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## Potential of reflected light microscopy as a non-invasive identification tool on wooden cultural artefacts-preliminary results

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#### Abstract

Species determination is a crucial step in wooden cultural artefacts diagnostic process. Not only it actually represents the key to physical and mechanical properties of a wooden object, but a precious instrument for its cultural understanding too. Scientific wood identification by microscopic analysis currently represents the most reliable and practicable technique. Since both this method is affected by biological limitations and most of the artefacts present sampling limitations, it is however impossible to predict end result accuracy and hence survey usefulness. Non-invasive alternatives to the common sampling procedure are hence preferable, when not necessary in the case of objects that do not permit sampling at all. Objective of this paper is to present some preliminary results of a study held in order to evaluate the usefulness of reflected light microscopy as a non-invasive identification tool.

Different surfacing and finishing treatments with reference to typical ancient manufacturing methods were reproduced on selected species. The visibility of anatomical features was then evaluated on the basis of a four level scale. Two indexes were created to evaluate both differences between treatments effects and single anatomical feature hardness of identification.

Surfacing affected anatomical features visibility at different degrees of severity depending both on adopted technique and species, while finishes partially improved or worsened it. Each anatomical feature demonstrated to have different susceptibility towards treatments, showing distinct identification hardness. For an evaluation of the reflected light microscopy potential as a non-invasive identification tool, a further study aimed to evaluate the efficacy of polarized and narrow-band filters has been fixed up.

## **1. INTRODUCTION**

The matter of species identification on wooden cultural heritage, which importance is stressed by the Italian standard UNI 11161 [12], is ruled in Italy by the standard UNI 11118 [11]. The common procedure consists in sampling, preparing the specimen and observing its anatomical features (Afs) at either the light or the scanning electron microscope.

This procedure is however invasive and thus affected by restrictions. On one side different typologies of objects, such as micro-sculpture or marquetry, do not allow its application, on the other against a damage on the object there is no guarantee about the result. Accuracy of identification depends in fact on different factors which have been well analysed by Wheeler & Baas [14] and that can be briefly summarised as: access to accessories information, such as geographical origin; sample dimension and state of conservation; access to bibliography and/or comparative samples; heterogeneity and range of variability inside the taxon.

Non-invasive wood identification is therefore an indisputable need for several wooden objects. The paper presents some preliminary results of a study that, through an evaluation of the effects of different surfacing and finishing typologies on the Afs visibility, aimed to evaluate the potential of reflected light microscopy as a non-invasive identification tool.

## 2. MATERIALS AND METHODS

Six species were selected: *Acer pseudoplatanus* (A), *Buxus sempervirens* (B), *Juglans regia* (J), *Olea europaea* (O), *Prunus avium* (P), *Pyrus communis* (Py). One 3 cm width quarter-sawn board of known origin was attentively selected paying attention to the absence of any defect for each species. From the heartwood of each board a 20 mm squared section lath was cut and then from each one 12 small cubes of approximately 20 mm length with perfect transverse (T), tangential longitudinal (TL) and radial longitudinal (RL) faces were set.

For each species 12 different treatment protocols were performed, resulting from the combination of four surfacing:

- two sanding treatments performed using a grain of 120 (S-) and 4000 (S+) respectively;
- two chip removal treatments performed using a really sharpened planer (CR+) and a traditional instrument called "rasiera<sup>1</sup>" (CR-) respectively;

and three finishing:

- shellac (Sh);
- wax (Wa);
- no finishes (Nf).

Afs visibility was evaluated through an Olympus BX51 optical microscope fitted with an Olympus TH4-200 reflected light source. As references for species anatomical descriptions we adopted [8], [9] and [10]. Terminology and survey principles followed the IAWA list of microscopic features for hardwood identification [5].

A scale of four levels was adopted to classify Afs visibility:

level A: the Af is visible and identifiable through all the specimen face;

level B: the Af is visible through all the specimen face but identifiable not throughout all the specimen face;

level C: the Af is visible through all the specimen face but hardly identifiable and only on rare portions of the specimen face;

level D: on all the specimen face the Af is not visible sufficiently clearly to be identifiable. Examples of Afs visibility levels are reported in Figure 1.

<sup>&</sup>lt;sup>1</sup> A metal foil sharpened on the edges.

Figure 1. Axial parenchyma strand length levels of visibility (TL face), from top left clockwise: level A (P, CR+, Sh); level B (O, S+, Nf); level C (Py, S+, Sh); level D (J, CR-, Nf). Scale bars level A and B = 150  $\mu$ m, level C and D = 75  $\mu$ m



Since only the A and B levels allowed Afs identification, Af identification hardness was expressed by means of an index called "Anatomical Feature Probability of Identification" (AFPI) calculated as:

$$AFPI = \frac{A+B}{A+B+C+D} * 100 \tag{1}$$

that is the percentage ratio between observations which led to identification and the total of performed observations.

On the basis of data elaboration, classes were then created to give an AFPI qualitative evaluation:

- low if  $\le 50\%$ ;

- good if >50% and < 75%;

- high if  $\geq$  75%.

Treatment effects were hence evaluated through an index called "Identifiable Anatomical Features" (IAF), calculated as:

$$IAF = \frac{\sum A + B}{\sum A + B + C + D} * 100 \tag{2}$$

that is the percentage ratio between identified Afs and the total of surveyed ones.

For statistical elaboration values were submitted to angular transformation ( $y = \arcsin \sqrt{\%}$ ) and P<0,05 significance level was adopted.

### **3. RESULTS AND DISCUSSION**

As shown by Ryan-Einot-Gabriel-Welsch Frest (REGWF) test (Table 1), IAF index was significantly different depending on the adopted technique. This, together with its values wide distribution amongst the four techniques, demonstrated how well they covered the potential range of variability in Afs visibility interference.

%		IAF	SD	REGWF	
Mea	n	35	28	-	
	<b>S</b> +	66	19	d	
Sumfacing	CR+	44	23	с	
Surfacing	CR-	23	11	b	
	S-	6	7	а	
	Α	28	19	a,b,c	
	В	23	19	a,b	
Species	J	52	37	с	
Species	0	43	34	b,c	
	Р	44	27	с	
	Ру	19	26	а	

Table 1. IAF mean value (%) and distinct respectively by surfacing and species is reported with standard deviation and REGWF test at the P<0,05 level. SD = standard deviation.

Significant differences merged too in IAF values if distinguished by species. REGWF test identified two distinct groups plus a third composed by "transition" species. Assuming that with a perfectly prepared sample IAF would be 100% for all species (this is in fact the result obtained with standard preparation for wood microscopic examination), different surfacing effects depending on species can explain these differences.

Looking at AFPI (Figure 2) a wide range of variability could be observed among Afs. Since this was verified also for Afs belonging to the same species, it proved that each Af had its own identification hardness regardless of species characteristics.

On the whole, more than  $\frac{3}{4}$  of all Afs resulted to have low probability of identification and none an high one. All Afs that were easier to identify, i.e. with an AFPI  $\geq$  50%, belong to the transverse face and this is in fact the anatomical section used by hand-lens identification methods (e.g. [1], [2], [3], [4], [6], [7], [13]).



Figure 2. Ranking of anatomical features observed in absence of finishes based on AFPI index (%).

Variation in IAF and AFPI values determined by the presence of Sh and Wa were then analysed in comparison with Nf ones. Table 2 reports IAF variation and final values with detail of positive (+), negative (-) and neutral (0) contributions.

 Table 2. IAF variation and final values due to shellac and wax interference. Mean values and distinct respectively by surfacing and species.

IAF (%)		-		0		+		Variation		Final value		REGWF	
		Sh	Wa	Sh	Wa	Sh	Wa	Sh	Wa	Sh	Wa	Sh	Wa
Surfacing	S+	5	18	85	82	11	1	+6	-17	72	49	с	b
	CR+	8	29	72	61	20	10	+13	-19	57	25	b,c	a,b
	CR-	4	11	76	82	20	8	+15	-3	38	20	a,b	а
	S-	1	1	75	93	23	6	+22	+6	28	12	а	а
Species	Α	2	8	73	89	25	3	+23	-5	51	23	b,c	a,b,c
	В	2	23	85	76	13	1	+10	-22	33	1	a,b	а
	J	5	19	70	63	25	18	+20	-1	72	51	с	с
	0	8	15	67	77	25	8	+17	-8	60	35	с	b,c
	Р	9	13	74	82	17	5	+9	-9	53	35	b,c	b,c
	Ру	2	10	90	87	8	4	+6	-6	25	13	а	a,b
Me	ean	5	15	77	79	19	6	+14	-8	49	27	-	-

Sh, with a mean variation of +14%, determined a remarkable improvement, instead Wa effect, with a -8%, was fairly negative. By the moment that neutral contribution were quite the same for both, this difference was due to a dissimilar proportion of positive and negative contributions, which appeared to be almost reversed between the two finishes.

If we look at values distinct by surfacing, IAF improvement determined by Sh increased as surfacing accuracy level decreased, that is why final values rose more appreciably according to the worst surfacing treatments. As regards Wa, except for CR+, IAF penalisation decreased with the surfacing accuracy level reduction, even with a positive IAF variation in correspondence of S-. Excluding CR+, both trends were mainly due to a decrease of negative contributions.

As confirmed by REGWF test, on the whole both Sh and Wa determined a reduction in IAF variability determined by surfacing: with Sh three groups were identified, while only two with Wa.

Summarising, as regards surfacing both Sh and Wa had an effect of IAF variability reduction, the first mainly thanks to a more marked improvement in the worst surfacing, the second mainly thanks to a more marked worsening in the better surfacing.

Distinguishing by species, even if variability inside both Sh and Wa effect was observed, REGWF test revealed that division among them remained quite similar as for Nf. Looking at final values, IAF raised over 50% in four species thanks to Sh, while with Wa only *Juglans regia* maintained a value greater than 50%.

As regards AFPI, in Figure 3 we can see that the balance of Sh interference was positive for more than one half of all Afs and negative only for "deposits in vessels". As a result there was an evident increase in the number of Afs which achieved at least a good AFPI (reported in square brackets), for a total of almost one half of them. Considering wax instead, from Figure 4 we can see that its interference was negative for most of the Afs; only "ray height", "ray width" and "fibre type (TL)" registered in fact positive variation.



Figure 3. AFPI variation (decreasing order) due to shellac interference, with detail of positive and negative contribution. AFPI final value is reported in square brackets.

Figure 4. AFPI variation (increasing order) due to wax interference, with detail of positive and negative contribution. AFPI final value is reported in square brackets.



## 4. CONCLUSIONS

The finest surfacing techniques referable to the manufacturing of artefacts do not reach the results provided by wood preparation for microscopic examination. These preliminary results show that even if the absence of a perfect surface preparation seriously affects the visibility of Afs at RLM and hence their probability of identification, the severity of this interference can be significantly different in relation to surfacing techniques, species and finishing.

In order to achieve an evaluation of the RLM potential as a non-invasive identification tool, a further study aimed to evaluate the efficacy of polarized and narrow-band filters has been fixed up.

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