# Assessing Accumulated Hard-tissue Debris Using Micro–computed Tomography and Free Software for Image Processing and Analysis

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## Abstract

**Introduction:** The accumulation of debris occurs after root canal preparation procedures specifically in fins, isthmus, irregularities, and ramifications. The aim of this study was to present a step-by-step description of a new method used to longitudinally identify, measure, and 3-dimensionally map the accumulation of hard-tissue debris inside the root canal after biomechanical preparation using free software for image processing and analysis. **Methods:** Three mandibular molars presenting the mesial root with a large isthmus width and a type II Vertucci's canal configuration were selected and scanned. The specimens were assigned to 1 of 3 experimental approaches: (1) 5.25% sodium hypochlorite + 17% EDTA, (2) bidistilled water, and (3) no irrigation. After root canal preparation, high-resolution scans of the teeth were accomplished, and free software packages were used to register and quantify the amount of accumulated hard-tissue debris in either canal space or isthmus areas. **Results:** Canal preparation without irrigation resulted in 34.6% of its volume filled with hard-tissue debris, whereas the use of bidistilled water or NaOCl followed by EDTA showed a reduction in the percentage volume of debris to 16% and 11.3%, respectively. The closer the distance to the isthmus area was the larger the amount of accumulated debris regardless of the irrigating protocol used. **Conclusions:** Through the present method, it was possible to calculate the volume of hard-tissue debris in the isthmuses and in the root canal space. Free-software packages used for image reconstruction, registering, and analysis have shown to be promising for end-user application. *(J Endod 2014;40:271–276)*

## Key Words

Biomechanical preparation, debris, free software, irrigation, micro-computed tomography, root canal system

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Since the first description of a smeared layer on instrumented root dentin (1), the concept of a smear layer has played a pivotal role in endodontic research and practice (2). The smear layer has been defined as a surface film of debris retained on dentin and other surfaces after instrumentation with either rotary instruments or endodontic files. It consists of dentin particles, remnants of vital or necrotic pulp tissue, bacterial components, and retained irrigant (3). Unfortunately, the results of previous studies were partially contradictory, and most of the clinical recommendations were based only on limited descriptive or semiquantitative *in vitro* scanning electron microscopic evaluations (2). On the other hand, Paqué et al (4) reopened an interesting discussion about the substantial accumulation of debris occurring after biomechanical preparation specifically in fins, isthmuses, irregularities, and ramifications of the complex root canal network. Hard-tissue debris accumulation has been considered a side effect of the cleaning and shaping procedures (4) and may be more clinically relevant than the smear layer because its sizable amount could easily harbor bacteria biofilm from the disinfection procedures. The assessment of hard-tissue debris accumulation has been made possible through the combination of nondestructive micro–computed tomography (CT) imaging and the development of robust image analysis and processing software (4, 5). Through micro-CT imaging, teeth can be scanned before and after cleaning and shaping procedures, and, with the aid of proper software, the image volumes resulting from both scanning procedures can be geometrically coregistered (ie, different sets of data can be transformed and integrated into 1 coordinate system) (6). This allows, to some measure, the identification of the dentin debris that was packed into the original root canal space after preparation. The rationale behind this approach has a simple basis, which was described first by Paqué et al (4) and was recently well defined by Robinson et al (5) as "pixels that were occupied by air and then became dentine must be debris."

Interesting findings of the effect of current cleaning and shaping procedures on the accumulation of hard-tissue debris has been shown in recent studies.

1. EDTA and passive ultrasonic irrigation reduced hard-tissue debris accumulation, but approximately 50% of the debris still remained in the root canal space (7)
2. The use of a hypochlorite-compatible chelator enabled reduction of hard-tissue debris accumulation (8)
3. Self-adjusting file systems (ReDent-Nova, Ra'anana, Israel) resulted in less hard-tissue debris accumulated in isthmus-containing root canal systems than rotary instrumentation with ProTaper (Dentsply/Maillefer, Ballaigues, Switzerland) and needle/syringe irrigation (9)

These findings need to be underscored because they were provided by methodologically sound experiments using micro-CT technology and image analysis. Thus, a point worth discussing is the recent methodologic shift in the study of hard-tissue debris accumulation. Therefore, some concerns regarding micro-CT technology must be pointed out considering that this is a high-cost, labor-intensive, and time-consuming procedure that demands an extended learning curve to get the required expertise to extract quantitative data. One of the reasons for the high cost of experimental procedures using this technology is related to the typically expensive proprietary software packages. This is one of the points that prevent the worldwide dissemination of this useful methodology.

Therefore, the aim of this study was to present a step-by-step description of a new method used to longitudinally identify, measure, and 3-dimensionally map the accumulation of hard-tissue debris inside the root canal space after biomechanical preparation using free software for image processing and analysis. Its advantages over proprietary image analysis software packages and its limitations are also carefully addressed.

## Materials and Methods

### Teeth Selection Criteria

This study was revised and approved by the Ethics Committee, Nucleus of Collective Health Studies (protocol no. 2223-CEP/HUPE). One hundred twenty human mandibular first and second molars with completely separated roots were obtained from a pool of extracted teeth. Teeth were extracted for reasons not related to this study and initially selected on the basis of digital radiographs taken in buccolingual direction to detect any possible root canal obstruction and to determine the curvature angle of the mesial root as described by Schneider (10). The curvature angle was measured using an open source image analysis program (Fiji v.1.47n; Madison, WI) (11), and only teeth with a mesial root with moderate curvature (ranging from 10°–20°) were selected. In addition, the inclusion criteria comprised only molars in which the final apical gauging of the mesial canals allowed for a size 10 hand file (Dentsply Maillefer, Ballaigues, Switzerland) to be placed to the working length. Moreover, the length of the specimens was standardized between 20 and 22 ± 1 mm to prevent the introduction of confounding variables, which might contribute to variations in the preparation procedures. As a result, 52 mandibular molars were selected and stored in 0.1% thymol solution at 5°C.

To attain an overall outline of root canal anatomy, these teeth were prescanned in a relatively low isotropic resolution (70 μm) using a micro–computed tomography scanner (SkyScan 1172; Bruker-microCT, Kontich, Belgium) at 70 kV and 114 μA. Based on the 3-dimensional (3D) models of this prescan set of images, 37 mandibular molars presenting a mesial root with a type II Vertucci's canal configuration system (12) with a large isthmus width between the mesial canals (7–9) were selected. After resection of the distal root at the furcation level, 3 teeth were randomly selected for the present study and scanned again at an isotropic resolution of 14.16 μm. The other teeth were kept for further use.

### Root Canal Preparation and Irrigation

The apexes of the 3 teeth were sealed with hot glue and embedded in polyvinyl siloxane to simulate the effect of apical gas entrapment in a closed canal system (13, 14) during root canal preparation. Then, to further streamline coregister processes, each tooth was placed coronal apically inside a custom-made epoxy resin holder (Ø = 18 mm) to smoothly fit it into the sample holder of the micro-CT device.

The specimens were randomly assigned to 1 of the 3 experimental approaches, and a flip of a coin was used to define which teeth would be treated with the following irrigation protocols:

1. 5.25% sodium hypochlorite (NaOCl) + 17% EDTA
2. Bidistilled water
3. No irrigation (positive control)

Teeth were prepared using a nickel-titanium reciprocation technique in a standardized way. Teeth were accessed, and the root canal patency was confirmed by inserting a size 10 K-file (Dentsply Maillefer) through the apical foramen before and after completion of root canal preparation. The working length was established by deducting 1 mm from the canal length. Reciproc R25 (VDW GmbH, Munich, Germany) was introduced into the canal until resistance was felt and then activated in a reciprocating motion generated by a 6:1 contra-angle handpiece (Sirona, Bensheim, Germany) powered by an electric motor (VDW Silver; VDW GmbH, Munich, Germany) using the preset configuration "Reciproc ALL." The instrument was moved in the apical direction using an in-and-out pecking motion of about 3 mm in amplitude with a 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 using reciprocating techniques performed all preparations.

For irrigation protocols 1 (5.25% NaOCl + 17% EDTA) and 2 (bidistilled water), irrigants were continuously delivered by a VATEA peristaltic pump (ReDent-Nova, Ra'anana, Israel) at a 2 mL/min rate connected to a 30-G Endo-Eze Tip (Ultradent Products Inc, South Jordan, UT) inserted into the canal without binding up to 2 mm from the apical foramen. Aspiration was performed with a SurgiTip (Ultradent Products Inc) attached to a high-speed suction pump. Between each preparation step, root canals were irrigated with 2 mL irrigant for 1 minute. As a result, a total volume of 20 mL 5.25% NaOCl (protocol 1) and bidistilled water (protocol 2) was used per root canal during biomechanical preparation. After root canal preparation, an additional rinse with 20 mL of the irrigant was performed for 10 minutes. Thus, in each protocol, a total volume of 40 mL irrigant was used per canal for a total time of 30 minutes. After this step, the smear layer was removed with 3 mL 17% EDTA (pH = 7.7) delivered at a 1-mL/min rate for 3 minutes. Then, all canals were dried with absorbent paper points (Dentsply Maillefer). For protocol 3, mesial canals were prepared without irrigant solution.

### Micro-CT Scans

High-resolution scans, before and after root canal preparation, were accomplished per tooth using the same selected parameters. The teeth were scanned (SkyScan 1172) at 70 kV, 114 μA, and an isotropic pixel size of 14.16 μm. The scanning was performed by 360° rotation around the vertical axis with a camera exposure time of 7,000 milliseconds, rotation step of 0.5°, and frame averaging of 5. X-rays were filtered with a 1-mm aluminium filter. A flat-field correction was taken before the scanning procedures to correct for variations in the pixel sensitivity of the camera. Images were reconstructed using NRecon v.1.6.3 (Brucker-microCT) with a beam hardening correction of 40% and ring artifact correction of 10, resulting in the acquisition of 700–800 transverse cross-sections per tooth in a bitmap format. The volume of interest was selected extending from the furcation level to the apex of the mesial root.

### Quantitative Image Analysis

For the quantitative analysis, the original grayscale cross-sectional images of the roots before and after preparation were processed with an interactive segmentation threshold to separate dentin and debris from the root canal space using the Seg3D v.2.1.4 software interface