

# Comparative Evaluation of Modified Canal Staining and Clearing Technique, Cone Beam Computed Tomography, Peripheral Quantitative Computed Tomography, Spiral Computed Tomography, and Plain and Contrast Medium–enhanced Digital Radiography in Studying Root Canal Morphology

Prasanna Neelakantan, MDS, Chandana Subbarao, BDS, and Chandragiri V. Subbarao, MDS

## Abstract

**Introduction:** This study investigated the accuracy of cone beam computed tomography (CBCT), peripheral quantitative computed tomography (pQCT), spiral computed tomography (SCT), plain (plain digi), and contrast medium–enhanced digital radiographs (contrast digi) in studying root canal morphology.

**Methods:** The root canal anatomy was analyzed in 95 teeth using CBCT, pQCT, SCT, plain digi, and contrast digi. After flushing out the radiopaque dye, access cavities were sealed, and the teeth were subject to the modified canal staining and clearing technique. The number of root canals (Vertucci classification and Gulabivala's additional classes) was calculated by three calibrated endodontists and two maxillofacial radiologists. Erroneous or unsuccessful identifications of root canals were statistically analyzed by one-way analysis of variance ( $p = 0.05$ ). **Results:** The modified canal staining and clearing technique identified an average of 1.8 root canals per mandibular central incisor, 2.3 per maxillary first premolar, 3.9 per maxillary first molar, 3.8 per maxillary and mandibular second molar, and 4.3 per mandibular first molar. CBCT and pQCT were erroneous in 0.29% and 2.05% cases, whereas SCT, contrast digi, and plain digi were unsuccessful in 15.58%, 14.7%, and 23.8%, respectively. There was a significant difference between all the methods ( $p < 0.05$ ) in the unsuccessful identification of root canals except between CBCT and modified canal staining and clearing technique where there was no significant difference ( $p > 0.05$ ). **Conclusions:** CBCT and pQCT were as accurate as the modified canal staining and tooth clearing technique in identifying root canal systems. (*J Endod* 2010; ■:1–5)

## Key Words

Cone beam computed tomography, contrast media, digital radiograph, modified staining and clearing, morphology, peripheral quantitative computed tomography, root canal, spiral computed tomography

Successful endodontic therapy stems from thorough canal debridement and effective filling of the root canal system, for which knowledge of morphology of the root canals is a critical prerequisite (1). Internal complexities of the root canal are genetically determined and have definitive importance in anthropology (2). These factors necessitate the identification of a method that accurately determines the root canal morphology.

There are numerous reports on the root canal morphologies of different populations, which is extremely important for an endodontist as well as general dental practitioners. Also of interest to us are the methods that have been used in these studies (3). The methods most commonly used in analyzing the root canal morphology are canal staining and tooth clearing (4–6), conventional radiographs (7–9), digital and contrast medium–enhanced radiographic techniques (10, 11), radiographic assessment enhanced with contrast media (12, 13), and more recently computed tomographic techniques (14–16). Canal staining and tooth clearing (CS) is generally considered the gold standard in these studies (4, 5, 17). A modification of this technique proposed by Weng et al (18) is accurate, allows the appreciation of intricate details, and is nondestructive. An ideal technique would be one that is accurate, simple, non-destructive, and, most importantly, feasible in the *in vivo* scenario. The gold standard methods CS and the modified method cannot be used *in vivo*.

The application of computed tomography (CT) scans in endodontics was first reported by Tachibana and Matsumoto in 1990 (19). A CT scan uses a fan-shaped beam and multiple exposures around an object to reveal the internal architecture of this object, thereby helping the clinician to view morphologic features as well as pathology from different three-dimensional (3D) perspectives. The distinct advantage of a CT scan is that it allows for 3D reconstruction of root canal systems. CT scanning has been suggested as the preferential imaging modality in difficult situations demanding localization and description of root canal systems because of its ability to render 3D information (20–22)

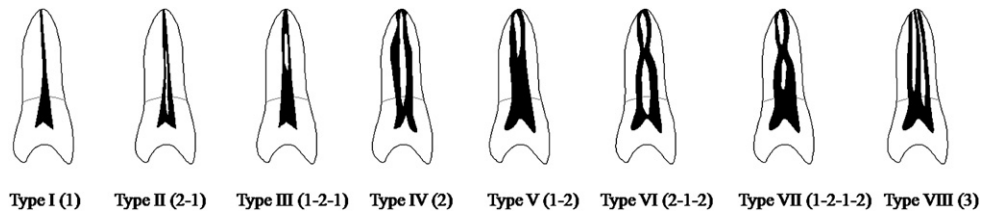
From the Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha University, Chennai, India.

Address requests for reprints to Dr Neelakantan Prasanna, Plot 1500, 16th Main Road, Anna Nagar West, Chennai, Tamil Nadu, India. E-mail address: prasu\_endo@yahoo.com.

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**Figure 1.** Classification of canal configurations according to Vertucci.

Cone beam computed tomography (CBCT) scanning or digital volume tomography (DVT) uses an extraoral imaging scanner to produce 3D scans of the maxillofacial skeleton at a considerably lower radiation dose than conventional CT scanning. CBCT scanning has been shown to be more accurate than digital radiographs in determining root canal systems. CBCT scanning has also been used *in vivo* in diagnosis and preoperative assessment (20, 23–26). Another computed tomography technique, peripheral quantitative computed tomography (pQCT), was originally introduced for bone mineral analysis. The only report on pQCT in studying root canal anatomy showed that this method offers accurate 3D reconstruction of the root canal systems (RCS) and analysis of endodontic procedures (16). The planar resolution of pQCT is approximately  $70 \times 70 \mu\text{m}$ , which is lower than  $\mu\text{CT}$ . Nevertheless, it might prove to be a nondestructive method of investigation at low cost and shorter scanning times. Spiral CT (SCT) has been used in several cases of diagnosis of aberrant root canal systems (27–32) as well as in the identification of the root canal morphology of Indian molars (33).

Despite a plethora of studies and clinical reports showing the canal morphology of different populations, the most accurate and ideal method is yet to be ascertained with scientific evidence. The hypothesis tested in this study was that there is a difference in the identification of the number of root canal systems between the modified canal staining and clearing technique (gold standard) versus CBCT, pQCT, SCT, contrast medium–enhanced digital radiographs, and plain digital radiographs. The aim of this *in vitro* investigation was to compare the efficacy of the six aforementioned methods in identifying root canal systems.

## Materials and Methods

A total of 95 recently extracted teeth (20 mandibular first molars, 20 maxillary first molars, 20 mandibular second molars, 20 maxillary second molars, 7 maxillary first premolars, and 8 mandibular central incisors) with intact roots and mature apices were collected from the Oral Surgery Department of the University and stored at 100% humidity. It was ensured that for every tooth type, the number of roots was standardized (mandibular incisors, 1; maxillary first premolars, 2; maxillary first and second molars, 3; and mandibular first and second molars, 2). The methodology was approved by the Institutional Review

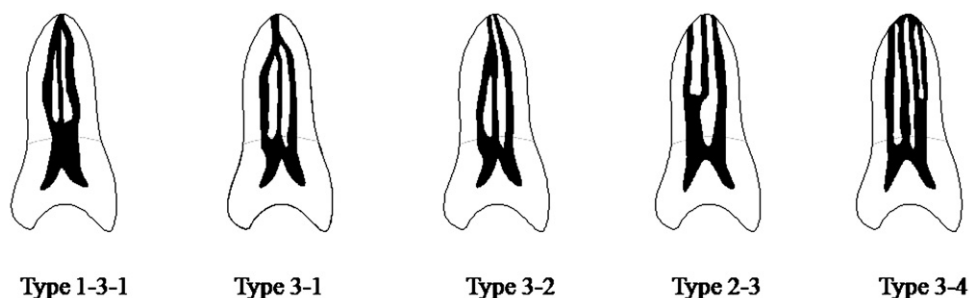
Board of the university. The teeth were washed under tap water immediately after extraction and stored in distilled water with thymol iodide crystals until the collection was complete. After this, the samples were washed thoroughly under tap water and immersed in 2.5% sodium hypochlorite for 30 minutes to remove adherent soft tissue.

## CT Scans

The teeth were randomly inserted into foam arches in close contact to each other to simulate their natural alignment in a dental arch. An acrylic facing was placed on the facial side to mimic soft tissue on the radiographs. All the teeth were scanned by a CBCT scanner (3DAccu-tomo; J Morita Corporation, Osaka, Japan), a pQCT scanner (Research SA+; Stratec Medizin-technik GmbH, Pforzheim, Germany), and a SCT scanner (GE Electricals, Milwaukee, WI) with constant thicknesses of  $125 \mu\text{/slice}$ ,  $250 \mu\text{/slice}$ , and  $650 \mu\text{/slice}$  respectively. The teeth were viewed both cross-sectionally and longitudinally. Volume-rendering and multiplanar volume reconstruction were performed using the Advantage Windows workstation (GE Systems, Milwaukee, WI).

## Digital Radiography with Contrast Medium

Following the scanning procedure, all the teeth were exposed to a radiograph using a digital radiography unit (DSX 730; Owandy Dental Imaging, France; and Kodak 2100 X ray unit; Kodak Dental Systems, Atlanta, GA). Access cavities were prepared in all the teeth. After gaining entry into the pulp chamber, the pulp tissue was extirpated with a fine barbed broach (Dentstly Maillefer, Ballaigues, Switzerland). The teeth were placed in 5% sodium hypochlorite for 30 minutes to dissolve the pulp tissue (10), washed in water, dried, and a water-soluble low-viscosity radiopaque medium (diatrizoate sodium, Hypaque; Amersham Health Inc, Princeton, NJ) was delivered into the root canals with a monoject syringe with a needle gauge 23 to 27 depending on the teeth and root canal size. The teeth were subjected to vacuum for 2 minutes (24 mm Hg), and a reapplication of vacuum was performed after 3 minutes. The penetration of the dye into the niches of the root canal system was enhanced by ultrasonication for 2 minutes. The teeth were placed in a model simulating the maxillary or mandibular arches, and radiographs were taken in the buccolingual direction using a digital radiography unit (DSX 730 and Kodak 2100). All radiographs (before



**Figure 2.** Additional classes of canal configurations according to Gulabivala.

**TABLE 1.** Average Number of Root Canals Identified with the Six Methods

Tooth	Conventional radiographs	CBCT	pQCT	SCT	Digital radiograph with contrast medium	Modified canal clearing and staining
Mandibular central incisor ( <i>n</i> = 8)	1	1.8	1.8	1.8	1.8	1.8
Maxillary first premolar ( <i>n</i> = 7)	2	2.3	2.1	2.1	2	2.3
Maxillary first molar ( <i>n</i> = 20)	3.1	3.8	3.8	3.3	3.2	3.9
Maxillary second molar ( <i>n</i> = 20)	3.1	3.8	3.7	2.7	3.5	3.8
Mandibular first molar ( <i>n</i> = 20)	2.8	4.3	4.2	3.4	3.1	4.3
Mandibular second molar ( <i>n</i> = 20)	2.8	3.8	3.3	2.9	2.7	3.8

CBCT, cone beam computed tomography; pQCT, peripheral quantitative computed tomography; SCT, spiral computed tomography.

and after introducing contrast medium) were taken in two horizontal angulations: 0° and 30°.

### Modified Canal Staining and Clearing Technique

The contrast medium was flushed out with water followed by 5.25% sodium hypochlorite and then rinsed again with water. The access cavities were sealed with light cure composite resin and light cured for 40 seconds. The modified method of canal staining has been described in detail elsewhere (18). Briefly, after immersion of the teeth in India ink, the teeth were placed in a hyperbaric oxygen chamber set to a pressure of 0.6 MPa for 2 hours. The method of decalcification and rendering the teeth transparent was adopted from Robertson et al (34). After 12 hours of drying, the teeth were decalcified in 10% nitric acid for 28 to 30 hours. The acid was changed after 24 hours and was stirred every 8 hours. The endpoint of decalcification was determined by taking periodic radiographs of five sample teeth. After thorough washing of the decalcified teeth in running tap water for 4 hours, the samples were dehydrated in ascending concentrations of ethanol (70%, 80%, 95%, and 100%) for 1 day, and the samples were rendered transparent by immersing in methyl salicylate for 2 days. The samples were analyzed using a magnifying lens (3×).

For all the methods, the evaluation was performed by three precalibrated and standardized endodontists and two oral and maxillofacial radiologists. To eliminate any bias, the evaluators were asked to analyze every slice (both cross-sectional and longitudinal) as well as the images obtained by multiplanar volume reconstruction to quantify the number of root canals. The number of root canals per tooth identified with modified canal staining and clearing technique was used as the gold standard to compare the number of root canals identified with CBCT, pQCT, SCT, contrast medium-enhanced digital radiographs, and digital radiography. The identification of root canal systems was done according to Vertucci's classification (4) and Gulabivala's additional classes (17) (Figs. 1 and 2). The total number of root canals was calculated for each tooth studied. In any canal system with divisions of the canal, the maximum number of divisions was considered as the number of canals. For example, type II canal system was considered as two canals, type 1-3-1 as three canals, and type VI as two canals.

The average number of root canals identified per tooth type was calculated for all the methods. Descriptive statistical analysis was per-

formed by calculating the percentage of root canals found from the modified canal staining and clearing method with those identified with CBCT, pQCT, SCT, contrast medium-enhanced digital radiographs, and digital radiographic images. Interrater agreement was measured between all three endodontist evaluators and two radiologists as well as between each pair of endodontist evaluators and the radiologists. Intrarater agreement was measured by having all three endodontists and the two radiologists evaluate one half of the CBCT, pQCT, SCT, contrast medium-enhanced digital radiographs, and digital radiographic images at each of two separate sessions. The percentage of unsuccessful identifications as compared with the modified canal staining and clearing method (0% unsuccessful) was statistically analyzed by one-way analysis of variance with a *p* value of 0.05 considered significant.

### Results

The average number of root canals identified by all the methods is depicted in Table 1. The percentage of root canals found from the modified canal staining and clearing analysis with those identified with CBCT, pQCT, SCT, contrast medium-enhanced digital radiographs, and digital radiographic images is given in Table 2. Table 3 presents the percentage of unsuccessful identification of root canals by all the methods analyzed in this study.

### Interrater and Intrarater Agreement

For the modified canal staining and clearing method, each pair of endodontist evaluators agreed with each other 100% of the time. For CBCT and pQCT, each pair of endodontist evaluators and radiologists agreed with each other between 98% and 100% of the time, whereas for SCT they agreed with each other 95% to 98% of the time. For digital radiography and contrast medium-enhanced digital radiographs, each pair of endodontists agreed with each other 80% to 83% and 86% to 89% of the time. Among all three endodontist evaluators and two radiologist evaluators, total agreement was found 100% of the time for the modified canal staining and clearing method, 99% for CBCT and pQCT, 98% for SCT, and 82% for digital radiography and 84% for contrast medium-enhanced digital radiographs.

Intrarater agreement results showed that the endodontist evaluators agreed with themselves 100% of the time for the modified canal

**TABLE 2.** Correct Identification of the Total Number of Root Canals by All the Methods

Evaluator	Digital radiograph (% correct)	CMDR (% correct)	CBCT (% correct)	pQCT (% correct)	SCT (% correct)	MCS (% correct)
Endodontist 1	80	88	99	100	98	100
Endodontist 2	83	86	100	98	96	100
Endodontist 3	82	89	99	98	95	100
Radiologist 1	80	88	98	99	98	100
Radiologist 2	80	87	100	99	96	100
All	82	84	99	99	98	100

CMDR, contrast medium-enhanced digital radiography; CBCT, cone beam computed tomography; pQCT, peripheral quantitative computed tomography; SCT, spiral computed tomography; MCS, modified canal staining.

## Basic Research—Technology

**TABLE 3.** Unsuccessful Identification of Root Canals in Individual Teeth in All the Techniques Compared with the Modified Canal Staining and Clearing Technique

Method	Missed one RCS in a tooth (%)	Missed two or more RCS in a tooth (%)	Total (%)
Digital radiograph	0	23.8	23.8
CMDR	0	14.7	14.7
SCT	0.58	15	15.58
CBCT	0.29	0	0.29
pQCT	0.88	1.17	2.05

CMDR, contrast medium–enhanced digital radiography; CBCT, cone beam computed tomography; pQCT, peripheral quantitative computed tomography; SCT, spiral computed tomography; RCS, root canal staining.

staining and clearing technique interpretation; 98% for CBCT, pQCT, and SCT; between 78% and 84% for digital radiographs; and 85% to 93% for contrast medium–enhanced digital radiograph interpretation. The evaluators failed to identify one or more root canals with digital radiographs in 23.8% of teeth, contrast medium–enhanced digital radiographs in 14.8%, SCT in 15.58%, CBCT in 0.29%, and pQCT in 2.05% of the teeth. There was a significant difference between all the methods ( $p < 0.05$ ) in unsuccessful identification of root canals.

## Discussion

Failure to identify extra canals is implicated as one of the most common reasons for the failure of endodontic treatment (1). The analysis of the root canal morphologies of teeth of different populations, ethnicities, and preoperative assessment of root canal systems is of importance in this regard. This study compared the efficacy of the six methods (modified canal staining and clearing, CBCT, pQCT, SCT, digital radiography, and contrast medium–enhanced digital radiography) in identifying root canal systems. In the absence of overlying bone, tissue, and anatomic features, it may not be possible to directly extrapolate the results of this *in vitro* investigation to the clinical scenario.

The canal staining and clearing technique is considered the gold standard method of studying root canal anatomy. We used the modified canal staining and clearing technique in this study. Although nondestructive and more accurate than the conventional staining and clearing method, the main disadvantage of this method is that it cannot be used *in vivo* (18). A method that has the accuracy of the canal staining and clearing technique and yet is clinically feasible is essential in endodontic practice.

Radiographs taken after the introduction of radiopaque contrast media are more useful than plain radiographs in the assessment of root canal anatomy (12). Hypaque is a tensioactive, water-soluble contrast medium with a specific gravity similar to sodium hypochlorite. It is an aqueous solution of two iodine salts: sodium iodine and diatrizoate meglumine. The low surface tension enables its penetration into the niches of the root canal system (12). This alteration of subject contrast induced by variations in transmission of the radiographic beam between the tooth and the contrast medium definitely improves the visibility of canal systems in comparison with conventional radiographs. Digital radiographs were used in this study because they are more accurate than conventional radiographs in analyzing root canal anatomy (10).

Exposing radiographs in two different horizontal angulations (30° shift and orthoradial position) provide additional information on root canal systems but show some amount of inherent distortion, which makes radiographic determination of some characteristics difficult. Our study showed that endodontists and radiologists only identified

86% to 89% of root canals as compared with the modified canal staining and clearing method. Passive injection of the contrast medium results in the entrapment of air bubbles, hampering proper visualization of apical anatomy (10, 12, 35). Despite the injection of contrast media under pressure in our study, it is possible that complete perfusion may not occur (35). This is probably the reason why this method failed to identify the intricacies of root canal anatomy. The clearing and staining technique has been shown to be more accurate than the radiographic technique with contrast medium (35). Our results are in agreement.

CT scanning is currently widely used in implantology, maxillofacial reconstruction, and endodontic diagnosis as well as for the assessment of canal preparation, obturation, and the removal of root fillings (20, 23, 27, 28). The main advantages of CT scanning are that it is nondestructive and allows 3D reconstruction and visualization of the external and internal anatomy of the teeth.

The results of our study showed that CBCT scanning was as accurate as the modified canal staining and clearing technique in identifying canal anatomy. CBCT scanning was erroneous only in 0.29% of the teeth examined. The differences between the results obtained by the evaluators were not significantly different when endodontists were compared with radiologists. However, in some instances of analysis of the CT images, radiologists were able to interpret the number of root canals better than endodontist evaluators, but this was not true for all endodontist evaluators. Nevertheless, the experience of the radiologists in the analysis of CT images may be an attributable factor. The endodontic evaluators and radiologists identified 98% to 100% of root canals as compared with modified canal staining and clearing method when CBCT and pQCT were used. The slice thickness for CBCT ranges from 80 to 200  $\mu$ . The slice thickness used in our study was 125  $\mu$ . The primary advantages of CBCT are significantly lower effective radiation dose, short exposure time (2–5 seconds), less expensive than conventional CT scanning, and highly accurate. Also, CBCT measurements are geometrically accurate because of the fact that the CBCT voxels (3D pixels containing data) are isotropic (20, 26). pQCT, with a slice thickness of 250  $\mu$  failed to identify 2.05% of RCS as compared with the gold standard. Also, the large pixel size as mentioned earlier is responsible for a low resolution, which may cause errors in the identification of intricate details of the root canal system. The advantages of pQCT are that it is economic, scanning times are shorter, and it allows mapping of multiple teeth at the same time (16). Our study showed that SCT did not identify 15.58% of root canals. The frequent errors were in differentiating type II canal systems from type III and 1-3-1 systems and in differentiating type IV canal systems from type 3-2. We attribute this to the slice thickness. The usual thickness of each slice in SCT ranges from 0.65 mm to 1.0 mm (33). In our study, it was 0.65 mm/slice. It is possible that this slice thickness does not offer sufficient reproducibility along the length of the canal (14). For example, type VI canal system may be misinterpreted as a type IV system if the fusion and subsequent division of the canals is covered in the same slice. Another main disadvantage of SCT is the increased radiation dose as compared with CBCT scanning (20).

## Conclusions

CBCT and pQCT are as accurate as the gold standard (modified canal staining and clearing technique) in identifying root canal anatomy. The application of 3D reconstruction based on CBCT and pQCT require further evaluation to validate its clinical application.

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