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치의학석사 학위논문

In-depth Morphology Study of  
Maxillary First Molar  
Mesiobuccal Root Canal System  
using Micro-Computed  
Tomography and Clearing  
Technique

상악 제1대구치 근심협측 치근에서의 Micro-  
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Technique를 사용한 형태학 연구 비교

2013년 2월

서울대학교 대학원  
치의학과  
이 기 욱

# In-depth Morphology Study of Maxillary First Molar Mesiobuccal Root Canal System using Micro-Computed Tomography and Clearing Technique

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이 논문을 이기욱 석사학위논문으로 제출함

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# In–depth Morphology Study of Maxillary First Molar Mesiobuccal Root Canal System using Micro–Computed Tomography and Clearing Technique

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## ABSTRACT

### Aim

Although clearing technique is generally considered to be the gold standard in root canal morphology study, it has limitations that complex root canal systems could not be fully revealed.

The aim of this study was to compare the clearing technique with micro-computed tomography (MCT) images using two different reformatting methods for in-depth study of human maxillary first molar mesiobuccal (MB) root canal morphology.

## **Methodology**

Eighteen extracted human maxillary first molar MB roots were scanned for MCT analysis (SkyScan, SkyScan b.v.b.a., Aartselaar, Belgium). Consequently, two-dimensional thin-slab minimum intensity projection images and three-dimensional images of these roots were constructed. Thereafter, all the 18 teeth were processed for clearing technique and then taken photographs of configuration images. One observer classified the images obtained by these three techniques using Weine's and Vertucci's classifications, respectively. The agreement of canal configuration types among three techniques was investigated. The incidence and location of accessory canals identified by these three images were also compared.

## **Results**

Among the three techniques, agreement on Weine's classification was 22.2% (4/18) and disagreement was 77.8% (14/18). Agreement on Vertucci's classification was 33.3% (6/18) and disagreement was 66.7% (12/18). In addition, Weine's type I (Vertucci's type I) configuration was the most frequent in clearing technique whereas Weine's type IV (Vertucci's type VI) configuration was the most frequent in

both MCT techniques. Accessory canals were more frequently observed in MCT techniques than in clearing technique.

## Conclusions

Canal configurations and accessory canals of maxillary first-molar MB roots could be more clearly identified by MCT with 2D and 3D techniques than clearing technique. MCT image can serve as gold standard for in-depth morphology study of complex root canal system.

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keywords: Accessory canal, Canal configuration, Clearing technique, MB root canal, Minimum injected projection, Micro-computed tomography

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# In-depth Morphology Study of Maxillary First Molar Mesiobuccal Root Canal System using Micro-Computed Tomography and Clearing Technique

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# I. Introduction

Knowledge of the anatomy of the root canal system is essential in endodontic success.<sup>1,2)</sup> Maxillary first molars have the most complex root and canal anatomy of the maxillary dentition,<sup>3)</sup> and the mesiobuccal (MB) root often has a separate root canal with separate root canal orifice or root canal ramifications.<sup>4)</sup>

A variety of methods have been used to study the anatomical variation of the complex root canal system. The result of root canal morphology study tends to be influenced by the method used for analysis, such as tooth clearing technique, X-ray, cone-beam computed tomography (CT) and micro-computed tomography (MCT). The most widely used conventional technique for root canal anatomy analysis was the tooth clearing technique. Examination of the transparent teeth with the dissecting microscope allowed the examiner to study in detail the morphology of the root canal system.<sup>5)</sup> However, a clearing technique was an invasive method for the tooth, in that the use of an acidic solution could damage the tooth structures. Another shortcoming of the tooth clearing technique was that it could not visualize small root canals because of limited penetration of dyeing solution into small and delicate root canal systems.

Radiography was another conventional method to study root canal system. Though radiography is a noninvasive technique to study root canal systems, radiographic images are not reliable



in the detection of multiple canals<sup>6)</sup> and lateral canals,<sup>7)</sup> and could not distinguish centrally placed apical foramina from those eccentrically located.<sup>8)</sup>

In recent years, MCT has allowed a detailed three-dimensional (3D) analysis of the internal root canal anatomy.<sup>5,9-12)</sup> MCT study with minimum intensity projection (MinIP) and 3D analysis offer greatly detailed information on the root canal systems. A curved thin-slab (TS), MinIP technique, which provides a two-dimensional view obtained by the ray projected perpendicular to the axis of the target, is an image-processing method used widely in medical research and clinical diagnosis.<sup>13,14)</sup> The curved TS-MinIP technique easily detects low attenuated structures with very small lumen, which were difficult to illustrate with conventional multiplanar reconstruction.<sup>15,16)</sup> In dental field, TS-MinIP imaging technique was first introduced for study of mesiobuccal root canal of maxillary molar. MinIP images could allow observations of the complexity of the root canal structures with greater accuracy and were suggested to be suitable to study root canal systems.<sup>17)</sup>

The aim of this study was to compare the accuracy of two of micro-computed tomographic images (TS-Min IP images and 3D reconstructed images) with those obtained by conventional clearing technique in studying morphology of the root canal system. The null hypothesis was that these three techniques were not different in their accuracy to visualize the morphology of the root canal systems.

## II. Materials and Methods

This study was carried out under the approval of the institutional review board (IRB) of Seoul National University Dental Hospital (ERI 12006). A total of 18 extracted maxillary permanent teeth, which were extracted for reasons unrelated to this study, were randomly collected. Selected specimens were intact and free of fractures, large restorations or gross caries. There was no history of previous root canal therapy. Teeth that had a single canal in the MB root were excluded. The specimens were placed in 3.25% sodium hypochlorite for five minutes, and then the remaining calculus was removed by using curette and ultrasonic scaler. After preparation, the teeth were scanned by micro-computed tomography. After scanning the specimens, teeth were processed to be transparent teeth using conventional clearing technique as described below.

### 1. MCT scanning

#### MCT scanning

The teeth were scanned by MCT scanner (SkyScan 1172, SkyScan b.v.b.a., Aartselaar, Belgium). Acquired image had a pixel size of  $31.8 \times 31.8 \mu\text{m}$ . The distance between each image was  $31.8 \mu\text{m}$ . From the volume of these images, a curved TS-MinIP image and 3D volume images were rendered for inspection of the canal systems.

## **Curved TS–MinIP image construction of MB roots and root canals**

Curved TS–MinIP images of 18 MB roots were constructed using OnDemand3D software (Cybermed Inc., Seoul, Korea) according to the following procedure. The volume image of the tooth was positioned so that the root apex faced upward. The slab thickness was dependent on the size of the canals. In this study, it ranged from approximately 0.5 to 1.0mm. Using the OnDemand3D software, a virtual ray was transmitted orthogonal to the curved slab (from mesial to distal direction), and the smallest gray value was recorded to obtain the MinIP image.

## **3D volume image construction of MB roots and root canals**

From the volume of the images, 3D models were rendered for inspection of the canal systems using OnDemand3D software (Cybermed, Seoul, Republic of Korea). To enhance visualization of the fine root canal structure, segmented volumes of canal structure were represented by an opaque red color and the external morphology of the root was rendered transparent.

## **2. Clearing technique**

Specimens were placed in small glass container and labeled for identification. Access cavities were prepared with a round bur. There was not any mechanical preparation except access

opening. A #10K file was placed in each canal to confirm apical foramen. Distobuccal root and palatal root were resected from teeth using disk to observe MB root directly.

Pulp tissue and organic debris were dissolved from root canal by immersing the teeth in 5% sodium hypochlorite for 12 hours at room temperature. Teeth were placed in running tap water for four hours to wash away remaining organic debris and sodium hypochlorite solution. The specimens were decalcified for five days in 5% nitric acid at room temperature. The nitric acid solution was changed every two days and agitated by hand two times each day. After the acid treatment, the teeth were placed in running tap water for two hours. Decalcified teeth were placed in acetic acid for 24 hours for enhanced dentin matrix. Then, the specimens were sequentially dehydrated in ascending concentrations of ethyl alcohol (70%–12 hours, 90%–6 hours, and 100%–6 hours). Solution volume allocated to each procedure was 40ml. Decalcified teeth were placed in a methyl salicylate solution for 24 hours. After treatment with methyl salicylate, teeth became cleared and hard.

India ink (Winsor & Newton, London, England) was used to visualize the root canal systems. India ink was injected into the pulp chamber with a 27-gauge needle on disposable syringe (KOREA Vaccine Co., Seoul, Korea). The ink then was drawn through the canal system by applying negative pressure at the apical end of the tooth with use of the central suction system. Suction tip was in contact with the root apexes. Excess ink was removed by using ethyl alcohol and brush. Photograph was

taken with a dissecting microscope (Olympus, Tokyo, Japan)

### **3. Comparison of mesiobuccal root canal morphology images obtained by TS Min-IP technique, 3D technique and clearing technique**

#### **Main root canal system classification**

Main root canal systems were compared between clearing technique and two MCT techniques. The classification of root canal configuration systems followed two classifications suggested by Weine and Vertucci (Figure 1).

#### **Accessory canal system**

Location and incidence of accessory canals were investigated in this study. An accessory canal is any branch of the main pulp canal or chamber that communicates with the external surface of the root<sup>1)</sup>. In addition~~al~~ to this definition of accessory canal, other criteria were considered to define main and accessory canals

- 1) Any anatomical structure that branched off from the main canal more than 3 mm upward from the apex with the egress located within the apical 3 mm was defined as another main canal. Others were considered accessory canals.<sup>19)</sup>
- 2) Root canal with a diameter below 0.15 mm is classified as accessory canal.<sup>20)</sup>

Incidence of accessory canals was measured in three parts of

root: 1) Apex to apical 3mm (apical part); 2) Apical 3mm to one-half of the distance between the root canal orifice and root apex (middle part); 3) one-half of the distance between the root canal orifice and root apex to root canal orifice (coronal part).

#### **4. Comparison of two micro-computed tomographic images and the images obtained by clearing technique**

After classification, agreement among the clearing technique and two MCT techniques were investigated. When teeth were classified as the same type in all three techniques, it was regarded that agreement among three techniques were made, and vice versa. In addition, classifications of root canal types according to Weine's classification and Vertucci's classification were compared. Incidence and location of accessory canals detected in three techniques were also compared.

### **III. Result**

#### **1. Agreement between the clearing technique and two MCT images**

A total of 18 teeth were compared. Agreement on Weine's classification was 22.2% (4/18) and disagreement was 77.8%

(14/18). Agreement on Vertucci' s classification was 33.3% (6/18) and disagreement was 66.7% (12/18).

Agreements on both classifications were 22.2% (4/18). Agreements on only one classification were 11.1% (2/18). Disagreements on both classifications were 66.7% (12/18).

## **2. Incidence of root canal type**

### **Weine' s classification**

Type I was most frequent in images obtained by clearing technique (type I: 38.9%, type II: 5.6%, type III: 5.6%, type IV: 22.2%). Type IV was most frequent in the images obtained by the two MCT images (type I: 0%, type II: 0%, type III: 11.1%, type IV: 16.7%). Multiple canals were more frequently observed in the two MCT images.

### **Vertucci' s classification**

Type I incidence was most frequent in the clearing technique (type I: 38.9%, type V: 22.2%, type III: 11.1%). Type VI incidence was most frequent in the MCT images (type VI: 22.2%, type V: 16.6%, type IV: 11.1%). More complex root canal types were observed in MCT images.

### **Unclassifiable teeth**

On Weine' s classification, incidence of unclassifiable teeth were 27.7% in clearing technique and 72.2% in MCT image. On Vertucci' s classification, incidence of unclassifiable teeth were

11.1% in clearing technique and 38.9% in MCT image. Unclassifiable configuration type were illustrated in Figure 3.

### **3. Accessory canal number and location**

A total of two accessory canals were found in clearing technique study. All of the accessory canals were in apical 3 mm. A total of 27 accessory canals were found in the MinIP 2D images and they were also located in apical 3 mm. A total of 37 accessory canals were observed in 3D images. Twenty-seven accessory canals were located in apical 3 mm, whereas 1 accessory canal was located in the center of the root. More accessory canals were observed in MCT images. Accessory canals were most frequently located in apical 3 mm.

## **IV. Discussion**

Thorough knowledge on the complex root canal anatomy and its frequent variations is an absolute necessity for a successful root canal treatment.<sup>21)</sup> For the study of root canal morphology, various methods, such as grinding teeth, clearing technique and cone-beam CT<sup>3,30,31)</sup> have been used. Owing to the characteristics and limitations of each method, the accuracy of tooth morphology analysis could vary. Clearing technique is the most commonly used method and generally has been considered as the gold standard.<sup>22)</sup>

In recent years, MCT has emerged as the latest technological



tool for studying the root canal system. Nielsen et al.<sup>23)</sup> demonstrated that MCT is a new innovative tool for reproducing tooth anatomy and endodontic research without tooth destruction. Bjørndal et al.<sup>24)</sup> used MCT to study the relationship between the external and internal macromorphology of the root complex. Peters et al.<sup>9)</sup> showed that high-resolution MCT accurately reproduced the root canal geometry of extracted maxillary molars. Fan et al.<sup>25-27)</sup> used MCT to study the intricate anatomic features of C-shaped mandibular second molar roots. Gu et al.<sup>17)</sup> reported that MCT study with MinIP images clearly showed fine anatomical structures such as loop, accessory canals and inter-canal communications. Furthermore, they suggested a more accurate morphology classification method for complex maxillary first molar MB root canal systems.

This study compared clearing technique with two MCT methods. The degree of agreement in the root canal classification among clearing technique and the two MCT methods was higher for Vertucci' s classification (33.3%) than for Weine' s classification (22.2%). The reason for the higher degree of agreement for Vertucci' s classification was that Vertucci' s classification has more variable types of canal morphology than Weine' s classification. Because Weine' s classification has only four types, some teeth were classified into unclassifiable type and regarded as disagreement.

More root canals were found in MCT images than the images obtained by clearing technique. MCT analysis of MB root canal

systems in 18 maxillary first molar teeth showed that 18 (100%) had multiple canals. Clearing technique analysis showed that 11 (61.1%) had multiple canals. The discrepancy in incidence of multiple canals stemmed from characteristic of the method and experimental fault.

Regarding the incidence of accessory canals, the image obtained by clearing technique showed only two accessory canals, whereas 27 accessory canals in TS–MinIP images and 37 in 3D images were observed. The difference of the incidence of accessory canals between the two MCT images might be due to the fact that 2D MinIP images did not reproduce the three–dimensional positions which made the chance for missing accessory canals. For example, the canals that run perpendicular to image plane could not be observed in MinIP technique. Accessory canals were able to be observed from various angles in MCT 3D images. But MCT 3D images were weak in observing faint structures. Therefore, using both MCT 2D image and 3D image may be very useful for in–depth morphology study of complex root canal systems with faint or calcified canals.

In this study, some teeth could not be classified when Weine’ s classification was applied. Incidences of unclassifiable teeth were higher in MCT images than in those obtained by clearing technique (clearing technique: 27.7%, MCT images:72.2%). When Vertucci’ s classification was applied, incidence of unclassifiable teeth was lower than when Weine’ s classification was applied (clearing technique: 11.1%, MCT

image: 38.9%). This result was attributed to the accuracy of MCT study and suggests that MCT is more a sensitive method to investigate complex root canal morphologies than clearing technique. Complex canal morphology was observed more in MCT images than in images obtained by clearing technique. Because of the complex root canal morphology, any currently available classification systems are not able to completely include all the root canals found in human maxillary first molars. In MCT study, unclassifiable teeth were lower when Vertucci's classification was applied because Vertucci's classification could cover more various canal systems.

In this experiment, complex root canal morphology was not observed in clearing technique. Although clearing technique has been used widely for the study of root canal systems, clearing technique has many disadvantages. First, tooth morphology tends to be distorted in clearing process, because the tooth structures are weakened in the demineralization process. The weakened tooth could be bent as a result of mechanical stimulation. Although the amount of distortion is slight, small structures like accessory canals or isthmus could be affected by slight distortion. This morphological distortion could result in closure or narrowing of accessory canals. In this experiment, it was observed in many cases that dyeing solution did not fully infiltrate in the small canals. When the root canals were severely narrowed in coronal portion, they could not be detected in clearing technique because of the limited dye penetration. To solve this problem, Yoshioka et al.<sup>19)</sup> removed

coronal dentine by toughing. This problem also could be evaded in modified clearing technique.<sup>28)</sup> However, root canal blockage resulting from narrowing and calcification of root canals below the size of dyeing solution grain size could not be solved with these modifications.

Cleghorn et al.<sup>29)</sup> in their review article, reported that the incidence of multiple canals of MB roots was different among the experimental methods in laboratory studies. Therefore, this study compared conventional clearing technique with two MCT methods and showed that MB root canal anatomy could be accurately observed in MCT methods. This result supports that the high incidence of multiple canals in recent MCT study stems from the accuracy and effectiveness of MCT in studying root canal morphology. If previous root canal morphology studies have used MCT analysis, there might be a high chance of multiple canals and thus statistical data might be changed.

## V. Conclusion

Although clearing technique is generally considered to be the gold standard in root canal morphology study, it has limitations that complex root canal systems could not be fully revealed. MCT with 2D MinIP and 3D techniques are more sensitive in the investigation of root canal morphology than clearing technique, and thus the combination of the two types of MCT images can be regarded as the gold standard for in-depth morphology study of complex root canal system.

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## Figure legends

Figure 1. Weine classification of four root canal configuration types and Vertucci classification of eight root canal configuration types

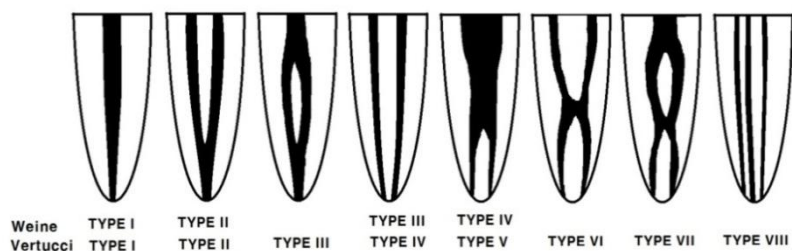


Figure 2. Three different images of one sample: clearing image (a), 3D volume image (b), and MinIP image (c).

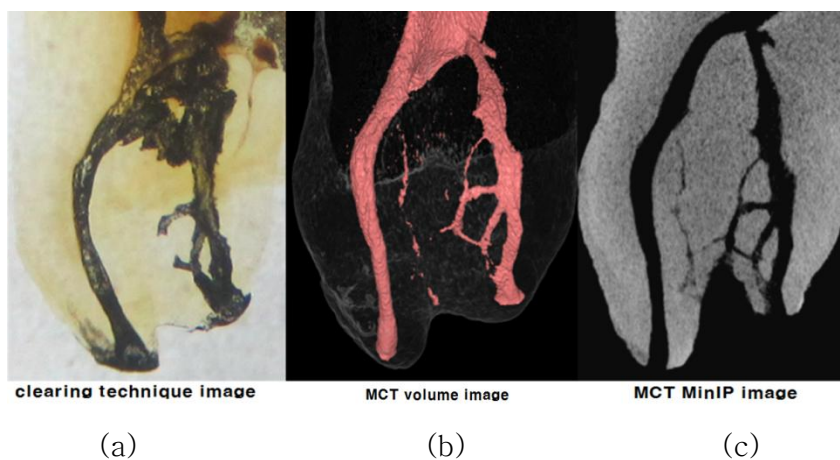
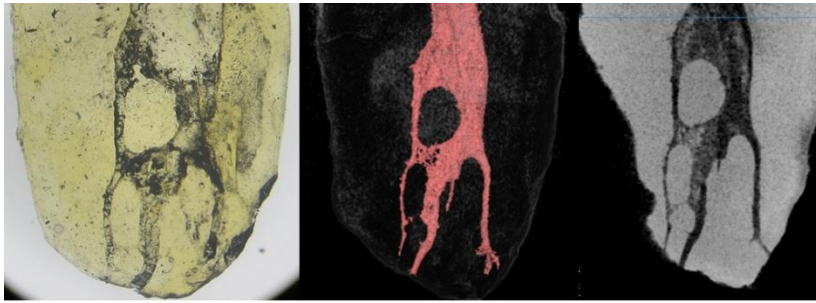


Figure 3. Three different images of one unclassifiable sample: clearing image (a), 3D volume image (b), and MinIP image (c).



clearing technique image

MCT volume image

MCT MinIP image

(a)

(b)

(c)

Table 1. Types and incidence of canal configurations of 18 MB roots classified according to the Weine classification.

Type of canal configuration					
	Type I	Type II	Type III	Type	NC*
Clearing techniqu Type of MCT study	7 (38.9%)	1 (5.6%)	1 (5.6%)	4 (22.2%)	5 (27.7%)
	0 (0%)	0 (0%)	2 (11.1%)	3 (16.7%)	13 (72.2%)

NC\*: Non -classifiable

Table 2. Types and incidence of canal configurations of 18 MB roots classified according to the Vertucci classification

	Type of canal configuration							
	Type I	Type II	Type III	Type	Type V	Type VI	Type VII	NC*
Clearing technique	7 (38.9%)	1 (5.6%)	2 (11.1%)	1 (5.6%)	4 (22.2%)	1 (5.6%)	0 (0%)	2 (11.1%)
Type of MCT study	0 (0%)	0 (0%)	1 (5.6%)	2 (11.1%)	3 (16.6%)	4 (22.2%)	1 (5.6%)	7 (38.9%)

NC\*: Non-classifiable

- 국문초록 -

# 상악 제1대구치 근심협측 치근에 서의 Micro-Computed Tomography 와 Clearing Technique를 사용한 형태학 연구 비교

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## 1. 연구목적

Tooth clearing method는 상악 제1대구치의 근심협측 치근의 해부학적 구조 연구 gold standard로 여겨져왔으나, 복잡한 근관의 형태가 모두 나타나지 않는 한계를 가졌다. 이 연구의 목적은 상악 제1대구치의 근심협측 치근의 해부학적 구조 연구에서 clearing technique과 두 가지 다른 방법으로 재구성한 영상을 사용한 micro-computed tomography (MCT) 영상을 비교하고자 한다.

## 2. 방법

18개의 인간 상악 제1대구치를 MCT(SkyScan) scanner를 사용하여 스캔하여 2차원적 thin-slab minimum intensity projection(TS-MinIP)영상과 3차원적인 영상을 구성하였다. 그 후, 모든 18개 치아를 탈회하고 투명화시킨 후 microscope를 사용하여 사진 촬영하였다. 한명의 관찰자가 Weine과 Vertucci에 의해서 제안된 기준에 의해 분류하였다. 3가지 영상에 의해 부근관의 빈도와 위치를 조사하였으며 비교하였다. 분류가 세가지 영상에서 모두 같았을 때 일치로 표시하였고 다르다면 불일치로 표시했다.

## 3. 결 과

3가지 방법으로 얻어진 영상을 Weine classification으로 비교하였을 때는 22.2% (4/18) 에서 일치 하였고 77.8% (14/18) 에서 불일치 하였다. Vertucci classification으로 비교하였을 때는 33.3% (6/18) 에서 일치 하였고 66.7% (12/18) 에서 불일치 하였다. clearing technique 에서는 Weine' s type I (Vertucci' s type I) 가 가장 많은 빈도로 관찰된 반면 두 가지 MCT technique에서는 Weine' s type IV (Vertucci' s type VI) 가 가장 많은 빈도로 관찰되었다. Accessory canals 은 clearing technique 보다 MCT techniques 에서 더 많이 관찰되었다.



#### 4. 결론

상악 제1대구치 근심협측 치근 해부학적 연구에서 근관 형태와 부근관은 cleared tooth에서 보다 MCT에서 더 자세히 확인할 수 있었다. MCT image 는 복잡한 치아 근관의 형태학적 연구에 있어서 gold standard가 될 수 있다.

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주요어 : Accessory canal, Canal configuration, Clearing technique, MB root canal, Minimum injected projection, Micro-computed tomography

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