



The stereomicroscope is a combination of two different microscopes to produce a three-dimensional image.

Microscopy techniques applied to leather

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The use of different types of microscopes and their application to the leather sector is of importance. Through these investigative techniques, the National Italian Leather Research Institute (SSIP) also offers additional specialist support aimed at verifying further crucial features in the leather sector, as in the case of the certification of authenticity of leather and hides, as well as the origin and characterisation of animal hair.

Through microscopic investigations it is possible to study the morphology of different areas of the same leather and how the orientation of the fibres is configured. This is beneficial for characterising the most used animal species, and for carrying out product evaluations on the leather, improving its use for the different intended applications.

With microscopy, not only is it possible to deepen the knowledge of the leather morphology, but also to obtain useful information to link the

structure of the skin to its physical properties. In many cases this microscopic examination is an essential method for identifying the origin of defects in finished leathers.

Optical microscopy

The most classic example of optical microscopy is the stereomicroscope (also referred to as stereoscopic microscope or stereoscope) consisting of the combination of two different microscopes (generally with reflected light), which allow observation from

slightly different angles producing a three-dimensional image as with direct vision, thus eliminating flattening effects.

Figure 1 shows a stereomicroscopic image of a piece of leather with the grain side intact and which has not undergone any mechanical surface treatment. It should be remembered that the term 'grain' refers to the external side of the skin after removal of the epidermis in the liming process and is characterised by a grain texture, i.e. by an arrangement of follicles specific to each animal species. Figure 2, instead,

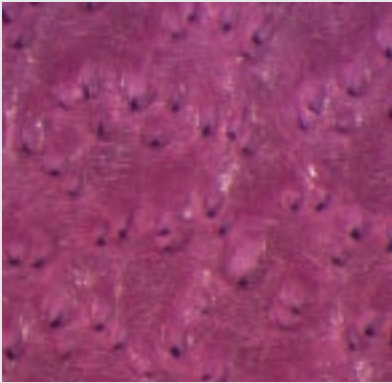


Figure 1: Follicle pattern of grain leather.



Figure 2: Synthetic "leather" surface.

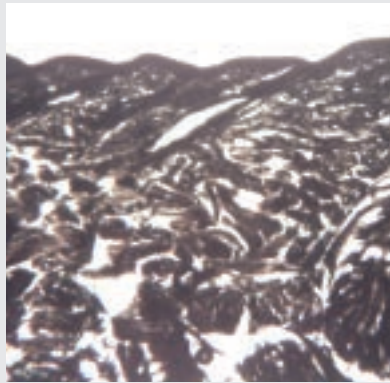


Figure 3: Image of Leather section.

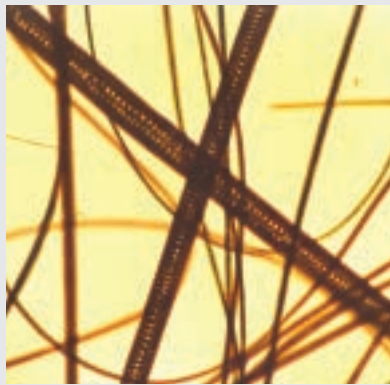


Figure 4: Internal medulla of animal hair.

has coarser fibres and three-dimensional interlacing. The phase-contrast microscope allows, in some cases, to characterise the nature of animal species among those most commonly used in the fur industry, as shown in *Figure 4*. The examination with the optical microscope shows the presence of the medulla inside the characteristic hair of each animal species. In this specific case it is rabbit fur, scientific name *Oryctolagus cuniculus*.

Electron microscopy

Scanning electron microscopy (SEM) is based on the sample-electron interaction and allows acquiring detailed knowledge of the skin structure, not only due to the possibility of obtaining significantly higher resolutions and enlargements compared to optical microscopy, but also because it allows a three-dimensional view of the sample, whose high magnification cannot be obtained with other techniques.

Due to the cases under examination and the problems, the use of both microscopes may be more useful. The introduction of the SEM has not, however, superseded the use of the optical microscope, which remains indispensable even for the observation of suitably coloured sections to highlight

shows an image of the surface of a synthetic substitute for leather on which the grain pattern has been recreated by a polyurethane print.

Another instrument in the field of optical microscopy is the phase-contrast microscope, a technique that converts phase shifts in light passing through a transparent specimen to brightness changes in the image. Phase shifts themselves are invisible but become visible when shown as brightness variations.

Figure 3 shows an image obtained with a phase-contrast microscope with a 30 μ thick section, obtained with a Cryomicrotome, in which it is possible to observe the morphology of leather from the grain to the flesh side or dermis.

The derma (or corium) represents 85% of the thickness of the skin and is therefore the most important part for tanning since it will become leather. The papillary layer (also called the flower or grain) represents the remaining part of the thickness of the dermis, and is the area in which the collagen fibres are more compact; the remaining part is called the reticular layer. The grain layer is characterised by thin fibres and is mainly arranged horizontally, while the flesh layer



Phase-contrast microscope.



SEM of SSIP mod. EVO MA 10 Zeiss

analysis of the cuticles of *Canis familiaris* and *Nyctereutes procyonoides*, has proved effective in distinguishing between the two species mentioned above, even where other modern technologies (including techniques based on DNA analysis) were not exhaustive, as was sometimes verified during the studies. Figures 7 and 8 show the SEM images of the characteristic hairs of the two species; it can be seen that *Nyctereutes procyonoides* hair is thinner than *Canis familiaris* hair (average thicknesses were, respectively, 0.025 mm and 0.15 mm). The cuticle pattern of the two species was also significantly different; the one related to *Nyctereutes procyonoides* was generally characterised by greater variability, with more elongated scales and sharper ends compared to what was found for *Canis familiaris*. Microscopy, together with other advanced diagnostic techniques, also offers analysis services to support research and development activities, as well as consulting and custom innovation services. ©

the presence of certain compounds present in the leather (grease, etc.) or morphological components. For three-dimensional vision at high magnifications, however, the SEM is still irreplaceable. Figure 5 shows a SEM image of a mould deposit on wet blue leather, while Figure 6 shows a detail of an *Aspergillus* mould.

The morphological analysis by SEM was particularly useful for the comparative characterisation of dog hair (*Canis familiaris*) and of murmansky (*Nyctereutes procyonoides*), whose fur is widely used in the clothing industry to produce hems in clothing items. This animal is also known as the 'raccoon dog' and belongs to the *Canidae* family but is similar to the American raccoon. However, this animal is identified by its own genus (*Nyctereutes*). The need for the recognition of this type of animal was also born after the issuance of the order of the Ministry of Health of 24 December 2002 which states 'the prohibition of using cat and dog skins for the production, marketing and import of hides, furs, clothing and leather goods made of or obtained, in whole or in part, from hides and furs of said animal species'.

The SEM characterisation, with particular reference to the comparative

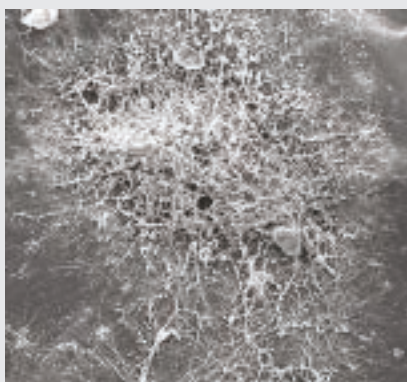


Figure 5: Wet blue mould deposit on wet blue leather.

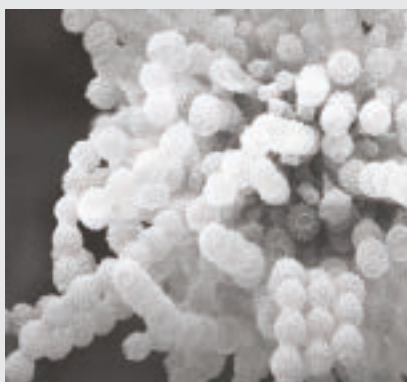


Figure 6: *Aspergillus* mould.



Figure 7: *Nyctereutes procyonoides*.

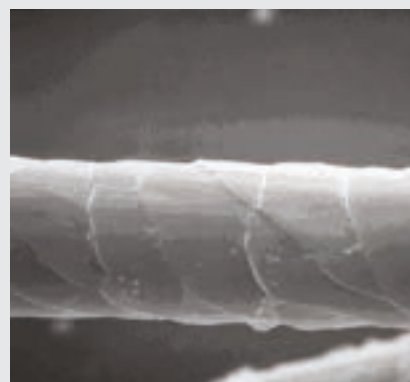


Figure 8: *Canis familiaris*.