*Paper C3*

# Advanced approaches in leather research using SEM microscopy X-ray probe equipped

**Claudia Florio**, Roberta Aveta, Gelsomina Tortora, Biagio Naviglio, Gianluigi Calvanese
Stazione Sperimentale Industria Pelli (SSIP), Via Nuova Poggioreale 39, Naples, Italy, Phone: 39-081-5979101, Fax: 39-081-265574, e-mail: g.calvanese@ssip.it

## 1. Introduction

In leather research of the past years, the early microscopy techniques, as the optical one, or the former electronic microscopy techniques, were widely utilized, mainly as initial approach, or as additional tool able to provide some useful information. Throughout the ages, the developments reached in microscopy technologies allowed to researchers the utilization of the modern techniques as the main, determining tool to get results in many research fields.

The modern scanning electron microscopy (SEM) technique we utilised in leather research, equipped with X-ray probe for the surface microanalysis of the samples, was the most needful and versatile tool in many applications.

The most interesting of these applications concerned: hair characterization, characterization of microorganisms involved in leather damage, investigation on leather defects and their causes, finishing characterization, surface treatment studies, diagnostic investigations of historic leathers for their valorization and conservation.

In the most of these applications a very little portion of sample was available for the analysis. In spite of this, the technique resulted comprehensive and able to solve the most problematic investigated cases. Particularly, SEM microanalysis is considered to be "non-destructive" indeed, as X-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly.

The study provided many interesting feedbacks, enforcing our knowledge on all the treated topics.

## 2. Materials and methods

The technique utilized is based on the electron-sample interactions and allows to reach information about the sample including external morphology and chemical composition. More in detail, The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of a given specimen. The signals deriving from electron-sample interactions reveal information about the sample. These signals include secondary electrons, backscattered electrons (BSE), photons (characteristic X-rays), visible light, and heat.

Secondary electrons and backscattered electrons are commonly used for imaging samples: secondary electrons are most valuable for showing morphology and topography on samples, while backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples, for example for rapid phase discrimination. X-rays are useful for microanalysis; their generation is produced by inelastic collisions of the incident electrons with electrons in discrete shells of the sample atoms.

The equipment was a Zeiss EVO MA10 scanning electron microscope equipped with a INCA X-act detector.

## 3. Results and discussion

### 3.1 Morphological studies

#### 3.1.1 Hair characterization

The study of many hair and their classification has been possible using morphological analysis. The characterization was based on some peculiar hair features; animal‘s coat is composed of several types of hair, the main components being the long and stiff hair, called *overhair* and the thinner, undulating and less stiff hair, called *underhair*. Both kind of hair are peculiar of a given animal and recognizable through the features of their main layers: *cuticula* (the outermost layer), *cortex* (the central layer) and *medulla* (the pit of hair). SEM morphological analysis is particularly decisive in *cuticula* characterization. More in detail *cuticula* is made up of a large number of overlapped scales of keratin and the technique allowed to identify:

- scale position relative to the longitudinal axis of the hair (transversal, longitudinal or internediete);
- *cuticula* patterns (petal pattern, dimond petal pattern, mosaic pattern, regular or irregular waved pattern and transitional pattern);
- structure of scale margins (smooth, rippled or frilled);
- distance between scale margins.

The technique was also utilized for the characterization of the hair cross-section, in order to esteem its size and shape, characteristic of each species. Furthermore, the cross section analysis allowed the identification of some important *medulla* features, with particular reference to the possibility of distinguish between unicellular *medulla* (single layer of medulla) and multicellular *medulla* (composed of two or more layers of cells).

One of the most interesting living matter requiring the use of the technique for hair characterization, concerns the identification of pets (dogs and cats) fur. This topic is particularly relevant due to an order of Italian Ministry of Health of 24/12/2002 concerning the prohibition on using dogs and cats for producing skins, furs, clothing and leather goods.

SEM morphological analysis was particularly used for characterization of dog (*Canis familiaris*) hair in comparison with those of murmansky (*Nyctereutes procyonoides*), whose fur is widely used in clothing market for edging of heads. This animal is known in English as “raccoon dog” and belongs to the *Canidae* family but is similar the American racoon. Nevertheless it is characterized by an its own genus (*Nyctereutes*). SEM characterization, with particular reference to the morphological comparative analysis of the *underhair cuticula*, from *Canis familiaris* and *Nyctereutes procyonoides*, allowed to distinguish between the two species, also were some other novel techniques (as the PCR, used for the DNA analysis) didn’t work, as sometimes happened. In figure 1 and 2 the SEM imagines of characteristic *underhair* from the two species are reported; it is possible to see that, thickness of the hair from *Nyctereutes procyonoides* is smaller than the one from *Canis familiaris* (average thickness resulted 0,015 mm and 0,025 mm respectively). Furthermore, *cuticular patterns* of the two species differ significantly; the ones from *Nyctereutes procyonoides* hair resulted ordinary characterized by more variability and normally its scales were more elongate and sharp than in *Canis familiaris*.

Other species from *Canidae* family were well characterized by the technique, as *Vulpes vulpes* (figure 3) as well as, more in general, many other species from *Carnivora* order, strongly attractive due to their sales value, as the *Mustelidae Mustela vison* (Figure 4).

Fig. 1: *Nyctereutes procyonoides* hair

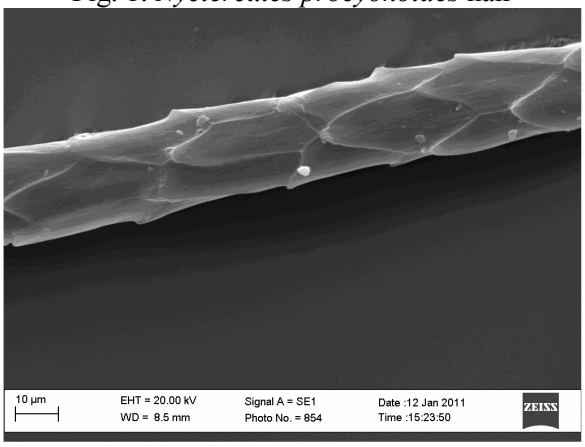



Fig. 2: *Canis familiaris* hair

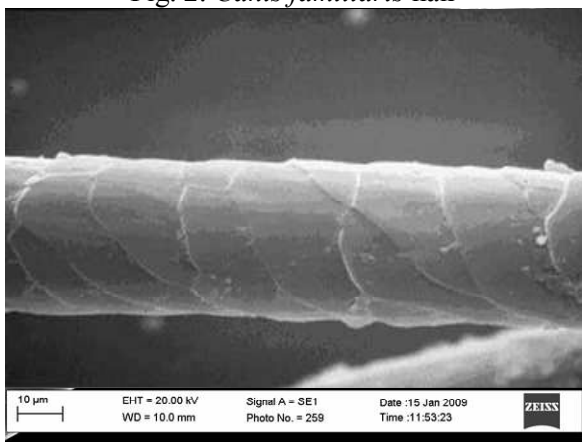



Fig. 3: *Vulpes vulpes* hair

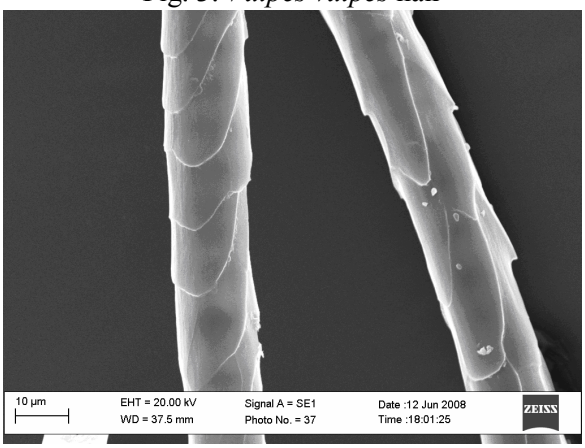
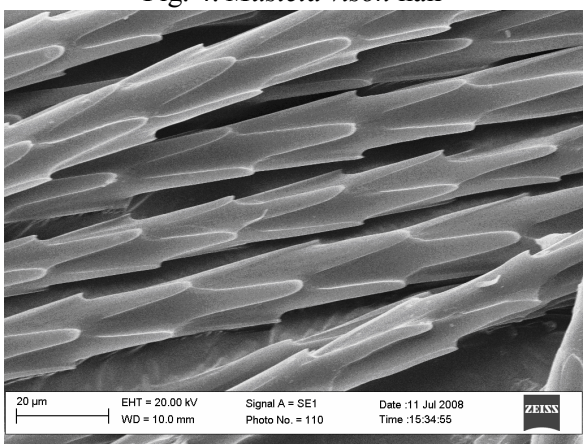



Fig. 4: *Mustela vison* hair




Furthermore, in recent years, a great attention is given from the market to the rabbit fur, that is even more utilized in clothing industry and that is more valuable when classifiable as *Oryctolagus cuniculus*. This species belongs to *Leporidae* family and is characterized by well definite features. As it is possible to observe in figures 5 and 6, these are more recognizable by the peculiar cross section, with particular reference to its shape and to the *multicellular medulla* identifiable, with five to eight longitudinal rows of cells. Relatively to the shape, cross section of *Oryctolagus cuniculus* resulted mainly "dumb-bell" shaped and concavo-convex (in some *underhair*).

### 3.1.2 Microorganism characterization

In leather production control of microorganisms presence is needed from the storage of rawstock to all the tanning process steps, until the finished good is reached.

SEM characterization, carried out on leather samples from the first wet-phases, up to the wet-blue, crust leathers and so on, showed a wide variety of microorganisms depending on the considered step of the tanning process. The typology and the amount of microorganisms found, indeed, resulted to vary according to some environmental factors, as pH, temperature, water activity, concentration of biocides and so on. Microorganisms found on leather belong both to *Prokaryota* (as *bacteria*) and to *Eukaryota* domain (as *moulds* and *yeasts*, from *Fungi* kingdom). More in detail, some bacteria were found, mainly, during the preservation phase and in steps characterized by an high alkalinity, as in liming, where pH reach values of 11-12; in this phase, some *bacteria* of *Bacillus* genus were found.

Most of *bacteria* found are able to damage leather, due to their proteolytic activity. Sometimes, also some pathogenic stock has been found, with particular reference to *Bacillus anthracis*, *Mycobacterium tubercolosis* or *Dermatophilius congelensis*.

Fig. 5: *Oryctolagus cuniculus* hair

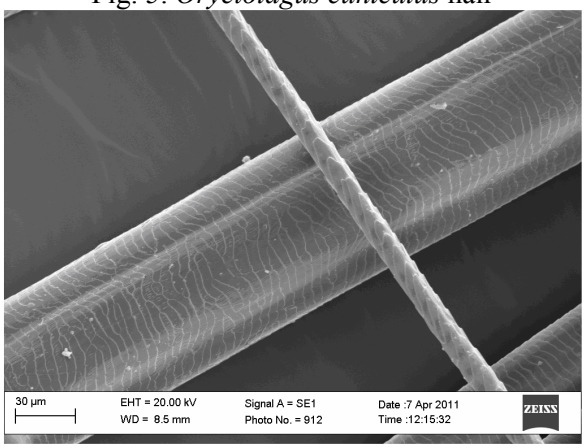
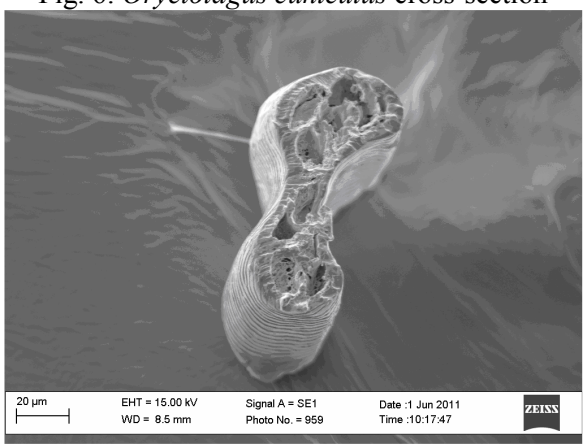
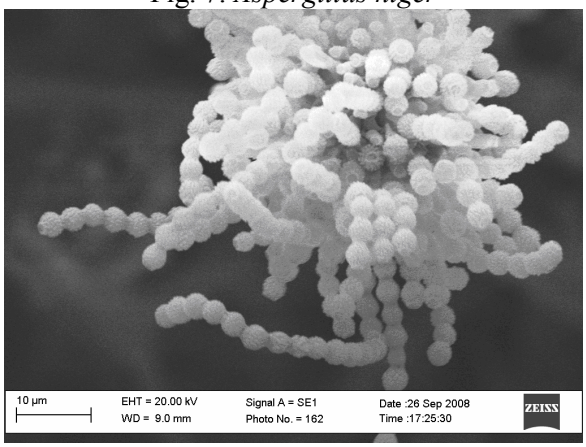



Fig. 6: *Oryctolagus cuniculus* cross-section




On the other hand, mainly at lower pH values (2-3), as in pickling, a wide set of *fungi* were found, with particular reference to *moulds*. They are recognizable through peculiar spores, as the moulds belonging to *Ascomycetes* class, with particular reference to the ones from genus *Penicillium* and *Aspergillus*. Most of these were found in pickled, wet-blue and finished leather and resulted to be resistant at high temperatures and to have a quick growth in presence of an high humidity degree. These *moulds* differ by colour as much as by apparence; normally ones from genus *Penicillium* are glassy or velvet looking and are green or blue coloured, while the ones from genus *Aspergillus* form yellow or black stains. Most of stains produced by moulds formations on leather among all the tanning process resulted to be blue, gray or black coloured. The blue formations have been normally identified as *Penicillium glaucum*, the gray ones as *Mucor mucedo*, while the black ones have been classified as *Aspergillus niger*. This last one resulted by far the most common identified on the leather samples analysed. It is well recognizable by its characteristic *conidium* with rounded spores strung together in chains (figure 7). This *mould* may cause serious damages to leather, due to great amount and type of enzymes it is able to produce. Finally, other than in leather, some moulds have been found, in chromate liquors too, as *Penecillium rubrum* (able to produce red stains on wet-blue leathers), when some saccharides, together with glycerol, are used to reduce dichromate.

Fig. 7: *Aspergillus niger*



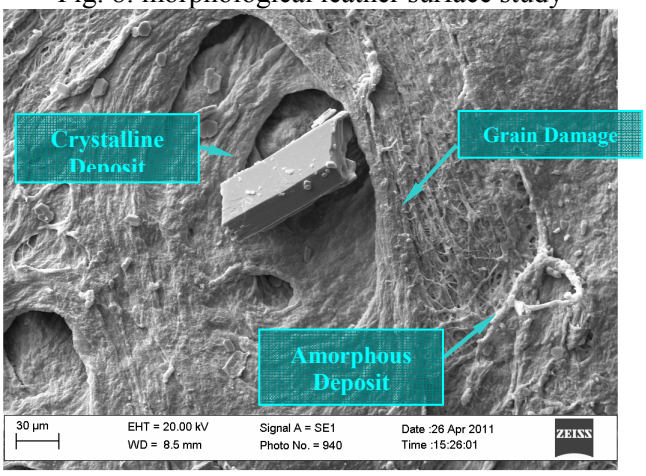
### 3.1.3 Morphological and Topographic analysis of leather surface

Many leather defects have been investigated using SEM morphological analysis. One of the most common application have been the study of some leather surface defects, having the macroscopic look of stains, but produced by local, microscopic grain damages. Furthermore, other surface defects have been investigated; the crystalline, rather than amorphous morphological look of deposits suggested their possible nature (saline, fatty, organic and so on) (figure 8).

Beside the study of defects, morphological analysis allowed the characterization of animal species, when it has not been possible by optical microscopy, that was in all those cases in which the leather surface was heavily damaged (as in historic leathers) or in slightly grounded leathers, where grain design was hardly recognizable.

Finally, the topographic analysis of leather surface have been useful, mainly, for finished leathers affected by peculiar defects, due to the presence of bumps, holes, bubbles (especially in patent leather), folds and halos.

Fig. 8: morphological leather surface study




## 3.2 Microanalysis

### 3.2.1 Leather defects investigation

This has been, by far, the main application of X-ray probe microanalysis, allowing to study many peculiar cases, identify the causes of leather damages and provide to tanners the tools to solve some technical inconveniences.

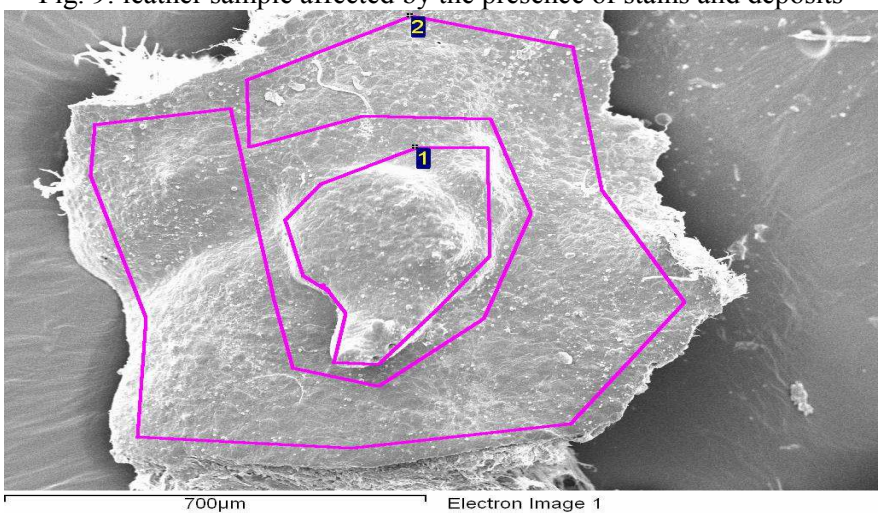
The presence of stains on leather surface, sometimes coupled with the presence of deposits, has been one of the most complained trouble by tanners and leather goods manufacturers.

Other technical problems investigated by microanalysis concerned some undesired features or damage of grain and leather structure, beside undesired non homogeneous absorption of dyes and/or chemicals.

The investigated stains were of different kinds: diffused, localised, point source shaped, more or less opaque, moist, fatty or wet looking, more ore less coloured, coupled or not with deposits, etc.
X-ray microanalysis showed that the most of coloured, point source shaped (or extremely localised) satins were caused by the presence of some metals, with particular reference to copper and iron, beside tracks of lead, zinc and others, especially in red, brown, black or blue coloured stains. That was

correlated to the possible formations of oxides; rameic and ferric oxide, for example, as well as lead oxide are red, while rameic sulphate is blue coloured. These elements may derive from external contacts with metallic parts of industrial machineries or other metallic tools utilised during the storage or from eventual contaminants in rinsing water utilised in the previous wet phases of the production process. In figure 9 and relative table 1 the microanalysis results of a sample affected by red and blue stains, coupled with deposits is reported; the results have been carried out as comparative microanalysis between stained and not stained regions of the sample. In figure 10 the X-ray spectrum of an isolated coloured deposit from another sample is reported.

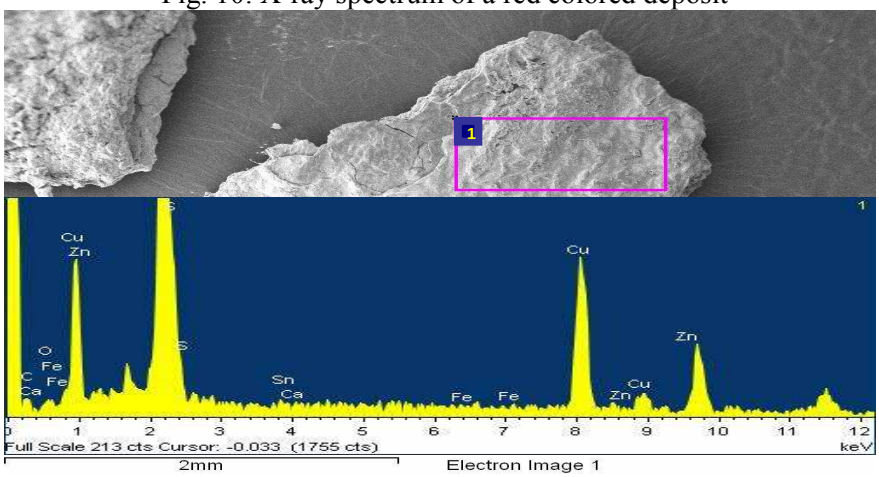
Fig. 9: leather sample affected by the presence of stains and deposits




Tab. 1: Average Surface Composition (%)
in presence of stains (Area 1) and in absence of stains (Area 2).

| | Element | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Carbon (C)** | **Azoth (N)** | **Oxygen (O)** | **Silicon (Si)** | **Titanium (Ti)** | **Sulphur (S)** | **Iron (Fe)** | **Copper (Cu)** | **Lead (Pb)** |
| **Area 1** | 37,58 | 28,37 | 11,26 | 2,52 | 11,52 | 0,55 | 1,04 | 3,45 | 3,71 |
| **Area 2** | 39,73 | 29,49 | 9,64 | 3,04 | 13,28 | 0,23 | 0,02 | 1,71 | 2,86 |

Fig. 10: X-ray spectrum of a red colored deposit




In vegetable-tanned leather, instead, the role of some metals was supposed to be more indirect, due to the possibility of formation of tannin-metal compounds. This hypothesis was confirmed for some dark,

point source shaped stains found in samples of vegetable leather, characterised by an high hydrosoluble content, with a significant presence of magnesium in the stained areas.

In other kind of defects, including grain and leather hardening or damage and irregular absorption of dyes, the presence of a significant percentage of calcium, sulphur and oxygen (correlated to the possible excess of calcium sulphate) suggested eventual errors in the first phases of tanning process, with particular reference to some beamhouse operations, as liming and deliming, with consequent worsening of leather absorption capability in the course of the further steps of the tanning process; in these cases a worse adsorption of chemicals, as tanning agents, fat-liquors and dyes may occur. In figures 11 and 12 the microanalysis results of a leather sample affected by surface bumps and by a scant absorption of dyes among the section is reported; figure 11 shows the surface of the sample, where a significant presence of calcium has been found, while figure 12 shows the distribution of calcium among the cross section of the sample, where a calcium barrier has been found in the upper side (grain side).

Fig. 11: calcium based deposits on leather

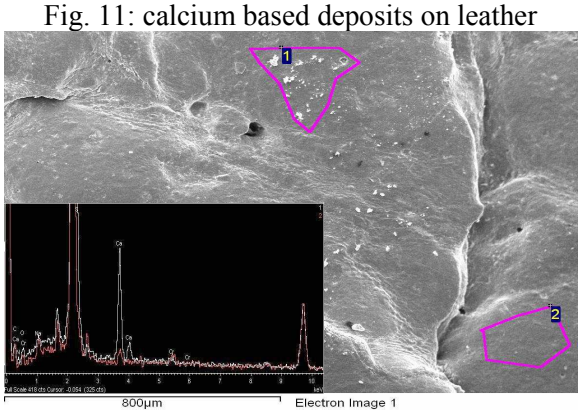
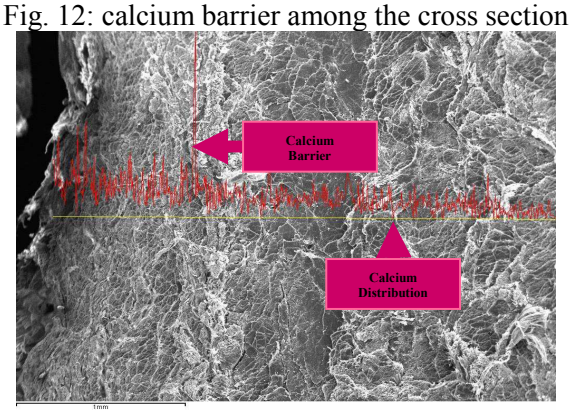



Fig. 12: calcium barrier among the cross section




In other cases of samples affected by an irregular absorbance of dyes and other chemicals, a relevant amount of chrome in stained areas has been found too; for the most of these cases a further in-depth examination suggested the formation of chrome soap.

Finally, in some cases, a relevant amount of surface sulphur content has been found, suggesting the possibility of eventual errors in the course of some steps of the tanning process, with particular reference to the hair removal one.

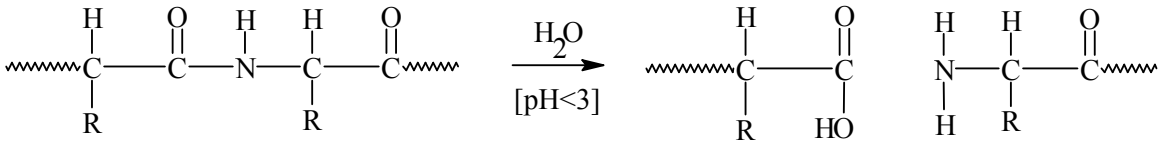
### 3.2.2 Cultural heritage applications

Reaching the past trough the future is just an apparent dichotomy. As a time travel needs its time machine, the more technology will be improved, the more it will be possible to sustain the value of the ancient items. Leather is one of the most versatile materials, so that it has been used since ancient times a for a great variety of uses. In recent years, the growing attention towards the problems of conservation of the antique handmade leathers needs more technological efforts in order to preserve their historic, artistic and cultural value.

SSIP has been widely involved in these efforts, firstly through dedicated studies to ancient bookbinding leathers, then to different other kind of other ancient items.

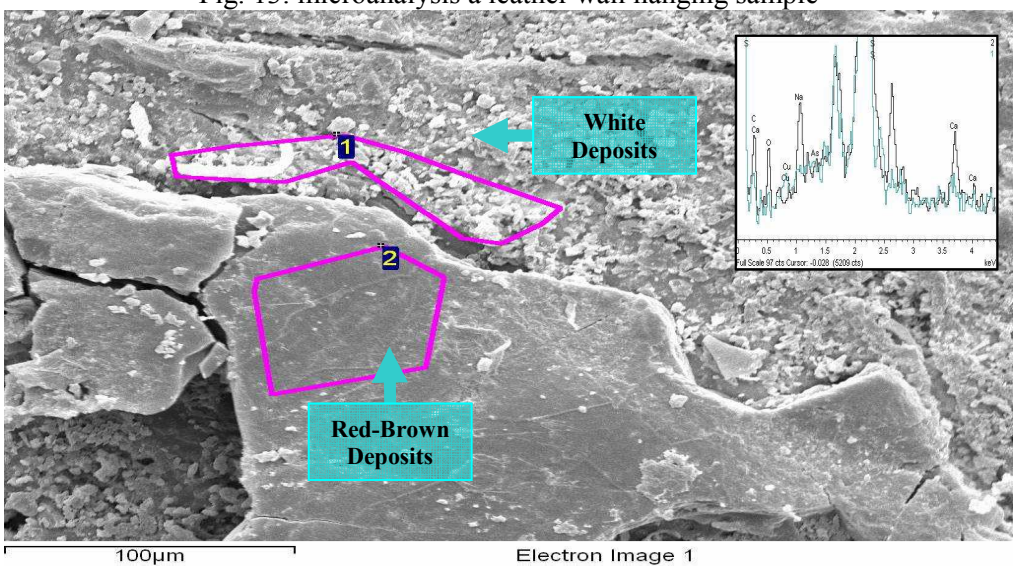
X-ray probe microanalysis resulted the most appropriate technology to this field, mainly due to its versatility and to its non-destructive character and the studies carried out with this technique allowed to increase the conservation time of antique handmade leathers and to planning adequate restoration works.

For the investigated cases, after the determination of the animal species, carried out with morphological examination, the microanalysis has been carried out for the determination of the tanning process employed and for the assessment of the type and degree of deterioration occurred. In the most of cases an oxidation process and an hydrolytic degradation, accelerated by acidic action of the polluting substances in the atmosphere, with particular reference to $SO_2$, have been identified as the main degradation mechanisms. The hydrolysis of peptide bond of proteins, indeed, is favoured by high humidity and low pH values:




In figure 13 and in table 2 an example of the results obtained with microanalysis of an investigated case is reported. The case concerned the conservation and restoration of a leather wall hanging from XVIII century, deriving from an Episcopal palace in Venice. The sample analyzed was a very narrow sized vegetable-tanned leather, as resulted from the low content of chrome found. It resulted heavily damaged and characterised by the presence of red stained regions on its surface. Furthermore, a large amount of white deposits, coupled with some brown-red ones were found. The white ones were small sized and granular shaped, while the brown-red ones were deposits characterized by a bigger size. Microanalysis shown that white deposits were characterised by an high content of sulphur, calcium and oxygen, suggesting they were calcium sulphate based, while the brown-red ones were characterised by a significant content of copper. Definitely the surface damage and the wide red stains were correlated to the degradation process known as "red rot", where some tannins (mainly the condensate ones) are subjected to a degradative oxidation and, on the other side, are able to adsorb a significant amount of atmospheric $SO_2$, with its possible further conversion in other substances able to damage the leather; more in detail, it may be converted in sulphuric acid, where this phenomenon may be favoured by metal contact, with particular reference to copper.

Fig. 13: microanalysis a leather wall hanging sample



Tab. 2: Average Surface Composition (%) of white deposits (Area 1) and of brown-red deposits (Area 2).

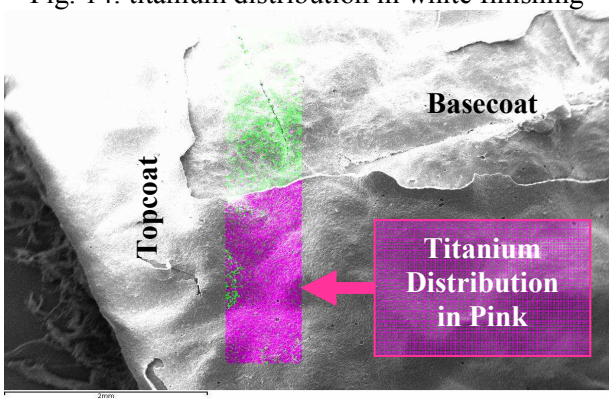
| | Element | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Carbon (C) | Azoth (N) | Oxygen (O) | Sodium (Na) | Sulphur (S) | Calcium (Ca) | Copper (Cu) | Arsenic (As) | Chrome (Cr) |
| Area 1 | 41,84 | 7,46 | 32,33 | 4,07 | 6,99 | 7,21 | 0,03 | 0,01 | 0,06 |
| Area 2 | 39,85 | 35,69 | 13,45 | 0,82 | 0,17 | 2,31 | 6,34 | 1,03 | 0,34 |

### 3.2.3 Finishing materials and treatment study

Microanalysis has been widely applied to the characterization of finishing materials and to monitoring the quality of surface treatments. The technique has been particularly useful for the characterization of laminated transfer films, made up of different thin layers; the study of these materials shown that the support layer was characterized by a significant content of aluminum, while the upper, variously colored one, resulted to contain tracks of other metals, as cobalt, copper and nickel, that need a particular attention, since they are under restriction for some eco-labels.

A particular application concerned the identification of titanium into white finishing layers, where topcoat and basecoat were both white colored, but not ever titanium based. This monitoring has been carried out, since, in a dedicate study, titanium oxide has been correlated to the possible insurance of a peculiar problematic of white upholstery leathers (figure 14).

Fig. 14: titanium distribution in white finishing




Finally the technique resulted particularly useful in monitoring the quality of hydrophobic surface treatments plasma induced. As, after these treatments, the formation of polysiloxane-like films was expected, the monitoring was carried out by determining the variation of the surface percentage amount of silicon when the applied operation conditions were changed. Carbon and oxygen percentage content were monitored too, in order to obtain further information on the formed species. In figure 15 the results of microanalysis are reported for two samples differently treated (A and B), showing differences in silicon surface percentage content. This monitoring allowed the planning of the most optimal parametric conditions for the treatments.

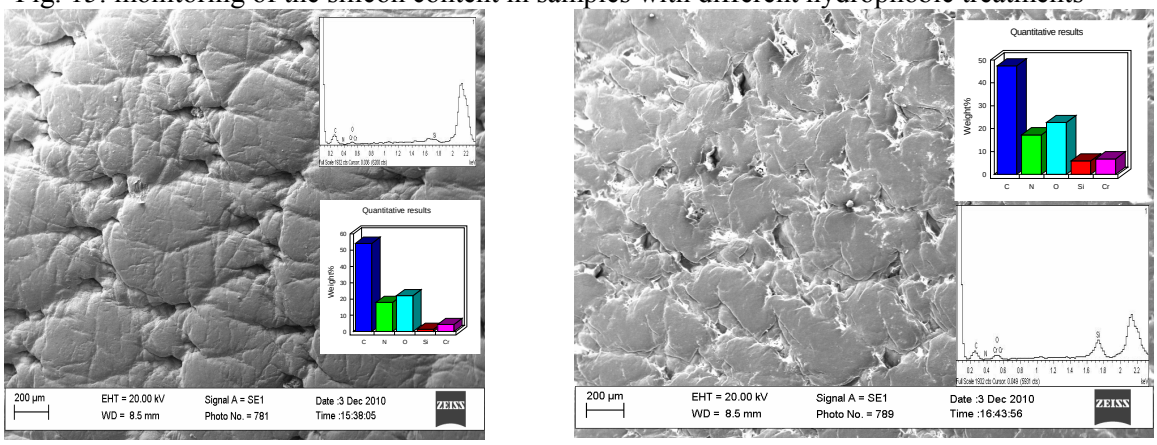
## 4. Conclusions

The technique has been successfully used in lots of interesting applications. Its flexibility allowed to reach relevant results in many fields.

Morphological analysis carried out with SEM microscopy was mainly applied to hair and microorganisms characterization, beside to morphological and topographic analysis of leather surface. Hair characterization resulted adequate and comprehensive for the classification of the animal species,

with consequent improving of the guarantied quality of the commercialized furs. Microorganisms characterization provided some precious information on the most diffused species among the different steps of the tanning process, suggesting appropriate and specific solutions to be adopted. Surface morphological and topographic analysis provided first qualitative information on surface defects origin, both on leather grain and on finishing coats.

Fig. 15: monitoring of the silicon content in samples with different hydrophobic treatments




**A** **B**

X-ray microanalysis resulted, by far, the main technique utilized in the last years, for the diagnosis of leather defects and the most effective one in the field of cultural heritage. Concerning the former application, the technique allowed to identify the causes of many peculiar leather defects, also providing information on the most critical phases of the production process for each kind of defect. In the most of cases, a critical role has been ascribed to the presence of some metals (as copper, iron, calcium, and so on) on defected items surface or cross-section. The letter application provided relevant information on the possible mechanisms causing the deterioration of antique handmade leathers, with consequent improving of their valorization and conservation strategies; more in detail, leather damage in ancient items has been correlated to some atmospheric conditions and polluting substances (as $SO_2$) and their acidic action, in association with the presence of some metal contaminants (as copper).

Furthermore, X-ray microanalysis has been widely employed for the characterization of finishing materials and for the monitoring of surface hydrophobic treatments, reaching encouraging results and allowing the optimal choice of the experimental conditions .

Finally, the technique yielded relevant results, improving our knowledge on the treated topics and suggesting strategies for the planning of further applications.

## 5. References

[1] B. Naviglio, G. Calvanese, R. Aveta, C. Florio, M. Romagnuolo. CPMC, 2009, 85 (3): 145-151.

[2] B. Naviglio, R. Aveta, C. Florio, G. Calvanese. I International Conference “Diagnosis for the conservation and valorization of cultural heritage” Napoli, December 9-10 2010 - CPMC, 2010, 86 (6): 341-353.

[3] C. Florio, R. Mascolo, G. Calvanese, B. Naviglio. X° AICC Conference, Serino, April 30 2010 - International Congress “dalla Pelle alla Moda”, Turbigo (MI), October 16 2010. CPMC, 2011, 87 (1): 3-15.

[4] B. Naviglio, R. Aveta, G. Comite. CPMC, 2003, 79 (2): 93-106.

[5] R. S., Thomson. JALCA, 2002, 97: 307-320.

[6] C. Florio; R. Mascolo; G. Calvanese; B. Naviglio. Beijing (CHINA) XXX IULTCS Congress, October 11-13 2009 - CPMC, 2009, 85 (6): 355-366.

[7] C. Florio; R. Mascolo; G. Calvanese; B. Naviglio. Leather International, 2010, 212 (4799): 24-28 - Tecnica Calzaturiera, November 2010: 42-44.

[8] G. Reich, J. Bradt, M. Mertig, W. Pompe. JSLTC, 1998, 82: 11-14.

[9] C. Florio; G. Calvanese; B. Naviglio; R. Mascolo; G. Grasso. CPMC, 2009, 85 (3): 131-143.

## 6. Acknowledgements

SSIP wish to tanks for his collaboration Prof. Vanni Tiozzo
Director of the Restoration Techniques Department of School of Fine Arts of Venice.
Curator of the restoration of the ancient wall hanging in leather we treated, taken from an Episcopal palace in Venice. Author of “Painted Leather in Venice”- School of Fine Arts of Venice - Seminars 2003.