1
Cross-section. Tannins in the wood cells.

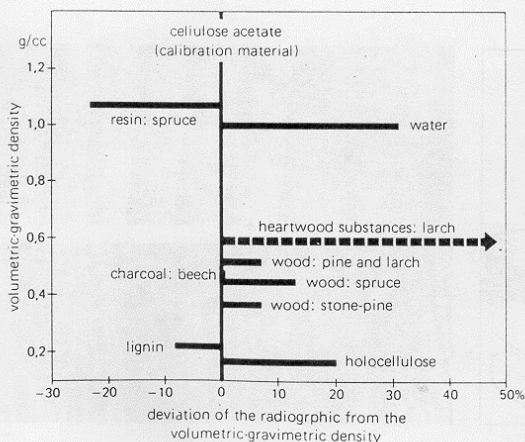


Tetrclinis sp. 100:1
Radial cut. Phenolic substances in the parenchyma cells.

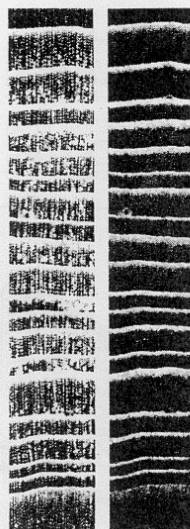


Pinus strobus ca. 400:1
Cross-section. Resin in the resin ducts.

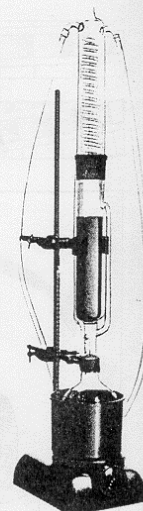
Extractives present in woods but not formed at the same time as the tree ring tissue.



The rates of X-ray absorption for different woods and wood substances in relation to cellulose acetate.



X-ray pictures of larch heartwood; left: before the extraction of heartwood substances; right: after the extraction of these substances.

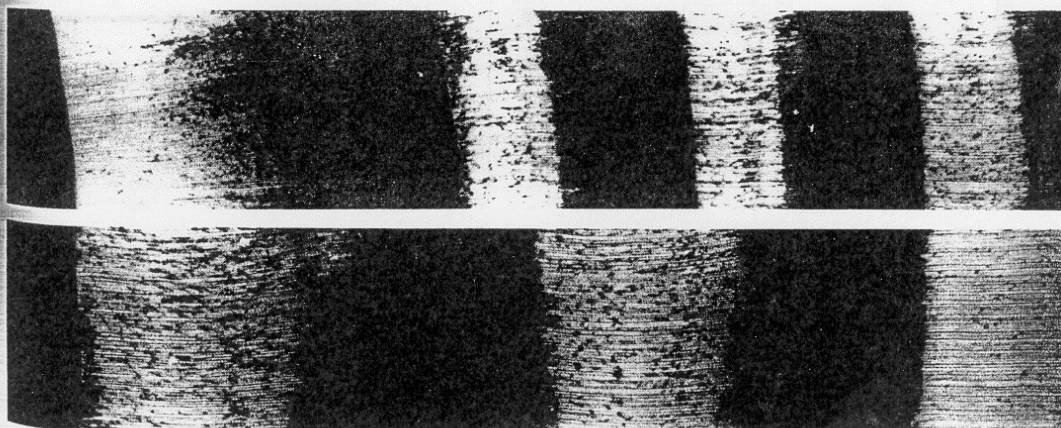
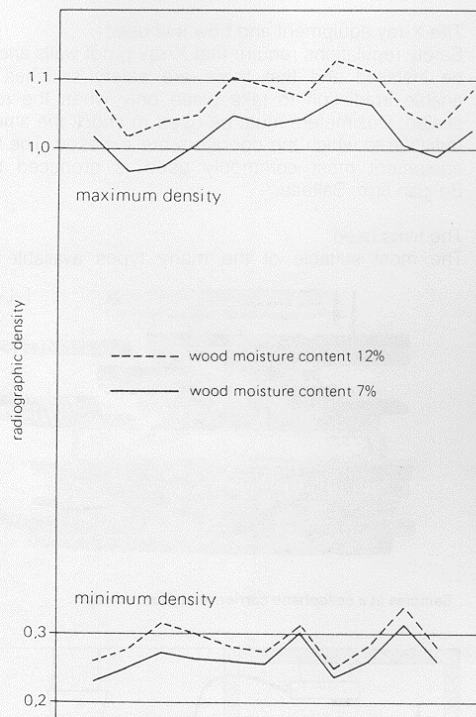


Extraction apparatus (Soxhlet)

Acclimatization

Where samples are to be radiodensitometrically analysed to obtain climatological information, the samples must be dried. This is because the distribution of water in a tree-trunk is irregular and water has a higher X-ray absorption than cellulose. The samples must all have the same water content if comparison of their absolute values is to be valid. Since wood is hygroscopic, its moisture content can be controlled by keeping it for some time at a fixed relative humidity. In the relatively damp European climatic zone wood is radiographed in conditions in which it may naturally reach hygroscopic equilibrium. In this case the samples are acclimatised at 20°C and 50% relative humidity. This gives a wood moisture content of approximately 9%. (The amount of water is taken as a proportion of the dry weight of the wood). Air-dry, 1.25 mm thick laths should reach hygroscopic equilibrium in one to two hours.

Maximum and minimum density curves for conifer wood. The water content has been adjusted to 12% and 7% respectively, and can be traced radiographically and quantified using densitometric techniques (Schweingruber *et al.*, 1978).



Positive of a radiogram, showing the irregular distribution of water in wood. (The dark patches are water.) The distribution is noticeably irregular, both in the individual cells and in larger areas. (5×) (Polge, unpublished).

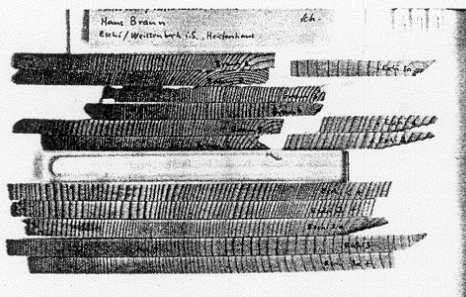
Radiography

The X-ray equipment and how it is used:

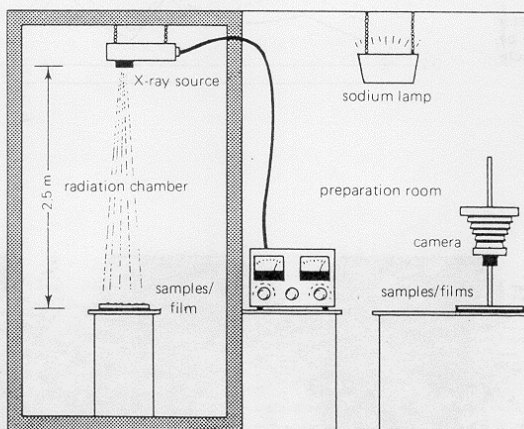
Safety regulations require that X-ray proof walls and doors be installed and that there are safety switches which enable irradiation to take place only when the room is sealed. Dosimeters must be used to check the amount of radiation to which the operators are exposed. The type of equipment most commonly used is produced by the Belgian firm, Balteau.

The films used:

The most suitable of the many types available is the



Samples in a cellophane carrier ready for radiation.

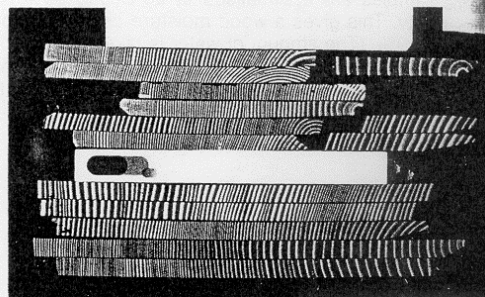


Plan of a radiation chamber. The radiation chamber is separate from the preparation and control rooms.

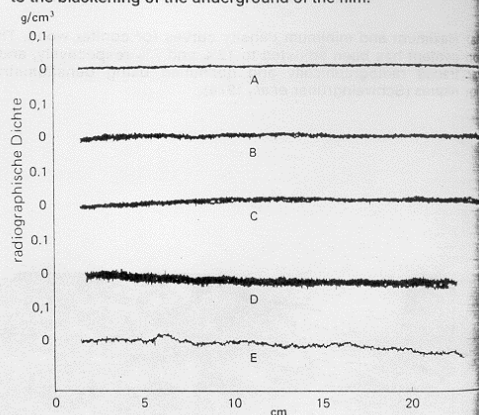
Kodak RP/M. This has a density scatter of only 0.007 over the whole film surface.

Developing the films:

Films which are designed for rapid processing take only 90 seconds in an automatic processor. The equipment that large hospitals have at their disposal is always kept in good condition and is therefore excellent for the development of radiodensitometric films.



A radiogram of these samples. Density 0 is taken to correspond to the blackening of the underground of the film.



Product	Emulsion coating	Voltage	Time	Scatter of density in g/cm³
A = Kodak RP/M	double sided	16 kV	10 min	0.007
B = 3M Medical X-Ray, Type S	double sided	14 kV	4 min	0.007
C = Kodak PE 4006	double sided	14 kV	4 min	0.007
D = Agfa CURIX RPI-PE-FW	double sided	13 kV	10 min	0.007
E = Kodak RP/SU	one sided	19 kV	8 min	0.007

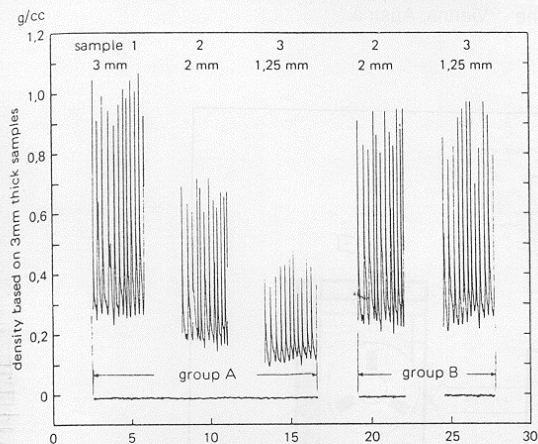
Density scatter in different types of film.

Film, coating, voltage, time, density scatter about the mean in g/cc (Lenz *et al.*, 1976).

Irradiation:

The X-ray source is 2.5 m above the film. This means that practically only parallel rays from the center of the X-ray tubes penetrate the samples. This ensures that the boundaries between the early and latewood are reproduced without blurring. The samples are in a cellophane carrier which is placed directly on the film.

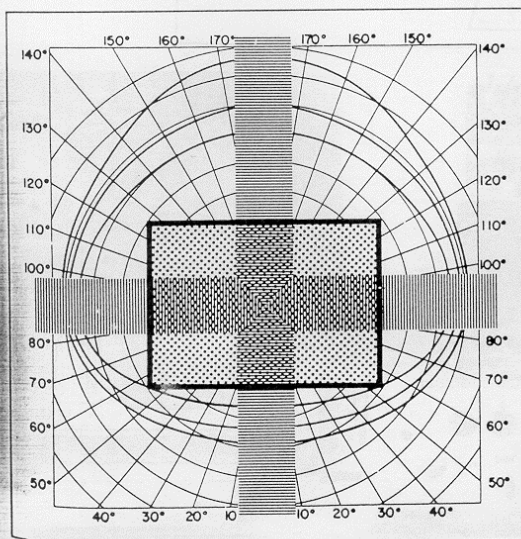
The center of the ray geometry is determined using a Geiger counter and the film itself. The duration of irradiation depends on the distance between the radiation source and the film: the longer the period of irradiation the sharper the contrast obtained. For this reason wood samples that are only 1.25 mm thick are irradiated at 11 kV and 20 mA for 90 minutes.



The influence of X-ray exposure time and sample thickness on radiographic contrast.

Group A: spruce samples 3 mm, 2 mm and 1.25 mm thick were uniformly exposed for 6 minutes. The density contrast falls with the decreasing thickness of the sample. The absolute density value (scale) is valid only for the 3 mm thick samples.

Group B: samples 2 mm and 1.25 mm thick were exposed for 25 and 180 minutes respectively. By prolonging the exposure time the contrast can be increased to approximate that obtained using the 3 mm thick samples.



Ray geometry of the X-ray tube.

A field with uniform irradiation intensity (isodose lines). By arranging the test films in a cross the areas receiving the same dosage of irradiation can be determined. The blackening of the film is practically uniform from the centre to the edge of the rectangle representing the surface of the film.

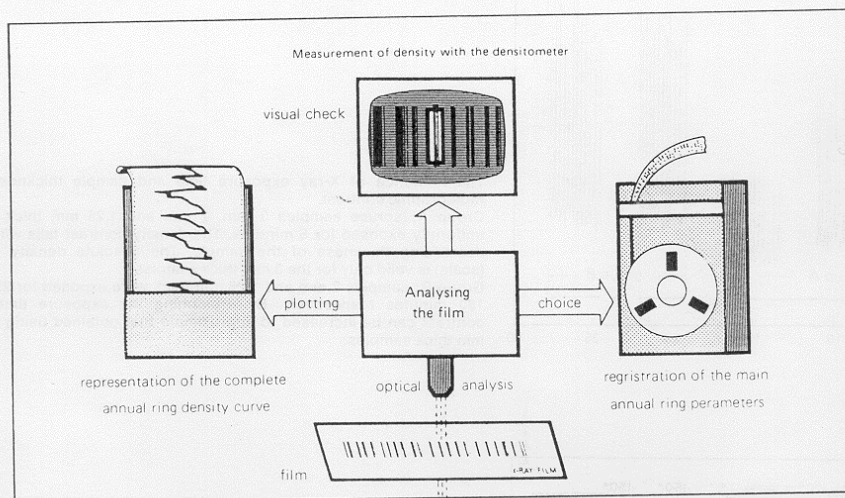
The radiographic-densitometric measurement of wood density

The optical density of the film is measured using a microdensitometer. The film of the radiographed samples rests on a movable table which passes over a light source with a slit-shaped aperture. The light signals of varying intensity are transformed by a potentiometer into electrical impulses. These in turn activate a strip-chart recorder. A magnetic storage unit registers selected values. The

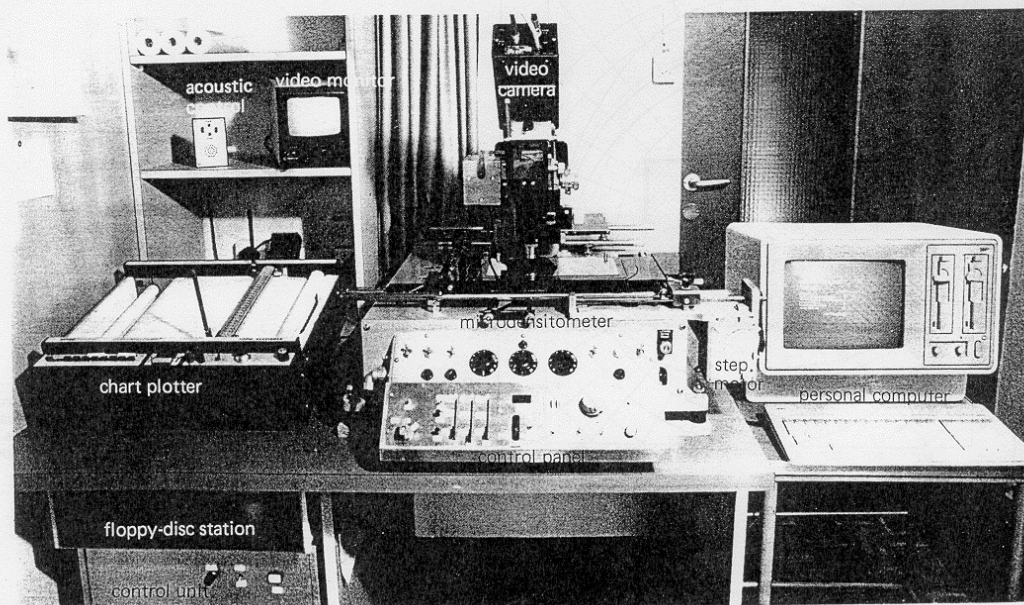
densitometer table, the rolls of paper for the strip-chart recorder and the tape-punch move in time with one another in response to signals transmitted from the control mechanism to the stepper motor.

A new, complete radiodensitometric apparatus is produced by Kutschenreiter, Siccaburgstrasse 64, A-1100 Vienna, Austria.

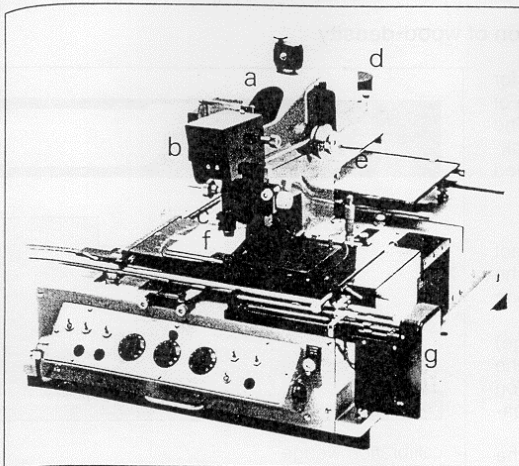
The equipment



Schematic representation of a densitometric equipment



Radiographic-densitometric equipment (System Birmensdorf 1983)



Microdensitometer (Joyce Loebel MK III CS)

- (a) housing with optical wedge
- (b) eyepiece
- (c) objective
- (d) potentiometer
- (e) plotter and recording table
- (f) specimen table
- (g) stepping motor.

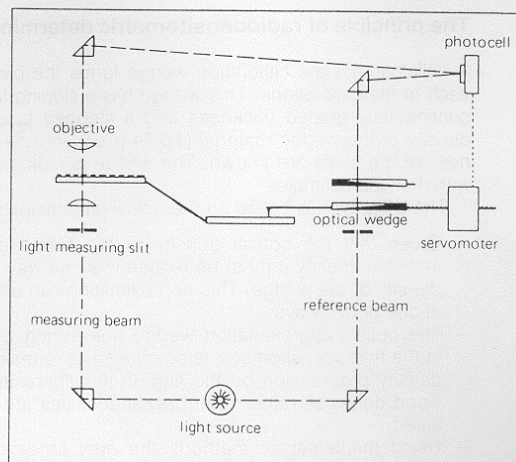


Diagram of a double-beam microdensitometer

The measuring and reference beams are switched alternatively through a light at a frequency of 50 Hz. This cell transforms the light into electric current.

A servomotor moves the optical wedge in such a way as to ensure that the values of both beams are equal. The amount of displacement is then registered directly by a pen or a potentiometer. The density curves can then be produced (Polge, 1966).

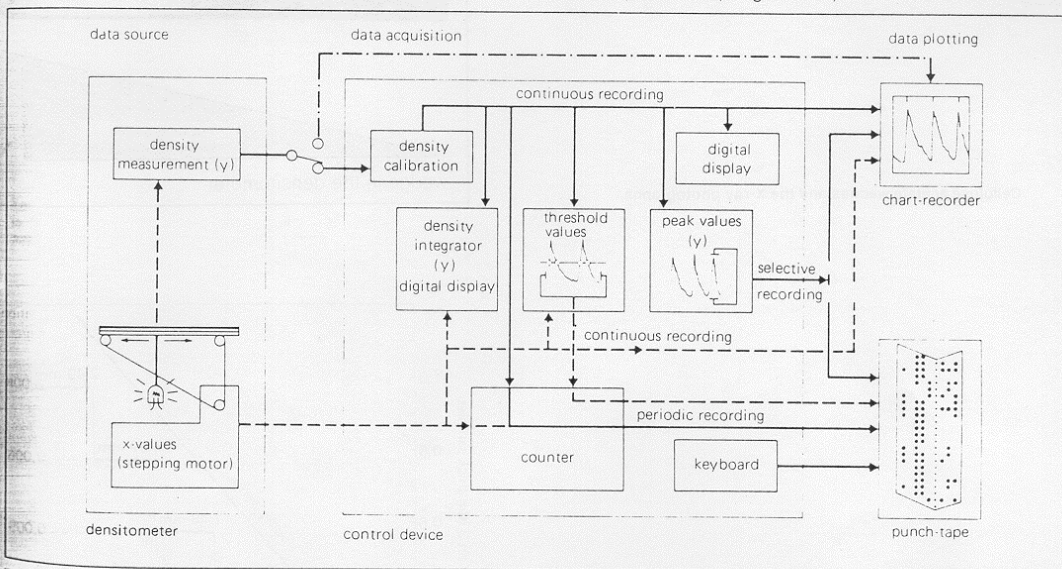


Diagram showing the functions of a data acquisition system

Impulses are sent from the densitometer to the control mechanism, the chart-recorder and the tape-punch.

The control mechanism can, in response to signals from the stepper motor, register the data in a continuous, periodic or selective form.

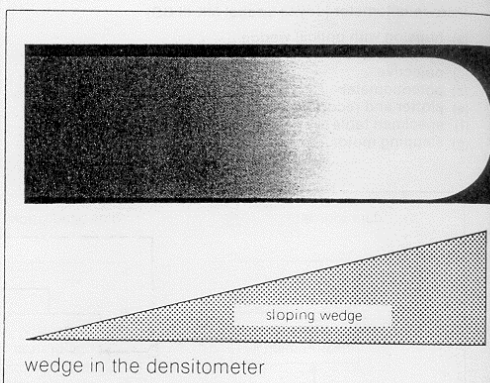
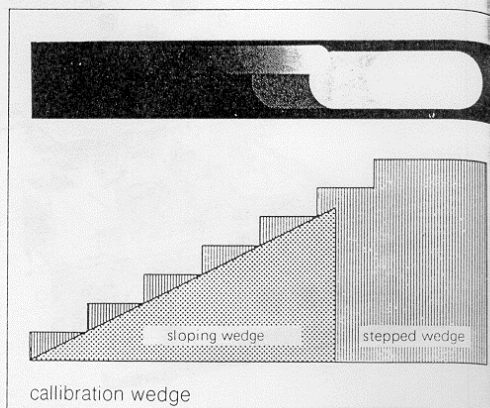
The chart-recorder continuously registers all the signals that it receives, in the form of a two-dimensional graph (analog values). The tape-punch registers data in digital form, and can do this continuously, periodically or selectively.

The principle of radiodensitometric determination of wood-density

A cellulose acetate calibration wedge forms the basis for each of the calibrations. This wedge has a sloping face of continuously graded thickness and a stepped face. The density of the wedge material (1.274 g/cc) and the thickness of the steps are known. The wedge is radiographed with the wood samples.

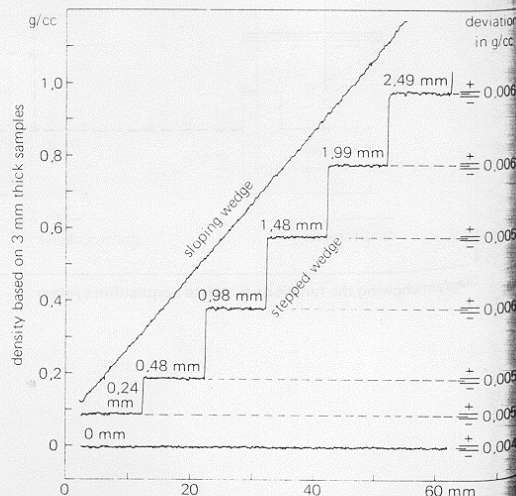
The calibration is based on the following principles:

- Given that the optical density of the film does not increase linearly it must be related in some way to the density of the wedge. This approximation can be done in one of two ways:
- The optical approximation wedge (measuring wedge) in the microdensitometer is so chosen as to match the density progression on the film. In this approximation wood densities rather than optical densities are measured.
- Using mathematical methods the grey tones of the measuring wedge in the densitometer are related to those of the cellulose wedge on the film. Using five density levels of the calibration wedge along a section of an ellipse, the wood density can be calculated from the optical density.



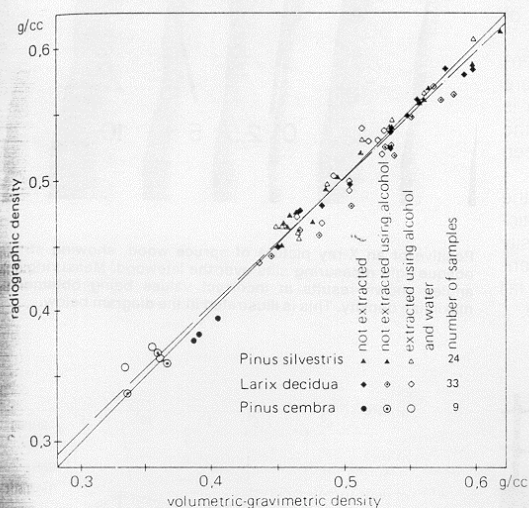
Cellulose acetate wedges and the X-ray photographs

Densitogram of a cellulose acetate calibration wedge irradiated at 17 kV for 6 minutes. The calibration relates to wood that is 3 mm thick. Here the deviation from the mean was not more than ± 0.006 g/cc. Where an optically non-linear wedge is inserted in the path of the reference beam of the densitometer or a mathematical transformation of the density curve is used, it is possible to obtain linear values for the wood density.



Density integrator

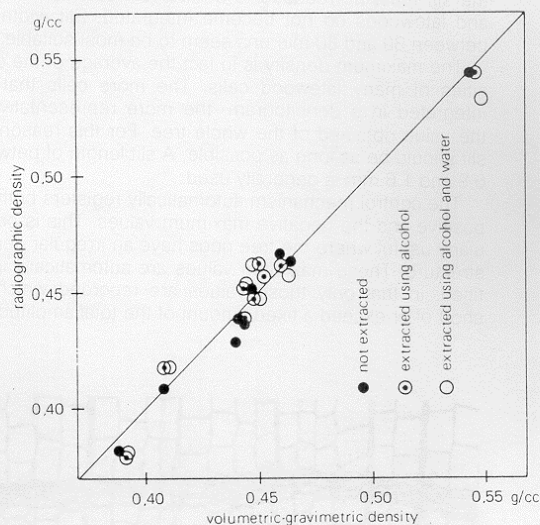
The values obtained using the densitometer must be integrated over a certain distance. This enables the density of the wood to be determined. Pieces of cellulose acetate and of woods that are commonly investigated are carefully shaped and then measured and weighed. The mean density is thus obtained. A null density is obtained by integrating the blank background of the film with the densitometer.



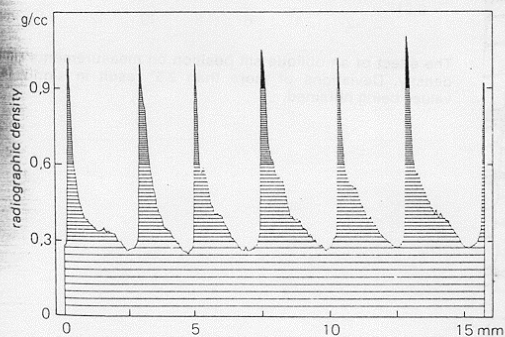
The relationship between the radiographic-densitometric and volumetric-gravimetric densities.

For Scots pine, larch and Swiss stone pine the radiographic density is too high by 7.2%, and a correction factor of 0.933 is used to approximate the absolute density.

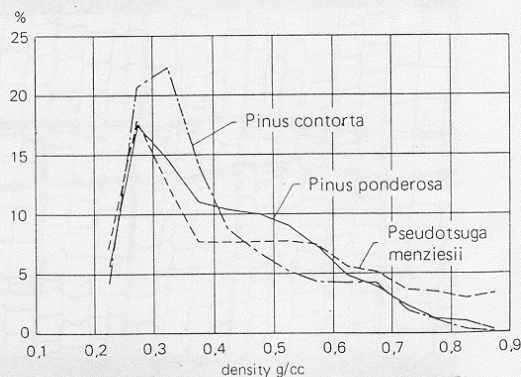
Comparison of the known volumetric-gravimetric densities of the cellulose acetate and of the wood with their known, volumetric densities enables the differing absorption rates of the materials to be determined. The value for the cellulose acetate can then be calculated. This means that the radiographic and the volumetric-gravimetric densities can be put on the same footing. The calculation of the correction factor is performed only once for each type of wood.



For spruce the radiographic density is too high by 12.9% and a correction factor of 0.886 is used.



The mean density above a given threshold can be calculated by dividing all the values measured at a distance of 10 microns by the number of steps. In this example, the mean density above a threshold of 0 g/cc is 0.42 g/cc. The mean density above the threshold 0.6 g/cc is 0.82 g/cc (Lenz *et al.*, 1976).



The percentage of different density levels in three types of wood (5 trees for each type). The average percentage of different levels can be obtained by integrating the density above different thresholds (Echols, 1973).

Measuring the density within the tree ring

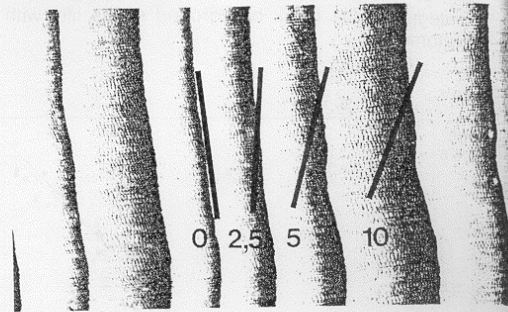
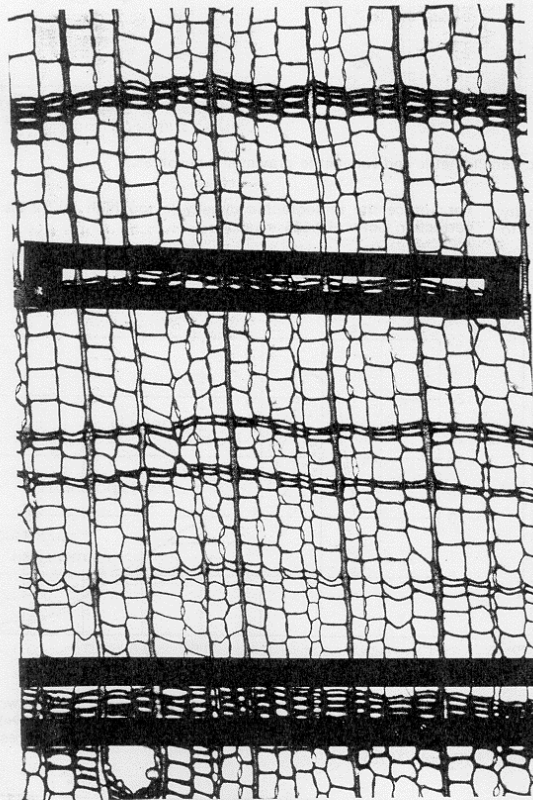
Measuring the minimum and the maximum density

In order to do this a densitometer must be available of a type where the form and position of the measuring slit can be adjusted. The maximum and minimum densities can be measured accurately only if the slit can be positioned parallel to the tree ring boundary. Failure to orient the slit correctly leads to inaccuracies in the results.

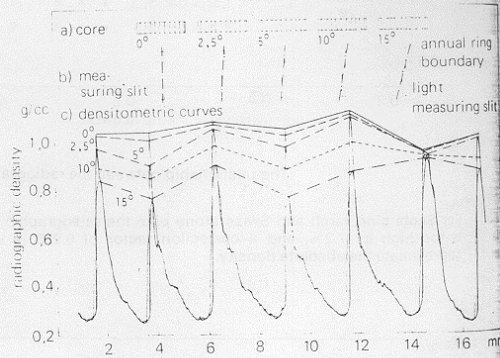
Where the tree rings are very narrow, the width of the slit must be reduced to a minimum so that the early and latewoods do not become integrated. Slit widths of between 30 and 60 microns seem to be most suitable.

The maximum density is in fact the average of the densities of many latewood cells. The more cells that are integrated in a densitogram, the more representative is the value obtained of the whole tree. For this reason the slit should be as long as possible. A slit length of between 0.8 and 1.6 mm is generally used.

The control mechanism automatically registers both the positive and the negative maximum values. This is particularly useful where the tree rings have an irregular density structure. These maximum values are automatically identified, in that only those values are recorded which fall short of or exceed a fixed amount of the total amplitude.



Positive of an X-ray picture of spruce wood, showing straight and oblique light measuring slits over the latewood. Measuring using the angles shown results in incorrect values being obtained for the maximum density. This is illustrated in the diagram below.



The effect of an oblique slit position on measurements of maximum density. Deviations of more than 2.5° result in significantly lower values being obtained.

Photomicrograph of larchwood. The measuring slits are drawn over the latewood. The narrow slit integrates about 30 cells, wider one about 120.

Measurement of early and latewood widths

For densitometric purposes earlywood and latewood are differentiated in one of two ways:

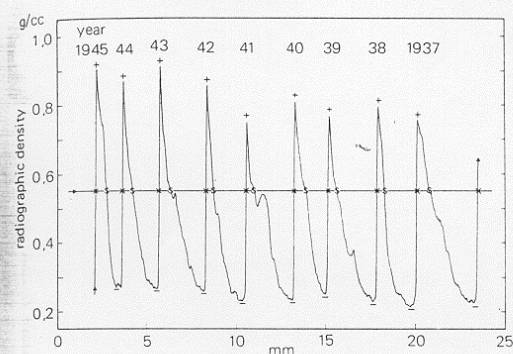
- Delimitation according to a predetermined density level (fixed threshold). This means that a particular density threshold is set and that where values exceed or fall below this threshold the boundary between the two woods is deemed to occur.
- Demarcation according to a density level that is related to the total density amplitude within a tree ring (floating threshold). Here the threshold varies from ring to

ring. It tends to lie around the mid-point between the maximum and minimum density.

Both these methods provide an answer to the problem of differentiating between early and latewood that is acceptable and technically feasible. The second alternative outlined here, that of demarcating in line with a floating threshold, would tend to reflect the biology of the tree better.

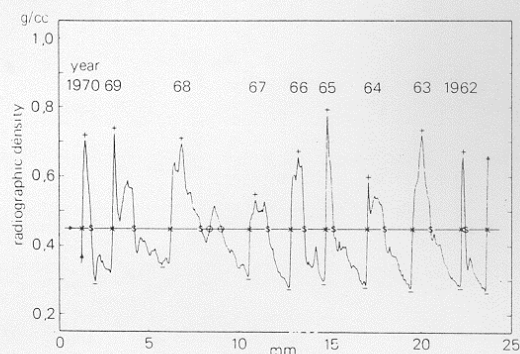
The diagrams and photographs illustrate measurement along a fixed threshold.

Selective data acquisition for curves with:



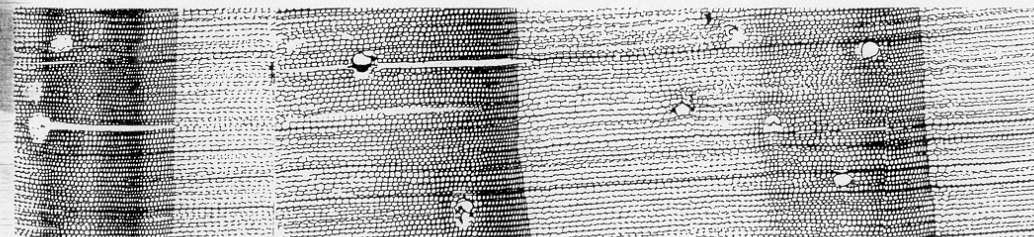
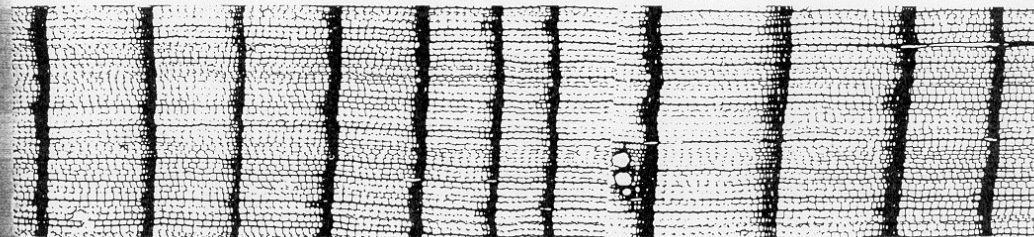
(a) regular density progression (*Abies alba*)

• = value higher than the threshold value
 S = value lower than the threshold value
 + = peak value



(b) irregular density progression (*Pinus strobus*)

— = lowest value
 ○ = non-registered value (on the ring for 1968)
 (Lenz *et al.*, 1976)



Tree-ring sequences illustrating regular and irregular density progression. Above: regular progression in larches (*Larix decidua*) from

the subalpine zone of the Alps. Below: irregular progression in Scots pine (*Pinus silvestris*) from the lowlands of northern Germany. Both $\times 25$.