Scanning and transmission electron microscopy in cultural heritage: State of the art

Sabrina Burattini,[1,2] Elisabetta Falcieri[1]

[1]Department of Biomolecular Sciences
[2]School of Cultural Heritage Conservation and Restoration, University of Urbino Carlo Bo, Italy

Corresponding author:Prof. Elisabetta Falcieri, Department of Biomolecular Sciences, University of Urbino Carlo Bo, Via Ca' Le Suore 2, 61029 Urbino (PU), Italy.
Tel. +39.0722.304284.
E-mail: elisabetta.falcieri@uniurb.it

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SUMMARY

The aim of this review is to analyse and report the importance of scanning and transmission electron microscopy applied to cultural heritage. Even if a lot of work has been carried out by chemical and micro-analytical methods, the contribution of the morphological study has been only recently focused. In particular, scanning electron microscopy (SEM), requiring easier specimen preparations, has been widely applied to the study of ancient wood, textiles, metal or lithic objects, giving precious information on their surface details, manufacturing or possible contaminating agents. On the other hand, transmission electron microscopy (TEM) analysis, more difficult due to sample embedding and sectioning, can provide important details mainly on wood and textile samples. Both techniques are becoming progressively more useful for both cultural heritage study and diagnosis

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Introduction

Archaeology, humanities, history, painting and sculpture have been studied for long by chemical, physical and biological analytical methods. In particular, the different samples, such as historical texts, manuscripts, paintings, sculptures, metal manufacts, gems, ancient coins, ceramics, stones and building surfaces, have been the objects of numerous studies, aimed at their characterization in terms of chemical and physical composition, as well as morphology and structure.

The choice of the analysing technique or of the complex of methods to utilise in the study of cultural heritage objects depends on the material a specific sample is made of and on the objective of its study. Three basic types must be considered: samples made of inorganic composites (stone, ceramics, glass, gems, metals and metal alloys), those formed by organic materials (parchment, paper, cardboard, wood, tissues, plants, bones, ivory or pigments) and those composed by organic and inorganic materials. Archaeological objects can be analysed in order to identify the composition of the materials, the fabrication technology, the eventual geographic areas of origin and, possibly, ancient populations life customs and style. The recovery of samples and their conservation are very important aspects and much care must be addressed to their preservation from the destructive effect of the environment, in particular from the action of bacteria, fungi or lichens (Montanari *et al.*, 2012; Sterflinger and Pinar, 2013; Caselli *et al.*, 2018). All these concerns suggest the opportunity, in cultural heritage study, to involve interdisciplinary scientific teams and to apply a multiple technical approach, both for the intrinsic characterization of the sample, and for diagnostic purposes. The morphological investigation appeared more recently, as well as the standardisation of specimen preparation techniques, quite delicate and necessarily different in the various conditions and object typologies (Carl *et al.*, 2014). A crucial point was also sample size, that, differently from chemical analysis, had to be reasonable, to allow a specimen orientation, finalized to its surface analysis or, eventually, sectioning (Nicolopoulos *et al.*, 2018).

Scanning electron microscopy

Scanning electron microscopy (SEM) is a well-known and consolidated method to describe in detail the surface of samples of both material and biological origin (Lee *et al.,* 2012; Kashi *et al.,* 2014; Moropoulou *et al.,* 2019). It has been applied to a variety archaeological objects or to similar experimental models (van Hoek *et al.,* 2001; Daniele *et al.,* 2007), and it is now used with powerful and innovative additional technologies which allow to enrich morphological information with chemical, physical and structural details (Jroundi *et al.*, 2017; Khan *et al.*, 2020).

Conventional SEM has been used since many years in the field of cultural heritage (Vasiliev *et al.*, 2016; Moropoulou *et al.,* 2019). Preferably, and to make it more resistant to subsequent treatments, the specimen must be chemically fixed and, if necessary, dehydrated. Before SEM observation and dependently on its composition, the sample can be carbon-coated and/or gold-sputtered (Heu *et al.*, 2019). Detection and characterization of specific microorganisms responsible for serious contamination of cultural heritage objects have been possible by means of this morphological approach, as demonstrated by numerous scientific reports.

Cultural heritage wood samples have been studied by a variety of authors (Blanchette *et al*., 2000; Timar *et al*., 2012; Kim *et al.*, 2018) and biodeterioration mechanisms, mostly due to fungi and bacteria, have been well characterized by SEM (Liu *et al*., 2017), also correlated to interesting and comparable experimental models (Chung *et al*., 1999; Hamed *et al*., 2012). A multidisciplinary diagnostic approach is frequently needed to evaluate the state of preservation of ships, furniture, foundation piles, in relationship with the different eras of origin and the peculiar species of wood. SEM morphological studies, when integrated with physical and chemical analyses, further provide a reliable evaluation of the preservation state (Macchioni *et al*., 2012).

A good contribution was given by SEM to the study of cultural heritage textile samples, with particular attention to their specificity (*e.g*., linen, jute, cotton, hemp, wool), fibre spatial organization (Singh *et al*., 2009; Teodonio *et al*., 2016) and, again, possible contamination. Experimental models (Bicchieri *et al*., 2019; Pinzari *et al.*, 2020) allowed the characterization of the different organic and inorganic components associated to material biodeterioration products, as well as the presence of contaminating microorganisms. In addition, conservation strategies of textiles in archaeological excavations could be highlighted by SEM specimen analysis in the different procedure steps (Ahmed *et al*., 2018).

Many metal ancient objects have been studied by SEM too. This approach represents indeed an important and fascinating possibility to have information about materials specificity, manufacturing processes and usage. When SEM is equipped with energy dispersive spectrometers (EDS) or wavelength dispersive spectrometers (WDS), the composition of the alloys, their phases and inclusions can be also identified (Piccardo *et al.*, 2013; Ghiara *et al.*, 2019). In particular, coins have been widely studied, because they represent an important information source on social and economic history of people which they are related to (Calliari *et al.*, 2015). In addition, in combination with chemical and metallurgical analysis, their production steps from melt alloy to final coin shape can be highlighted. Roman coins of the imperial ages (Conventi *et al.*, 2017; Di Fazio *et al.*, 2019) as well as middle age ones (Martorelli *et al*., 2018) have been the object of a variety of studies. SEM of metal ancient nails was also carried out and allowed their characterization (Cornacchia *et al*., 2020). More recently, this technical approach has been applied to the study of some cast iron-made street furniture, frequently neglected but very significant, being an artistic witness of the style and manufacturing (de Ruggiero *et al.,* 2018). The morphological study, correlated to chemical analysis, of lithic artefacts used as stone tools (*e.g.*, pestles, axes, millstones, arrows) can provide crucial information on cultural and socio-economic aspects in history and prehistory. In particular, a number of works have been published on obsidian artefacts, widely distributed along Mediterranean coasts and islands (Acquafredda *et al.*, 2019). Both of archaeological and geological interest are a number of researches (Mini *et al.*, 2016) addressed at the characterization of stones utilized in the production of containers for food cooking, as well as for melting pots, ovens and other tools. Biological colonization, in the relationship to deterioration state of ancient walls, bricks and cements, has been the object of a number of works, in which SEM, together with X-ray diffractometry and EDAX microanalysis, represented the most important technical approach (Franchi *et al*., 2017; Zhao *et al.*, 2019). Together with X-ray fluorescence, SEM has been diffusely used to study painting techniques in pictures of different eras. These non-invasive or micro-invasive methods allow in fact to identify pigments and various components of specific pictorial layers (Volpin and Fedrizzi, 2017), as well as artist's *modus operandi* (De Luca *et al*., in press). Materials utilized in plastering of canvas manufacts have been also investigated by SEM (Burattini *et al*., 2014; Bader *et al.*, 2014).

Transmission electron microscopy

Transmission electron microscopy (TEM), even if used since long in biomedical and material sciences (Salucci *et al*., 2017; Giordano *et al*., 2019), is relatively poorly utilized in cultural heritage (Reza *et al*., 2015). Nevertheless, it gives, differently from SEM, the possibility to investigate the inner part of the specimen, in terms of pure morphology but also of structure and chemical composition, when additional techniques as cytochemistry or immunocytochemistry are used (White *et al*., 2011). To access the core of the sample, its thin sectioning is required (Wineya *et al*., 2014; Miranda *et al*., 2015), which must be obtained in block or, more frequently, preceded by a resin embedding aimed at its convenient hardening and plasticity (Belu *et al*., 2016). Therefore, because of the difficulty of procedures, its use is relatively recent in cultural heritage. Wood has been occasionally investigated by TEM and technical procedures have been reported by some authors. Wood samples can be sectioned at cryogenic or at room temperature (Reza *et al*., 2014), as well as stained with the most common TEM staining solution. Wood ultrastructure is now relatively well known. Nevertheless, little has been carried out on archaeological objects. Bacterial decay has been investigated in

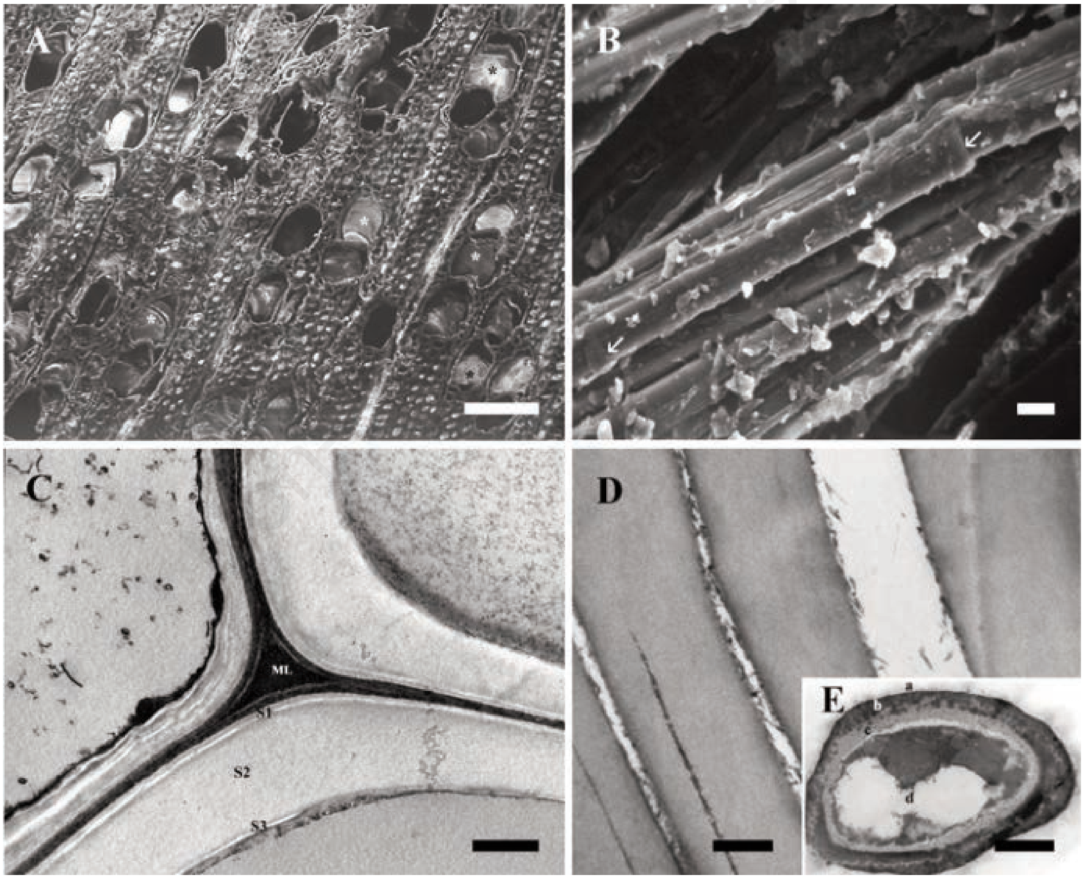

Figure 1.Wood (A,C) and textile fiber (B,D,E) samples observed at SEM (A,B) and TEM (C,D,E). In A a poplar wood fragment is visible, showing single or grouped tracheae. Some of them contain tylose (*), a sugary substance occcluding *vasa* after a trauma. Linen cilindric fibers appear in B, presenting, if longitudinally observed, peculiar bulges called knots (↗). In a cross section of poplar wood specimen (C) the primary wall (S1), the secondary one (S2, S3) and the lamella mediana (ML) appear at TEM. D and E show cotton fibers in longitudinal and cross section, respectively. In (E) the external cuticle (a), the primary cell wall (b), the secondary one (c) and the central canal can be identified (d). Scale bars: A) 100 µm; B) 50 µm; C) 1 µm; D) 0.5 µm; E) 1 µm.

ancient foundation piles, with particular attention to lignin modification at the initial stages of wood degradation by bacteria (Blanchette *et al*., 1989; Rehbein *et al*., 2013; Bjerregaard Pedersen *et al*., 2014). Conventional TEM, immunolabelling and cryo-SEM have been applied to the characterization of cotton fibres by Singh *et al*., 2009. A case study of green and white ancient Roman glass tesserae has been reported by Nicolopoulos *et al*. (2018), in which TEM orientation imaging, in combination with 3D precession diffraction tomography, has been utilized. Figure 1 shows some representative images of wood (A,C) and textile (B,D,E) cultural heritage samples, analysed by means of SEM (A,B) and TEM (C,D,E).

Concluding remarks

TEM and SEM application to cultural heritage are becoming an important contribution both to archaeological object study and diagnosis. The scientific literature reports progressively more numerous investigations including electron microscopy, frequently supported by physical and chemical analysis, on historical texts, manuscripts, paintings, sculptures, metal artefacts, gems, ancient coins, ceramics, stones and building surfaces. The fabrication technology, the material composition and structure, the possible geographic areas of origin and even ancient populations customs can so be identified with a growing precision. Finally, a particular role must be assigned to ultrastructural analysis in highlighting object conservation and in the identification of possible, frequent and harmful contaminating agents.

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Figure panel B belongs to the thesis "Il restauro della tela raffigurante la Madonna con il bambino, San Giuseppe e San Giovannino" by Maria Veronica Soro.

Figure panels D and E are a presented in the thesis "La produzione pittorica giovanile di Renato Bruscaglia. Analisi delle opere, indagini diagnostiche, proposte di conservazione" by Micòl Migani.

All these works have been discussed at the end of the courses of the School of Cultural Heritage Conservation and Restoration, University of Urbino Carlo Bo, Italy.

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