Original Research
Endodontics

How does nano-focus computed tomography impact the quantification of debris within the root canal system?

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2023.vol37.0059

Submitted: March 1, 2022
Accepted for publication: January 5, 2023
Last revision: March 2, 2023

Abstract: The aim of this study was to compare the quantification of hard-tissue debris by using micro-computed tomography (micro-CT) and nano-focus computed tomography (nano-CT) after root canal instrumentation. Ten mandibular molars containing an isthmus in the mesial root were scanned in a SkyScan 1172 micro-CT device with a voxel size of 12.8 µm and in a NanoTom nano-CT device with 5.5 µm. The mesial root canals were irrigated with 5 mL of saline solution at the orifice level, instrumented with Reciproc R25 files and a second scanning was performed by micro-CT and nano-CT devices for post-instrumentation images. DataViewer software was used for registering the pre- and post-operative micro-CT and nano-CT images. The root canal and the debris were segmented for quantitative analysis of the volume of the canal and volume of debris using CTAn software. Statistical analysis was performed using the T test for comparison between volume of the canal after instrumentation and volume of debris in both image modalities. The level of significance was set at $p < 0.05$. Nano-CT images showed higher values of debris when compared with micro-CT ($p < 0.05$) after root canal instrumentation. No difference was observed between the volume of the root canal after instrumentation in the two imaging methods used ($p > 0.05$). Nano-CT technology can be recommended as a more precise method for quantitative analysis of hard-tissue debris. Moreover, in Endodontic research it is a promising method, as it is capable of providing higher spatial and contrast resolution, faster scanning and higher image quality.

Keywords: Anatomic Variation; Endodontics; Root Canal Preparation; X-Ray Microtomography.

Introduction

Chemomechanical preparation of the root canal system is of the utmost importance for successful endodontic treatment.[1] However, accumulated hard tissue debris formed during the cutting action of instruments on the root canal walls may be packed into the anatomic complexities of the root,[2,3] hampering disinfection and complete filling of the entire root canal system.[2-6]

Methods for root canal cleaning have been extensively investigated over the last decades, including evaluation of smear layer and debris

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removal from root canal walls by means of scanning electron microscopy (SEM).[7-9] Nevertheless, SEM is a qualitative score-based method[10] which uses 2D projections[11] and its use is limited to single-rooted teeth with straight roots.[12] Moreover, it provides a limited view of the location and distribution of the debris into the system.[5,8]

Micro computed tomography (micro-CT), has been widely used for three-dimensional (3D) assessment of root canal surface cleaning, of all types of teeth, including those with complex anatomy.[2,3,4-6,12-16] In contrast with SEM, micro-CT is a non-destructive method that allows the sample to be evaluated before and after treatment.[2,12] With the aid of dedicated software for image analysis, it is possible to obtain quantitative data by measuring the volume of ultra-small structures such as debris formed after root canal instrumentation.[5,10]

Nevertheless, for accurate visualization and quantification of ultra-small structures, aspects such as high spatial resolution and contrast are required.[17] Therefore, nano-focus computed tomography (nano-CT) systems have become the gold standard imaging modality for visualization of bone structure or voids in filling material.[17-19] In comparison with micro-CT devices, they achieve a maximum spatial resolution in the submicrometer range[20,21] since they have smaller focal spot and voxel sizes and increased signal-to-noise ratio (SNR), tube voltage and tube power.[20,21] These devices also provide images equivalent to histological sections, revealing differences between calcified and noncalcified structures,[19-22] with the advantage of lower scanning time duration than micro-CT.[19] Moreover, nano-CT devices are equipped with flat-panel detectors (FPD) that generate image quality that is superior to that of charge-couple (CCD) devices, providing distortion-free images, high sensitivity to X-Rays and excellent contrast resolution.[23]

Nano-CT has been widely used in biomedical and biomaterial sciences,[11,21,22,24-27] and its research applications are emerging in different fields of dentistry.[18,28-33] It is noteworthy that Nano-focus Computed Tomography is the gold standard in the field of Operative Dentistry,[28] on the other hand, needs to be more explored for laboratory studies in Endodontology. To date, there is no study using nano-CT images for assessing the quality of root canal cleaning relative to debris quantification.

Therefore, the aim of this study was to explore the potential of 3D nano-focus-CT as a tool for quantitative assessment of hard tissue debris formed after root canal instrumentation, in comparison with micro-CT as the validated method. The null hypothesis was that there would be no differences between nano-focus-CT and micro-CT for debris analysis.

Methodology

Tooth selection

This study was approved by the local Research Ethics Committee (protocol 86782418.5.0000.5417). Human mandibular molars, extracted for reasons not related to this study and stored in 0.1% thymol solution, were scanned in a micro-CT device (SkyScan 1174; Bruker, Kontich, Belgium) with 22.9 µm voxel size, 50 kV, 800 µA and 1.0 step rotation, in order to search for mesial roots containing isthmus. Teeth with incomplete rhizogenesis, internal or external resorption, root fractures, C-shaped or calcified root canals and endodontic treatment were excluded. For the sample calculation the G*Power v3.1 for Mac (Heinrich Heine Universität Düsseldorf) was used and the Wilcoxon-Mann Whitney test of the T test family was selected. Data acquired in a previous study that evaluated the detection of isthmus in mandibular molars was used,[34] and the effect size in the present study was established (= 1.36). The alpha type error of 0.05, a beta power of 0.80, and a ratio N2/N1 of 1 were also stipulated. A total of 9 samples per group were indicated as the ideal size required for noting significant differences. Considering 10% of risk of sample loss, 10 teeth with mesial roots with type I canal, according to Vertucci´s[35] and connected by a single and continuous isthmus, i.e., type V according to Hsu & Kim[36] were selected.

Micro-CT and Nano-focus-CT pre-operative images

Subsequently, the teeth selected were scanned in a high-resolution micro-CT device (SkyScan 1172, Bruker, Kontich, Belgium) to acquire pre-operative images. The scanning parameters were set at 100 kV, 100 µA, 360° of rotation, 0.5 mm Al filter, 0.7 step rotation and an isotropic voxel of 12.8 µm, resulting in a scanning time of 62 minutes. The system included a charge-coupled device detector (2000 X 1332 pixels).

For nano-focus CT imaging, the same teeth were scanned in higher resolution (5.5 µm) using the Phoenix NanoTom S device (GE Measurement and Control Solutions, Wunstorf, Germany), equipped with a flat-panel detector (2304 X 2304 pixels). A tungsten target was used, and voltage and current applied were 65 kV and 320 µA, respectively, with an exposure time of 500 ms and 0.5 mm Al filter. The fast scan mode was used, which is a frame averaging of 1 and no image skip, resulting in a scanning time of 20 min.[19]

Table 1 shows the technical characteristics of micro-CT and nano-CT devices used.

Silicone molds were created to smoothly fit into the sample holder of the micro-CT and nano-CT, to allow the teeth to be scanned in the same position during pre-and post-operative image acquisition in each device.

Preparation of teeth

Coronal access was performed by using 1014 and 3082 diamond burs (KG Sorensen, Cotia, Brazil). The working length was established by introducing a 10-K file until its tip was visible at the apical foramen, and the working length was set at 1.0 mm short of this measure. Tooth apices were sealed with hot glue and embedded in silicone-based polyvinyl siloxane impression material (Zetaplus, Zhermack, Italy), to simulate the effect of apical gas entrapment in a closed canal system during root canal preparation.[13]

The root canals were instrumented with Reciproc R25 files (VDW GmbH, Munich, Germany) in reciprocating motion driven by an electric motor (VDW Silver; VDW GmbH, Munich, Germany) in the configuration "Reciproc ALL". The instrument was moved in the apical direction using an in-and-out pecking motion of approximately 3 mm in amplitude, with light apical pressure. After 3 pecking motions, the instrument was removed from the canal and cleaned. A single operator with expertise in performing root canal treatment with the use of reciprocating techniques, performed all the preparations. During instrumentation, the canals were irrigated at the orifice level with a total of 5 mL saline solution per canal using a 30-G Navitip needle (Ultradent, South Jordan, USA), as described by Leoni et al.,[14] in order to allow debris to accumulate within the root canal system. Then, each canal was slightly dried with one absorbent paper point (Reciproc R25 paper points, VDW GmbH, Munich, Germany).

Table 1. Specifications and parameters of the devices used for debris evaluation.

Variable	Micro-CT	Nano-focus-CT
Current	100 µA	320 µA
Tube voltage	100 kV	65 kV
Tube power (maximum)	10 W	15 W
Scanning time	62 min	20 min
Voxel size	12.8 µm	5.5 µm
Filter	0.5-mm Al	0.5-mm Al
Rotation	360°	360°
Detector	Charge-couple device (CCD)	Flat-panel detector (FPD)
Camera (Image size)	2000 X 1332	2304 X 2304
Spot size	< 5 µm	< 0.9 µm

After instrumentation, the teeth were scanned for the second time in micro-CT and nano-CT devices for assessment of debris formation in the root canal, using the same parameters as described for pre-operative images.

After scanning, the micro-CT images were reconstructed using NRecon software (1.6.9.8; Bruker micro-CT) with a beam-hardening correction of 60% and ring artifact correction of 8, in bitmap (BMP) format. The nano-CT images were reconstructed using the Phoenix datos|x software (GE Measurement and Control Solutions, Wunstorf, Germany) according to the manufacturer´s instructions, and based on reconstruction of previous images, including beam-hardening, ring artifact correction, and optimal contrast limits.

Image analysis

The pre- and post-operative micro-CT and nano-CT images were registered by using DataViewer v.1.4.4 software (SkyScan, Kontich, Belgium), in order to ensure that images were in the same position before and after canal instrumentation.

Afterwards, the imagens were exported to CTan v.1.12 software (Bruker, Kontich, Belgium) for quantitative analysis. The original grayscale cross-sectional images of the roots were processed with an interactive segmentation threshold to separate dentin and debris from the root canal space, by changing the range of gray levels, which resulted in a binary image composed only of black (empty spaces) and white pixels (dentin and debris). The region of interest consisted of the area of the mesial canals and the isthmus and the volume of interest was selected extending from the furcation level to the apex, set by integration of all cross sections. Canal spaces before and after instrumentation were segmented from dentin and co-registered by an automatic superimposition process. Debris in segmented canal spaces was removed using a morphological operation and the intersection between pre- and postoperative images was achieved by a morphological subtraction operation. Quantification of debris was calculated considering the difference between nonprepared and prepared root canal space using post-processing procedures (Figure 1). The total volume of the canal after instrumentation and total volume of debris were calculated and expressed as mm^3.

The normality of data was tested using the Shapiro-Wilk test, and showed that the data were normally distributed. For comparisons between the groups, the unpaired t-test was used. The level of significance was set at $p < 0.05$. Analyses were performed by using GraphPad Prism 5 (GraphPad Software Inc, La Jolla, USA).

Results

No statistical difference was found between the volume of the root canal after instrumentation in the two imaging methods used for evaluation ($p > 0.05$) (Table 2).

Whereas when the volume of debris was compared between micro-CT and nano-focus-CT, statistical difference was found, with higher values for nano-focus-CT ($p < 0.05$) (Figure 2) (Table 2).

Figure 3 illustrates the larger amount of debris detected inside the root canal system in Nano-CT images, compared with images acquired with Micro-CT, in all root canal thirds.

Discussion

Debris formation after root canal instrumentation and its removal have been extensively studied by means of micro-CT imaging with different resolutions in terms of voxel size, ranging from 8.6 µm to 20 µm.[2-4,6,12-15] Recently, no significant difference among 5 µm, 10 µm and 20 µm voxel size was demonstrated to evaluate dentin debris after root canal preparation.[37] Therefore, a voxel size of 12.8 µm was selected for image acquisition with micro-CT in this study. However, there is still a lack of data in the literature about the impact of image resolution on visualization of dentin debris after root canal instrumentation.

The NanoTom S device used in this study has a nanofocus x-ray tube and stabilized ultra-precision mechanisms down to the submicrometer voxel scale. Due to their relatively high voltage and nano-focus spot, nano-CT systems allow high resolution

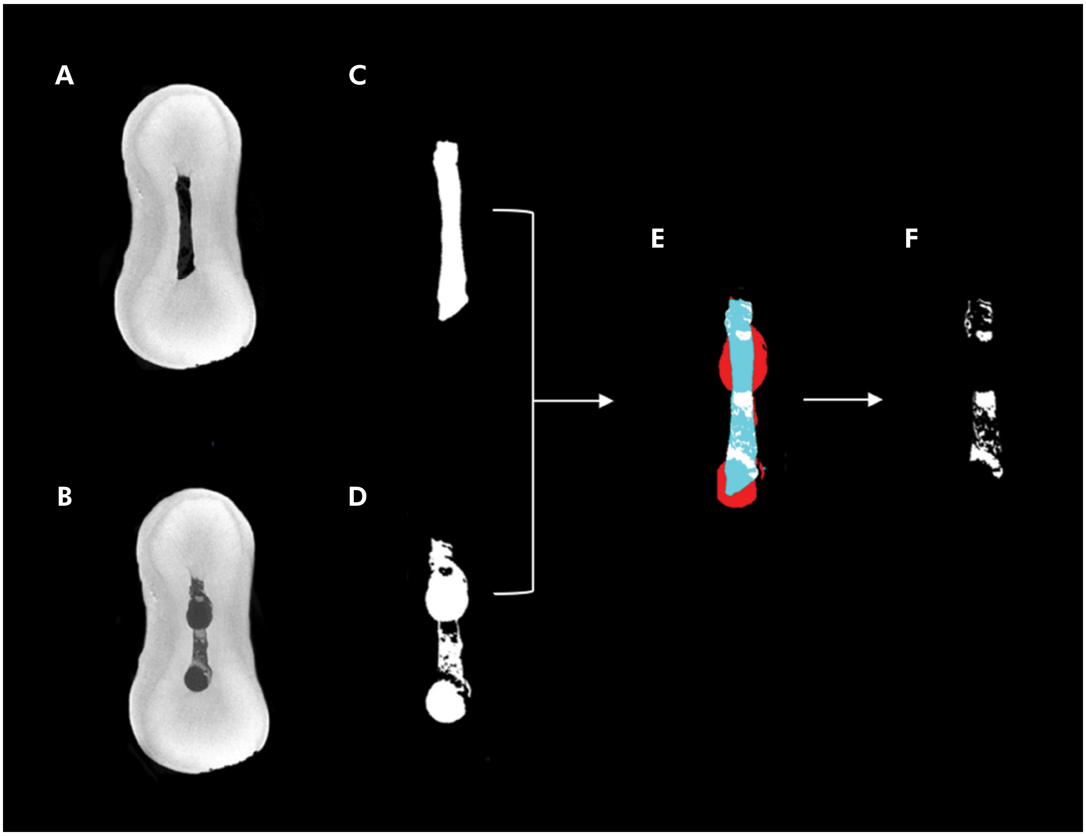

Figure 1. Methodological operations used for image analysis: (a) Nano-CT pre-operative image of a representative sample; (b) Nano-CT post-instrumentation image of same sample; (c) segmentation of root canal (pre-operative image) and (d) post-instrumentation image; (e) superimposition of segmented canal before and after instrumentation; (f) segmented hard tissue debris for volume quantification.

Table 2. Median, minimum, maximum values (mm^3), mean and standard deviation of the volume of canal and debris after instrumentation (Unpaired T-test).

Variable	Canal volume (mm^3)		After instrumentation	
	Median (min-max)	Mean ± SD	Median (min-max)	Mean ± SD
Micro-CT	10.08 (9.09–12.66)^A	10.53 ± 1.28^A	0.90 (0.53–1.25)^B	0.88 ± 0.22^B
Nano-CT	10.72 (9.64–13.18)^A	11.13 ± 1.29^A	1.11 (0.74–2.00)^A	1.19 ± 0.36^A

Different superscript letters in each column indicate statistical differences between groups ($p < 0.05$).

images to be captured with voxel sizes down to 200-300 nm.[21,27,30] Relatively large objects can be scanned in nano-CT devices, however, to achieve a submicrometer resolution the samples must be very small or limited to a small region of interest.[21,27,29,38] One limitation of the present study was that for the purpose of assessing debris in the entire length of the mesial root, it was not possible to apply a nanometric resolution.

Although it was possible to achieve the voxel size obtained in nano-CT scans with micro-CT devices in this study, previous studies have shown that nano-CT displays greater detail when compared with micro-CT.[28,31] Voxel size is crucial to obtain accurate results,[18] however, this is not the only aspect related to the better quality of nano-CT images. The increased power enables greater penetration of the beams into hard tissues, consequently

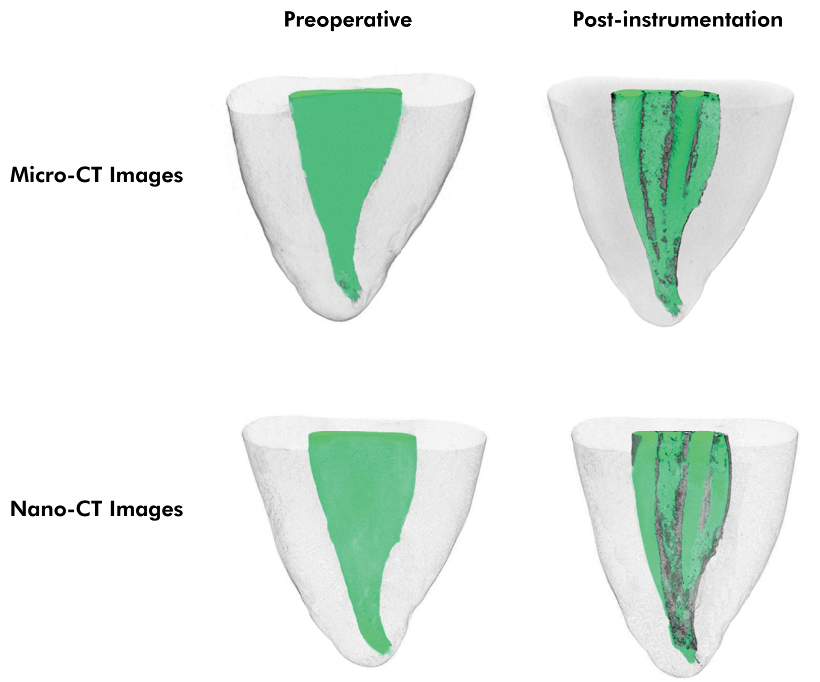

Figure 2. 3D model of a representative sample before and after root canal instrumentation in both image modalities, highlighting the larger amount of debris in nano-CT image compared with micro-CT, mainly in the area of the mesial root isthmus of the mandibular molar. Accumulated hard-tissue debris particles are depicted in black.

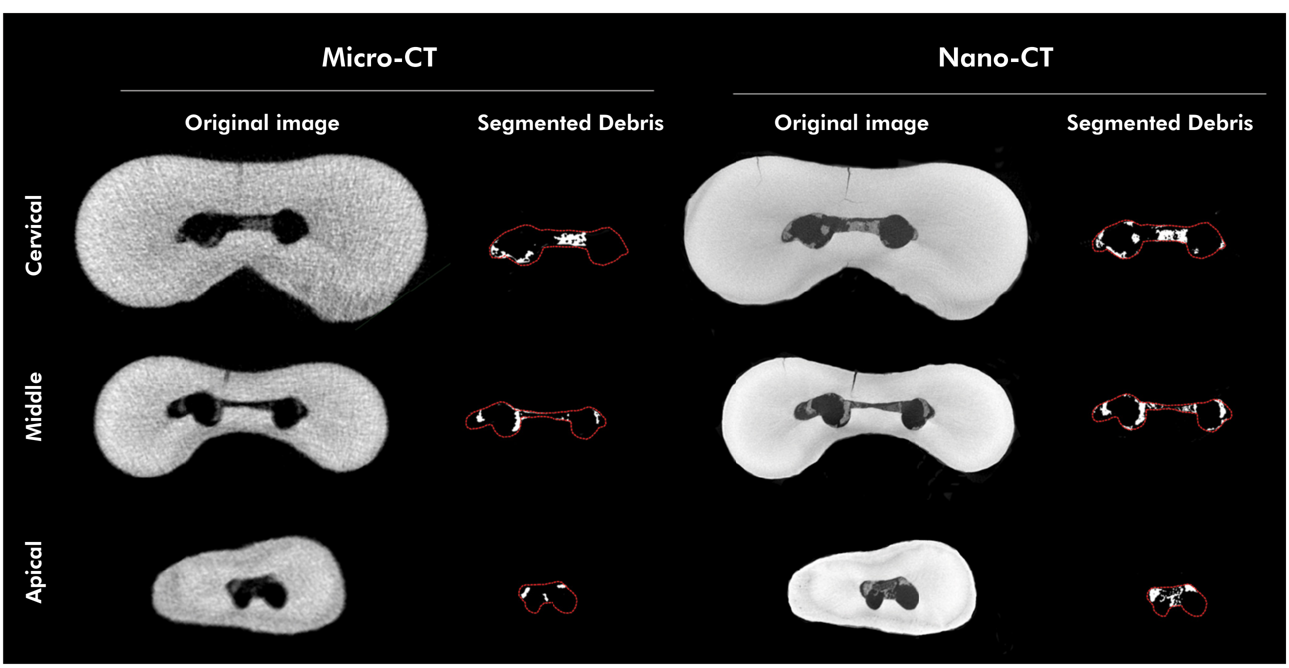

Figure 3. Nano-CT and micro-CT images of a representative sample showing the mesial root and segmented debris after canal instrumentation. Note that in nano-CT images contrast of the image is enhanced and debris can be visualized more clearly. Debris particles were accumulated in all root canal thirds, especially in the isthmus area.

increasing the ability to distinguish between materials and structures.[31] Furthermore, the use of a nano-focus spot significantly improves image quality for samples scanned with voxel sizes of 5–15 microns.[26] In the present study, in nano-CT images acquired with a resolution of 5.5 µm it was possible to identify a larger amount of debris than could be identified in micro-CT images with a voxel resolution of 12.8 µm. Thus, the null hypothesis was rejected. Similar results have been found in previous studies that compared nano-CT imaging with micro-CT. An investigation of external cervical tooth resorption showed that nano-CT images acquired with a resolution of 7 µm were more effective than micro-CT with 15 µm, resulting in more detailed representation of tooth structures, with excellent image quality, comparable with histological images.[19] A voxel size comparison in the detection of voids inside a root canal filling material also showed a higher number of voids when using nano-CT images with a resolution of 5 µm, than using micro-CT images with 11.2 µm.[18]

It should be borne in mind that different spatial and contrast resolutions of tomographic images have higher effects on evaluations of very small structures than on those of larger structures.[39] Our results showed significant difference in debris analysis between micro-CT and nano-focus-CT, while in the analysis of a larger structure, such as root canal volumes, the two scanning methods performed similarly. Structures that are very small, such as particles of debris, may be missed without the use of a high-resolution and contrast enhanced method. The precise location of debris is particularly important in cases of irregularities, such as isthmus since the complete cleaning of the root canal is clinically challenging when these complex anatomical features are present.[40] The accurate localization and quantification of debris by using 3D imaging in endodontic research is of utmost importance to evaluate the efficacy of different cleaning methods and irrigation procedures used in the clinical practice, especially in cases with a complex anatomy. The potential use of the nano-CT imaging in identifying ultra-small structures has been suggested in dental research, since a certain number of voids inside an endodontic filling material have not been detected in micro-CT images.[31] Similarly, nano-CT has shown to be more accurate than micro-CT in revealing micro-porosities in cured dental resin composites.[28]

Another important advantage of the nano-CT system is the possibility of reduced scanning time compared with micro-CT.[26] The fast scan tool available in the nano-CT device used in this study made it possible to perform data acquisition of a sample in only 20 minutes, without losing image quality.[19,22] This is especially important in studies that have multiple steps and require pre and post procedure scans. Micro-CT parameters used for this type of scan usually result in 1-hour scanning time for each sample. Whereas nano-CT images generate larger image volume and consequently their image processing is more time consuming.[18,26,32] In the present study, different steps such as image registration, segmentation and debris quantification were performed during the analysis for both imaging modalities, however handling nano-CT procedures took substantially longer.

Another limitation of this study was selection of the threshold values during the image analysis. This binarization is a manual process that entails processing the range of grey levels to obtain an image formed of black/white voxels only.[18] This process may affect the appearance of the debris and consequently the measurement accuracy in both image modalities used.[41] In view of this fact, the images were carefully analyzed and the threshold values selected for this study were those that most faithfully represented the accumulated hard tissues debris in each image modality.

Although nano-CT has been shown to be a promising tool, especially due to its higher spatial and contrast resolution, faster scanning and higher image quality, the difficult access to this cutting-edge technology and high cost may limit its use in Endodontology. Nevertheless, with new perspectives of contrast agents and staining protocols that allow the visualization of cells and soft tissue components in mineralized tissues of the tooth,[42] further studies are necessary to evaluate the possibility of new applications of nano-CT, such as evaluation of biofilm and bacteria among the debris, or in regenerative Endodontics, since it is able to assess soft biopolymeric scaffolds seeded with stem cells.[11]

Conclusions

In nano-CT images, it was possible to identify a larger amount of debris in comparison with micro-CT images. Therefore, nano-CT imaging can be recommended as a more precise method for quantitative analysis of these ultra-small structures.

Acknowledgements

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico/National Council for Scientific and Technological Development (CNPq) (150046/2018-9).

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