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Present and future in the use of micro-CT scanner 3D analysis for the study of dental and root canal morphology

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Summary. The goal of the present article is to illustrate and analyze the applications and the potential of microcomputed tomography (micro-CT) in the analysis of tooth anatomy and root canal morphology. The authors performed a micro-CT analysis of the following different teeth: maxillary first molars with a second canal in the mesiobuccal (MB) root, mandibular first molars with complex anatomy in the mesial root, premolars with single and double roots and with complicated apical anatomy. The hardware device used in this study was a desktop X-ray microfocus CT scanner (SkyScan 1072, SkyScan bvba, Aartselaar, Belgium). A specific software ResolveRT Amira (Visage Imaging) was used for the 3D analysis and imaging. The authors obtained three-dimensional images from 15 teeth. It was possible to precisely visualize and analyze external and internal anatomy of teeth, showing the finest details. Among the 5 upper molars analyzed, in three cases, the MB canals joined into one canal, while in the other two molars the two mesial canals were separate. Among the lower molars two of the five samples exhibited a single canal in the mesial root, which had a broad, flat appearance in a mesiodistal dimension. In the five premolar teeth, the canals were independent; however, the apical delta and ramifications of the root canals were quite complex. Micro-CT offers a simple and reproducible technique for 3D noninvasive assessment of the anatomy of root canal systems.

Key words: microcomputed tomography, three-dimensional imaging, root canal anatomy.

Riassunto *(Prospettive future e attuali orientamenti nello studio tridimensionale dell'anatomia dentaria ed endodontica con micro-TAC)*. L'obiettivo di questo articolo è stato quello di dimostrare la validità della microtomografia computerizzata (micro-CT) per visualizzare, analizzare e studiare l'anatomia dentaria ed endodontica *in vitro*. Sono stati selezionati 15 differenti elementi dentari umani estratti per motivi ortodontici o parodontali, selezionati tra primi molari dell'arcata superiore con un secondo canale nella radice mesiovestibolare (MB2), tra primi molari inferiori in cui la radice mesiale avesse un cosiderevole grado di curvatura in senso mesiodistale o tra premolari con una o due radici aventi un'anatomia apicale particolarmente complicata. L'hardware utilizzato per la scansione è stato un micro tomografo computerizzato (SkyScan 1072, SkyScan bvba, Aartselaar, Belgium) mentre il software per la visualizzazione e l'analisi tridimensionale dei dati è stato ResolveRT Amira (Visage Imaging). Gli autori hanno ottenuto le ricostruzioni tridimensionali ottenute da analisi micro-CT di 15 elementi dentari umani estratti. È stato possibile osservare dettagliatamente sia l'anatomia endodontica fine che l'anatomia radicolare esterna degli elementi dentari analizzati. Tra i cinque molari superiori analizzati, in tre casi il canale mesiobuccale secondo (MB2) era confluente con il canale mesiobuccale principale, mentre negli altri due campioni dello stesso elemento analizzati essi erano indimìpendenti con forami apicali separati. Tra i molari inferiori due dei cinque analizzati avevano un unico canale nella radice mesiale di forma estremamente appiattita in senso mesiodistale. Tra gli elementi premolari analizzati, i canali se doppi erano indipendenti ma spesso hanno dimostrato possedere una complicata anatomia apicale con delta e ramificazioni molto intricate. La microtomografia offre una tecnica semplice e riproducibile per l'analisi tridimensionale non invasiva dell'anatomia endodontica.

Parole chiave: microtomografia, *imaging* tridimensionale, anatomia endodontica.

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INTRODUCTION

The complexity of the root canal system has always been a challenge for endodontists and researchers. One of the most important study was performed by Walter Hess [1], who injected the root canals with a specific ink and visualized the huge amount of variables and complexities of root canal systems, publishing the first extensive report on this subject. Later, other studies [2-5] have examined the anatomy of root canals in permanent teeth using different methods and techniques. In those studies, however, all the methods were not very detailed and/or caused more or less irreversible alterations to the samples. Some examples of these changes are the preparation of consecutive ground sections on extracted teeth [6-9], rendering the surrounding hard tissues transparent through decalcification after permeation of dyes [10-14] and the removal of all surrounding tissue from casts of the root canals with Wood's metal, celluloid or resin [15-18]. Radiographic techniques also have been used to obtain a two-dimensional image [19-23]. Some of these techniques are complicated and time-consuming, and many difficulties can be encountered during their execution, introducing artifacts and distortion of the internal anatomy of the specimens. Furthermore, these techniques do not allow for the observation of the external and internal anatomy of teeth in three dimensions at the same time. Three-dimensional methods for the morphological study of teeth are replacing the more limited two-dimensional techniques. Historically, several techniques have been described for visualization of the 3D anatomy of root canals in human teeth. This usually has been done by reconstructing the image derived from tracings of the contours from serial cross-sections of the specimens, as the first study cited, published by Hess [9, 24-29]. It must be underlined that in the process of making the sections, the specimens are destroyed, and an accurate image cannot be obtained owing to the thickness of the sections.

Computed tomography (CT) images can be formed from planar slices through objects. These can be physical sections, optical sections or CT reconstructions [30] The development of X-ray computed transaxial micro-tomography, or micro-CT, has gained increasing significance in the study of hard tissues [31-35]. Significant improvements in both software and hardware reduced section thickness from conventional CT ranges (approximately 1.5 millimeters) [36] to those for micro-CT systems: 81 micrometers [30], 34 μm [37] and 12.5 μm [38]. The miniaturized CT technique, with a resolution of 100 μm, has proven to be useful as a nondestructive technique for 3D reconstruction of teeth *ex vivo* [39-42]. It is anticipated that a section thickness of 5 μm may be attainable for *ex vivo* investigations in the near future [39]. The feasibility of clinical CT studies of human teeth was suggested initially by Tachibana and Matsumoto [43] in 1990. Micro-CT has been used to observe the structure of bone [44-47] to measure enamel thickness in teeth [48] and to measure surface areas and volumes of teeth [37, 39, 40, 42, 49, 50]. Root canal instrumentation techniques have been studied using micro-CT [30, 36, 38, 51-56]. Additionally, it has been used for research in restorative dentistry [57, 58]. To date, micro-CT is not available for use in a daily clinical setting; however, the technology of cone beam CT is developing and diffusing rapidly in dental field [59]. Cone beam computed tomography (CBCT) has been introducted to obtain threedimensional information of the maxillofacial district, including the teeth and their surrounding tissues [60, 61]. This technology produces excellent quality with a significantly lower effective radiation dose compared with conventional CT. Periapical disease may be detected with better predictability using CBCT compared with conventional periapical radiographs and the true size, extent, nature and position of periapical and resorptive lesions can be determined. It is possible to study for endodontic diagnosis and treatment plan the presence of root fractures, root canal anatomy aberrations and the nature of the alveolar bone topography [60, 61].

Animal *in vivo* studies have shown micro-CT imaging to be a rapid, reproducible and noninvasive method that produces results comparable with those of histological sections [62] and that 3D analysis of micro-CT images has a high correlation with 2-D cross-sections of periradicular lesions [63]. In addition, micro-CT allows assessment of micro-structural features as well as subregional analysis of developing lesions [63]. Micro-CT has potential application in preclinical *training* of students with regard to tooth morphology and endodontic procedures. The advantage of using this approach is that it can show internal and external dental anatomy and the results of the treatment exercise before endodontic procedures are attempted in the clinic.

The aim of this work was to demonstrate the applicability of micro-CT in the analysis of the macro-morphology of teeth through the use of a 3D imaging software that permits all possible visualization, virtual sectioning, and rendering of the raw micro-CT data, which is an excellent tool for the study and teaching of dental and endodontic anatomy.

MATERIALS AND METHODS

Specimen selection and preparation

We selected 15 permanent teeth for the micro-CT analysis from a pool of extracted teeth. After extraction, the teeth had been cleaned in 5 percent sodium hypochlorite solution for 24 hours, washed under running water, blotted dry and stored in a saline solution. We selected five maxillary first molars, five mandibular first molars and five premolars. The criteria for selection were the following:

- each tooth had to have fully formed apexes, no restorations with intact crowns and no defects or caries;
- the maxillary first molars had to have a second canal in the mesiobuccal root;
- the mandibular first molars had to show evidence of a mesial root that had a significant angle of curvature (± 90 degrees), or the presence of difficult anatomy as isthmuses, communications or recurrent root canals;
- the premolars had to have a single canal with an intricate apical anatomy (numerous secondary canals).

We selected the specimen holder of the micro-CT according to the specimen size and necessary magnification range; throughout our study, we used a specimen holder with a diameter of 15 mm. We fabricated a custom attachment from vinyl polysiloxane for each tooth to exactly fit the specimen and the specimen holder of the micro-CT machine. This attachment allowed a precise repositioning of the specimen in the scanning system along the z-axis (error < 1 voxel) with minimal rotational error (< 1 degree). The analysis of each sample consisted of two stages requiring approximately 4 hours in all: 2 hours for the scanning and 2 hours for the reconstruction procedure.

Scanning of the sample

The hardware device we used in this study was a desktop X-ray microfocus CT scanner (SkyScan 1072, SkyScan bvba, Aartselaar, Belgium). We completed the scanning procedure using 10 watts, 100 kilovolts, 98 micro-amperes, a 1.0 mm aluminum filter and x 15 magnification, resulting in a pixel size of 19.1 µm x 19.1 µm. During acquisition we saved hundreds of 2D projections through 180 degrees of rotation in digital format on a computer disk. To gain a 3D perspective, we then transformed the data stored as projections into new two-dimensional images (axial cross-sections) with a pixel size of 19.1 µm x 19.1 µm and a slice thickness of 13.0 µm. The 3D image is achieved by juxtaposition of 2D images of adjacent slices. A computer software analysis system recorded the data to realize a 2D image of absorption coefficients. The use of a charge-coupled device detector allows the production of images with micrometer-sized resolution. We then stored these data for later use. After completion of the scanning procedure, we replaced the samples in the saline solution.

3D reconstruction

The reconstructed axial cross-sections have a 1024 x 1024-pixel (floating point) format. A typical cycle of data collection for reconstruction contains shadow image acquisitions from 200 to 400 views with object rotation of more than 180 degrees. For the reconstruction of complete 3D objects, a serial reconstruction of axial cross-sections can be used. It consists of one acquisition cycle followed by an "off-line" reconstruction of the complete 3D object in a 1024 x 1024 resolution for a maximum of 1024 layers. Typically, these are cone-beam reconstructions. After the serial reconstruction, axial cross sections of the object, as well as a construction of a 3D object's realistic view with possibilities to "rotate" and "cut" the object model can be displayed on the screen. From the reconstruction results, it is possible to reconstruct 3D objects with the use of an external software ResolveRT Amira (Visage Imaging).

RESULTS

We rendered a 3D reconstruction of example specimens from the pool of the teeth analysed. The 3D models showed, in great detail, the anatomy of root canals from different angles. The maxillary first molars had three roots with four canals. In three of the five cases, the secondary mesiobuccal root canal joined the mesiobuccal root canal, while in the other two cases they were completely separate.

The mandibular first molars had a mesial and distal root. *Figure 1A*, *B* is a 3D reconstruction of a mandibular molar viewed from the apical side

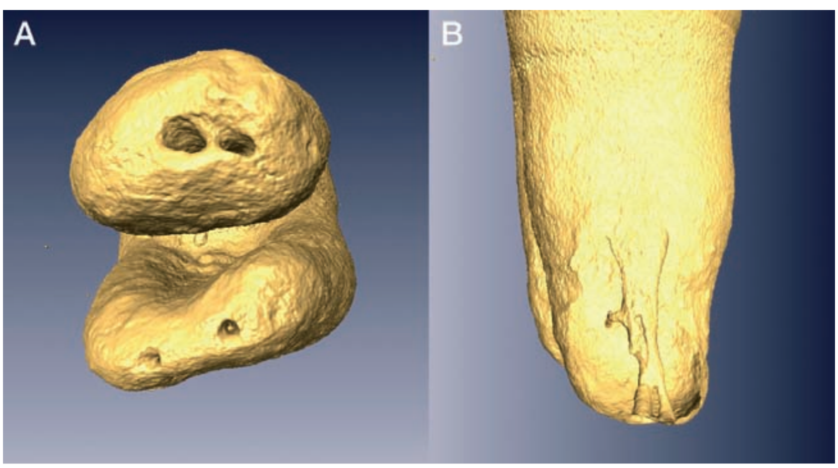

Fig. 1 | *A) Apical view of the apexes of a first lower molar with multiple apical foramina in distal apex. B) Distal root of the same first lower molar sectioned in distal view, it is possible to see the ramification in a double apical foramen of the single distal root canal and a lateral canal that opens in the mesial aspect of the apical third of this root.*

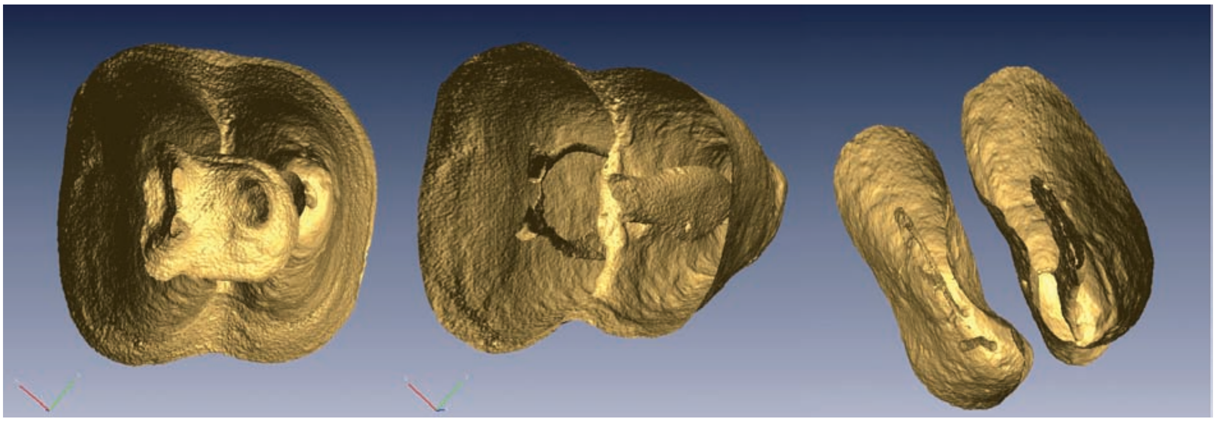

Fig. 2 | *Lower molar sectioned at different levels. From left to right, a section at the level of the pulp floor where it is possible to see the three root canal orifices, a section under the pulp floor where it is possible to note the oval anatomy of the single distal canal and the round shape of the two mesial root canals, a section at the medium-apical level where there is a lateral canal that opens in the mesial aspect of the distal root and he complex ramifications of the mesial canals.*

(Figure 1A), and the distal side *(Figure 1B)*. In *Figure 1B* the root has been virtually sectioned to visualize the complicated apical anatomy of the distal root canal, that has a bifurcation in the last millimeters of its course.

Figures 2A-C represents the same mandibular molar virtually sectioned at the level of the floor of the pulp chamber *(Figure 1A)*, at the medium third *(Figure 1B)* and at the apical level *(Figure 1C)*. The mesial root is characterized by two root canals, which had a curve in the distal and mesiobuccal senses, in the apical third of the distal root, the single flat-ribbon shape root canal has a big lateral canal that opens in the region ofthe furcation *(Figure 2C)*.

In *Figure 3A*, *B* the mandibular molar has been virtually sectioned in a bucco-lingual sense in both distal *(Figure 3A)* and mesial *(Figure 3B)* roots. It is possible in these sections to clearly visualize the anatomy of the root canals in the bucco-lingual view, that is commonly unaccessible in the clinical radiographic projection. The distal canal has an oval orifice that tends to become flat and ribbon shape in the medium third region. This anatomical feature could determine difficulties in the preparation and obturation of the buccal and lingual extensions of the anatomy. Mesial root canals have a curvature in the BL verse that usually is not possible to be visualized in the bucco-lingual verse.

Figure 4 is an example of the 3D imaging, showing one of the specimens among maxillary first molar that were scanned from a buccolingual and a mesiodistal views *(Figures 4A and 4B)*. In this tooth in particular the secondary canal, commonly

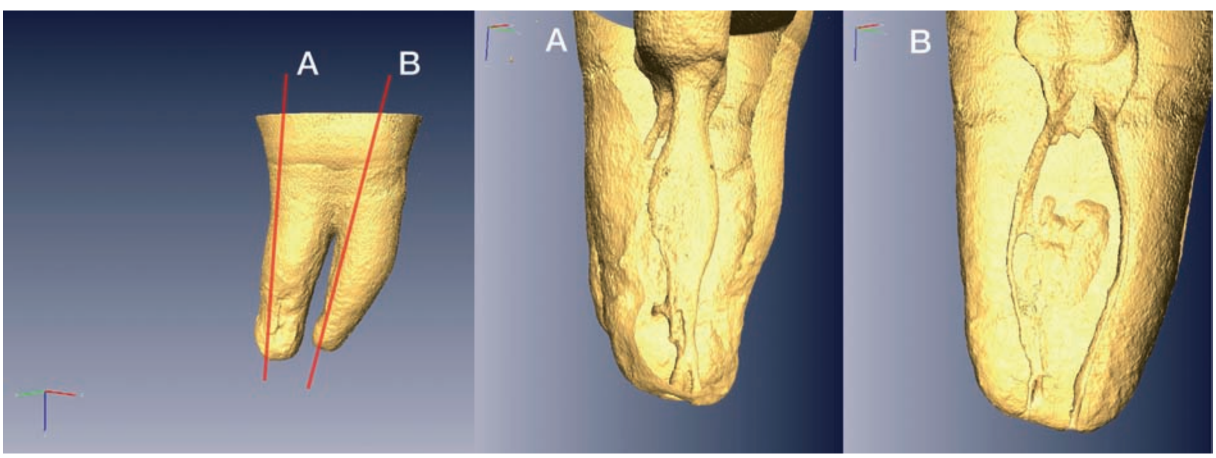

Fig. 3 | *A, B) Bucco-lingual sections of the mesial and distal roots of a first lower molar. In Fig. 3A it is evident the multiple portal of exit of the distal root canal and the shape that tends to be long oval in the medium third. In Fig. 3B it is possible to note the bucco-lingual curve trajectory of the mesio-buccal and mesio-lingual canals.*

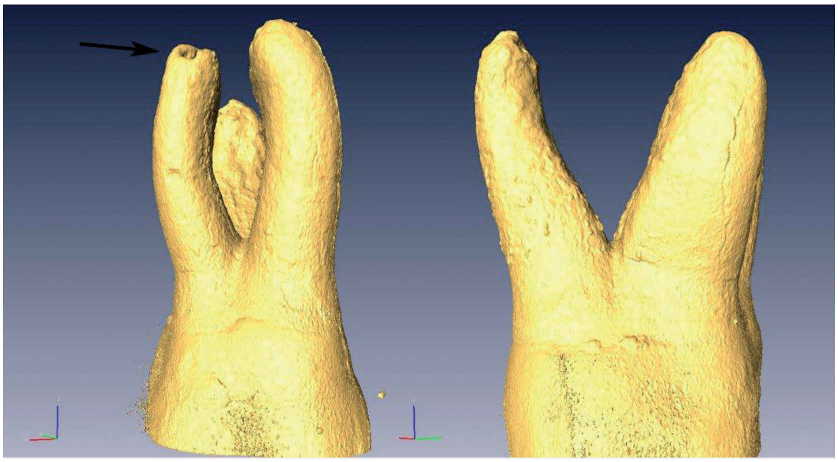

Fig. 4 | *Bucco-lingual (left) and mesio-distal (rigth) views of a first upper molar with three roots. it is possible to appreciate (arrow) the apical resorption of the bucco-distal root, probably due to periapical pathology.*

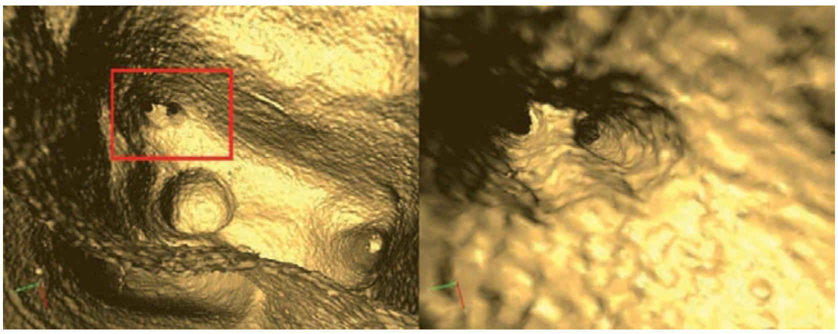

Fig. 5 | *Panoramic and particular of the root canal orifices of a first upper molar, the mesio-buccal and MB2 root canals divides deep in the mesial root, this can determine difficult localization of the MB2.*

named MB2 origins, 2 to 3 mm below the level of the pulpal floor inside the orifice of the main MB root canal *(Figure 5)*. This is a location that often presents a clinical challenge in terms of finding and recognizing the presence of the MB2 and its negotiation to the foramen, which in this case had multiple portals of exit *(Figure 6)*.

Comparing *Figures 6 and 7* it is possible to appreciate how the tridimensional imaging can wonder clinicians about the complexity of the root canal anatomy. In *Figure 6* it is possible to appreciate the extreme complexity of the anatomical interactions between the "so called" mesiobuccal main root canal and the so called MB2. In *Figure 7* it is represented what often is visual-

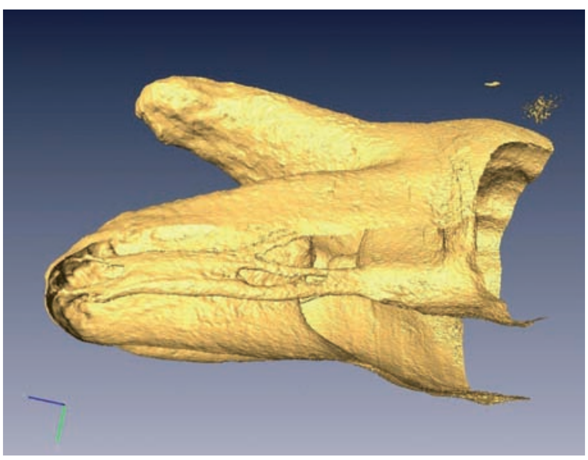

Fig. 6 | *Mesio-distal section of the mesial root of a first upper molar, it is shown the complex anatomy of the mesio-buccal root canal space flattened shape with multiple communications.*

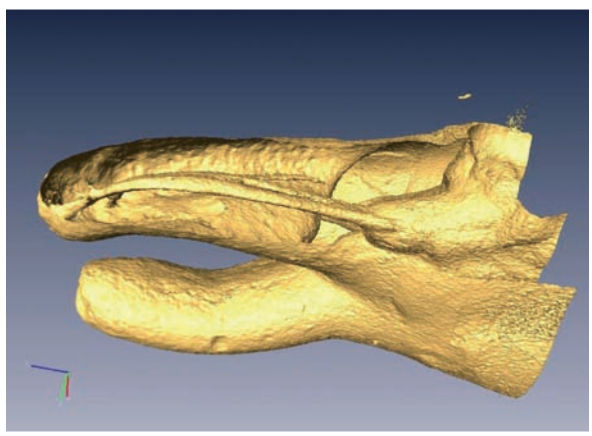

Fig. 7 | *Bucco-lingual section of the same root of Fig. 6, this cut evidences the common radiographic projection, in which it is possible to observe the curvature of the mesio-buccal root canal space, but where the complexity of this extremely difficult root canal space is missed.*

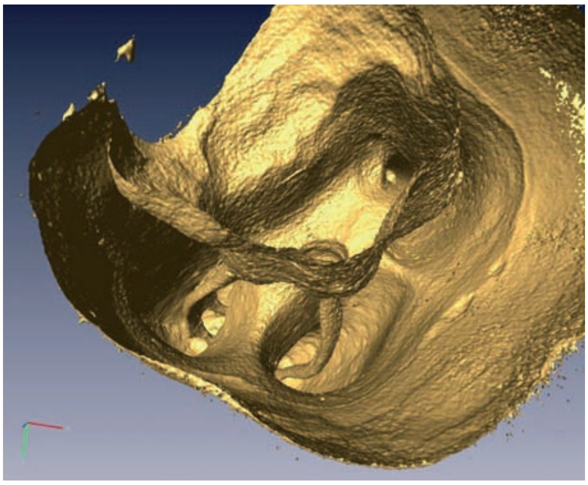

Fig. 8 | *Coronal view of the buccal roots of a first upper molar, in which it is possible to appreciate their convergent curvatures.*

ized by clinical radiology, a single entity that could be a single or multiple root canals, often intended as a circular tube that runs from the orifice to the portal of exit located in the area of the apex. The reality is more complex because despite the presence of two orifices, the pulp space in the mesiobuccal root can be defined as a unique space with multiple interactions and ramifications that is complex to be defined as a "canal" entity.

Figure 8 shows the curves in the space of both the distal and mesial buccal roots.

Figure 9 is a separate rendering of the root canal system of the palatal root canal seen by the inside and outside views. The extremely complex apical anatomy, as shown in the example figures selected for this paper publication represents a considerable challenge when cleaning, shaping and three-dimensionally filling the root canal system are required for endodontic treatment. The presence of an apical delta is not an uncommon occurrence, even in a single, straight root canal. The presence of an apical bifurcation and four separate apical foramina with multiple communications can further complicate all phases of endodontic treatment. What these samples have in common is the presence of even the smallest details, all reproduced from the original anatomy, thus demonstrating the ability of the micro-CT technique to reproduce three-dimensionally the internal and external anatomical features of teeth.

DISCUSSION

One of the most used system to evaluate and compare pre- and post-operative endodontic treatment, has been the Bramante technique, original or slightly modified [24-29], which allowed to visualize the anatomy of root and root canals. With this technique the authors made serial cross-sections of teeth which were traced in the contours, of the pulp cavities and of the canals of each section. Images were analyzed and measured, in most cases by sumperimposition procedures. More recently, they also entered the data into a computer to produce a 3D image. However, owing to inherent limitations of the technique, such as the width of the slices (0.5-0.7 mm), detailed observation was not possible. In a study in which investigators used histologic slides to reconstruct a 3D image, models of dentin and the root canal systems were produced [28].

In the authors' opinion, however, the above mentioned methodology is not precise and detailed, mainly due to the low resolution of the acquired data. An alternative method fan beam CT was introduced. Gambill and colleagues [36] produced CT images of teeth to compare three endodontic instrumentation techniques. They constructed the images from 1-mm slices; however, reproducibility was not consistent along the length of the root canals. Using micro-CT with a pixel size of 127 µm, Nielsen and colleagues [40] demonstrated that it was possible to reproduce tooth anatomy accurately using a noninvasive technique. Shibuya and colleagues [64] reported that the measurements obtained by this method indeed were accurate. Rhodes and colleagues [30] compared the internal root canal space and the external surface area using reconstructed micro-CT images and video-digitized images. They found that there was a highly significant correlation between the micro-CT and video-digitized images. Balto and colleagues [62] and von

Fig. 9 | *Apical view of the apexes of a first upper molar (left) in which multiple portals of exit are present in the surfaces of the three roots and particular of the internal anatomy of the palatal root in which an apical delta is present.*

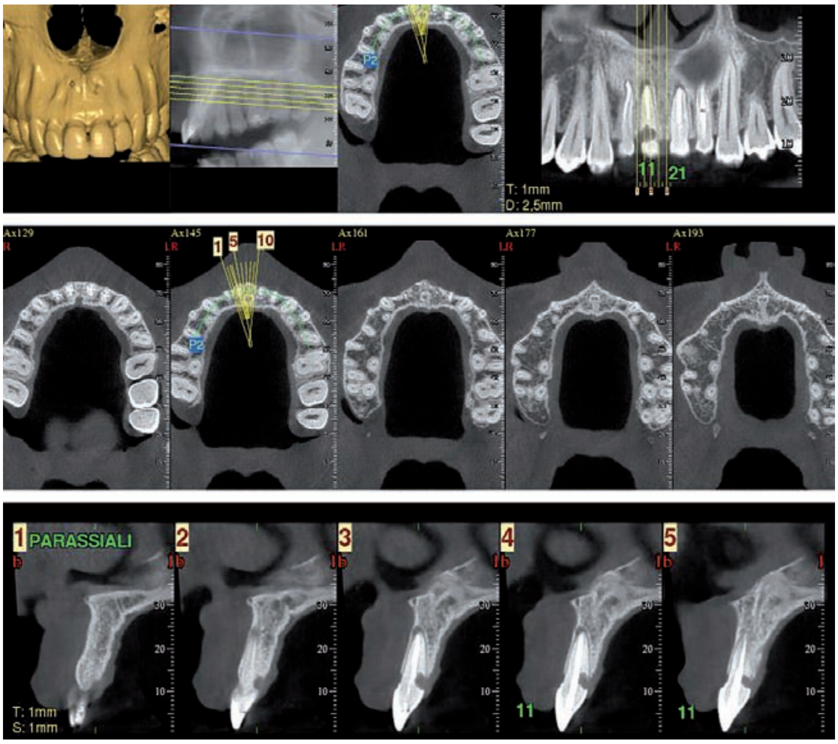

Fig. 10 | *CBCT scan of an upper arch, the root canal anatomy of all the teeth is clearly visible in the coronal sections. The right first incisor presents an external cervical resorption that is localized in the palatal side of the root. In the sagittal sections view the extension of the pathological process is clearly seen.*

Stechow and colleagues [63] demonstrated *in vivo* that micro-CT imaging yields results that have a high correlation with data obtained from histology. The nondestructive approach in our study made it possible to achieve a 3D analysis of the external and internal macro-morphology of the root complex using a spatial resolution of 13 µm between tomographic slices. It appears that this method is a highly useful tool for studying the external and internal anatomy of teeth. Threedimensional knowledge of root canal anatomy is of great importance, since it allows the transfer of information obtained from laboratory experiments to a clinical setting. One of the advantages of this method is that the dentist can observe the internal anatomy of teeth from different angles. In a pilot study, it was possible, by rotating the sample 360 degrees, to qualitatively assess the effect of root canal instrumentation in molars. Furthermore, it was possible to tilt and rotate the image while areas of interest were magnified. All of these possibilities can be important for clinicians, because through proper use of light, color and texture, a better understanding of dental anatomy as a whole can be achieved. Root canals can be imaged separately or with the tooth superimposed, thus showing the orientation of root canals within a tooth. Not only can this technique be a useful educational tool, it can also have far reaching implications for clinical dentistry. Though we have not discussed this possibility in this article, it is also possible to obtain an animation, showing movement of each tooth around all of its axes. This proved to be a helpful method of visualizing tooth morphology. Several published studies in the dental literature discuss areas that may benefit from micro-CT. For example, the morphogenesis of carious lesions and the development of subjacent tertiary dentinogenesis [53] have been examined and interpreted on the basis of invasive 2D data [65-67]. The technique reported here is not suitable for clinical use, but CBCT systems have been introduced for imaging hard tissues of the maxillofacial region [68]. CBCT is capable of producing submillimeter resolution (ranging from 400 µm to as low as 125 µm) with images of high diagnostic quality. The scanning times (10-70 seconds) and radiation dosages reportedly are as much as 15 times lower than those of conventional CT scans.

Although the CBCT principle has been in use for almost two decades, only recently have affordable systems become commercially available. An increase in availability of this technology provides the clinician with an imaging modality that is capable of achieving a 3D representation of the maxillofacial region with minimal distortion *(Figure 10)*. These systems are promising and eminently more suitable than micro-CT scans, which are limited to *ex vivo* applications only and are not suitable for patient care. Nevertheless, micro-CT is a powerful tool for research and preclinical education in fundamental procedures of endodontic treatments, as well as for clinicians and researchers who desire to study dental anatomy in great detail. Micro-CT offers exciting potential; however, current imaging times (two hours for scanning a sample and two hours for the reconstruction) are long. The equipment is expensive, and the 3D reconstruction requires a high degree of computer expertise.

CONCLUSIONS

The data for the present study clearly show that micro-CT offers a noninvasive reproducible technique for the 3D assessment of root canal systems and can be applied quantitatively as well as qualitatively. Due to the fact that CT is nondestructive, it is possible to analyze root canals before, during and after endodontic instrumentation. Internal and external anatomy can be demonstrated simultaneously or separately. A significant amount of information can be gleaned from the scans and slices can be recreated in any plane, while data can be presented in 2D or 3D images. Therefore, its use is not limited to root canal morphology, but can be widely used in dentistry. Applications to restorative dentistry and other areas are now becoming extensive. In the endodontic area, the micro-CT technology is an exciting tool for experimental endodontology and can produce detailed informative images of the anatomy of teeth. The technique is not suitable for clinical use, but it can become a powerful tool for research. It can also allow for better preclinical *training* in fundamental procedures of endodontic treatments, and it gives clinicians and researchers who desire to study dental anatomy in detail and want to investigate the effect of procedures on the endodontic space a new means of doing so.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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