Detection of Regional Variation of Bone Mineralization in a Human
Mandible using Computed Tomography

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ABSTRACT

Objective: The objective of this study was to examine computed tomography’s ability to assess the regional variation of bone mineralization in a human mandible.

Methods: Ten mandibular sections from cadavers (81.5±12.1 yrs) were scanned using micro-computed tomography (micro-CT) with 27.2 micron voxel size and cone beam CT (CBCT) with 200, 300, and 400 micron voxel sizes. In addition, fifteen clinical CBCT images from young patients (18.9±3.3 yrs) were identified. After segmentation of bone voxels, the 3D alveolar bone (AB) and the basal cortical bone (CB) regions were digitally isolated. A histogram of gray levels, which is equivalent to degree of bone mineralization, was obtained from each region of the CT images. Mean, standard deviation (SD), coefficient of variation (COV), fifth percentile low and high of AB and CB regions were obtained. Percentage differences of the gray level parameters between AB and CB were then computed.

Results: The AB region had significantly lower Mean, Low$_5$ and High$_5$ but significantly higher SD and COV than the CB region for all CT images (p<0.016). The percentage difference of all gray level parameters was not significantly different between CBCT images with different voxel sizes (p>0.111). However, all parameters were significantly lower for the old cadaver group than for the young patient group (p<0.001).
**Conclusions:** CBCT and micro-CT provide comparable results in the assessment of regional variation of bone mineralization in the human mandible. The percentage difference relative to an internal reference (CB) can be used to examine different CBCT images for both cross-sectional and longitudinal comparisons.
DEDICATION

Dedicated to my family and everyone who has helped me achieve this great accomplishment.

Thank You!
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I would like to sincerely thank all those participants who made this study possible. Your service is appreciated by all. I would like to acknowledge Stephanie Gans for her great effort in every aspect of this project. Dr. Mathew Ames also deserves my gratitude for his previous research. I would also like to acknowledge the members of my thesis committee. I would like to thank Dr. Kim for all of his guidance and inspiration during my research. I would also like to thank Dr. Firestone for his timely and helpful advice and Dr. Johnston for all of his assistance with the statistical analysis. I would also like to thank Delta Dental for their modest financial support of this project. Dr. Amanda M. Agnew at Division of Anatomy, College of Medicine of the Ohio State University also deserves acknowledgment for providing the human cadaver mandibles. Finally, I would like to thank my mother and father for all of their love and support.
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CHAPTER 1

INTRODUCTION

Bone Density Anatomy and Physiology

Healthy human bone must be both stiff and flexible simultaneously in order to meet specific physiological functional demands. Bone must be strong enough to bear loading yet elastic enough to resist fracture (Seeman 2003). Both of these physical properties are achieved by contrasting the unique three-dimensional architecture and mineral composition of bone (Curey 1969).

The three-dimensional structural properties of bone contribute greatly to its mechanical properties. Cortical bone, which is highly mineralized and lamellar in composition, provides rigidity and stiffness. Trabecular bone, on the other hand, is more variable in structure and mineralization providing necessary flexibility (Seeman 2003). Individual human bones have a unique composition of cortical and trabecular bone due to the physiologic stresses and functions they serve.

The mineral composition of bone is altered by modulating the concentration of the calcified hydroxy-apatite in the triple helix of the type I collagen matrix (Seeman 2003). The organic matrix consists of approximately 90% collagen and 10% non-collagenous
proteins. Excessive mineralization of this collagen matrix increases stiffness, decreases flexibility and leads to fracture. While under-mineralized bone is too flexible and also leads to fracture. Therefore, depending upon location, disease free human bone is approximately 65% mineralized in order to resist fracture (Seeman 2003).

The three-dimensional macro and micro architecture of healthy human bone is dynamic; continually changing due to the forces borne. Modeling events result in a net change of bone volume due to the uncoupled resorption and formation of bone tissue (Martin et al 1998). Bone modeling is evident throughout life as bone tissue atrophies and hypertrophies during growth, development, aging, disease and repair (Huja and Beck 2008). Bone remodeling; by contrast, is the coupled process of bone formation following resorption without a net change in bone volume (Ruffoni et al 2007; Seeman and Delmas 2006). Healthy human bone has a baseline of bone remodeling activity. The resorption and formation of bone occurs at different points in time and involves bone tissue mineralization that progressively develops and changes over time (Ruffoni et al 2007).

The remodeling activity of bone is the biological determination of mineralization (Grynpas 1993). Remodeling consists of 6 different phases; resting, activation, resorption, reversal, formation and resting (Brouwers 2008). Bone surfaces and cells are inactive during the resting phase. Activation begins with the conversion of inactive osteoclasts into active bone resorbing cells (Brouwers 2008). Resorption proceeds as osteoclastic cells release hydrogen ions through the action of carbonic anhydrase ($\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{HCO}_3^- + \text{H}^+$), dissolving the mineralized bone matrix into $\text{Ca}^{2+}$, $\text{H}_3\text{PO}_4$, $\text{H}_2\text{CO}_3$, water and degraded collagen protein (Väänänen et al 2000). Reversal, when osteoclastic activity ceases, marks
the end of bone resorption. Bone formation begins with the creation of an organic osteoid by osteoblasts, consisting mainly of Type I collagen, forming the basic structural unit (BSU). Primary mineralization of this osteoid begins shortly thereafter and proceeds rapidly until approximately 50 - 70% of the BSU is mineralized with Ca$^{2+}$ within 5 to 10 days (Brouwers 2008). Secondary mineralization of the BSU follows a slower and gradual maturation process as the final mineralization is achieved over a period of 3 - 6 months (Boivin and Meunier 2003). Remodeling ends, just as it begins with a resting period of cellular inactivity. The fact that individual BSU’s are being resorbed and created at different times continuously; the heterogeneity of the mineralized matrix reflects the degree of bone turnover and average age of the bone matrix (Roschger et al 2008; Ruffoni et al 2007).

**Regional Variation of Human Mandible Mineralization**

The human jaw bones are very unique due to the functional demands placed upon them. As tooth bearing structures, the anatomy and physiology of these bones are quite different from other cranio-facial structures due to forces of mastication (Ames et al 2010). These forces originate from the muscles of mastication and are disseminated from the teeth and periodontal ligament to the alveolar bone and finally to the basal bone of the jaws. Masticatory forces produce stress and strain within the bone which leads to fracture micro-damage and the stimulation of bone remodeling (Seeman and Delmas 2006).

Increased remodeling activity leads to a decrease in bone density as mature, more mineralized bone is replaced with immature, less mineralized bone matrix (Ruffoni et al
2007). Due to masticatory functional demands, a remodeling gradient exists in the mandible (Tricker et al 2002). This gradient leads to regional variation in bone mineralization. The alveolar process (AB) has higher rates of remodeling and decreased mineralization while the basal bone (CB) has decreased remodeling rates and a higher degree of mineralization (Ames 2010; Chun 2009; Park et al 2008).

Currently, what are the best methods available to study bone density in the human mandible? Two-dimensional radiographic techniques such as, fractal dimension analysis (FD), have been used to quantify the architecture of bone and to determine bone fragility (Hua et al 2009). However, with the advent of three-dimensional computed tomography, better results are achievable. Bone mineral density (BMD) has been defined to be the mineral content within an apparent volume of bone including the porosity, bone marrow, and bone matrix (WHO 1994). The tissue mineral density (TMD) of bone represents mineral content contained only within the matrix of bone. It was indicated that BMD alone cannot fully explain the risk of bone fragility. On the other hand, it was suggested that tissue mineral density distribution is an important parameter in determining the strength and elastic mechanical properties of bone (Yao et al 2007). Additional research has also demonstrated a strong correlation between the determination of tissue mineral density and the micro-architecture of bone (Teo et al 2006). Currently, the evaluation of tissue mineral density appears to be best standard in determining bone density; therefore the degree of bone mineralization, (DBM), equivalent to tissue mineral density (TMD) will be examined in this study.
Orthodontics over the last 100 years has primarily relied upon panoramic and cephalometric radiographs in order to diagnose and treat malocclusions. Unfortunately, these radiographs have never provided quantifiable diagnostic information regarding the bone density of the jaws (Horner et al 1997). However, during the last decade with the advent of cone beam computed tomography, CBCT, accurate three-dimensional information regarding bone density and structure has become available (Marquezan et al 2011). The additional information provided by CBCT could prove to be very beneficial in the clinical practice of orthodontics. Patient specific bone density data could lead to a more patient specific approach in orthodontic treatment planning, biomechanics, force levels, and retention regimens (Mah et al 2010).

Unfortunately, the data provided by CBCT has currently been underutilized by practitioners because of a lack of significant research regarding bone density determination. Most of the clinical CBCT studies thus far have focused upon linear measurements in approximating the amount of bone volume surrounding various structures from CBCT scans (Suomalainen et al 2008; Veyre-Goulet et al 2008). These studies have demonstrated that CBCT is indeed very accurate and reliable in determining geometric relationships (Baumgaertela et al 2007; Agbaje et al 2007).

However, linear measurements are an inherently limited method in bone density determination due to the fact that they ignore the degree of bone mineralization and the three-dimensional structure of the bone itself. Fortunately, previous research has
demonstrated that CBCT is strongly correlated with two-dimensional FD determination of boney architecture (Hua et al 2009). In addition, several studies have also demonstrated that CBCT determination of bone mineral density is comparable to other methods, including the gold standard for osteoporosis diagnosis, dual-emission x-ray absorptiometry, (DEXA), as well as medical grade multislice helical computed tomography, (MSCT), (Marquezan et al 2011; Naitoh et al 2009; Nomura et al 2009).

Inspite of these correlations with other methods, questions remain regarding the accuracy and reliablity of CBCT gray level values (Hua et al 2009). Several factors related to the physics of a CBCT scanner are responsible. CBCT has a larger beam width than the conventional MSCT. This causes a non-uniform angular distribution of the x-ray beam intensity known as the heel effect, which leads to decreased beam uniformity (Hua et al 2009). In addition, CBCT machines have a single, non-collimated, detector, whereas MSCT have multiple, collimated detectors. As a result, CBCT scans have a larger amount of scattered x-rays than conventional spiral CT which leads to more image artifact. The excess scatter also affects the low-contrast detectability of structures (Endo et al 2001). Moreover, the scatter and artifacts in CBCT are more pronounced near non-homogenous tissues with reduced density (Yoo 2006). Finally, beam hardening is a phenomenon resulting from the increase of mean energy of the x-ray beam when it passes through an object. Because of beam hardening, certain structures such as soft tissue can alter bone gray level values in CBCT scans (Hua et al 2009). Due to these limitations, additional research is necessary so that accurate and reliable data regarding bone density can be generated from CBCT images (Mah et al 2010).
Micro-Computed Tomography

While CBCT is a relatively recent diagnostic imaging tool in clinical practice, micro-computed tomography (micro-CT) has been widely used to obtain high resolution images for small animal models and in-vitro human specimens for decades (Bouxsein et al 2010). Micro-CT provides precise and accurate bone density determination because of the quality of the resolution capacity of approximately 20 micrometers (Muldet et al 2004). In fact, micro-CT has become the gold standard for degree of bone mineralization determination and has been shown to be as accurate as histology sections (Muller et al 1998).

Unfortunately, in order to obtain the high degree of resolution with micro-CT, large amounts of radiation must be used thus making it unreasonable for clinical practice. However, CBCT uses much lower levels of radiation and thus can be used on humans clinically. Unfortunately the decreased radiation dose of CBCT produces a tenfold decrease in resolution capacity; i.e. 20 micrometers versus 200 micrometers. To date, only one previous study has attempted to compare micro-CT to CBCT (Maret et al 2010). Their in-vitro investigation compared the accuracy of tooth size determination and found that CBCT provided sufficient accuracy for clinical practice (Maret et al 2010). However, gray level comparisons were not attempted. Therefore, in order to understand the quality of the diagnostic information regarding bone density provided by CBCT, a correlation with micro-CT must be determined.
Thesis Objective

The objective of this thesis is to develop a novel method of degree of bone mineralization distribution analysis that can be applied to CBCT clinical images (Ames 2010). In the laboratory, the DBM distribution of alveolar bone (AB) and control bone (CB) in human cadaver mandibles will be tested using CBCT and the gold standard, micro-CT. This comparison will allow for our degree of bone mineralization analysis to be applied to clinical CBCT images of our patients. The establishment of an accurate and reliable method of clinical CBCT analysis will facilitate further comparisons of bone density clinically.

Specific Aim

The specific aim of this study is to determine if the regional variation of degree of bone mineralization distribution in the human mandible is evident with CBCT both in-vitro and in-vivo.
CHAPTER 2

MATERIALS AND METHODS

Comparison of Micro-CT and CBCT In-vitro

The first part of this investigation was to evaluate our novel method of degree of bone mineralization in the laboratory; comparing our analysis of CBCT scans with the gold standard of micro-CT using the same human mandibles. Therefore, ten mandibles were obtained from cadavers (7 males and 3 females, mean 81.5±12.1 yrs) that were provided by Division of Anatomy, College of Medicine of the Ohio State University. The mandibles were dissected from the skull and removed of all non-bone tissue. Individual teeth were randomly selected for analysis (4 left canines, 1 right canine, 2 left premolars and 3 right premolars). All teeth with dental restorations were excluded so that the enamel mineralization could be analyzed. The exclusion of dental restorations also meant that any scatter radiation artifacts created by these restorations would not affect our analysis of the mandibular bone. The teeth were sectioned using a low speed saw (Isomet, Buehler, IL USA) with two parallel diamond blades butting under water irrigation (Fig. 1). The 10 mm specimen cuts were made in a bucco-lingual direction and perpendicular to the occlusal plane. The specimens were stored at -21°C until utilized.
The mandibular specimens were then scanned using a 3D Micro-CT scanner (Skyscan 1172-D, Kontich, Belgium) with 27.2 micrometer voxel resolution. The same scanning conditions (70 kV, 141 μA, and 20 minute scanning time) were applied for all specimens. These same specimens were also scanned by a cone beam-CT (CBCT) scanner (iCAT, PA) at 200, 300 and 400 micron voxel sizes under the same scanning energy (120 kV and 5 mA) but with different scanning times (26.9 seconds, 8.9 seconds, and 8.9 seconds for images with 200, 300 and 400 micron voxel sizes, respectively). These resolutions were chosen because they are the most common scanning ranges used in clinical practice.

Both data files containing the raw CT images were then imported into Image J (National Institutes of Health, USA) (Fig. 2a) and saved in tagged image file format (.tif extension). CT attenuation values were maintained and reported as voxel gray level values (Fig. 3). Image segmentation of bone voxels from non-bone voxels using a heuristic algorithm introduced in previous studies was completed (Ames et al 2010; Yeni et al 2005; Zauel et al 2004). With only bone remaining in the images, isolation of the region of interest (ROI) in the mandibular segment could proceed.

In order to study the degree of bone mineralization in the ROI, the teeth must first be isolated from the bone (Fig. 2a). With the micro-CT images, any connections between the teeth and alveolus were manually removed in Image J (Kim et al 2004; Ames 2010). Due to the decreased CBCT resolution; the voxels of tooth and periodontal ligament were not as clearly defined as those in the micro-CT image (Fig. 2b). Therefore, Livewire®, a semi-automated segmentation software was used to isolate the teeth for the CBCT images
(Agbaje 2004) (Fig. 2c). The teeth were then three-dimensionally dilated by 39 voxels. By overlapping the dilated teeth back onto the ROI within the mandibular segment of the original images, a 3D alveolar bone region (AB) within 1 mm from the tooth roots was obtained (Fig. 2).

The basal cortical bone region (CB) was determined by the opposite process, erosion of the internal and external bone surfaces producing a core region of basal cortical bone. The AB regions were subtracted from the original images and the remaining mandibular bone was three-dimensionally eroded by 22 voxels (0.6 mm) from periosteal and endosteal surfaces (Fig. 2). CB represents a core region of basal cortical bone at which normal bone remodeling occurs. The CB region excludes the marginal basal bone at which a rapid bone turnover due to active bone formation occurs (Huja and Beck 2008).

**Clinical CBCT**

With our method confirmed in the laboratory; the degree of bone mineralization analysis of clinical CBCT images from dental patients began. Following the Institutional Review Board (IRB) approval at the Ohio State University (Protocol #: 2011H0128), 15 CBCT images were selected from a previously existing database of 350 CBCT scans taken during routine clinical practice at the College of Dentistry. Those clinical CBCT images were taken using the same CBCT scanner as the cadaveric specimens scanned previously. These young patients (7 males and 8 females, mean 18.9±3.3 yrs) were all healthy individuals with no reported medical complications or current medications at the time of
scanning. Mandibular left first premolars were identified from each CBCT image. Once again, all restored teeth were excluded.

The voxel size of the clinical CBCT images was 300 micron for 14 images and 400 micron for 1 image. The AB and CB regions in these clinical 3D CBCT images were identified following the same process used for the CBCT images of cadaveric specimen. However, additional steps had to be added to our analysis in order to remove the background gray levels that were not present in our in-vitro scans. This was accomplished through the envelope function in ModelPrep (Finite Element Method, FEM processing program). Each of the clinical CBCT images, enveloped at 1200, resulted in the isolation of the ROI without any background noise within or outside the mandibular bone.

**Statistical Analysis**

The gray level of each bone voxel, which is equivalent to degree of bone mineralization (DBM), was obtained in the process of bone voxel segmentation (Fig. 3a). The gray level histograms of AB and CB were used to determine mean value (Mean) that was computed by dividing the sum of gray level values by the total count of voxels in each region (Table. 1). Standard deviation (SD) of the gray level histogram and coefficient of variation (COV=SD/Mean) were obtained to account for the variability of gray levels. Low and high gray levels (Low$_5$ and High$_5$) were determined at the 5$^{th}$ and the 95$^{th}$ percentiles of voxel counts in the histogram (Table. 1).

Percentage difference (%) of the gray level parameters between AB and CB ((AB-CB) / ((AB+CB)/2)x100) was then computed for each CBCT image (Table. 2). A paired t-
test was used to compare the gray level parameters between AB and CB regions in the same individual image for all micro-CT images (27.2 micron voxel size) and CBCT images (200, 300, 400 micron voxel sizes and clinical) (Table. 1). Paired t-testing was also used to compare the percentage differences of gray level parameters between micro-CT and CBCT images of cadaver specimens (Fig. 4). A linear regression was used to test correlations of the percentage differences of gray level parameters with voxel sizes of CBCT images of cadaver specimens. Finally, the percentage differences of gray level parameters based on the CBCT images (300 micron voxel size) of old cadaver group were compared with those of young patient group using analysis of variance (ANOVA). Significance was set at $\alpha \leq 0.05$. 
CHAPTER 3

MANUSCRIPT

Detection of Regional Variation of Bone Mineralization in a Human Mandible using Computed Tomography

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Abstract

Objective: The objective of this study was to examine computed tomography’s ability to assess the regional variation of bone mineralization in a human mandible. **Methods:** Ten mandibular sections from cadavers (81.5±12.1 yrs) were scanned using micro-computed tomography (micro-CT) with 27.2 micron voxel size and cone beam CT (CBCT) with 200, 300, and 400 micron voxel sizes. In addition, fifteen clinical CBCT images from young patients (18.9±3.3 yrs) were identified. After segmentation of bone voxels, the 3D alveolar bone (AB) and the basal cortical bone (CB) regions were digitally isolated. A histogram of gray levels, which is equivalent to degree of bone mineralization, was obtained from each region of the CT images. Mean, standard deviation (SD), coefficient of variation (COV), fifth percentile low and high of AB and CB regions were obtained. Percentage differences of the gray level parameters between AB and CB were then computed. **Results:** The AB region had significantly lower Mean, Low₅ and High₅ but significantly higher SD and COV than the CB region for all CT images (p<0.016). The percentage difference of all gray level parameters was not significantly different between CBCT images with different voxel sizes (p>0.111). However, all parameters were significantly lower for the old cadaver group than for the young patient group (p<0.001). **Conclusions:** CBCT and micro-CT provide comparable results in the assessment of regional variation of bone mineralization in the human mandible. The percentage difference relative to an internal reference (CB) can be used to examine different CBCT images for both cross-sectional and longitudinal comparisons.
Introduction

Masticatory force applied on teeth is directly transferred to the alveolar bone (AB) region stimulating more remodeling than other oral bone regions (Ames et al., 2010; Ohtani et al., 2008). The active modeling and remodeling of AB region is also observed during tooth movement due to orthodontic treatment, bone healing after implantation and periodontal bone disease (Cavallaro et al., 2009; Deguchi et al., 2008; Meikle, 2006; Nanci and Bosshardt, 2006). While bone modeling refers to the uncoupled resorption and formation of bone tissue, bone remodeling is the coupled process of bone formation following resorption (Martin et al., 1998; Ruffoni et al., 2007; Seeman and Delmas, 2006). The resorption and formation of bone occurs at different points in time and involves bone tissue mineralization that progressively develops and changes over time (Ruffoni et al., 2007). As such, the active modeling and remodeling at the AB region results in various tissue mineral distributions within the mandible.

As degree of bone mineralization (DBM) accounts for the amount of mineral content at the tissue level of bone, the DBM distribution directly reflects biological activities (resorption and formation) in the process of bone remodeling (Boivin et al., 2009; Roschger et al., 2008). While many studies suggested possible tools to assess the DBM, destructive specimen preparation (histology, backscattered electron imaging and microradiograph)(Boivin and Meunier, 2003; Follet et al., 2004; Roschger et al., 1998), and non-destructive but high radiation dose requiring micro-computed tomography (micro-CT) limited the usage of these methods to the laboratory (Burghardt et al., 2011; Ritman, 2011). On the other hand, usage of clinical cone beam CT (CBCT) in the dental setting has
rapidly increased over the past several years (Scarfe and Farman, 2008). While many previous studies indicated that the CBCT can assess bone mineral using the CT attenuation values of CBCT image, its clinical applicability is still controversial (Hsu et al., 2010; Hua et al., 2009; Katsumata et al., 2009; Naitoh et al., 2009).

The objective of this study was to examine whether the clinical CBCT can assess the regional variation of bone mineralization in a human mandible. Thus, the aims of this study were to determine if 1) the clinical CBCT and the higher resolution micro-CT provide comparable results to detect the regional variation of DBM parameters in the same CT image and 2) if percentage difference relative to an internal reference can be used to compare the DBM parameters between the different CT images. The micro-CT and CBCT images of human cadaveric mandibles were used to address these aims. In addition, CBCT images obtained from patients were used to test applicability of the new DBM analysis methods clinically.

Materials and Methods

Ten human mandibles were obtained from cadavers (7 males and 3 females, mean 81.5±12.1 yrs) that were provided by Division of Anatomy, College of Medicine of the Ohio State University. Individual teeth were randomly selected for analysis (4 left canines, 1 right canine, 2 left premolars and 3 right premolars). All teeth with dental restorations were excluded to avoid X-ray artifacts. After removal of soft tissue, the mandibular bone was sectioned using a low speed saw (Isomet, Buehler, IL USA) with two parallel diamond blades under water irrigation. The 10 mm thick slice of specimen was made in a bucco-
lingual direction and perpendicular to the occlusal plane (Fig. 1). The specimens were stored at -21°C until utilized.

After thawing at room temperature, specimens were scanned by a micro-CT scanner (Skyscan 1172-D, Kontich, Belgium) with the scanning and reconstruction voxel sizes at 27.2 micron. The same scanning conditions (70 kV, 141 μA, and 20 minute scanning time) were applied for all specimens. These same specimens were also scanned by a CBCT scanner (iCAT, PA) at 200, 300 and 400 micron voxel sizes under the same scanning energy (120 kV and 5 mA) but with different scanning times (26.9 seconds, 8.9 seconds, and 8.9 seconds for images with 200, 300 and 400 micron voxel sizes, respectively), which are the scanning range used in clinical practice. Bone voxels of those CT images were segmented from non-bone voxels using a heuristic algorithm as described in the previous studies (Ames et al., 2010; Zauel et al., 2004).

After segmentation, mandibular bone regions were identified following the image process described in a previous study (Ames et al., 2010). The teeth in the three-dimensional (3D) micro-CT image were digitally separated from the mandible segment using imaging software (ImageJ, National Institutes of Health, USA) (Fig. 2a). Then, the isolated teeth images were three-dimensionally dilated by 39 voxels. The dilated teeth images were overlapped on the separated mandible segment providing a 3D alveolar bone region (AB) within 1 mm from the tooth roots (Fig. 2a,b). A basal cortical bone region (CB) was determined by three-dimensionally eroding the separated mandible segment by 22 voxels (0.6 mm) from periosteal and endosteal surfaces of basal bone. This isolation process provided a core region of basal cortical bone at which normal bone remodeling
occurs excluding the marginal basal bone at which a rapid bone turnover due to active bone formation is observed (Huja and Beck, 2008).

As the resolution of a CBCT image is much coarser than that of micro-CT image, the voxels of tooth and periodontal ligament in the CBCT image were not as clearly distinguishable as those in the micro-CT image. Thus, Livewire®, a semi-automated segmentation software was used to isolate the teeth in CBCT images following the procedure introduced in the previous study (Agbaje et al., 2007). After the removal of teeth, the 3D AB and CB regions were identified in the CBCT images corresponding to those regions in the micro-CT images (Fig. 2c). The alveolar bone (AB) region was isolated within approximately 1 mm from root surface and the core region of basal cortical bone (CB) region was located below the tooth root and approximately 0.6 mm away from periosteal and endosteal borders of the mandibular bone base using Image J.

Following the Institutional Review Board (IRB) approval at the Ohio State University (Protocol #: 2011H0128), 15 CBCT images were selected from a previously existing database of 350 CBCT scans taken during routine clinical practice at the College of Dentistry. Those clinical CBCT images were taken using the same CBCT scanner as the cadaveric specimens scanned in the current study. These young patients (7 males and 8 females, mean 18.9±3.3 yrs) were all healthy individuals without any medical complications or medications at the time of scanning. Mandibular left premolar tooth regions were identified from each CBCT image. Once again, restored teeth were excluded. The voxel size of the clinical CBCT images was 300 micron for 14 images and 400 micron
for 1 image. The AB and CB regions in these clinical 3D CBCT images were identified following the same process used for the CBCT images of cadaveric specimen.

The gray level of each bone voxel, which is equivalent to degree of bone mineralization (DBM), was obtained in the process of bone voxel segmentation. The gray level histograms of AB and CB were used to determine mean value (Mean) that was computed by dividing the sum of gray level values by the total count of voxels in each region (Fig. 3a). Standard deviation (SD) of the gray level histogram and coefficient of variation (COV=SD/Mean) were obtained to account for the variability of gray levels. Low and high gray levels (Low\textsubscript{5} and High\textsubscript{5}) were determined at the 5\textsuperscript{th} and the 95\textsuperscript{th} percentiles of voxel counts in the histogram. Percentage difference (%) of the gray level parameters between AB and CB ((AB-CB) / ((AB+C\textsubscript{B})/2)x100) was then computed for each CBCT image (Table. 2). A paired t-test was used to compare the gray level parameters between AB and CB regions in the same individual image for all micro-CT images (27.2 micron voxel size) and CBCT images (the cadaveric images at 200, 300, 400 micron voxel sizes and the clinical images) (Fig. 4). Paired t-testing was also used to compare the percentage differences of gray level parameters between micro-CT and CBCT images of cadaver specimens. A linear regression was used to test correlations of the percentage differences of gray level parameters with voxel sizes of CBCT images of cadaver specimens. Finally, the percentage differences of gray level parameters based on the CBCT images (300 micron voxel size) of old cadaver group were compared with those of young patient group using analysis of variance (ANOVA). Significance was set at ≤0.05.
Results

The histograms of all gray level parameters were successfully obtained from the 3D CT images of the alveolar bone (AB) and the basal cortical bone (CB) regions (Table. 1 and 2). The gray level histogram of micro-CT images resembled that of CBCT images (Fig. 3b and c).

The AB region had significantly lower means of Mean, Low5 and High5 but significantly higher SD and COV than the CB region (p<0.016) (Fig. 2 and Table 1). These results were consistently observed for all of micro-CT and CBCT images independent of the scanning voxel sizes (200, 300, and 400 microns), and the old cadaver and the young patient groups. There was no gender effect evident (p>0.101).

Means of the percentage differences of all gray level parameters between AB and CB regions were not significantly different between micro-CT and CBCT images (p>0.069) except Low5 that had significantly higher percentage difference for micro-CT images than that for CBCT images (p<0.009) (Fig. 4). The percentage differences of SD, COV and Low5 decreased with increasing voxel sizes having weak but significant correlations with voxel sizes ($r^2<0.425$ and $p<0.014$) while those of Mean and High5 had not significant correlations ($p>0.078$). However, means of the percentage differences of all gray level parameters were not significantly different between CBCT images at the range of voxel sizes examined here (p>0.14).

The clinical CBCT images of the young patient group had significantly higher means of the percentage differences of all gray level parameters than the 300 micron voxel size CBCT images of the old cadaver group (p<0.001) (Fig. 4). The negative values of
percentage differences indicated that the AB had lower Mean, Low₅ and High₅ than the CB while their absolute values were higher for the young patient group than those for the old cadaver group.

Discussion

A significant regional variation of the gray level parameters, which are equivalent to the degree of bone mineralization (DBM) parameters, were found in the human mandible and this result was consistent between the in vitro micro-CT images and the CBCT images with different voxel sizes that have been used in clinic. This finding suggests that the DBM analysis based on the clinical CBCT scanner can provide comparable results with that based on the high resolution micro-CT scanner. It is likely that a masticatory demand stimulated more active bone remodeling in the AB region resulting in the decrease in DBM (gray level Mean, Low₅ and High₅) and the increase in DBM variability (gray level SD and COV) when compared with those in the CB region where normal bone remodeling would occur. Values of the percentage difference of gray level parameters between AB and CB regions were not affected at the range of clinical voxel sizes (200, 300 and 400 microns) of CBCT images. This result suggests an analysis of percentage differences is a possible method to compare the distribution of bone mineralization between CBCT images taken under different scanning conditions. The substantially lower percentage difference of gray level parameters found in the old cadaver group than that in the young patient group likely resulted from a decrease in bone forming activities while increasing tissue mineralization in the AB with age. The current results
support that non-invasive clinical CBCT can be used to provide useful information to estimate changes in the degree of jaw bone tissue mineralization, which can account for the progress of dental treatments in a patient.

DBM parameters have been used to estimate the alteration of bone tissue mineral distribution resulting from bone remodeling (Roschger et al., 2003; Ruffoni et al., 2007; Yao et al., 2007). The mineralization of bone at the tissue level correlates with the mechanical properties of bone at the macro level (Follet et al., 2004). As such, analysis of DBM parameters was proposed to aid the diagnosis of bone disease (Roschger et al., 2008). The DBM distribution was analyzed using a thin section of bone specimen for microradiographs or quantitative backscattered electron imaging (Follet et al., 2004; Roschger et al., 1998). Although the high resolution images of bone surface allowed for accurate analysis of bone mineral distribution, these methods require a destructive process during specimen preparation, two-dimensional analysis on the surface of bone, and a small local region of interest. Instead, some studies used micro-CT images for DBM analysis because it provided non-invasive 3D scanning of whole bone specimen (Ames et al., 2010; Yao et al., 2007). While the micro-CT based DBM parameters could provide a valid result in animal studies, its high radiation exposure limits its clinical application for scanning patients. The clinical CBCT image includes gray level of voxels while scanning patients with lower radiation. Thus, it was proposed to use the CBCT image for bone mineral density measurement (Naitoh et al., 2007; Nomura et al., 2010). However, the reliability of CBCT in assessing DBM had not been investigated. In the current study, we examined the regional variations of DBM parameters measured by the micro-CT as the gold standard to
evaluate those measured by CBCT because the micro-CT based analysis was anticipated to provide more accurate results due to its much higher resolution (27.2 micron voxel size) than that of CBCT (larger than 200 micron voxel size). We found that the micro-CT and CBCT based analyses provided consistent results for the regional variation of every gray level parameter. This finding suggested that the micro-CT and CBCT based gray level analyses of the bone mineralization at the hard tissue level are comparable.

Other studies have demonstrated that CBCT can assess bone mineral density of jaw bone (Hsu et al., 2010; Naitoh et al., 2009; Nomura et al., 2010; Norton and Gamble, 2001). However, it was noted that the CBCT based bone mineral density measures varied depending on scanning conditions (Kwong et al., 2008; Loubele et al., 2008). As such, caution should be used to compare the bone mineral density values between different CBCT images. In the current study, we proposed two ways of analysis to obtain a better interpretation of results from CBCT based bone mineralization measurement. First, the absolute values of DBM parameters in different oral regions can be compared only using the same image in which gray levels of bone tissue were attenuated under the same scanning and reconstruction conditions. Second, only the relative values of gray level, like the percentage differences in the current study, can be compared between different CBCT images by using an internal reference, like the CB in the current study, which is included to compute the relative value.

A substantial amount of the masticatory force applied to teeth is transmitted to the alveolar bone (AB) through the periodontal ligament (Ona and Wakabayashi, 2006; Poiate et al., 2009). This masticatory functional demand on the AB region stimulates active bone
remodeling (Ames et al., 2010; Ohtani et al., 2008). More bone turnover due to the bone remodeling increases newly forming bone regions and decreases pre-existing old bone regions (Busse et al., 2009; Marcus, 1996). As the newly forming tissue has less mineral content than the pre-existing old tissue, the mean DBM is decreased in the actively remodeling region. Tissue mineralization is a long-term process and it takes years to complete full mineralization (Ruffoni et al., 2007). Thus, the variability of DBM changes in the newly remodeled region depending on the status of mineralization after bone remodeling. The Low$_5$ in the current study accounts for DBM at the newly forming bone region and the High$_5$ represents DBM at the pre-existing old bone region. We hypothesize the current results that demonstrated lower Mean, Low$_5$ and High$_5$ but higher variability in the AB region than those in the CB region reflects the active bone turnover in the AB region under the masticatory stimulus. As the current three dimensional (3D) CT image based analysis allowed for identifying the 3D AB region completely surrounding the periodontal ligament that outlines the tooth structure, more comprehensive results could be obtained compared with the 2D histological and micro-radiography analysis. This is the first study analyzing the DBM parameters using the 3D CT image of human mandible. The DBM analysis technique verified using the CBCT images for cadaver specimens could be applied to examine the clinical CBCT images.

The percentage differences between AB and CB for gray level variability and Low$_5$ decreased with increasing voxel sizes. This finding likely resulted from the courser voxel size and reduced image resolution which decreased the gray level contrast between voxels. In particular, the finding that the means of Low$_5$ were significantly different between
micro-CT images and other CBCT images indicated that the high resolution micro-CT can more precisely segment bone voxels from non-bone voxels than is possible with the lower resolution of CBCT. Due to the periodontal ligament, the AB region has border lines between bone and non-bone voxels while the CB inside the basal cortical bone does not have a similar demarcation of non-bone voxels. Thus, the Low5 of AB region has lower values because a higher resolution image can provide a more detailed gradient of the low gray levels than a lower resolution image which provides the averaged gray levels. On the other hand, the gray levels of CB region with much less non-bone voxels would be less influenced with changing image resolution. In light of this aspect, it can be hypothesized that the significantly higher percentage difference of Low5 in the micro-CT image than those in the CBCT images could arise from a decrease in the Low5 values of AB region of the micro-CT image.

Similarly, the percentage differences for Mean and High5 that were less affected by the non-bone voxels showed more consistent values between CT images independent of voxel sizes. Except for the Low5, the percentage differences of all other gray level parameters were comparable between the micro-CT and CBCT images with different voxel sizes for the same cadaver specimens. Most importantly, all of those parameters between CBCT images were not significantly different. This result indicates that the effect of imaging resolution on comparison of gray level parameters can be reduced when the relative gray levels were compared between different CBCT images scanned at the clinical range of imaging voxel sizes (200, 300 and 400 microns) as examined in the current study.
The clinical patient CBCT images were obtained using the same CBCT scanner utilized for the human cadaver specimens. The clinical patients and human cadaver mandible specimens represent young and elderly human groups, respectively. It has been observed that bone forming capacity of the alveolar bone decreased with age (Jager, 1996). This reduced capacity for alveolar bone formation likely decreases the forming rate of new (less mineralized) bone region, while more mineral is added to the pre-existing (more mineralized) bone regions resulting in the increased mean value and decreased variability of DBM in the AB region with aging. From the gray level histogram comparisons of the AB and CB regions (Fig. 3b and Fig. 3c), we hypothesize that the aging process progressively transforms the gray level distribution of the AB region to be more similar to that of the CB region. The current findings supported this age dependent change in DBM distribution because all of the percentage differences of gray level parameters between AB and CB regions for the old cadaver specimens (81.5 yrs) were lower than those for the young clinical patients (18.9 yrs).

The limitation of this study may be that correlations of the absolute values of DBM parameters between micro-CT and CBCT images were not examined. It was very difficult to exactly match the same regions of interest between the CT images of the mandibles obtained from the two different scanners due to their more than 7 fold difference in voxel sizes. Additionally, not only the voxel size but also the scanning voltage, current and duration were also different between micro-CT and CBCT. These substantially different resolution and scanning conditions limited the direct comparison of absolute values between images obtained by the two CT scanners.
In conclusion, the CBCT can detect the regional variation of bone mineralization in the jaw bone providing comparable results to the micro-CT. This finding indicated that alteration of tissue mineral distribution, which is a consequence of biological activities in jaw bone, can be detected using the individual clinical CBCT images. The percentage difference relative to an internal reference can be used to examine different CBCT images for both cross-sectional and longitudinal comparisons. These CBCT based analyses for gray level parameters further promotes the validity of assessment of bone density changes that is required for dental processes including orthodontic tooth movement, alveolar bone loss in periodontitis, jaw bone augmentation before implantation and bone regeneration after oral surgery.

Acknowledgements
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References


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the young patient group likely resulted from a decrease in bone forming activities while increasing tissue mineralization in the AB with age. Non-invasive clinical CBCT can be used to provide useful information to estimate the degree of alternation of jaw bone tissue mineralization, which can account for the progress of dental treatments in a patient.

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However, the reliability of CBCT in assessing DBM had not been investigated. In the current study, we examined the regional variations of DBM parameters measured by the micro-CT as the gold standard to evaluate those measured by CBCT because the micro-CT based analysis was anticipated to provide more accurate results due to its much higher resolution (27.2 micron voxel size) than that of CBCT (larger than 200 micron voxel size). We found that the micro-CT and CBCT based analyses provided consistent results for the regional variation of every gray level parameter. This finding suggested that the micro-CT and CBCT based gray level analyses are comparable to account for the bone mineralization at the hard tissue level.

Other studies have demonstrated that CBCT can assess bone mineral density of jaw bone (Hsu et al 2010; Naitoh et al 2009; Nomura et al 2010; Norton and Gamble 2001). However, it was noted that the CBCT based bone mineral density measures varied depending on scanning conditions (Kwong et al 2008; Loubele et al 2008). As such, caution should be used to compare the bone mineral density values between different CBCT images. In the current study, we propose two ways of analysis to obtain a better interpretation of results from CBCT based bone mineralization measurement. First, the absolute values of DBM parameters in different oral regions can be compared only using the same image in which gray levels of bone tissue were attenuated under the same scanning and reconstruction conditions. Second, only the relative values of gray level, like the percentage differences in the current study, can be compared between different CBCT images by using an internal reference, like the CB in the current study, which is included to compute the relative value.
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The percentage differences between AB and CB for gray level variability and Low$_5$ decreased with increasing voxel sizes. This finding likely resulted from the courser voxel size and reduced image resolution which decreased the gray level contrast between voxels. In particular, the finding that the means of Low$_5$ were significantly different between micro-CT images and other CBCT images indicated that the high resolution micro-CT can more precisely segment bone voxels from non-bone voxels than is possible with the lower resolution of CBCT. Due to the periodontal ligament, the AB region has border lines between bone and non-bone voxels while the CB inside the basal cortical bone does not have a similar demarcation of non-bone voxels. Thus, the Low$_5$ of the AB region has lower values because a higher resolution image can provide a more detailed gradient of the low gray levels than a lower resolution image which provides the averaged gray levels. On the other hand, the gray levels of CB region with much less non-bone voxels would be less influenced with changing image resolution. In light of this aspect, it can be hypothesized that the significantly higher percentage difference of Low$_5$ in the micro-CT image than those in the CBCT images could arise from a decrease in the Low$_5$ values of AB region of the micro-CT image. Similarly, the percentage differences for Mean and High$_5$ that were less affected by the non-bone voxel segmentation showed more consistent values between CT images independent of voxel sizes. Except for the Low$_5$, the percentage differences of all other gray level parameters were comparable between the micro-CT and CBCT images with different voxel sizes for the same cadaver specimens. Most importantly, all of those parameters between CBCT images were not significantly different. This result indicates that the effect of imaging resolution on comparison of gray level parameters can be
reduced when the relative gray levels were compared between different CBCT images scanned at the clinical range of imaging voxel sizes (200, 300 and 400 microns) as examined in the current study.

The clinical patient CBCT images were obtained using the same CBCT scanner utilized for the human cadaver specimens. The clinical patients and human cadaver mandible specimens represent young and elderly human groups, respectively. It has been observed that bone forming capacity of the alveolar bone decreased with age (Jager, 1996). This reduced capacity for alveolar bone formation likely decreases the forming rate of new (less mineralized) bone region, while more mineral is added to the pre-existing (more mineralized) bone regions resulting in the increased mean value and decreased variability of DBM in the AB region with aging. From the gray level histogram comparisons of the AB and CB regions (Fig. 3b and Fig. 3c), we hypothesize that the aging process progressively transforms the gray level distribution of the AB region to be more similar to that of the CB region. The current findings supported this age dependent change in DBM distribution because all of the percentage differences of gray level parameters between AB and CB regions for the old cadaver specimens (81.5 yrs) were lower than those for the young clinical patients (18.9 yrs).

There are some limitations that should be discussed. First, correlations of the absolute values of DBM parameters between micro-CT and CBCT images were not examined. It was very hard to exactly match the same regions of interest between the CT images of the mandibles obtained from the two different scanners due to their 7 fold difference in voxel sizes. Additionally, not only the voxel size but also the scanning voltage, current and
duration were also different between micro-CT and CBCT. These substantially different resolution and scanning conditions limited the direct comparison of absolute values between images obtained by the two CT scanners. Second, the comparability of percentage difference of gray level parameters was examined only for the CBCT images of the same cadaver specimens. This analysis method for relative values was introduced to compare CBCT images taken at different voxel sizes and scanning time points clinically. Thus, it was not necessary to compare the parameters with those of the micro-CT images that were only used in vitro. Nevertheless, when the percentage difference of gray level parameters was compared between micro-CT and CBCT images, most of the comparisons were not significantly different.

In conclusion, the CBCT can detect the regional variation of bone mineralization in the jaw bone providing comparable results to the micro-CT. This finding indicated that alteration of tissue mineral distribution, which is a consequence of biological activities in jaw bone, can be detected using the individual clinical CBCT images. The percentage difference relative to an internal reference can be used to examine different CBCT images for both cross-sectional and longitudinal comparisons. These CBCT based analyses for gray level parameters further promotes the validity of assessment of bone density changes that is required for dental processes during orthodontic tooth movement, AB loss in periodontitis, and AB augmentation in implant dentistry.
CHAPTER 5

BIBLIOGRAPHY


APPENDIX A: FIGURES & TABLES
Figure 1. Pre-molars Sectioned for Micro-Ct and CBCT Scanning.
Figure 2. Isolation of AB and CB Regions.

a) AB and CB regions of a human mandible, b) isolation of those regions using 3D micro-CT image, and c) using 3D CBCT image for the same specimen.
Figure 3.  **Histogram Analysis.**

a) DBM parameters determined using a histogram of gray level values, b) comparison of gray level histograms between AB (gray line) and CB (black line) regions using 3D micro-CT image, and c) using 3D CBCT image for the same specimen.
Figure 4. Percentage difference of gray level values between micro-CT and CBCT images; (27.2, 200, 300, 400 micron voxel size images for old cadaver group and clinical images for young patient group). The negative values indicated that AB had lower Mean, Low5 and High5 than CB. The error bars represents standard deviation of each parameter. The gray level parameters were not significantly different between CT images (p>0.14) except *; p<0.009 for Low5 between micro-CT and other CBCT images for cadaver group and **; p<0.001 for all gray level parameters between clinical and other CBCT images for cadaver group.
<table>
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<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>COV</th>
<th>Low5</th>
<th>High5</th>
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<td>AB</td>
<td>CB</td>
<td>AB</td>
<td>CB</td>
<td>AB</td>
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<tr>
<td>Micro-CT</td>
<td>2288.37 ± 289.42</td>
<td>2788.12 ± 213.36</td>
<td>561.96 ± 161.61</td>
<td>312.17 ± 59.05</td>
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<td></td>
<td>p&lt;0.001</td>
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<td>200 micron</td>
<td>2102.83 ± 117.56</td>
<td>2473.87 ± 59.00</td>
<td>291.98 ± 52.79</td>
<td>190.20 ± 46.55</td>
<td>0.14 ± 0.02</td>
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<tr>
<td></td>
<td>p&lt;0.001</td>
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<td>300 micron</td>
<td>2134.38 ± 104.31</td>
<td>2462.24 ± 175.00</td>
<td>234.79 ± 43.13</td>
<td>172.72 ± 40.75</td>
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<td>p&lt;0.002</td>
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<td>400 micron</td>
<td>2144.82 ± 129.60</td>
<td>2460.89 ± 105.85</td>
<td>229.08 ± 41.08</td>
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<td>Clinical</td>
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<td>2519.79 ± 96.77</td>
<td>281.60 ± 33.85</td>
<td>82.79 ± 34.81</td>
<td>0.16 ± 0.03</td>
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Table 1. Regional Variation of Gray Level Values for Micro-CT and CBCT Images (27.2, 200, 300, 400 voxel sizes).
<table>
<thead>
<tr>
<th></th>
<th>P_Mean</th>
<th>P_SD</th>
<th>P_COV</th>
<th>P_GL High</th>
<th>P_GL Low</th>
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<td>Micro-CT AB</td>
<td>-20.11 ± 10.87</td>
<td>53.67 ± 24.53</td>
<td>70.99 ± 30.68</td>
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<td>-16.32 ± 4.73</td>
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<td>-14.05 ± 10.05</td>
<td>34.71 ± 17.45</td>
<td>47.92 ± 23.71</td>
<td>-7.71 ± 8.31</td>
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<td>CBCT 400 AB</td>
<td>-13.84 ± 8.07</td>
<td>29.79 ± 22.65</td>
<td>42.84 ± 25.57</td>
<td>-8.16 ± 6.50</td>
<td>-18.25 ± 11.06</td>
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<td>-37.14 ± 6.43</td>
<td>111.20 ± 28.81</td>
<td>133.89 ± 23.94</td>
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Table 2. Percentage Difference (%) of Gray Level Values for Micro-CT and CBCT Images (27.2, 200, 300, 400 voxel sizes).