Micro-preparation of wooden samples to study the
distribution of natural glue systems in wood

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Abstract

Wood properties at macroscopic scale depend on its structure at microscopic scale. Currently, there are few data concerning the microscopic-macroscopic relationship, and this is why here is detailed and developed a method of wood slicing with a thickness of 3 microns, in order to visually observe the microstructure and in some cases its interactions with adhesives (when present), and establish possible links with the mechanical properties of a given specie. This method implementation is based on an already existing method. The sample is soaked in a resin via the succession of dehydration steps, then cured by heating in the oven. According to various tests, the resin used by the University of Freiburg proved to be the best, whereas at the level of the blade used for the samples slicing, the use of a diamond blade with a preslicing using a metallic blade of profile D, proved to be the most conclusive regarding the quality and fineness of slicing, as well as less wear on the diamond blade. Indeed, cuts of 3 microns can be obtained, thus allowing excellent microscopic observation, which can be applied for an accurate study of the microstructure in different projects.

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1 Introduction

The distribution of glueing systems and their influence on the mechanical and sorptive properties of wood is of high interest. Especially in the field of restoration, the stabilization of the wood structure with polymers is often necessary to remain historical and cultural objects. So far, there are no data available, which describe the fixation and distribution of polymers at the cell level. In order to make the polymers visible in the wood, different ways of micro-analysis are possible: light microscopy, SEM, AFM. For each analysis, the sample preparation is the fundamental key for a successful investigation. Not only for studying the distribution of glueing systems, also for the characterization of degradation in wood, so more on a biological point of view, very thin sectionning (i.e. < 6 microns) for light microscopy observation proves to be useful.

In the frame of this master project, a reproducible procedure for sample preparation for the development of a very thin sectionning method for transmission light microscopy analysis will be developed and tested.

1.1 Objectives

To start, reference wood samples for light microscopy without any degradation according to the classical state of the art (thickness 18-20µm) will be prepared. It will be followed by preparation of reference wood samples for light microscopy with a thickness of 3µm, using a rotary microtome and an adapted pretreatment including the microstructure stabilisation. Such a method has to be developed.

Then, preparation of wood micro-slices for light microscopy with a high level of degradation including the stabilisation of the microstructure before the cutting (thickness 3µm), and impregnation of wood samples with different binding/glueing systems, preparation of micro-slices (3µm, 18-20µm), analysis of the glue distribution based on the fluorescence microscopy.

In parallel to the light microscopy research, preparation of micro-samples for SEM analysis, investigation and description of the glue distribution in the cell tissue and on the cell wall.

In the end, the following goals are targeted:

- Find a suitable method for very thin (3µm) wood micro-slicing, based on the ones already existing.
- Assess the cuts quality differences between metal blade and diamond blade.
- Produce good quality microscopic pictures for visualizing the glue distribution in the wood and if possible the glue-cell wall interaction, or the possible degradation patterns, depending on the studied samples.

A short introduction to wood anatomy and microscopy is presented in State of the art, but to have a broader view concerning the classical cutting techniques and results one can refer to [2]...
1.2 State of the art

1.2.1 Wood anatomy

Wood is a natural hygroscopic material which presents hierarchical levels of complex organization from the macro to the nano scale. Its porous nature as well as its very different levels of organisation influenced by external conditions make it a very anisotropic material. Due to wood anisotropic nature, it is necessary to know about its structure in order to understand what happens and why, at the macroscopic level.

![Figure 1: Woods specific gravities [3]](image)

Wood is also a light material, which thus simplifies handling and implementation. Indeed, compared with concrete or steel, having relative densities of approximately 2.5 and 8 respectively, wood have a relative density (also called specific gravity) ranging from 0.2 up to 1.3, it’s a 1 to 5 factor which enables it to adapt to many uses. Fig. 1 gives their values for some common wood species. One can see that hardwoods generally have a higher specific gravity.

Although biodegradable, wood has an impressive durability over time, that for example plastics, which are also biodegradables, have not.

**Wood classification and microstructure**  Wood species can be identified by their macroscopic characteristics, and are grouped into two major classes, which are the gymnosperms also called **softwoods** and the angiosperms also called **hardwoods**. Softwoods characteristics and organisation are much simpler than for hardwoods, with the presence of only 2 different cells type, the tracheids and the parenchyma cells. The tracheids, which can be oriented either axially or radially, have the fonction of conduction and mechanical support, whereas the parenchymas have the role of distribution and storage of the nutrients. Softwoods also present
an important feature to identify its type, it is the presence of **resin canals**. The specie can have large, numerous, evenly spaced, solitary resin canals, or more small, infrequent, sporadically spaced, tangentially-grouped resin canals or no canals at all [4], as can be seen in Fig. 2. By comparison, hardwoods present more different types of cells, they have tracheids as well (also called fibres), vessels and parenchyma cells. Vessels ensure the conduction of sap and nutrients, whereas fibres mainly ensure the mechanical support, and parenchymas, as well as in softwoods, have the role of distribution and storage of the nutrients [5].

![Figure 2: Resin canal distribution in softwood species: Pinus Ponderosa presenting many resin canals (left), Picea Sitchensis presenting few resin canals (middle), Cedrus Libani presenting no resin canal](image)

Both in softwoods and hardwoods, tracheids are long and thin and constitute the major part of the wood. Their composition varies with external conditions, i.e. during the first growth period (spring in our temperate climate) the tracheids are shorter and thin walled, and their lumen (the cell core) is wide for easy and quick conduction, whereas in the second growth period, in summer, before starting the dormancy period, tracheids are longer and thick walled, having a small lumen. These layers of thin or thick walled cells are respectively called **earlywood** and **latewood**, and form the two of them the annual growth ring of the tree, as explained in [6].

Due to the tracheids length and wall thickness differences, latewood is denser and harder than earlywood, thus this and also the variety of features and organisation influence the mechanical properties, which are explored in the *Mechanical properties* part.

To the softwoods and hardwoods two classes can be added also the **Tropical woods**, which present some similar organisation to hardwoods, but the main difference is that tropical woods don’t have growth rhythm like european woods: indeed, in tempered regions, as previously said, years are alternated with spring-summer growing season and autumn-winter dormancy season, due to weather and conditions variation, thus giving rise to an annual growth, and this cyclic pattern leaves a serie of annual rings in wood. Regarding tropical woods, growth is punctuated by alternation of rainy season and dry season happening several times a year, leaving also an inconspicuous or totally absent ring growing limit.
Wood strength and structure stabilization  Wood strength is mainly ensured by the fibres present in the wood, which as previously said are much more thick-walled than the vessels or the parenchym cells and have smaller lumens. Indeed, wood porous nature and elements specific orientation make it a very anisotropic and so complicated structure to handle. Not only concerning wood performances for structural applications, also when performing cuts, one has to think about the particular wood structure, in order not to destroy the cell and be able to conduct specific observations. For this purpose, it is necessary in some cases, when performing very thin cuts, to fill wood pores with a stable material such as resin. Before curing and under vacuum, resin can easily flow and fill the pores, and is then hardened by heating. Somehow, not every resin is suitable for such a purpose, and this will be investigated later on, in Resin investigation section.

1.2.2 Microtomy

![Microtome apparatuses](image)

Figure 3: Apparatuses used for microtomy

The microtome in an apparatus designed for handcutting of wood and can be seen in Fig. 3, under two different forms: in Fig. 3(a) as a sliding microtome and in Fig. 3(b) as a rotary microtome.

**Sliding Microtome**  Samples to be cut generally don’t exceed 10 mm in length and width, and 20 mm in height. The microslice thickness can be manually defined for each microslice or predefined in order to make series of the same and constant thickness. The microtome blades used are generally removable blades which are made of stainless steel and different caliber. The ones generally used for our classical technique are of caliber N35. The blade is placed in clamps and can be changed when not sharp anymore. The cutting movement is illustrated in Fig. 4(a) and goes horizontally from one edge of the sample to the other, goes back in reverse direction for reloading and so on.
Rotary Microtome  Samples to be cut generally don’t exceed 5mm in length and width and 10 mm in height. Especially when cuts are performed using a diamond blade, whose width is 8 mm. Here also the microslice thickness can be defined for each microslice, and series of the same and constant thickness can also be made. The main difference with the sliding microtome is that in the rotary microtome it is the sample which is moving, while the blade stays still, as illustrated in Fig. 4(b). Also, cutting can be proceeded manually or electronically via a motor moving the sample for a defined speed and slices thickness. Different types of knives can be used here, metallic ones, made of stainless steel, which are much more stable than the removable blades from the sliding microtome, and just need to be sharpened from time to time, and also diamond knives, which duration over time is even better, but somehow also need to be sharpened after a while.

1.2.3 Transmission Light Microscopy (TLM) principle

Light microscopy is based on lenses assembly so as to obtain a bigger representation of a sample. The magnifying effect is thus increased, and total magnification is found by multiplying each one of the lens magnification together. This way, magnifications of up to 2000x can be obtained [9]. The first lens is called the objective, and produces a magnified image of the sample when this one is placed at a smaller distance than the double of the focal length of the objective. This magnified image is formed in the intermediate image plane. Then a second lens called the ocular or eyepiece magnifies the image formed by the objective. This is illustrated in Fig. 5(a). Objective plus ocular form the classical light microscope, but other lenses like a condenser or a tube lens are sometimes added to increase microscope performances. The term transmission in Transmission light microscopy means that the light for sample observation comes from behind the sample and goes through it. It supposes to have thin samples for allowing observation.

Magnification is not the only important parameter for observation, resolution is also very
important and corresponds to the amount of light diffracted by the sample, that is collected by the objective. The objective has to collect as much as possible of this diffracted light, in order to have the sharpest image as possible. This resolution is named the aperture of the objective.

For a more detailed explanation, one can refer to [9]

1.2.4 Fluorescence microscopy

Fluorescence consists of light emission due to excitation of the electrons of a sample’s molecule. Incident light reaches the sample, which molecules will absorb photons energy, and thus be in an electronically excited state. When the electrons go back to their ground state, they emit radiative energy under the form of light, called fluorescence, or in certain case phosphorescence (see Fig. 6). Fluorescence is characterized by the emission of a photon very rapidly, and this is due to the fact that electrons jump from one state to another, without intermediate coupling, in contrast to phosphorescence. The light can be emitted at different wavelength, thus presenting different fluorescence colors. This is dependant on the fluorochrom properties of absorption and emission.

Incident light reaching the sample and emitted light from the sample can be regulated via a filter cube, via the association of an excitation filter who lets pass defined wavelengths of the incident light, a dichromatic mirror, and a suppression filter who lets pass defined wavelengths of the sample’s emitted light.

1.2.5 Scanning Electron Microscopy

Principle In conventional optical microscopy, visible light reacts with the sample, and the reflected photons are scanned by sensors or by the human eye. In electron microscopy, the light beam is replaced by a primary electron beam that strikes the surface of the sample and reemitted photons are replaced by a spectrum of particles or radiation: secondary electrons,
backscattered electrons, Auger electrons or X radiation. These particles or radiation provide different types of information on the material from which the sample consists of, and thus gives a very good appreciation of the sample topography. One can appreciate the depth, the relief, which is not permitted by light microscopy.

**Method of use** The SEM has to be handled with precaution, because any wrong step can easily damage the machine. The SEM used in this project is a TM-1000 Tabletop microscope made by Hitachi and of maximal magnification 10’000, having a back scattered electron detector. The first step is to place the sample on the holder that will go into the machine. Once the sample is placed, it is necessary to adjust the sample height, using the height checker given with it. The sample must be under the checker bar, and approximately 1mm under. Once the machine is powered on, one has to wait for the red light next to the text *Air* to be still, and not blinking, to open the compartment and place the sample. The compartment closed again, press the button *Exchange* to engage the pumps and create vacuum in the chamber. Wait until the green light next to the text *Ready* is still, then the electron beam can be switched on using the given program on the computer.

Once observation is finished, switch off the electron beam and press again the *Exchange* button, wait until reaching the state when the red light is still and get off the sample.
2 Method development

In this section will be exposed and discussed the techniques and means implemented for the establishment of a procedure for producing good quality 3µm wood slices, and use it for various projects.

2.1 Sample preparation according to Fink (University Freibug) [1]

A method for very thin slicing already exists at the University of Freiburg, Germany [1], which gives good results despite it is a time consuming method. The first goal here is to be able to reproduce this current existing method for wood micro-slicing, and establish a reproducible written process. For this, a precise description of the different steps and material used will be performed in order to allow other people to learn the method and go on with further work or projects.

To study the different steps importance, the method will be parallely performed entirely and with missing steps. The preparation differences will be described in the paragraph 2.1.2. Materials. The final results will be studied to see if it is posible to modify the method in order to make it less time consuming and cheaper.

2.1.1 Embedding procedure

Sample’s pre-embedding preparation  Prepare a fixation solution of the following composition. For 1L solution :

- 500 mL buffer solution (solution A+B see below)
- 160 mL glutaraldehyde (25%)
- 340 mL distilled water

Solution A
0.2M Na₂HPO₄·2H₂O (di-Natrium Hydrogenphosphate hydrate) (177.99 g/mol)=35.6 g/L
With di-Natriumhydrogenphosphate 7-hydrate 26.8g in 500mL distilled water. 
or
With Natrium-phosphate dibasic waterfree 14.2g in 500mL distilled water.

Solution B
0.2M KH₂PO₄ (Potassium dihydrogen phosphate) (136.09 g/mol)=27.2 g/L=6.8 g/250mL=3.4g in 125mL distilled water.

Buffer solution
375mL solution A (=75%) + 125mL solution B (=25%) mixed, the final pH solution must be between 7.2-7.4, and can be corrected by adding solution A or B.

Put the sample into a pot filled with solution like in Fig. 7(a), and put under vacuum. Every 2 hours, release vacuum and start again for half a day, and then leave under vacuum for a
night (vacuum pump can be shut down). When the sample fall at the bottom of the pot, the sample is ready for the next step.

Put the sample in normal water 3x for 2h (change the water in between). Sample must always stay wet.

**Dehydration**  The following steps happen with the sample in rotation, in device in Fig. 7(b).

- 1/2 day in 50% isopropanol
- 1/2 day in 70% isopropanol
- 1/2 day in 90% isopropanol
- 1/2 day in 100% isopropanol
- 1/2 day in 1:1 isopropanol:embedding solution
- 1/2 day in only embedding solution
- 1/2 day in only embedding solution
- over 1 night in embedding solution + 0.4% catalysor A and 0.2% catalysor B

**Cuts support preparation**  This preparation can be done parallely to the pre-embedding procedure. First, prepare the glasses that will receive the slices. Melt 2.5g gelatin in 500mL water for 20 mins at 60°C, with stirring. When the mix has cooled down, add 7.5 mL potassium dichromat solution (3g potassium dichromat in 100mL water). Be extremely careful, since potassium dichromat is very toxic! Plunge the glasses 5 mins in the Gelatin+Potassium solution, and then put them for 40 mins in oven at 80°C. Throw the gelatin+potassium solution in a special container.
**Embedding**  The sample is embedded in gelatin capsules of corresponding size. Half-fill the capsule with embedding solution+catalysor, put the sample in the capsule, and finish filling the capsule with the solution. Put in oven at 60°C for 1 day.

**Slicing**  Once the samples are cured, remove the gelatin capsule and cut the top of the sample to make it flat, using a razor blade. Polish the cut surface with fine grain. Place the sample in the rotary microtome clamps like in Fig. 8(a), and with a D profiled blade (chisel shaped) made of steel, do a first cutting (9µm, until it is flat). Put the sample 1 day at 100% humidity (very important!), then cut with a C profiled blade (wedge-shaped) made of steel or a diamond knife (1 to 3 µm). In order to manage the cuts handling, it is necessary to add a water bath at the cutting reception.

To get the best results, play with the microtome features.

(a) Embedded wooden sample placed in the rotary microtome clamps  (b) Wood 3 microns slices drying on 60°C hot plate

Figure 8: Steps of the slicing process

Then, using a pinsel, place the cuts on the previous gelatined glasses, wetted, and dry on hot plate at 60°C like in Fig. 8(b). One generally places 6 to 7 cuts per glass.

**Colouring**  Plunge the glasses with the cuts on it in :

- 1/2 day - 100% isopropanol
- 2 mins - 80% isopropanol
- 2 mins - 60% isopropanol
- 2 mins - 30% isopropanol
- 2 mins - H₂O to dissolve the embedding material.
Then plunge the slices 12 hours in a solution of 2% Safranin-O (12g in 600mL H₂O) and 1% Acriflavin solution (6g in 600mL), which have been stirred and filtrated, and dry in hot air for 30 mins.

Finally, embed in resin as for the light microscopy procedure, as described in [2].

2.1.2 Materials

The following sets of samples were prepared, undergoing different types of preparation, listed from 1 to 6.

1) Embedding according to reference procedure 2.1.1

2) Sample not put into fixation solution. Put directly into normal water, as in Sample’s pre-embedding preparation end of paragraph.

3) Sample is not stirred during Dehydration

4) Sample doesn’t go for dehydration step in 90% isopropanol in Dehydration

5) Sample doesn’t go for the 2nd time step in only embedding solution in Dehydration

6) Combination of 2), 3), 4) and 5).

The first material set was composed as follow:

- Pinus spp. normal, preparation 1)
- Pinus spp. naturally degraded, preparation 1)
- Pinus spp. normal, preparation 2)
- Pinus spp. naturally degraded, preparation 2)
- Pinus spp. normal, preparation 3)
- Pinus spp. naturally degraded, preparation 3)
- Pinus spp. normal, preparation 4)
- Pinus spp. naturally degraded, preparation 4)
- Pinus spp. normal, preparation 5)
- Pinus spp. naturally degraded, preparation 5)
- Pinus spp. normal, preparation 6)
- Pinus spp. naturally degraded, preparation 6)
Maybe due to the size of the samples, some final unexpected problems appeared, like crystallization at the sample surface (maybe due to uncomplete impregnation), and the final results were not as expected. Thus, a second row of samples being smaller in size have been prepared, to make sure to have a good pores impregnation with the resin.

The second material set was:

- Picea abies - naturally degraded - preparation 1)
- Picea abies - naturally degraded and exposed to weathering - preparation 1)
- Picea abies - naturally degraded - preparation 2)
- Picea abies - naturally degraded and exposed to weathering - preparation 2)
- Picea abies - naturally degraded - preparation 3)
- Picea abies - naturally degraded and exposed to weathering - preparation 3)
- Picea abies - naturally degraded - preparation 4)
- Picea abies - naturally degraded and exposed to weathering - preparation 4)
- Picea abies - naturally degraded - preparation 5)
- Picea abies - naturally degraded and exposed to weathering - preparation 5)
- Picea abies - naturally degraded - preparation 6)
- Picea abies - naturally degraded and exposed to weathering - preparation 6)

2.1.3 Results, discussion and cutting tips

Slices from every prepared samples were made and studied under the light microscope, and since no noticeable difference could be seen, only pictures for showing the quality and details sharpness will be showed, and no pictures comparison between the samples will be made.

The method quality is clearly visible in Fig. 9, where details can be clearly seen. In the middle-left of the picture a pit with its perfectly cut and in place thorus can be seen, with great details. So far, it was never possible to observe a pit with its thorus still in place using the classical method. Another noticeable improvement, is the color differenciation between the different parts of the wood structure: here, the lignin rich middle lamellae appear bright red, in comparison with the fibres cell walls which are verging on pink, because of the lignin poorer concentration, thus rendering the limit between the 2 easily visible.

Also in Fig. 10 the cut quality is very clear, degradation is easily visible in the cell wall. The type of voids can be assessed, and thus the type of fungi which is responsible for the degradation. Using such pictures, research could be pushed on as far as via a program,
numerically determine the void percentage present in an image and quantitatively analyse the amount of degradation, allowing comparison between different wood species for example, or creating a curve tendency along with time exposure, and compare these curves for different coating types.

The comparison between this very thin wood slicing method of 3 microns and the classical process for producing 20 microns thick wood slices is quickly done, with just a glance at Fig. 11(a), Fig. 11(b) and Fig. 12(a), Fig. 12(b), details appreciation and sharpness are way much better in Fig. 11(a), Fig. 12(a) displaying the very thin wood slicing method.

Somehow, even if final results of the 2nd material set were all good, some little things were noticed during preparation. Those very small details while performing the cutting and preparing the samples for cutting proved to be very important and each step should be carefully performed. Feelings of the performer while cutting should also be noted down, for later reference, because one time impressions might help for a next performance. The following problems and conclusions were pointed out.

**Stirring** Concerning stirring, it was realised that it is a step that should be kept for being sure to have homogenised solution. Indeed, if embedding solution with catalysors mix is kept for too long, it was seen that after curing, the solution at the top in the embedding capsule was not hardening and staying more or less liquid, chewy. Since it was not clear whereas this was due to just the solution age or the solution age coupled with uncontinuous stirring of the solution, it is decided among all the possible removable steps, to keep stirring the samples, since it also doesn’t influence on the process time. Stirring the samples also helps reducing dehydration time, since this way, the exchange between the wood sample and the solution is accelerated due to the active exchange between the sample and the solution.

**Swelling and resin cristallization at the sample surface** This swelling and resin crystallization can be observed in Fig. 13(a) and Fig. 13(b). This might affect the cut stability, due to voids in the resin. Indeed, one must be careful when proceeding for the cutting. It is necessary to check at the sample surface if there are some resin voids, which can be due to this sample swelling and resin cristallization, or else, because these voids might not influence the cutting itself, but will influence the cut stabilization on the gelatined glass. The cuts will more easily detach when plunged into the isopropanol-water bath for the resin dissolving steps. If the samples don’t detach, these separations will render anyway observation more difficult.

**Cutting** Cutting speed proved to be important for the microstructure quality. There is no precise defined speed or protocole to be respected, and the cutting speed must be evaluated by the performer via the microscope installed on top of the microtome, and different tests must be done to reaffine the method. Each sample is unique and needs its own method adaptation. For easier cut handling, the following Cutting tip should be followed : between each cut, dry
Figure 9: Cut of *Pinus spp.* of 3µm thickness, using diamond blade. Quality is very good.

Figure 10: Cut of *Pinus spp.* degraded by fungi of 3µm thickness, using diamond blade.
Figure 11: Comparison of picture quality between 3 and 20µm at few degraded spots

Figure 12: Comparison of picture quality between 3 and 20µm at very degraded spots

Figure 13: Resin crystallization: see the whiter spots at the sample surface
the sample using your thumb (or any other finger). Because your skin is slightly greasy, it will render the sample’s surface more hydrophobic and your cut will swim on the water bath surface, thus being much easier to catch.

**Glass plate sticking**  After some failure concerning glass plate slices sticking, that is to say when slices happened to detach from the plate after cutting and placing them on the gelatin support glass, it was realized that it is necessary to previously wet the support glass covered with the gelatine, in order to dissolve this gelatine that will impregnate the upcoming slices and then dry again with the slices, thus obliging them to stick on the plate.

**Coloration**  Coloration solutions shouldn’t be kept for more than 4 days, otherwise, due to proliferation of bacteria, the slices plunged into the solution won’t color as good as normally and will get additional “impurities” or elements that hinder microscopic observation. The second material set was not as coloured as wanted (pale colour) and the slices were slightly destroyed due to the embedding, but it was still possible to observe them and assess their quality and differences between each others. Depending on the wood specie, coloration time can vary, and leaving the sample 1/2 day in the solution should be enough for every type of sample. However, lesser time for coloration, up to only 2h, is sometimes enough, and the decision should be left to the appreciation of the performer.

**Impurities presence**  Another problem which was encountered, was the presence of impurities. Indeed, because the glass is covered with gelatine, not only the samples stick better, but also everything coming around, and the elements present in the water on the glass plate. These impurities can be seen in Fig. 9, somehow, they don’t bother observation too much. So far, the best to avoid these impurities, is to place the freshly new cut slices in a new water bath (i.e. not the one at the slice’s reception) for a minute before placing them on the glass plate.

**Influencing parameters**  Among all that have been said, one must also consider some less evident parameters, that might lead to failure, eventhough the process and other tips were respected. Some more parameters which are not mentionned here because not yet observed might also influence the result.

- Mood of the performer
- Weather and room conditions
- Type of sample
- Size of sample

Keep in mind this method is a lot based on the performer’s feelings.
2.2 Resin investigation

2.2.1 SPURR resin

**Materials**  SPURR resin is produced using 4 components: ERL 4221 (a cycloaliphatic epoxide resin), D.E.R. 736 (Diglycidyl ether of polypropylene glycol), NSA (Nonenyl Succinic Anhydride) and DMAE (Dimethylaminoethanol).

The following wood species have been embedded in the SPURR resin, with the mention *dehydrated* meaning the sample went into 1:1 then 3:1 ethanol:water mix and then in pure ethanol for 1/2 day each. *coloured* means the sample was plunged into a bath of ethanol plus safranin.

- Oak sapwood dehydrated
- Oak sapwood non-dehydrated
- Oak heartwood dehydrated
- Oak heartwood non-dehydrated
- Robinie dehydrated
- Spruce dehydrated
- Chestnut dehydrated
- Pine degraded dehydrated
- Larch dehydrated
- Chestnut dehydrated coloured
- Larch dehydrated coloured

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<tr>
<td><strong>Components</strong></td>
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<td>ERL 4221</td>
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<td>D.E.R. 736</td>
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<tr>
<td>NSA</td>
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<tr>
<td>DMAE</td>
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<tr>
<td>Cure schedule at 70°C</td>
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<td>Pot life</td>
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</tbody>
</table>
Protocol  Mix and stir ERL, D.E.R. and NSA after weighting the quantity specified in Table 1, according to the desired medium consistency. At the last moment, add the DMAE and stir again. Plunge the samples into the resin, then install the vacuum device in order to impregnate them. Leave approximately 30 mins under vacuum for the impregnation. Place the impregnated samples in the mould provided for this purpose, and cure at 70°C in oven for 8 hours. For a more detail technical sheet, check [10]

Figure 14: *Quercus spp.* embedded in SPURR resin. The resin marks can be seen inside the vessels

Results  Cuts of 6 microns thickness were managed, via the use of metal blades, which was considered an interesting start. Dehydration process didn’t seem to influence the cutting itself, nor did it influence the sample final quality. The firm quality of the resin was the first one tried since it is the standard/medium one, and it seemed to be too hard for cutting. Indeed, the resin was not cut smoothly but rather by small steps as can be seen in Fig. 14. On the contrary, the soft quality resin was also tried and appeared to be too soft for cutting, especially with the removable blades, producing slices with different thicknesses like in Fig. 15.

The major problem concerning the SPURR resin was how to dissolve this resin after slicing and before glass embedding for microscopic observations. It is mentionned in [11] that *The resins Epon, Araldite, and Spurr are soluble in acetone but not in ethanol*. So dissolving in acetone was performed, for different timesteps up to 1 day and a half, but the final result was not satisfying. Somehow, some of the resin was still present in the vessels or fibres, and SPURR resin impregnation was then not selected for further studies.
2.2.2 Cactus juice resin

Cactus juice resin was an attractive resin alternative, due to its handling ease and preparation speed. Steps like wood boiling can be additionally performed before resin embedding, to make the wood softer for cutting. Cuts of 6 microns were managed, but, cactus resin has a granular aspect like most of the resins, which makes the observation difficult. There was then a need to find a way to dissolve the resin afterwards. Unfortunately, once cured, cactus juice cannot a priori be dissolved. Ethanol and acetone were tried for the dissolution but were unsuccessful. So far, nothing was found to dissolve the cactus juice resin. In the case something was found, this resin would be a good alternative. Finally, the combination of the following problems was a deciding factor for not using this resin in the developed method.

- **Problems**  
  Not enough hydrophobic. When cutting, the wood slice falls at bottom of the water bath, rendering it very difficult to handle and in addition to that, the slice curls (because of this non-hydrophobicity). Not dissolvable after curing, rendering observation difficult and not interesting.

2.2.3 Polyethylene Glycol (PEG) resin

PEG solution, or resin, is a polymer mix in which chains length can be tailored for obtaining the desired properties. Unlike most resin, it has a waxy texture and it is very soft. It is already much used in wood slicing [12]. Since the steps for impregnation into PEG solution...
are quite short (approximately 1 day), the wood piece after embedding is still very hard and stiff, the wood fibres have not been softened. This wouldn’t be a big problem if after drying the PEG matrix was also hard and stiff (shouldn’t be too stiff though for the cutting), but it is not the case, PEG has a waxy texture that is easily cuttable and the difference of consistency and stiffness between the matrix and the wood itself make the cutting close to impossible. This texture and consistency difference doesn’t allow very thin slicing, it will yield hard and unhomogeneous cutting conditions, the step between the two phases destroy the eventual slice’s stability.

2.3 Knives investigation

2.3.1 Disposable metallic blades

Different disposable metallic blades have been tried, and they all presented the same main problem. Due to the fact the wood is not previously boiled, (or when it is, it then has to be dried which thus annihiates the benefits of the boiling) it is too hard for the blades concerning their stability. The blades are also easily damageable. Thus, for very thin sectionning, it is a big problem: for 3 microns cutting, the slice thickness will vary between 0 to 15 microns, from one slice to another, or even amongst a same slice (see Fig. 15). For less thin slicing, around 10 microns, the following results and comments could be made, with differentiation of the blades:

SEC 35 - R35 - N35 The 35 blade family showed similar results. 10 microns cuts were easily managed, but the results under the microscope were not very good: the cut marks could be seen in the resin as if printed in it (see Fig. 14) and it looked like some bubbles were present in the resin.

Dur A Edge With the Dur A Edge blade, cuts of 10 microns were difficultly managed, 1 on 2 was breaking/folding during the slicing or was non existant, the one following being thus much thicker than 10 microns.

Globally, due to their instability and easily damageable profile, removable blades were considered as non-satisfying for very thin slicing via resin embedding, eventhough the 35 family blades allows good quality cuts for thickness superior or equal to 10 microns.

2.3.2 Metal knives

Metal knives presented a very good stability to resin embedded wood slicing, due to their profile. Eventhough their stability is good, that the produced cuts showed a constant thickness and were remaining as whole for being placed on the glass plate, the quality of the cuts under the microscope was bad, at least for cuts with a thickness < 6 microns. The final cut quality might be due to the performer technique, so theoretically it would be posible to produce good quality cuts of 3 microns using these knives. Somehow, in our case, these knives were considered good for a precutting for diamond knife slicing, in order not to wear the diamond knife too quickly.
2.3.3 Diamond knife

Diamond knife cutting stability is unrivaled. It is by far the best knives for very thin slicing, and with a great precision, but has a cost. Thus the diamond knife should be used only for the final slicing, in order not to see wear appearing too fast. These knives can effectively present signs of wear after repeated use. As previously mentioned, a precutting using a metal knife of profile \( D \), then followed by the final cut with diamond knife is the best solution so far.

2.4 Final preparation method for wood micro-slices

Depending on the samples dimensions, times can be reduced by half. The limit is set at the following size 3x3x5. If samples are bigger, the following procedure should be applied as such, otherwise if the samples are smaller, times for dehydration can be reduced by half.

Put the sample in normal water or directly in 100% isopropanol (start directly from step 3) in the list below) if the sample is already very dry, for 1/2 day under vacuum. The first 6h release vacuum every 2h and then leave it under vacuum for night.

Figure 16: Stirring device

The following steps happen with the sample in rotation, in device in Fig. 2.4. Sample must always stay wet.

Dehydration

- 1) 1/2 day in 50% isopropanol
- 2) 1/2 day in 70% isopropanol
- 3) 1/2 day in 100% isopropanol
- 4) 1/2 day in 1:1 isopropanol:embedding solution
- 5) 1/2 day in only embedding solution
- over 1 night in embedding solution + 0.4% catalyser A and 0.2% catalyser B
Cuts support preparation This preparation can be done parallelly to the pre-embedding procedure. First, prepare the glasses that will receive the slices. Melt 2.5g gelatin in 500mL water for 20 mins at 60°C, with stirring. When the mix has cooled down, add 7.5 mL potassium dichromat solution (3g potassium dichromat in 100mL water). Be extremely careful, since potassium dichromat is very toxic! Plunge the glasses 5 mins in the Gelatin+Potassium solution, and then put them for 40 mins in oven at 80°C. Throw the gelatin+potassium solution in a special container.

Alternatively, the same preparation without adding potassium dichromat can be made. Here, the potassium dichromat is supposed to enhance the wood coloration, but no real difference could be observed between with and without. Even if no difference was observed, it cannot be guaranteed that there is effectively no difference.

Embedding The sample is embedded in gelatin capsules of corresponding size. Half-fill the capsule with embedding solution+catalysor, put the sample in the capsule, and finish filling the capsule with the solution. Put in oven at 60°C for 1 day.

Slicing Once the samples are cured, remove the gelatin capsule and cut the top of the sample to make it flat, using a razor blade. Polish the cut surface with fine grain. Place the sample in the rotary microtome clamps like in Fig. 8(a), and with a D profiled blade (chisel shaped) made of steel, do a first cutting (9µm, until it is flat). Put the sample 1 day at 100% humidity (very important!), then cut with a C profiled blade (wedge shaped) made of steel or a diamond knife (1 to 3 µm). In order to manage the cuts handling, it is necessary to add a water bath at the cutting reception.

Then, using a pinsel, place the cuts on the previous gelatined glasses, wetted, and dry on hot plate at 60°C like in Fig. 8(b). One generally places 6 to 7 cuts per glass.

Colouring Plunge the glasses with the cuts on it in:

- 1/2 day - 100% isopropanol
- 2 mins - 70% isopropanol
- 2 mins - 50% isopropanol
- 2 mins - 30% isopropanol
- 2 mins - H₂O to dissolve the embedding material.

Then plunge the slices 6 hours in a solution of 2% Safranin-O (12g in 600mL H₂O) and 1% Acriflavin solution (6g in 600mL), which have been stirred and filtrated, and dry in oven at 60°C, for approximately 15 min.

Finally, embed according to the following. Since the slices have been dried, the steps of dehydration from the classical embedding procedure can be jumped, and the glass plate can be directly placed into xylene solution, for 5 min, and then ready to be embedded according to [2]
3 Method validation: application extended to various projects

3.1 Fir and linden species investigation concerning gelatin impregnation and interaction with wood cell walls, for art and historical objects conservation

The goal in this project is to be able to describe some gelatine distribution inside the wood, and also manage to see if the gelatine penetrates the wood cell walls or not, and if yes, assess gelatine penetration depth into the cell walls.

3.1.1 Materials

The mix of the fluorochrome and the gelatine was done by the HKB in Bern, using the following components:

- **Gelatine** Typa A, bloom 180
- **Fluorochrome** Fluorescein-Isothiocyanat (FITC)

The fluorochrome and gelatine foils obtained after mixing those two components, were dissolved in water according to the following proportions: 20%, 15% and 5%.

Two wood species were selected for impregnation, *Tilia spp.* as a hardwood and *Abies spp.* as a softwood. Each sample specie was impregnated according to the 3 different gelatine mix of 20%, 15% and 5% and the samples sizes were as described in *Final AHB wood embedding method*, which means inferior or equal to 5x5x10. A list of the produced samples is given below, for more clarity:

- Tilia 20%
- Abies 20%
- Tilia 15%
- Abies 15%
- Tilia 5%
- Abies 5%

The fluorescence behaviour observation was done under UV light exposure, using a filter system A having excitation wavelength BP340-380, dichromatic mirror RKP400, and suppression wavelength LP425.

3.1.2 Results and discussion

The different samples enumerated above in the *Materials* section have been studied here, with their different gelatine concentration impregnation, in order to find the most suitable consistency and the right fluorescence brightness for observation. For this, and for studying
the gelatine distribution and penetration in the wood, 3 different slices thickness have been produced, 20, 8 and 3 microns.

Samples have also been prepared with and without coloration, plunging the samples in a bath of safranin+ethanol for 6 hours. Since coloration is not ameliorating the results quality, it was decided to continue without coloration, in order not to unnecessarily loose time.

20µm samples 20µm cuts came out perfectly, for 5 or 15% fluorochrome content, and it is already possible to see very well the gelatine distribution in Abies spp. Fig. 17 and Fig. 18 as well as in Tilia spp. Fig. 20 and Fig. 21, even at different gelatine concentration. However, because of the quite high thickness, it’s not possible to have sharp interfaces between wood cell walls and the glue, and thus know if the glue has penetrated the wood cell walls, at least at high magnification, in Fig. 19. Reaching a magnification of 1000x, image details are too blurry, see Fig. 19, but quality is anyway still very good. Up until 400x, amazingly good pictures can be made.

Comparing 5 and 15% fluorochromed gelatine samples, it comes out that impregnation is much more visible in 15%. At 5%, there is interference between the gelatine fluorescence and the fluorescence of the wood lignin like in Fig. 21.

Figure 17: Abies spp. 20 microns thick slice impregnated with fluorescent gelatine. Distribution of the gelatine is clearly visible
Figure 18: *Abies* spp. 20 microns thick slice impregnated with fluorescent gelatine. Magnification 400x

Figure 19: *Abies* spp. 20 microns thick slice impregnated with fluorescent gelatine. At magnification 1000x, details are blurry
Figure 20: *Tilia* spp. 20 microns thick slice impregnated with fluorescent gelatine. Few gelatine is present, but it is still visible.

Figure 21: *Tilia* spp. 20 microns thick slice impregnated with fluorescent gelatine. Due to the low amount of gelatine, fluorescence is less strong and thus wood lignin fluorescence is visible.
8-16µm samples Using the sliding microtome, cuts as thin as 8µm were managed, sometimes being slightly damaged, but with good quality in some places. Due to the blade instability (removable blades), the thickness cannot be assured to be 8 microns, and might reach up to 16 microns in some cases. With this thickness, better sharpness couldn’t really be achieved (see Fig. 23), and at that thickness, using the sliding microtome, the wood structure already appears to have been a little bit destroyed, there are some cell wall debonding or even breaking as seen in Fig. 22. The pictures which were made are somehow of global good quality, and can be used for observation.

Figure 22: *Abies spp.* 8 microns thick slice impregnated with fluorescent gelatine. Cell walls are delaminating with the gelatine
Figure 23: *Abies spp.* 8 microns thick slice impregnated with fluorescent gelatine. Pits are filled with the gelatine. At magnification 1000x image is blurry
Using the diamond knife mounted on the rotary microtome, it was possible to produce cuts of 3µm thickness. The amount of embedding material is not enough for allowing cut stability and so the handling was difficult. Since the gelatin is soluble in water, the procedure was done using ethanol bath instead of water, or even no bath at all and using just half a drop of water for putting the slice on the glass plate, instantaneously dried at 60°C for not dissolving the gelatine. Since wood was not fully impregnated with the gelatine, the cuts didn’t stay at the bath surface but rather drawn.

Finally, with the combination of this second embedding of the wood piece, cuts of 3µm were produced for Tilia specie. For Abies specie, cuts were always destroyed, and were not worth observing. Still, Tilia cuts quality is not so good, vessels are often deformed or even broken Fig. 24. Where it is not too destroyed, pictures at high magnification 100x could be made, with an improved sharpness compared to the 2 previous thickness as seen in Fig. 25.

After producing the cuts and if going deeper into details, the followings remarks/problems were stated. For very thin slicing, i.e. 3 microns, higher gelatine concentration appeared to be better, because of the combination of 2 factors. First one is that, due to the sample impregnation with gelatine, embedding in a second medium is not possible, or at least there won’t be penetration into the wood cell, because the pits and cells lumens are already partially filled with the gelatine. The second factor is that, since the stabilizing resin cannot penetrate the wood, the gelatine that filled the wood needs to fulfill the criteria of the stabilizing resin for allowing a good cut stability, and managing to produce a slice. With a low gelatin concentration, i.e. inferior to 15%, and after drying, not enough material was left in the wood structure, thus rendering impossible 3 microns cutting. Indeed, if the pores are not filled and the resin consistency is not appropriate when cutting, the slice curls up, and is unusable. At high gelatin concentration, i.e 15 to 20% (20% is the maximum gelatin solubility in water here), the problem was somehow reduced, due to the higher amount of gelatine present in the wood, increasing stability for cutting, and few cuts of 3 microns were produced for Tilia spp., as previously stated.

**Influence of gelatine concentration on microscopic observation** 15% or 20% fluorochrome doesn’t change much the observation, but to the contrary, 5% fluorochrome makes the contrast between the wood and the gelatine more difficult to see. Indeed, wood also shows a fluorescence phenomena, which is diminished when the amount of fluorochrome is increased. On the other hand, with a high amount of gelatine and so a high amount of fluorochrome, fluorescence is so strong, that details are also not sharp anymore. It is in some cases not possible to tell if what is seen is the gelatine itself or a halo due to the strong fluorescence effect. So a compromise between too high and too low fluorescence has to be find, and for this, research must be pushed further.
Figure 24: *Tilia spp.* 3 microns thick slice impregnated with fluorescent gelatine. Wood structure is damaged.

Figure 25: *Tilia spp.* 3 microns thick slice impregnated with fluorescent gelatine. Picture sharpness is good.
3.2 6000 years old *Quercus Pubescens* wood microscopic analysis, from wooden piles found in Biel’s lake.

Prehistoric piles of oak specie have been discovered in Biel’s lake, on the heritage site of Twann commune, and were dated through the use of dendrochronology, as old as 5000 to 6000 years old [13]. Having such old wood available in our region, was a good opportunity to have a closer look at the microscopic features, see the degradation evolution along the centuries, and study wood conservation in water. Three parts were distinguished in this wood, the sapwood, light brown and very weak, and the heartwood, dark close to black, which could be distinguished furthermore into two different parts. The external one is rather soft, easily crushable and looks degraded. The inner part, the core, is very hard and close to perfectly conservated. Those two parts will subsequently be named soft heartwood and hard heartwood respectively.

![Figure 26: Bielersee wood piles parts description](image)

An important shrinkage is observed between the wet and dried phases of bielersee wooden piles. Due to the structure difference of degradation, water absorption in the wood structure varies much, and thus the shrinkage percentage from one part of the wood to the other. It is illustrated in Fig. 27: the sapwood shrinks the most, followed by soft and then hard heartwood.

![Figure 27: Bielersee wood piles shrinkage](image)
3.2.1 Observations

Sapwood

**Transverse** The structure is here very much destroyed and nearly not recognisable. The vessels don’t have their original round shape anymore, they have a lense shape, due to the wood compression. The whole structure itself is being deformed in an ondulated way as can be seen in Fig. 28. When having a look at high magnification (above 400x), one can differentiate the different parts of the structure, that is to say the compressed fibres structure Fig. 29 and the parenchmys cells aerated structure Fig. 30. What is left of the structure seems to be the lignin rich middle lamellae, which collapsed or even merged together after the cellulose matrix of the cell walls was eaten by bacteria. This assumption would concord with the research of [14], [15], [16] in which wood degradation happens in anoxic environments (which would be the case for the wood parts buried in the ground), where bacteria are the main wood degraders. Three types of bacteria are distinguished, namely erosion, tunneling and cavity bacteria, depending on their decay pattern. According to [17], these bacteria degrade the cell wall by producing troughs within it and therefore the middle lamella usually remains intact. It would also concord with our observation that under UV light using a filter system A having excitation wavelength BP340-380, dichromatic mirror RKP400, and suppression wavelength LP425, these triangular shaped parts of the structure present a fluorescent behaviour, which would indicate high and even the highest concentration of lignin of the microstructure. Also, different colorations can be observed. Looking at Fig. 30, one can distinguish what seems to be the middle lamellae structure as discussed above, bright red, and more to the inside of the cells what remains of the initial cell walls cellulose matrix, darker red.

**Radial** The radial cut confirms what can be said from the transverse cut. The parenchmys cells constituting the rays are deformed Fig. 31, and thin walled as can be seen in Fig. 32.

**Tangential** Here also, one can see in Fig. 33 the melted fiber structure, with the one cell wide rays crossing the structure. The parenchmys cell walls in one cell wide rays are mostly destroyed in Fig. 33, but are not completely crushed that is to say, the cell space is conservated. In larger rays, Fig. 34, parenchym cells look even better conservated, despite some cell walls breakdown.
Figure 28: *Quercus Pubescens* sapwood transverse section global structure. The vessels have a lens shape.

Figure 29: *Quercus Pubescens* sapwood transverse section melted fibres structure.
Figure 30: *Quercus Pubescens* sapwood transverse section parenchym cells structure. The bright red parts are the lignin rich lamellae.

Figure 31: *Quercus Pubescens* sapwood radial section global structure. Parenchym cells from the rays are visible in the middle.
Figure 32: *Quercus Pubescens* sapwood radial section parenchym cells structure. Ray parenchym cells

Figure 33: *Quercus Pubescens* sapwood tangential section melted fibres structure. The uniseriate ray parenchym cell-walls are mostly destroyed
Figure 34: *Quercus Pubescens* sapwood tangential section parenchym cells structure. Some cell-walls are broken, despite a rather good conservation
Soft heartwood

**Transverse** Like for sapwood, the global structure has changed a lot, but the parenchymas cells are somehow still distinguishable even though they are much deformed. Indeed, 2 types of deformation can be distinguished: the slightly deformed parenchymas cells in Fig. 35 and the "melted" fibers structure which can be seen on the left and right side in Fig. 36 (with the ray parenchymas cells in the middle), and which is barely not recognizable anymore. The darker spots in this melted structure signal the closed cells lumens, and are dark due to the "eaten" or degraded cell walls. Like in the sapwood, most of the structure is composed of the lignin rich middle lamella, after the cellulose was degraded.

**Radial** Extractives are easily distinguishable, they have a smooth texture and a characteristic light brown color. Somehow, due to the extractives presence, it is sometimes not easy to distinguish the eventual other elements or organisms. Here, some fungi seem to be present, indeed the round elements that are in the parenchym cells look like fungi hyphae in Fig. 37, or biofilms. The parenchym cells from Fig. 38 also have one of this round hyphae sporophore or biofilm microorganisms, and what might be aerial hyphae along the cell walls (cells seem to have double layered walls with connections between them).

Also, concerning the wood change of color in oak heartwood, soft or hard, from light brown to darker brown up to black, it is due according to Krutul [18], to extractives (particularly tannins) which react with the iron present either in the soil or the water, as reported by Sandak [17].

**Tangential** In Fig. 39 the vessels have their straight shape, the one cell wide parenchym rays are deformed, in which most of the parenchym cells are filled with some dark material (it is better seen in Fig. 40), which as said in radial direction paragraph could either be fungi hyphae, extractives, biofilms or bacteria slime.
Figure 35: *Quercus Pubescens* soft heartwood transverse section parenchym cells. The thin walled parenchym cells are quite deformed.

Figure 36: *Quercus Pubescens* soft heartwood transverse section fibres and ray. The fibres lumen have completely disappeared.
Figure 37: *Quercus Pubescens* soft heartwood radial section parenchym cells ray. The ray parenchym cells are filled with extractives and maybe bacteria slime.

Figure 38: *Quercus Pubescens* soft heartwood radial section parenchym cells. Separations between the cell-walls are visible.
Figure 39: *Quercus Pubescens* soft heartwood tangential section global structure. Vessels are not crushed and rather straight.

Figure 40: *Quercus Pubescens* soft heartwood tangential section parenchym cells. Parenchym cells are filled with extractives and maybe fungi hyphae or bacteria slime.
Hard heartwood

**Transverse**  Concerning the structure, it is globally very well preserved as seen in Fig. 44. Cells still have their round shape (no real deformation, see Fig. 43) but at high magnification (above 400x) one can see the cell walls are very thin (degradation can also be seen by the cell walls color change). What is then left of the cell walls is often cracked as in Fig. 45. Sometimes it’s even some entire cell walls parts missing Fig. 42 (cell wall + middle lamella). Where the cell wall is degraded or "eaten", color is much darker, and sometimes, brown material is present inside the cell for example in Fig. 46, which are extractives and also maybe fungi hyphae, or bacteria’s slime in comparison with degradation mentionned and illustrated in [19]. Presence of the extractives in heartwood can explain also why sapwood is more degraded than heartwood. The role of extractives is generally to protect wood from biodegradation [17] and sapwood have lesser amount of these substances than heartwood, thus being much more sensitive to degradation [15].

Another pattern that appears a lot is delamination of the cell walls from the middle lamellae. This delamination is visible due to the cutting, but happens only if the cells are weak enough, so when they have been degraded. Here, delamination might even be present before the cutting, since it appears on different sides of the cell and not necessarily in the cutting direction.

In the end, the following specific degradations can be observed: erosion of the parenchyma cells, as can be seen in Fig. 42, delamination of the fibers cell walls and of the parenchyma cell walls, as in Fig. 41, total break of the parenchyma cell walls, as in Fig. 42.

The parenchyma cells erosion can be due to bacteria or fungi. To identify these, the degradation pattern is important. But the degradation pattern here could be assumed to be due to erosion bacteria, like the ones found in the Baltic sea in [19]. Since the environment of both is very different it is not possible to conclude that it is due to erosion bacteria. These degradation patterns can be apprehended via the use of electron microscopy, which help to complete what can be seen via optical microscopy. This erosion of the vessel or cell walls can be particularly seen in Fig. 42.

**Radial**  Some interesting features appeared in radial cut, if one look closely at Fig. 47, in the fibers lumen, a wavy element going the whole length of the fiber appears. It will be more described in tangential section, since it was more easily seen in there. In Fig. 48, erosion of the parenchyma cell wall can be well seen: the inside of the walls is not smooth and presents some cavities which are not pits. The brown material also present into the cells are extractives, which might also be mixed with residue of the degraded cell walls or bacteria slime, since it has a granulated texture.

**Tangential**  In tangential section, in Fig. 49, one can see the presence of an ondulated "animal" present in many fibers lumen, which doesn’t look like any fungi specie or bacteria, which might have grown within the tree before it was cut, or otherwise came from the soil or water from Biel’s lake. In Fig. 50, a specimen can be more closely seen. It seems to have a
head with 3 anchor points to feed onto the cell walls. As well, in Fig. 51, part of a much bigger specimen, which somehow looks a bit different from the one of Fig. 50, can be seen. On each ondulation that goes into the cell wall, one can see 2 small antennas which must be anchor points to feed.

Figure 41: *Quercus Pubescens* hard heartwood transverse section presenting a lot of delaminated parenchym cells
Figure 42: *Quercus Pubescens* hard heartwood transverse section presenting destroyed and eroded parenchym cell walls

Figure 43: *Quercus Pubescens* hard heartwood transverse section fibres being nicely preserved
Figure 44: *Quercus Pubescens* hard heartwood transverse section global structure. At this magnification 100x, deformation can nearly not be seen.

Figure 45: *Quercus Pubescens* hard heartwood transverse section presenting cracked cell walls.
Figure 46: *Quercus Pubescens* hard heartwood transverse section. Parenchym cells are partially filled with extractive or degraded

Figure 47: *Quercus Pubescens* hard heartwood radial section global structure. The fibres lumens are filled with some wavy element
Figure 48: *Quercus Pubescens* hard heartwood radial section presenting a ray parenchym cells, which are eroded and filled with extractives and maybe fungi hyphae or bacteria slime.

Figure 49: *Quercus Pubescens* hard heartwood tangential section global structure. The parenchym cells are very well preserved, as well as the fibres.
Figure 50: *Quercus Pubescens* hard heartwood tangential section, presenting some "animal" in a fibre

Figure 51: *Quercus Pubescens* hard heartwood tangential section, presenting parts of some "animals" in some fibres. It seems to be anchored in the fibre cell-wall
3.2.2 Conclusion

This *Quercus Pubescens* wood specie structure shows a very good state of preservation, and some very interesting degradation patterns. Over 6000 years, the wood core is nearly undegraded. Due to this, it is possible to see some special "animal" specie that might be present in the tree all these years ago, or have come while the wood was evolving in the soil, under water. Some biological researchs concerning Biel’s lake soil composition, water composition, and the nature of this animal should be conducted through in order to say more about that topic. Next interesting step would be to compare the wood structure from piles that were under anaerobic conditions, in soil, and from piles under aerobic conditions, in water. It also allows to study wood degradation comparison between freshwater and saltwater, based on researchs that were already made about shipwrecks evolution in baltic see for example [19]. Some more precise studies can and should then be conducted.
### 3.3 Weathering and fungi degradation observation in *Picea Abies*

Wood weathering and degradation by fungi is of great concern in the field of construction, especially concerning aesthetics. There is still a lot to research about the mechanisms and depth of degradation, the coupling of weathering with degradation, and how to hinder it. Special coatings are being produced and applied for UV or fungi protection, and help much for wood protection, but do not completely prevent the degradation and thus need some ameliorations. Very thin wood slicing for microscopic observation can offer a visual determination of degradation patterns and depth, and is a promising research method in this field.

#### 3.3.1 Materials

Three different samples of *Picea abies* are being studied, based on their different degradation exposition, for a given time, using two different preparation methods:

- No degradation
- Wood exposed to fungi for 28 months
- Wood exposed to UV light for 12 months
- Wood exposed to both fungi and UV light for 28 months

It is here not a problem to compare the degradation patterns even for different time exposure for the following reason. Concerning UV degradation, i.e. degradation of the lignin, the degradation pattern reached after 12 months of exposure is the same as after 28 months. Indeed, once delignified, the cellulose matrix begins to detach, and lignin degradation can go further, as if newly exposed. Exposure was performed under UV lamps for shorter time than specified on the samples, but in a way to reproduce the given outdoor exposure time.

#### 3.3.2 Results and discussion

**Fungal degradation**  At rather low magnification, wood seems to be nearly undegraded Fig. 52. When looking more closely, one can clearly see the fungal degradation pattern. At 1000x magnification, Fig. 53, the wood cell-walls present a color differentiation, due to the ratio cellulose/lignin in this part. The fungi eat the cellulose matrix, living a highly lignin concentrated structure, which in the picture Fig. 53 appears to be dark red, instead of light red/pink. The degradation really goes along the inside edge of the cell, and doesn’t dig holes in the structure.
Figure 52: *Picea abies* surface degraded by fungi. The structure looks like it is preserved

Figure 53: *Picea abies* surface degraded by fungi. The degradation is visible along the inside edge of the cell, in dark red
**UV degradation**  The limit between the UV degraded wood surface and the deeper undegraded wood part appears very clearly, and calculations of degradation depth can be measured with a certain ease. Two types of degradation allow to identify the degradation depth: first, the color change from red to light-brownish-pink, which is due to the lignin degradation by the UV, and second, the delamination of the cell wall as highlighted in Fig. 55, also due to the weakening of the lignin rich middle lamellae. This color change and delamination are visible due to the thinness of the slice. Delamination can be better seen in Fig. 56, space in between the cell walls appears clearly.

In Fig. 54, a crack is present at the wood surface, and one can see the degradation follows the crack, thus meaning the crack was present before exposure or happened while exposed.

Eventhough the aim here was to study the UV degradation, it seems that after a while fungi developed and attacked the wood. Some hyphae or spores can be seen in every picture, they are these big brown dots. This would explain why the wood cell-walls are so thin. A systematic study of the evolution of the degradation pattern needs to be performed in order to have an idea about the degradation evolution and would be an interesting future project.

![Image of wood degradation with crack and delamination](image_url)

**Figure 54:** *Picea abies* surface degraded by UV. Degradation penetration goes deeper where a crack is present.
Figure 55: *Picea abies* surface degraded by UV. Delimitation of the degradation depth is clearly visible, based on color change and cell-wall delamination.

Figure 56: *Picea abies* surface degraded by UV. The cells partially keep their shape, but delamination is everywhere.
Fungal and UV degradation  Prolonged exposition of wood to UV and fungi proves to weaken it very much. Indeed, the wood cell delamination combined with cell wall thinning induces an easy collapse of the cells as in Fig. 57, and make their detachment very easy, this is why the observed degraded layer thickness was much thinner (3-4 cells thick, Fig. 58) than what it was for just one degradation type (6-7 cells thick, for example in Fig. 55).

Figure 57: *Picea abies* surface degraded by UV and fungi. The degraded cells layer is completely crushed.

It is finally clear that this 3 microns slicing method opens doors concerning the study of degradation evolution, that will be further developed in upcoming projects.
Figure 58: *Picea abies* surface degraded by UV and fungi. Most of the cells at the surface have detached, the degradation being thus smaller, with thinned and delaminated cell-walls.
3.4 Anatomical wood features and glue distribution observation at glue joints in wood industry

Polyurethane glue is one of the glue the most used in industry, and understanding the link between its distribution at two wood pieces interface, or also inside the wood fibres, and its mechanical properties is currently a great challenge.

3.4.1 Materials and preparation

Two different wood species have been prepared for this observation. A little piece of the wood taken from the glue joint of two wood glued parts of _Fagus sylvatica_ and _Picea abies_ species was prepared according to the method described in this report under Final AHB wood embedding method.

Like for the section _Fir and linden species investigation concerning gelatin impregnation and interaction with wood cell walls, for art and historical objects conservation_ with the gelatine impregnation, the fluorescence behaviour observation was done under UV light exposure, using a filter system A having excitation wavelength BP340-380, dichromatic mirror RKP400, and suppression wavelength LP425.

3.4.2 Results and discussion

![Image](image.png)

Figure 59: _Fagus sylvatica_ polyurethane glued sample. The polyurethane shows a fluorescent behaviour, which makes it very distinguishable from the wood structure. The glue line is visible
The polyurethane glue shows a fluorescent behaviour under exposition to UV light, and it is of valuable interest. Using this property coupled with very thin slicing, allows to observe with very good precision the glue line of some study samples. In *Fagus sylvatica*, Fig. 59, the glue line appears to be very thin, the cells which are at this glue line are not much damaged. There is not so much glue present at the two wood piece connection. The glue has rather penetrated into the wood vessels, and in some cases into the wood fibres. This can be more closely viewed in Fig. 60, where the inside of the wood vessels are lined with glue or even completely filled with the glue. Details of the wood-glue interface are sharp and of good quality.

Figure 60: *Fagus sylvatica* polyurethane glued sample. Details are bright and sharp

Not just concerning the glue distribution into the wood, the interaction between the glue and the wood cell-walls can already be studied: in Fig. 61, where a glue lined vessel is present, looking at the upper part of the vessel where the glue has detached from the cell-wall, one can see the delamination is not due to the detachment of the glue from the cell-wall, but from the breaking or delamination of the cell-wall itself. This would mean the bond between the glue and the wood cell-wall is a priori stronger than the intrinsic strength of the wood cell-wall.

The glue distribution also seems to vary much from one wood species to another. In *Picea abies*, illustrated in Fig. 62, the glue line is much more visible, because accumulation of the glue at the two wood pieces interface is large. The wood cells at the interface are much more destroyed and crushed than for *Fagus sylvatica* thus maybe retaining and trapping more glue, which could be either due to the wood species or maybe some slight differences con-
cerning polishing. For drawing any conclusions regarding that, a deeper study should be performed. Also, from Fig. 62, the glue doesn’t seem to penetrate much into the wood in *Picea abies*. Only the cells very close to the glue line are filled with the glue. This might be due to the fact that softwoods have no or few vessels, which are the main canals for transportation. Glue penetration into the fibres is surely more difficult, and so is its exchange between the fibres.

Figure 61: *Fagus sylvatica* polyurethane glued sample. The polyurethane seems to have a stronger bond with the cell-wall than the cell-wall has itself, because part of it is still attached to the glue after delamination

For further investigation a futur link between the vessel occupation percentage or distribution and the mechanical behaviour at macroscopic scale could be established.
Figure 62: *Picea abies* polyurethane glued sample. Cells at the glue line are crushed, unlike the ones in *Fagus sylvatica*
4 Conclusion

After studies of the various means at our disposal, the conclusion of this project is that despite it is still a rather lengthy process, the very thin wood slicing method developed in this project based on Professor Fink’s method from Freiburg University in Germany, is a promising method for a large variety of projects, it can be applied not only for the observation of wood-glue distribution and interaction but for all types of projects where visual description of wood microscopy is necessary or helpful. The method consists of first dehydrating the wood samples, and then embed them into a special resin for having a certain wood stability when performing 3 microns cutting using a diamond blade mounted on a rotary microtome. The slices are then dried in oven at 60°C and embedded for transmission light microscopy observation. The time necessary to produce these microscopy samples is of approximately 1 week.

Here, the light review performed for glue-cell-wall interaction, 6000 years old wood microstructure and features and mechanisms of biological weathering degradation patterns studies, was from far not a complete scientific study, but rather a first foot in some interesting possibilities, and showed the potential of this method in these fields, as well as opening doors to many new projects, or as a complement to already existing projects. This method can be easily completed through coupling with SEM observation for verification or addition of details.

Also, this method mainly reveals its scope and interest when associated to macroscopic scale studies, such as the study of wood mechanical properties. Indeed, the microscopic study displays, unfolds its value when it enables a better understanding of phenomena at a scale that one can perceive, i.e. at macroscopic scale.
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References


