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Preface

In the last millennium, the advent of electron microscopy and the constant evolution of histological techniques convinced everyone that these techniques were able to meet any requirement of in vitro *research as regards morphological investigation of the structure and possible alterations of a specific sample.*

The scanning electron microscope, also known as SEM, provides information on the structure, composition and properties of layers' surfaces in solid samples, with a mean resolution of a few nanometers.

SEM was invented in early 30s by the German scientist Ernst Ruska (1906-1988) who developed together with Max Knoll a prototype that had a far more better resolution than an optical microscope. Later, Ruska contributed with Siemens to the implementation of a hi-fidelity well-functioning SEM instrument, although this was created in the first decades of the 20th century.

In the 40s, the instrument was used in biology researches for the first time by the Electron Microscopy Institute of Berlin. In fact, after World War II, the Institute was re-built and carried on studies and researches until 1954, when the first electron microscopy distributed worldwide, in thousands of items, was produced.

The first reliable observations were made on viral sub-microscopic particles placed on thin films, then coloured in negative, starting in 1954.

Since then, electron microscopy has been widely applied to investigate different kinds of structures, from thin cells to tissues and intercellular matrices, in a very short time.

In the 1950s, a new era began for morphologists, the improvement of fixation techniques keeping pace with the development of hi-penetration power resins, able to polymerize in more elastic and resistant support, and with ultra-microtomy, able to section internal parts of samples to investigate, by means of glass and, later on, diamond blades.

At the end of these pioneering years, the use of electron microscopy in diagnostics and researches decreased, since the results obtained through this older technique weren't so satisfactory at high resolutions.

Instead, electron microscopy shows a number of innovative features, compared to more classical techniques. First of all, it does not destroy any type of tissue, resulting in a better preservation of sample architecture and morphology, as well. It also reduces efforts and time in sample preparation, which can be considered an added value.

Histology, widely known nowadays as the study of the microscopic anatomy of cells from different tissues, became a scientific discipline in the second half of the 19th century, thanks to the development of the cell theory by Matthias Jacob Schleiden and Theodor Schwann in 1838. Schleiden recognized the importance of the cell structure as the basic functional unit in biologic organisms and, from this great discovery, started investigating microscopic structures of organs, studying and classifying the different features of cells from different tissues.

In the same years, the nervous tissue was studied, identifying sensory nerves and motor nerves in the spinal marrow, detecting different muscle tissues, describing for the first time the neuron's anatomy, the model of impulse transmission and the structural and functional unit of a neuron itself. However, a very important role in histology was played by Camillo Golgi who, using this technique, contributed to the discovery and definition of the cellular structure named after him.

*In the new millennium, with the developing of new electron techniques and the evolution of dedicated software, object reconstruction by means of data acquired in different ways (*i.e. *X-rays, ultrasounds, etc.), through virtual algorithms for virtual models, has become a useful resource in various fields, and also in biomedical research.*

Human beings generally think in two dimensions, probably following an educational pattern showing images in a non-three-dimensional way. Three-dimensional concept has been recently introduced to represent reality supported by new technologies.

To reduce this problem, over the last 20 years, instruments created to obtain qualitative and quantitative three-dimensional information on images have become ever more user-friendly, compared to more traditional bi-dimensional ones, since human brain needs to be trained to 3D thinking.

This is the main feature of three-dimensional microtomography, an innovative technology reproducing 3D images of a sample that doesn't need to be treated or reconstructed for investigation, providing more reliable results since the sample itself is not altered. Other techniques such as electron microscopy and histology, in fact, usually require to treat the sample before observation, influencing its conditions and providing not so reliable results. Furthermore, to observe internal structures it is necessary to cut samples with predictable consequences, an important issue in current research.

Micro-tomography allows original sample preservation in order to run various tests on the same object and observe, at last, the differences between its initial and final condition. This procedure was impossible with traditional in vitro *tests because it was necessary to construct different sets of samples, for electronic microscopy studies or histological ones.*

X-ray micro-tomography (micro-CT) is a non-destructive non-invasive technique for three dimensional characterization of different materials in medicine, material science, biology and tissue engineering. It is widely used since it can provide ever more effective quantitative and qualitative information on 3D morphology of analyzed samples. Micro-CT is a radiographic 3D imaging technique, similar to conventional computed tomography (CT), used in medical and manufacturing fields, but while CT shows images at a maximum spatial resolution of 1 mm, micro-CT goes down to 1 micron.

Micro-CT is used in many studies in order to analyze the details of the original structure of different materials, biomaterials and devices, focusing on dimensions, shape, internal imperfections of density, total porosity by the distribution and dimension of pores or gaps and their possible interconnections.

Microtomographic imaging and analysis is now one of the most important challenges for observation techniques in biomedical research.

The main objective of Istituto Superiore di Sanità (ISS) in new technologies applied to health is to guarantee the effectiveness of new tools to support in vitro *and* ex vivo *experimental studies, mostly to assure the safety of subsequent* in vivo *clinical trials and applications in humans. At the same time, ISS researchers have the task to study and lead the research, promoting new technologies and encouraging their use after extensive studies.*

In the last few years, with the increasingly spread of X-ray microtomographic technique as a more complete observational investigation with 3D imaging and analysis, it has been necessary to carry out a large amount of studies to improve several medical and biomedical field applications. Thanks to the support provided by various funding from different research projects, it has been possible to start work and obtain the first results from the use of this technique.

ISS research group now is ready to carry out experimental work on different samples, comparing also the results obtained with other traditional techniques (SEM, histology, etc.).

In the wake of pioneering studies carried out with different universities of Rome, according to scientific collaboration agreements with engineering and medicine faculties, ISS research group is still involved in current microtomographic investigations in the biomedical field with other Italian universities, European and international research institutions.

By means of this collection of articles published in Annali dell'Istituto Superiore di Sanità *monograph, authors' intention is to show the large amount of work developed in different research studies.*

The first article of this issue suggests the precious contribution of the microtomographic technique to tissue engineering, providing three-dimensional information about scaffold structure characterization during research and development phases.

The second article aims at showing, qualitatively and quantitatively, the trabecular tissue structure in different pathologies that affect the bone. This study, about the characterization of human bone, is carried out within the framework of a scientific collaboration agreement between ISS and the Faculty of Engineering, Sapienza University of Rome.

The third article is on the use of the 3D microtomographic technique in displaying a new and more precise morphology of dental root canals, suggesting the future goal of building a new atlas to learn more about tooth's internal structure.

The usefulness of the 3D microtomographic analysis to evaluate the efficiency of new endodontic treatment techniques compared with old ones is explained by Angerame et al. *article.*

The article by Sinibaldi et al. *points out a new and* ad hoc *software program developed to explore and reconstruct the internal structure of a complex human anatomy as tooth root canals.*

A comparison among three different laboratory instruments based on the same 3D microtomographic technology, that is spreading in dental clinical practice, has been shown in another article of this monograph by a European group. The obtained results have been performed evaluating root canals of monkeys' molars.

Another important topic of ISS research group working with microtomography is implantable medical devices. The seventh article evidences the reliability, up to a resolution of a few microns, of different connections of internal dental implant geometrical designs by 3D measurements and evaluation.

The next article reports a very important and interesting study carried out with 3D microtomographic technique on bone and biomaterials. A morphometric characterization example of a biomaterial implanted in human bone is described in the article by Meleo et al. *titled "Microtomographic and morphometric characterization of a bioceramic bone substitute in dental implantology".*

The following article by Bedini is on the results of fatigue wear testing on agonist/antagonist teeth. Sample-specific features and alterations in different couples of natural vs prosthetic teeth and natural vs *natural teeth have been assessed by 3D micro-CT before and after mechanical testing. Some prosthetic tooth devices subjected to biomechanical fatigue wear testing have also been analyzed and compared to human tooth samples.*

The two-fold feature of the non-destructive microtomographic technology is not only to obtain 3D structure imaging and analysis of an object but, whenever possible, to investigate expected structure alterations induced by various type of stress on the same sample. Using this approach, the problem raised by the preparation of a lot of sample, as needed for traditional observation analysis, has been overcome.

Another application of the microtomographic technique is described in the article proposed by Sawicka et al.*, for the assessment of root resorption damage due to orthodontic stress mainly on the teeth of young patients.*

A contribution from one international group that widely uses the microtomographic technique is given in the article by Perilli, from University of Adelaide, Australia. This author proposes a high-resolution 3D microtomographic analysis of a human vertebra by means of a high-performance in vivo *micro-CT instrument for preclinical research.*

The last article aims at showing an application for displaying and quantifying possible gaps among composite-adhesive materials and dental tissue in conservative dental restoration procedures. Today, this topic is poorly represented in literature but the results obtained seem to be very encouraging to continue carrying on this study on a large amount of samples for a statistical validation.

In this monograph, all articles are a source of contribution in the scientific debate on the use of 3D microtomography as a new approach in observational techniques, compared to traditional analytical methods.

More specifically, the final goal of this collection of articles is to support the use of the microtomographic technique in the biomedical field, and to show the role of the ISS group, that is doing a great research work in Italy in collaboration with several national universities and also some European and international groups.

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