INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE
ISSN: 2067-533X Volume 12, Issue 2, April-June 2021: 349-360 www.ijcs.ro

LIMITATIONS AND OBSTACLES ON WOOD IDENTIFICATION FROM SCULPTURES - ANALYSIS OF A SET OF GILDED AND POLYCHROMED FLEMISH ARTWORKS FROM THE 15th CENTURY

José Luis SILVA[1], Carolina Sofia BARATA[1], José PISSARRA[2,*]

[1] Department of Art and Restoration, Portuguese Catholic University, Porto, Portugal.
[2] Green UPorto - Sustainable Agrifood Production Research Centre and Department of Biology - Faculty of Sciences, University of Porto, Portugal.

Abstract

Wood identification through its internal anatomy is a complex and time-consuming process, often leading to taxonomic results at genus level (sometimes only family). The identification of the wood support of artworks contains specific methodological requirements and various limitations that are normally not applied with the common histological methodology. Preparation of wood for microscopic examination requires samples with approximately 1 cm^3. This is a volume that can conflict with ethical issues of intervention, namely the scale of the piece itself, the aesthetic and structural integrity or even the commercial value of the artwork. Because of these, besides other factors such as the presence of metal leaf, polychromies and varnishes, collecting usually becomes impractical. Also, the absence of any crack, fracture or perforation where collecting could be facilitated, or the presence of degraded or already intervened areas allowed for collecting, all contribute to hamper the identification of wooden artworks. In this paper, which is intended mainly for artwork restorers, we describe the alternatives and solutions that were explored to circumvent the limitations on the wood processing for identification, which allowed us to analyse and characterize a set of Flemish 15th century polychromed and gilded sculptures.

Keywords*: Flemish art; 15th Century sculpture; Gilding; Polychrome; Wood identification; Preservation and restoration.*

Introduction

The identification of wood through its internal anatomy is a visual exercise at the microscopic level that requires the skill to recognize basic differences in the cell and tissue structure. The identification resorting to microscopy, either light microscopy or scanning electron microscopy, is a procedure demanding dedication, time, technical and economical capacities and the consciousness that wood samples can usually be taxonomic identified to genus (sometimes only to family). Applications of wood identification span out from biology, pharmaceutics, palaeobotany, archaeology and history of art to forensic science and customs, timber industry and structural engineering, industry of decorative arts and conservation and restoration [1].

The methodology used to study wood anatomy requires at least one wood sample with an average size of 1 cm^3 [2-4]. Because of the orientation of the xylem cells in the stem the sections cannot be cut randomly from a wood piece and three plans of orientation must be detected: the cross section (transversal to the wood axis), the radial section (along an imaginary

* Corresponding author: jpissarr@fc.up.pt

longitudinal plan passing through the centre of the trunk and the tangential section (which does not cross de centre of the trunk but is tangential to the grows rings) [5, 6]. It is through the analysis of the characteristics of the various cell types constituting the wood (form, size, disposition, organization of the different elements), that we attempt to identify the species or, when it is not possible to reach that level, the genus to which the sample belongs. After softening in boiling water (for 1 - 2 hours) or in a mixture of 96% alcohol, water, and glycerine (1:1:3), the wood cube will, in normal circumstances, allow high quality sections with thicknesses between 8 and 20µm, 15µm in general [6].

With the referred sample volume most of the species' characteristics are available within the sections analysed, resulting in high quality sections with great amount of information for species or at least the genus identification.

Another potential with larger volume samples is the possibility to collect more sections if or when any of the defining elements referred in the literature for wood identification is missing from previous sections.

Limitations of collecting samples from artworks

When dealing with artworks, is common sense that the samples collected from the wooden supports must be very small. This limitation has to do with the size of the artwork and with ethical, aesthetic, structural and commercial value issues, but also, it is important to consider the emotional relation between the artwork and the owner, which can be in specific situations, the main limitation related with the sample volume.

Invariably, the sample size acquires less importance when dealing with large volume artworks as altarpieces from which, due to its size and also for having so many hardly visible areas, collecting is greatly facilitated. In the same category, the big scale sculptures, especially the hollowed ones that were originally made to be placed against altarpieces or walls, provide abundant collecting areas. Often the interior of furniture can be accessed for collecting. On the contrary, sample collecting is hindered in paintings on wood, small volume and freestanding sculptures or entirely polychromed/gilded artworks. In the specific case of paintings on excessively thin boards the collecting cannot be carried out without structural implications, particularly for the polychromy present on the face. Small volume, entirely polychromed or gilded and freestanding sculptures, have extremely limited or even inexistent collecting areas that in most cases are reduced to the inferior face of the base of the sculptures or any pre-existing perforation for sculpture standing. These two options have their own limitations, being in the first case the common continuous crushing due to the movement (tension stress) of the piece during its lifetime. This factor can modify the appearance of the cells shape, distribution and organization, until the complete destruction of the internal wood structure. In the second case, the perforations are usually very narrow, which create big difficulties in the separation of the wood sample from the object itself, plus that the recognition of the elements orientation in the sample is impossible until the sample has been collected, sectioned and prepared for microscopic analyses.

Beyond the collecting area limitations, the main factors that create difficulties to the achievement of viable samples for the purpose of wood species identification are the presence of polychromies and metal sheet, which are only a problem when its presence covers the artwork completely.

Fungal infected areas can result in viable samples depending on the type of fungi present (Chromogenic fungi or Saprophytic fungi) and the level of that infection.

The xylophagous insect infestation has always a considerable destructive impact in the internal structure of the wood. This impact is most evident when analysing the wood at the microscopic level (which is essential for scientific wood identification), where the galleries have a colossal proportion compared to most of the wood elements. As explained further ahead, this is not a guarantee of failure when it comes to wood identification.

Finally, the collecting of samples from areas previously intervened for conservation and restoration and with remaining intervention products as waxes, consolidation resins and filling pastes are of no utility for identification purposes.

For the present work, the collecting from the defined sculptures had to be limited to one sample per piece, with the smallest volume possible and had to be carried out in areas that would not affect the structural, aesthetic and commercial value of the artwork.

Suggestions for collecting viable wood samples from artworks

Collecting small-volume samples (ca. 3 mm^3)

Theoretically, small samples can be collected from a wide range of artworks. However, is very important to recognize the orientation of the wood elements *in situ* either with a hand lens or with a stereo microscope. For instance, the rays, when identified, indicate whether the sample collected will be a tangential or radial section. This cautious collecting optimizes further sectioning and avoids wood wasting (Fig. 1). The main drawback of this option is the fact that we do not know if the sample is viable until it is analysed.

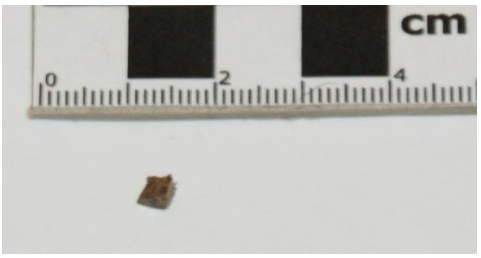

Fig. 1. Small wood sample for histological processing and further identification

Collecting samples from areas affected by fungi

Because the pieces stay untouched for long periods of time in the same place and in the same environmental conditions without any preventive conservation routine as those outside the museological environment, the presence of degrading fungi is often common in art objects [7].

Fungal infections are divided in two different types that have different implications for the wood identification: the chromogenic fungi (also called brown rot) cause aesthetical changes of the wood surface without relevant damage in the wood structure; on the other side, the saprophytic fungi (white rot) cause modifications in the internal structure of the wood and may, in some cases, result in unviable identifications [8, 9]. Therefore, the success of this task is limited by the nature of the infection itself. Nonetheless, when art works are attacked by fungi wood samples must be embedded for histological processing and further sectioning (see Appendix for information on embedding media).

Collecting samples from areas affected by xylophagous insect infestation

This pathology is probably frequent when dealing with wooden art objects (Fig. 2). In addition to its frequency, it is also the most destructive factor (Fig. 3), hindering the wood identification, and might cause serious implications in the structural integrity of the work itself.

The impact of the xylophagous infestation on the wood structure is always very relevant when analysing its microstructure. The galleries formed (Fig. 4) can effectively preclude the wood identification. However, if we collect several small samples, after preparation and observation using the light microscope, we can assemble the images to obtain an annual ring for example. Concretely, if in the cross section of one sample we obtain only the winter parcel of the ring, in the next sample we can get the spring parcel of the ring and thereby, continuing the assembly is possible to obtain sufficient information to achieve the identification.

Fig. 2. An artwork deeply attacked by xylophagous insects as seen by the numerous holes

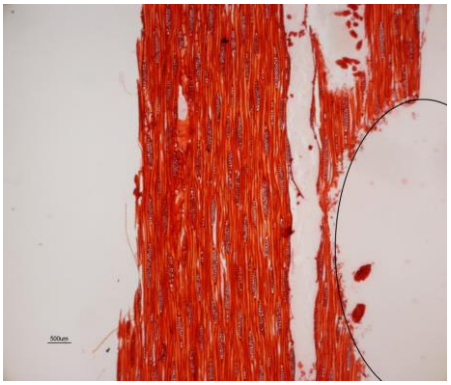

Fig. 3. Wood of *Castanea* sp attacked by a xylophagous insect. The encircled region of this tangential section corresponds to the hole drilled by the insect

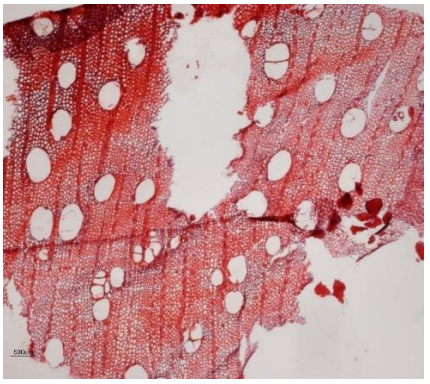

Fig. 4. Cross section of *Castanea* spp wood attacked by a tunnelling insect. Note the large hole in the middle of the section and other peripheric missing parts completely different from the well-defined pores (vessels)

Collecting samples from already intervened areas

The collecting of samples from already intervened areas is a very common ground, when working with samples resulting from artworks. There is always a real hypothesis of the presence of remaining products from previous interventions of restauration or conservation. Most commonly, these products are waxes, consolidation resins and filling pastes.

In the presence of waxes the problem is that this material can create real difficulties to the penetration of the embedding resins. In this case, the collected sample can be boiled in a hydrocarbon compound until all the wax is diluted and removed from the sample. With the same methodology, samples that contain great concentrations of consolidation resins can be subjected to all the subsequent identification methodology after its removal by submerging the sample in the corresponding solvent.

Sometimes it is not viable to sample because the hidden parts appropriate for sampling are filled with cement and filling pastes; therefore, unless avoiding these areas when colleting is impossible to identify (Fig. 5).

Collecting samples from carbonized areas

Usually when dealing with an artwork affected by fire, the only area in which the proprietary allows the collecting of samples is precisely the charred area, for its lost assumption. However, the carbonization has no influence on the internal structure of the wood, other than the volume reduction of the piece [10]. The fragility of the samples collected is, in most cases, higher than in any others, which requires a more careful packaging for transportation and makes its embedding in resin unavoidable.

Fig. 5. Missing parts reconstructed with a filling cement

Fig. 6. Example of a small wood fragment collected for identification

Collecting sample slices

There are situations when the collecting of three-dimensional portions of wood, for smallest as they can be, is not possible. In these cases, the collecting of thin wood slices can result in viable samples. For this, it is important to identify previously the orientation of the wood elements, being the main reference in this case the rays. This methodology is viable using a chirurgical scalpel (Fig. 6), in which the samples collected with an approximate thickness of a millimetre, are enough to obtain several microtome woods sections.

Sample preparation for microscopy

The analysis of small-scale samples requires it embedding in resin to allow handling and sectioning to prepare slides suitable for detailed wood observation. We have used several resins that are listed in the Appendix, but our experience has shown that LR White impregnates the woods better. As an alternative to the embedding methodology, we can proceed to the analysis of samples using scanning electron microscopy (SEM), a technique that offers both advantages and disadvantages. Obviously, the main advantage is the 3D effect and the magnifications scales that this technique allows. On the other hand, the opaque resulting image does not permit reading elements that are visible through the transparency of the sample.

Case study – analysis of 15th century Flemish sculptures

When speaking of the 15th century Flemish art usually oil painting on wood most often comes to mind. However, sculpture was alongside with painting a major contributor in commercial exchange and therefore the dissemination of what is known as Flemish Art. Nevertheless, the sculpture art of the Flemish Renaissance is poorly known, the Flemish sculptor was in a much greater extent than the painter the unknown author of its time, and unlike painters a large percentage of the sculptures remain still unknown or even imbued with an almost absolute identity haze.

The growing fame of the works produced in Flanders, as well as the increased demand and popularity, led to the abundance of altar panels (single or triptychs) carved and painted, with an extremely organized and somehow standardized structure and design. This development was due to the prolific export of these sculptural pieces to France, Iberian Peninsula, Canary and Balearic Islands, Germany and Scandinavian countries. Antwerp and Brussels were the two main centres of production of this sculptural typology, highlighting Malines in figurines manufacturing according to Bruneau *et al* [11]. The same author mentions also that the major woods used for these pieces were oak (*Quercus* spp.), walnut (*Juglans* spp.) and some fruit trees. Whilst the altarpiece panels in southern Germany were usually made from linden (*Tilia* spp.), in the alpine regions the choice fell on the pine wood (*Pinus* spp.). The selection of the wood species had much to do with the intended visibility of the structures. While the best quality woods were selected for the piece itself, the lower quality woods were used for less important parts, such as pedestals [12].

The use of local woods is common to several European locations, example of this are several Spanish altarpiece structures with clear Flemish and northern European influences, sculptured on species such as walnut and oak (common in Northern European art), but also chestnut (*Castanea* spp.), poplar (*Populus* spp.), pine (*Pinus* spp.), cedar (*Cedrus* spp.) and cypress (*Cupressus* spp.). The North European species typically used, could also be ordered from Northern Europe [11] although it is deductible, if they were locally available, they were collected from these locals.

In general, the only criterion for the choice of woods by the artists was its ease of acquisition. Therefore, it is surprising that species from the local flora had been rarely used, such as elm (*Ulmus* spp.), mountain ash (*Sorbus* spp.), alder (*Alnus* spp.), beech (*Betula* spp.) or maple (*Acer* spp.), even when they have the necessary work qualities [13]. After a thorough analysis of the supports used by European artists from 12th to 16th century, Marette [13] concluded that the same varieties of wood are found in the same school or region.

In this paper, which was written not for wood anatomy experts but for those dealing with artworks restoration, we describe the limitations and problems we faced when reducing the scale of the samples and still maintaining their viability as to the identification of the wood species. Using this methodology, we succeeded to characterizing a group of Flemish 15th century polychromed and gilded sculptures contributing, thereby, to the scientific and artistic knowledge of this sculptural typology.

Materials and Methods

The group of Flemish 15th century polychromed and gilded sculptures consisted of religious sculptures representing Our Lady of Calvary, Saint Bartholomew, Our lady with the boy in majesty and Saint John the Evangelist.

The wood samples were collected as previous described in Bernal *et al.* [14, 15] with minor modifications. Briefly, samples were collected as carefully as possible from hidden and unpainted areas using a scalpel (the size of the fragments ranged from 3 to 5 mm in length, and 1 to 3 mm in width. Considering the minute size of the samples for microtomy, they were, therefore, embedded in London Resin White (LR White) with the following procedure. Samples were dehydrated through a graded ethanol series of 25, 35, 50, 70, 90 and 100% (v/v) of ethanol at 10 min. intervals. The pieces were then incubated in a series of LR White resin and ethanol (1:3, 1:2, 1:1, 2:1, 3:1, 1:0) at room temperature overnight. The embedded pieces were polymerised at 60ºC in LR White for 24 h in capped vials. Sections with 10μm were cut on a sliding microtome, stained with safranin and permanently mounted with Entellan New (Merck) between slide and cover slip. After drying they were examined under the light microscope.

Descriptions followed the recommendations of the IAWA Committee [16]. Plant species were identified using wood anatomy textbooks [3, 10, 17], INTKEY databases [18], and the Wood Identification On-line Database [19]. The identifications were done at the most detailed level achievable, if possible, at the species level.

There is a big variety of resins available, having all of them pros and cons for the same end.

After sectioning, staining and mounting between slide and cover slip, sections are examined under the light microscope.

The resins used for the embedding of woody material for microscopic analysis are broad, being, the most frequently cited in the specialized bibliography:

Results

Following this methodology, it is possible to obtain samples with a wide range of quality and information. The resulting images of the alternative collecting methods exposed, often

don't offer aesthetically appealing images, but they can clearly be used for wood identification. Using thoughtful handling we succeeded to identify the wood of the following artworks.

The sculpture of Our Lady of Calvary was made of *Quercus* spp. wood (Oak) (Fig. 7A).

The diagnosis characteristics were vessels arranged in a diagonal and/or radial pattern, exclusively solitary (Fig. 7B); rays of two distinct sizes; rays uni- and multiseriate; multiseriate rays up to 30 cells wide (Fig. 7C); simple perforation plates (Fig. 7D).

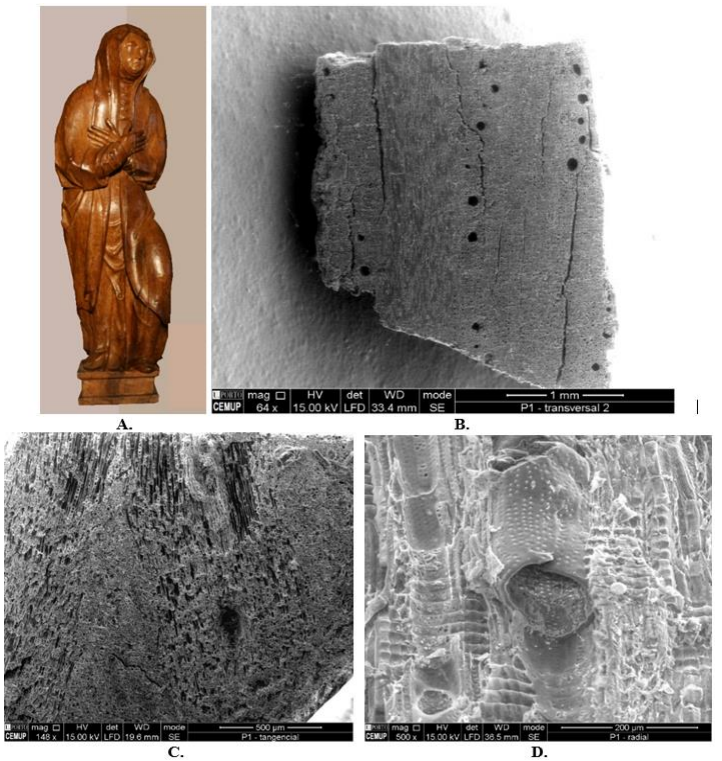

Fig. 7. Sculpture of Our Lady of Calvary made of *Quercus* spp. analyzed by scanning electron microscopy: **A.** The image of the statue; **B.** Cross section showing vessels arranged in a diagonal/radial pattern, exclusively solitary; **C.** Tangential section depicting rays of two distinct sizes. Rays Uni- and multiseriate. Very wide multiseriate rays, up to 30 cells wide; **D.** Radial section showing a vessel with simple perforation plates

The Saint Bartholomew sculpture (Fig. 8A) was carved on *Tilia* spp. Wood (Linden). The histological characteristics leading to the identification were wood with growth ring boundaries distinct; diffuse-porous; vessels in short (2–3 vessels) radial rows and in clusters; apotracheal parenchyma generally in short, uniseriate, oblique to tangential bands, often also terminal; rays flare along growth ring boundaries (Fig. 8B); rays of two distinct sizes; uniseriate rays generally composed of upright cells; rays multiseriate 2–4 cells wide; rays homocellular (Fig. 8C); conspicuous spiral thickenings in vessels (Fig. 8D).

The sculpture of Our Lady with the boy in majesty (Fig. 9A) was made of *Juglans regia* wood (Walnut). The diagnosis characteristics were distinct growth rings; diffuse-porous to semi-ring-porous; pores solitary or in radial rows of 2 to 4 cells (Fig. 9B); rays generally bi- to 4-seriate, rarely uni- and 5-seriate (Fig. 9C); rays homogeneous (Fig. 9D), occasionally slightly heterogeneous with one to several rows of square marginal cells.

The Saint John the Evangelist sculpture (Fig. 10A) was made also on *Juglans regia* wood as evaluated by the histological characteristics: distinct growth rings; diffuse-porous to semi-ring-porous; pores solitary or in radial rows of 2 to 4 cells; apotracheal parenchyma, both

diffuse and in short, tangential bands (Fig. 10B); rays homogeneous (Fig. 10C and D), occasionally slightly heterogeneous with one to several rows of square marginal cells.

Fig. 8. Saint Bartholomew sculpture made of *Tilia* spp wood:
A. The image of the statue; **B.** Cross section - Wood with growth ring boundaries distinct. Diffuse-porous. Vessels in short (2–3 vessels) radial rows and in clusters. Apotracheal parenchyma generally in short, uniseriate, oblique to tangential bands, often also terminal. Rays flare along growth ring boundaries; **C.** Tangential section - Rays of two distinct sizes. Uniseriate rays generally composed of upright cells. Rays multiseriate 2–4 cells wide. Rays homocellular; **D.** Radial section - Conspicuous spiral thickenings in vessels

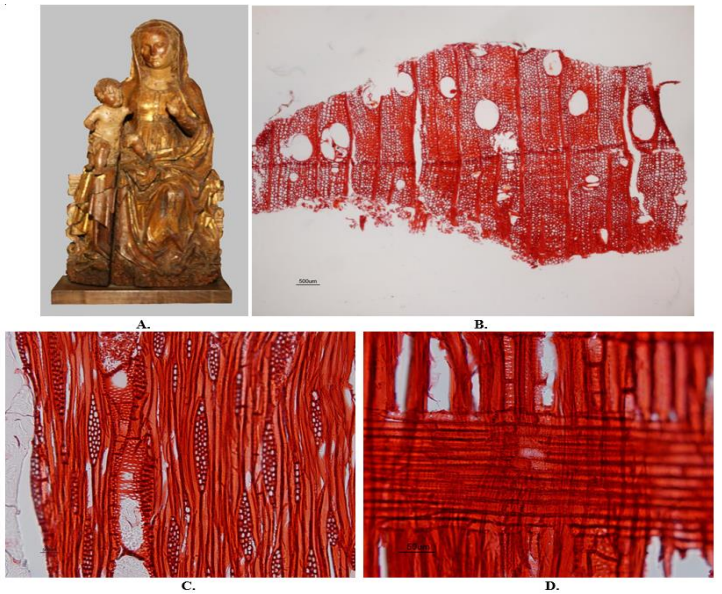

Fig. 9. Sculpture of Our Lady with the boy in majesty made of *Juglans regia* wood:
A. The image of the statue; **B.** Cross section. Distinct growth rings; diffuse-porous to semi-ring-porous; pores solitary or in radial rows of 2 to 4 cells; **C.** Tangential section. Rays generally bi- to 4-seriate, rarely uni- and 5-seriate; **D.** Radial section. Rays homogeneous, occasionally slightly heterogeneous with one to several rows of square marginal cells

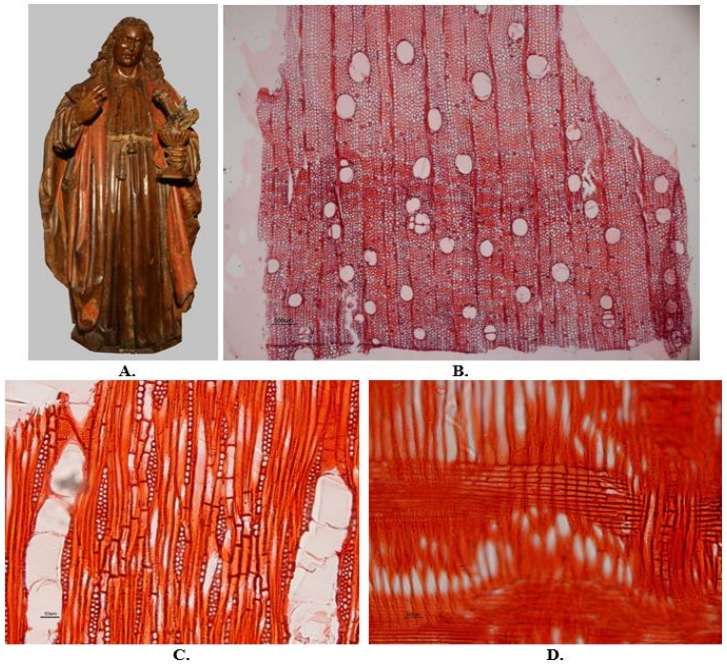

Fig. 10. Saint John the Evangelist sculpture made as well of *Juglans regia* wood:
A. The image of the statue; **B.** Cross section showing a distinct growth ring (note the limit of a growth ring at the bottom of the section); diffuse-porous to semi-ring-porous wood; pores solitary or in radial rows of 2 to 4 cells; apotracheal parenchyma, both diffuse and in short, tangential bands; **C.** Tangential section. Rays generally bi- to 4-seriate, rarely uni- and 5-seriate; **D.** Radial section. Rays homogeneous, occasionally slightly heterogeneous with one to several rows of square marginal cells

Discussion

The 15th century was a period of strong commercial growth between Portugal and Flanders and the Flemish art always had a particular impact and acceptance in Portugal. The sculptures in question match with the definition of the Flemish 15th century sculpture in what matters the sculptural, iconographic, aesthetic and formal language, however, all the sculptures present different factors and levels of degradation and were object of profound conservation and restoration interventions throughout the years. These constraints (degradation and restoration interventions), present a concrete challenge to the wood identification processes as explained ahead.

The areas most affected by the several degradation factors, are concentrated, as common in these situations, in the back and base of the pieces, being also these areas the most indicated to wood samples collecting for their less visible characteristics. In addition to this, all the sculptures present one or more degradations factors, as the presence of xylophagous insect infestation, fungal infections, structural wood breakdown, waxes, consolidation resins and filling pastes.

In this work we can conclude that the collecting of samples from areas that present different levels of degradation can still be used for wood identification. Nevertheless, this option obliges a more carefully handling of the collected samples because of their degraded status, their small scale, and all the embedding procedures needed.

The sample scale reduction's point is a work in progress since it's related to the wood in question. If we do the collecting from a piece that is constituted by a wood that presents the vessels visible to the naked eye (in opposition to a diffuse porous wood with small vessels), the collected sample must be obligatorily larger.

All the collected samples were embedded with the acrylic resin *LR White* (*Medium grade. London Resin Company Ltd*.) which presented good results. However, it is a resin that demands an oven for 10 to 12 hours for full polymerization, and, if left in the oven for too long or at a higher temperature, it becomes too rigid to provide good sections.

Therefore, Butyl-methylmethacrylate (*Leica Historesin Embedding kit*) was also tested as a simpler embedding medium since it polymerizes at the room temperature (see Appendix).

Once again, the result was very positive, allowing the preservation of the internal structure of the wood sample and resulting thereby in viable histological sections.

Conclusions

A set of polychromed and gilded sculptures dating back to the Flemish 15th century was analysed and characterized. These figures consisted of a group of religious sculptures representing Our Lady of Calvary, Saint Bartholomew, Our Lady with the boy in majesty, Saint John the Evangelist. Collecting of small-scale wood samples without damaging the sculptures allowed to identify the woods as *Quercus* spp. (Our Lady of Calvary), *Tilia* spp. (Saint Bartholomew), and *Juglans regia* (Our lady with the boy in majesty and Saint John the Evangelist).

The wood identification allowed the characterization of the original materials, which, correspond to the species that are normally identified when it comes to this sculptural typology, selecting the same wood for further restoration and conservation treatments and adding significant value to the pieces. We intend to implement this methodology in the Department of Art and Restoration at Portuguese Catholic University, as a tool for systematic wood identification of Portuguese wooden artworks. A database that researchers may consult and update will be created, in order to allow a panoramic view of the artist's choices in our country over time.

Acknowledgments

The authors gratefully acknowledge Helena Moreira (Department of Biology - Faculty of Science, University of Porto, Portugal) for technical assistance, Lília Esteves (José de Figueiredo Institute, Portugal) for the suggestions and support and Arlindo Silva (Department of Art and Restoration, Portuguese Catholic University, Porto, Portugal) for the xylotheque vouchers and support over the years.

This research was supported in part by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/05748/2020 and UIDP/05748/2020.

References

[1] A. Ghavidel, T. Hofmann, M. Bak, I. Sandu, V. Vasilache, *Comparative archaeometric characterization of recent and historical oak (Quercus spp.) Wood*, **Wood Science and Technology, 54**(5), 2020, pp. 1121-1137 . doi: 10.1007/s00226-020-01202-4

[2] L.G. Esteban, A.G. Casasús, C.P. Oramas, P.P. Palacios, **La Madera y su Anatomía. Anomalías y defectos, estrutura microscópica de coníferas y frondosas, identificación de maderas, descripción de espécies y pared celular,** Ediciones Mundi-Prensa, Madrid, 2003.

[3] J.G. Hather, **The Identification of the Northern European Woods. A Guide for Archaeologists and Conservators**, Archetypre Publications Ltd, 2000.

[4] H.G. Richter, L.M.Burger, **Anatomía da Madeira,** Livraria Nobel S.A., São Paulo, 1991.

[5] A. Ghavidel, J. Gelbrich, A. Kuqo, V. Vasilache, I. Sandu, *Investigation of Archaeological European White Elm (Ulmus laevis) for Identifying and Characterizing the Kind of Biological Degradation,* **Heritage, 3**, 2020, pp. 1083-1093.

[6] S. Carlquist, **Comparative Wood Anatomy. Systematic, Ecological, and Evolutionary Aspects of Dicotyledon Wood,** Springer-Verlag, Germany, 1988.

[7] A. Ghavidel, A. Scheglov, V. Karius, C. Mai, A. Tarmian, W. Vioel, V. Vasilache, I. Sandu, *In-depth studies on the modifying effects of natural ageing on the chemical structure of European spruce (Picea abies) and silver fir (Abies alba) woods*, **Journal of Wood Sciences, 66**, 2020, Article Number: 77. 10.1186/s10086-020-01924-w

[8] A. Ghavidel, R. Hosseinpourpia, H. Militz, V. Vasilache, I. Sandu, *Characterization of Archaeological European White Elm (Ulmus laevis P.) and Black Poplar (Populus nigra L.),***Forests, 11**, 2020, Article Number: 1329.

[9] B.R. Hoadley, **Identifying Wood. Accurate Results with Simple Tools,** The Tanton Press, Inc., Newtown, 1990.

[10] F.H. Schweingruber, **Wood Structure and Environment,** Springer-Verlag, Berlin, 2007.

[11] P. Bruneau, M. Torelli, X.B. Altet, **Sculpture – From Antiquity to the Middle Ages – From the Eighth Century BC to the Fifteenth Century, Part. I**., Taschen, Koln, 2006.

[12] M.J.G. Lopez, A. Le Gac, E.B. Rabelo, A. Barreiro, R. Baglion, *POLICROMIA – A escultura policromada religiosa dos séculos XVII e XVIII. Estudo comparativo das técnicas, alterações e conservação em Portugal, Espanha e Bélgica***, Congresso Internacional de Escultura Religiosa: Instituto Português de Conservação e Restauro**, Lisboa, 2002, pp. 261-284.

[13] J. Marette, **Connaissance des primitifs par l'étude du bois du XIIe au XVIe siècle,** 6ª ed. Éditions A. & J. Picard & CIE. Paris, 1961.

[14] R. Bernal, A. Valente, J. Pissarra, *Wood identification of 18th century furniture – Interpreting wood naming inventories,* **International Journal of Conservation Science, 2**(3), 2011, pp. 165-178. http://www.ijcs.uaic.ro/pub/IJCS-11-15-Bernal.pdf.

[15] R. Bernal, A. Valente, J. Pissarra, *Eighteenth century technological efficiency: The reuse of Brazilian sugar chest wood in Portuguese cabinet manufacture,* **International Journal of Conservation Science, 2**(4): 217-228. http://www.ijcs.uaic.ro/pub/IJCS-11-19-Bernal.pdf.

[16] * * *, *IAWA List of Microscopic Features for Hardwood Identification,* **IAWA Bulletin n.s. 10**(3), 1989, pp. 219-332. IAWA Committee.

[17] T. Hamann, E. Smets, F. Lens, *A comparison of paraffin and resin-based techniques used in bark anatomy,* **Taxon, 60(3)**, 2011, pp. 841-851.

[18] * * *, https://www.delta-intkey.com/www/data.htm

[19] * * *, https://www.wood-database.com/wood-articles/wood-identification-guide/

[20] * * * http://www.emsdiasum.com/microscopy/technical/datasheet/14380_LR_white.aspx

[21] * * *, http://www.sigmaaldrich.com/catalog/product/sigma/62661?lang=pt&region=PT&gclid=CLyO4MXsrMYCFSQFwwodhKYLbA

[22] * * *, http://www.sigmaaldrich.com/catalog/product/aldrich/235865?lang=pt&region=P

[23] N. Chaffey, **Wood Formation in Trees – Cell and Molecular Biology Techniques.** Taylor & Francis, N.Y., 2002.

[24] C.C. Baskin, E.W. Chester, J.M. Baskin, *Deep Complex Morphophysiological Dormancy in Seeds of Thaspium-Pinnatifidum (Apiaceae)*, **International Journal of Plant Sciences, 153**(4), 1992, pp. 565-571. DOI: 10.1086/297080.

[25] * * *, http://www.fpl.fs.fed.us/documnts/fplrn/fplrn056.pdf

[26] B.E. Causton, R. Gillett, and L.J. Philpotts, *A new embedding resin*. **Proceedings of the Royal Microscopical Society,** 1980 Suppl. **15:**pt.4, 21.

[27] L.R. Gjovik, *The application of fluorescence microscopy to the study of the permeability of aspen, (Populus tremuloides Michx)*, **MSc. Thesis**, University of Minnesota, 1961.

Received: May 18, 2020
Accepted: February 23, 2021

Appendix

In this appendix, intended for those not acquainted with plant histology, we list several embedding media for further xylotomy of small wood samples.

The media used for embedding woody material for microscopic analysis are broad, being the most frequently cited in the specialized bibliography. A few are listed.

LR White

Causton, Gillett and Philpotts [26] developed this non-toxic acrylic resin, widely applied for both light and electron microscopies. It has a very low viscosity, which allows it to infiltrate deeply into the wood tissue. After polymerized, the sections become hydrophilic. The commercial synonymous designation is Acryl resin LR white [20, 21]. It cures in four distinct methods: UV irradiation (365nm wavelength); chemical with accelerator; microwave and in temperatures between 60-65°C [20]. In the last method, if the time exceeds 12 hours, the resin might become too hard to allow good sections.

In his studies, Hamann *et al.* [17] concluded that within the hard resins, the LR white has a higher impregnation capacity of the wood tissues than other resins on the market, such as the EponTM (Hexion, Rotterdam, The Netherlands) or Technovit® (Heraeus-Kulzer, Wehrheim, Germany).

Butyl-methylmethacrylate

Commercially referred as BMM, it's a translucent resin less dense than water and insoluble in it. After addition of the hardener, the total polymerization requires about 12 hours at room temperature, allowing sections from 6 to 30µm [22].

Chaffey [23 based on the studies of Baskin *et al.* [24], states that the use of waxes for inclusion becomes unsatisfactory when it is important to preserve the most delicate tissues present in the sample. As a satisfactory alternative he refers BMM.

Celloidin

It is a pure form of nitrocellulose and it is commonly used for the inclusion of rotten wood samples. It preserves the wood structure and can therefore be considered for the embedding of very soft woods or fungal infected samples.

The major setback in the process is the long preparation period that this technique involves (up to two months for a fully embedded sample) [25].

Paraffin

It is even more effective than Celloidin for the embedding of samples with high levels of decay. For *S. Carlquist* [6] the paraffin selected should have a melting point between 59-61ºC. On the other hand, Hamann *et al.* [17] adds that he uses the Peel-a-wax paraffin (Electron Microscopy Sciences, Hatfield, Pennsylvania, U.S.A.), which has the melting point between the 62 and 64°C, not recommending the use of waxes with a melting point below 60ºC.

To the hardening protocol, it is added that the samples embedded should first harden at room temperature for several hours and then be placed in a refrigerator at temperatures between 0 and 5°C for several days [25].

Polyethylene glycol

It is a polymer formed from ethylene glycol. The use of polyethylene glycol (PEG), has the advantage that it can be use in wet or green wood, reducing the distortion problem due to the dehydration and it's a rapid method that requires minimal handling of the specimen.

The creator of the method, Gjovik [27] points out that special techniques such as cooling the blade of the microtome or softening, or cooling of the included sample did not show significant improvement in the histological obtained sections. He also concluded the possibility of obtaining acceptable sections by reducing the time of all steps of the protocol in 15-20 minutes. For histology, the PEG selected should have a molecular weight of 1450 [25].