Evaluation of Bone-Implant Integration: Efficiency and Precision of 3 Methods

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Computer-assisted planimetry, computer-assisted lineal analysis, and point-counting stereology have been compared with respect to their reproducibility and the time required to analyze bone-implant integration. Sections of 6 threaded dental implants selected from a bone augmentation experiment for their wide range of new bone formation were analyzed by each method 3 times. The bone density and percentage of osseous integration were evaluated at 4 sites around each implant section. It was found that computer-assisted planimetry demonstrated a modest but significantly greater variance ($P < .05$) in bone density estimates when compared to the computer-assisted lineal analysis and point-counting methods. Computer-assisted planimetry requires a different method of measuring each parameter and separate fields of view to evaluate fields distant from the implant. However, this can all be accomplished with line probes, as in computer-assisted lineal analysis, which extend from the implant surface into the surrounding alveolar bone. Whereas computer-assisted planimetry requires a separate identification of the perimeter of each field to be analyzed (next to and distant from the implant), computer-assisted lineal analysis allows expansion of the field to be evaluated without creating a new field of view. Also, following a limited learning curve, both point-counting and computer-assisted lineal analysis required less time to complete than did computer-assisted planimetry.
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During the last 10 years, implant dentistry utilizing root-form implants has become a strong and rapidly growing clinical science. What was formerly considered an experimental solution to tooth loss has now become a predictable restorative alternative. Root-form dental implants are available in either threaded or cylindric shape, are fabricated from either inert alloys or ceramic materials, and are offered with a variety of surface characteristics. The implant-bone interface has been described in several reviews.[1–3] *Osseointegration* is the term used to denote the intimate contact of bone to the implant surface. Clinical success, the clinical manifestation of histologic osseointegration, has been defined by immobility when special mechanical testing devices are activated.[4,5] Histologic assessment of osseointegration is usually made on ground sections as described by Donath.[6] Initially, linear measurements were made directly with an ocular eyepiece grid. More recently, stereologic measurements have been made on digitized images with the assistance of computer software packages. However, methods based upon stereologic principles and those depending upon direct measurement have not been compared systematically with respect to precision and efficiency.

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Quantitative evaluation of parameters that characterize bone-implant integration depends upon obtaining appropriate histologic sections. These can only be made by plastic embedment of specimens, followed by slicing with a diamond saw and then grinding to the desired thickness. This precludes the analysis of multiple sections, since only 1 or 2 sections can be obtained from 1 implant. Moreover, the plane of that section is either frontal (facial to lingual) or parasagittal (mesial to distal). It is understood that estimates of bone density (bone volume/tissue volume) or osseointegration (percentage of implant surface closely apposed to bone) may be biased because they can only be obtained from a single ground section from each root-form implant specimen. Nevertheless, the assessment of these parameters is still useful when comparing the effectiveness of different treatments.

Three methods are available for the evaluation of osseointegration (OI) and bone density (BD). The first is that of point and line intersection counts. In 1979, Weibel[7] clearly showed that point counting was the most efficient manual method of estimating area fractions. However, this was before computers capable of image analysis were widely available. Furthermore, such comparisons have not been made for complex analyses, as in when both area fractions and boundary lengths are to be estimated. In that case, point counts are used to estimate area fractions, and line intersection counts are used to estimate boundary lengths. A second method is the use of line probes (computer-assisted lineal analysis) to estimate both parameters simultaneously, and in this situation it has been reported that the efficiency of their use might then equal or exceed point counting.[8] A third method is that of automated image analysis, which is now readily available for computer-assisted planimetry. It is desirable to know which of these is the most precise and efficient method of estimating BD and OI. Consequently, this study was designed to compare variances of estimates of interthread osseointegration and bone density using 3 methods: computer-assisted planimetry (CAP), point counting (PC), and computer-assisted lineal analysis (CALA).

Materials and Methods

Specimen Preparation and Selection. To evaluate the precision and efficiency of 3 different methods of histometric analysis, ground sections from block specimens containing 6 threaded dental implants were selected from a study designed to evaluate different bone augmentation devices in 5 dogs. The protocol for that study was approved by the Loma Linda University Animal Care Committee. A marginal osseous dehiscence defect was created in the coronal facial marginal aspect of the mandibular alveolar ridge, and various regenerative materials filled the space. Three months later, the dogs were sacrificed, and the specimens were removed and processed for routine mineralized ground section histologic analysis.[6] Four regions around each root-form implant were analyzed. Histologic evaluation revealed a wide range of responses in the coronal portion of the facial aspect of the implants, amounting to 1.1 to 4.2 mm^2 of new bone. Six of the 30 implants available were selected to provide this variation so that the methods of osseointegration analysis and intrathread bone density could be tested under conditions that would give a wide range of outcomes. It must be noted that the actual values of the estimated parameters are unknown, and this study will therefore be limited to describing the precision of the different methods of analysis.

Histometric Methods of Analysis. The analysis of osseous integration and bone density for each of the 6 implant specimens was accomplished in triplicate, by each of 3 methods, on 4 geographic regions around each implant (Fig 1). The regions were defined as follows: coronal facial interface (CFI) and coronal lingual interface (CLI), respectively (4 grooves between Peaks 1 to 5), and apical facial interface (AFI) and apical lingual interface (ALI), respectively (2 grooves between Peaks 7 to 9). Thread number 1 was defined as the first peak that is at least one-fourth of the height of the highest peak.

For all of the methods, the image of the region to be analyzed was digitized and displayed on the computer screen using ImagePro Plus (version 1.2; Media Cybernetics, Silver Springs, MD), at a final magnification of 60×. The chosen region was then enclosed in an area-of-interest rectangle, and that region was replicated and rotated so that the shadow of the implant was oriented vertically and to the left side of the image. Only the tissue within the grooves of the implant and a 50- to 100-μm border beyond the implant were included in the analysis (Fig 1). Two parameters (BD and OI) were evaluated for each region (Fig 2).

Osseointegration with Computer-Assisted Planimetry. The accepted instruction for this commercially available software was followed. Mainly, a macro was created that duplicated the rectangular region of interest, extracted the red channel, obtained the histogram of that channel, and then

duplicated the red-channel image. A threshold of the second red-channel image was then selected that ideally created a binary image, with the implant black and the tissues white. The interface between them was then measured using the autotrace feature, and the length was manually recorded in a spreadsheet. The total length of the interface occupied by bone or free tissue was then obtained by manually tracing the least abundant interface (the other was obtained by calculating the difference). These were entered in the spreadsheet for the computation of OI.

Bone Density with Computer-Assisted Planimetry. The total tissue area was estimated from the ImagePro histogram by selecting a lower threshold that excluded the implant. Then the bone area was obtained by selecting an upper threshold that excluded the lighter tissue areas. These values were recorded in the spreadsheet for the computation of BD. The scale factor was 154 pixels per millimeter.

Osseointegration with Point Counting. For this analysis, a transparency of a lattice, consisting of 2 sets of orthogonal lines 0.083 mm apart in the object plane, was placed in front of the computer screen over the image of interest. Using a 2-component manual counter, each line that crossed the implant-tissue interface was evaluated as to whether it was free or bound to bone. These counts were recorded in a spreadsheet, and OI was computed as the percentage of lines intersecting bone-implant interfaces.

Bone Density with Point Counting. Using the same transparency as for OI, the intersections of the lattice that lay over the tissue within the groove were each evaluated as to whether they were over soft tissue or bone. These counts were also recorded in the spreadsheet and BD was computed as the percentage of intersections falling on bone.

Osseointegration and Bone Density with Computer-Assisted Lineal Analysis. A locally created application program, Ribbon, was used to place a series of systematically spaced horizontal lines (each 2 pixels wide), one by one, on a vertically oriented image selected for analysis. In this study, the lines were spaced 0.065 mm apart in the object plane, and the first line was placed randomly within that distance from the top of the image. Keyboard entries, followed by mouse clicks, recorded the lengths of the line segments that crossed the various features. The first feature in every case, the implant and its interface, was recorded as free or bound, depending upon its association with bone. The program created a comma-delimited text file that recorded the total length and the number of line segments on each feature. Consequently, for this method, the data for OI and BD were acquired simultaneously. When this file was opened in a spreadsheet, the appropriate computations were easily carried out to identify the desired parameters. For BD, the percentage of line lengths falling on bone was computed. For OI, the percentage of lines that crossed

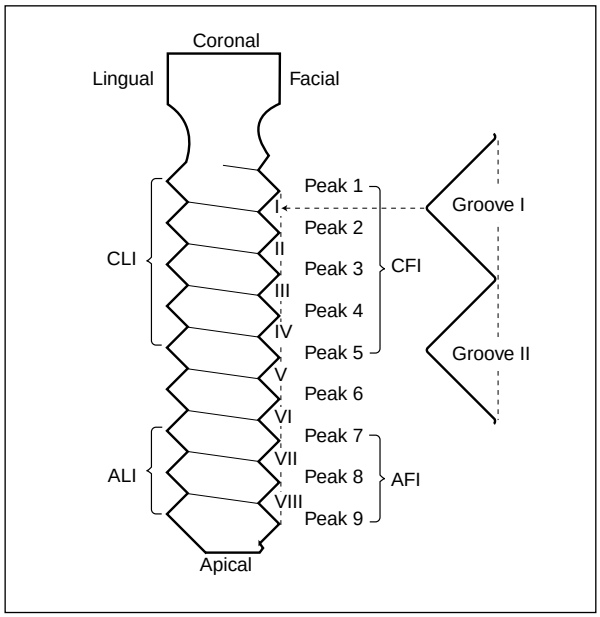

Fig 1 Description of the areas for histometric analysis. CFI = coronal facial interface; CLI = coronal lingual interface; AFI = apical facial interface; ALI = apical lingual interface.

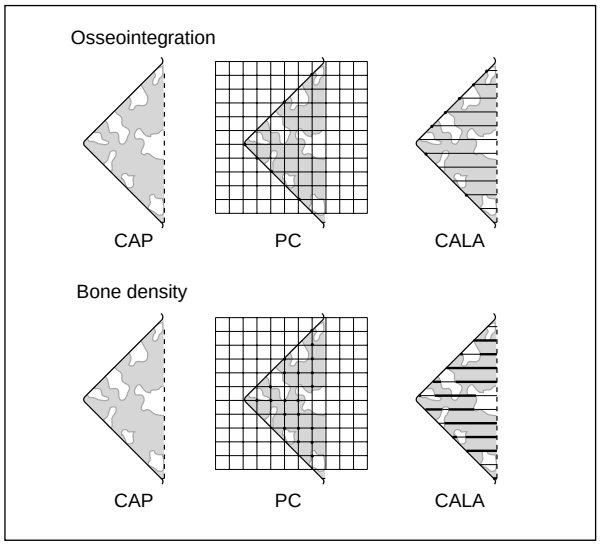

Fig 2 Description of the 3 methods of histometric analysis. Shaded areas represent sampled bone. CAP = computer-assisted planimetry; PC = point counting; CALA = computer-assisted lineal analysis.

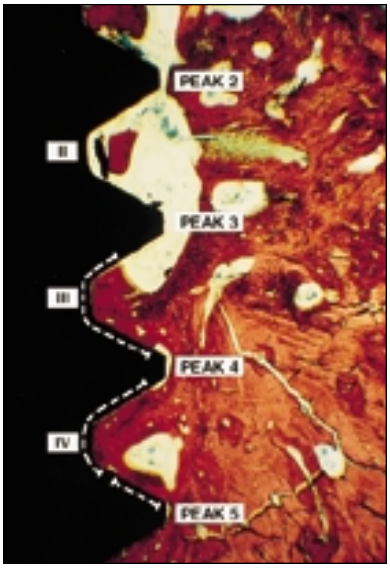

Fig 3 Histologic ground specimen, Van Gieson stain. Interpeak distance is 0.59 mm. Coronal facial interface representing a range of osseointegration (OI) and bone density (BD) in grooves II, III, and IV; corresponding peaks are identified. Osseointegration is represented in grooves III and IV by the dashed lines. Notice the absence of OI in groove II. Moderate bone densities are found in grooves III and IV, whereas groove II is an example of low bone density.

the implant interface attached to bone was computed. In addition, a raw data file consisting of the data obtained for each line was created.

Partial Data Set: Computer-Assisted Lineal Analysis. A partial subset of the raw data file was obtained to determine how the variance changed as the number of probe lines was reduced. To accomplish this, a special program was written in Visual Basic that read the file line by line, extracting the desired data from every line; every other line; or every third, fourth, or eighth line.

Sequence of Methods. All 4 regions of each implant, from all 6 specimens, were evaluated by 1 method, and this was repeated twice to give triplicate evaluations before continuing on to the second and third methods. For each method, the time required to evaluate the last 3 specimens was recorded during the third replication. The author most experienced in the 3 histometric techniques accomplished the analysis.

Statistics. Each method of analysis provided 3 estimates of the 2 parameters (OI and BD) for 4 sites on each of 6 implants. In all, 432 separate estimates were made from 216 images. The mean and standard deviation of each triplicate estimate were computed. To evaluate the precision of the 3 methods, the coefficient of variation (CV = mean/SD) from each triplicate was calculated. Based on these CVs, the statistical comparison of the 3 methods with respect to their precision was performed by applying the nonparametric technique known as the Friedman test. A significance level $\alpha = 5\%$ or less was taken as significant.

Results

An example of the range of histometric osseointegration and bone density is presented in Fig 3. The data for pooled, apical, and coronal bone density triplicate measurements are presented in Table 1. Mean apical densities from the 3 different methods of analysis ranged between 82 and 86%. More variability, by experimental design, was found in coronal percentages (59 to 68%). Osseointegration measurements are recorded in Table 2. Mean apical osseointegration ranged from 74 to 78%, whereas coronal mean percentages ranged between 37 and 47%. When these data were subjected to comparisons of coefficients of variation, bone density measurements in the coronal and pooled apical and coronal areas showed significant differences between computer-assisted planimetry and each of the other 2 methods (Table 3). Box plots of CVs for bone density and osseointegration are presented in Figs 4a and 4b.

In the computer-assisted lineal analysis, the line spacing was chosen so that more data would be obtained than might be required to achieve the desired precision. In an effort to test this, the line-by-line raw data file was used to determine the results when fewer lines were used. The computer program used to accomplish this was tested by comparing the data obtained using all the data lines with the data obtained directly from the computer-assisted lineal analysis program. Then the results of using only every other line, every third line, every fourth line, and then every eighth line were compared with those obtained using the full data set. The fractional data are summarized in Table 4. The Friedman test, which analyzed the coefficients of variation, showed significant differences between the full bone density data set and all the partial sets ($P < .05$; Table 5). A similar difference was noted for the full and partial data set for osseointegration ($P < .05$).

In an attempt to establish the time required to complete each of the methods of analysis, a set of 6 slides was evaluated 3 times using each of the 3 methods of analysis. Rather than evaluating each slide 3 times before going on to the next slide, the entire set was evaluated once before repeating the

Table 1 Summary of Estimated Bone Density Value for 3 Methods

Site/method	n	Median	Mean	SD
Pooled				
CAP	72	80.5	77.0	17.3
PC	72	76.5	70.9	19.3
CALA	72	75.9	70.9	19.2
Apical				
CAP	36	88.3	86.2	9.6
PC	36	83.3	82.4	12.0
CALA	36	83.6	82.9	11.3
Coronal				
CAP	36	70.4	67.8	18.5
PC	36	64.8	59.4	18.5
CALA	36	65.4	58.9	18.1

SD = Standard deviation; CAP = computer-assisted planimetry; PC = point counting; CALA = computer-assisted lineal analysis.

Table 2 Summary of Estimated Osseointegration for 3 Methods

Site/method	n	Median	Mean	SD
Pooled				
CAP	72	59.5	55.8	25.2
PC	72	67.7	62.5	22.2
CALA	72	61.1	58.7	21.3
Apical				
CAP	36	76.0	74.5	14.3
PC	36	77.1	77.8	12.3
CALA	36	73.7	73.5	12.8
Coronal				
CAP	36	33.9	37.2	19.1
PC	36	49.6	47.3	19.3
CALA	36	40.9	43.8	17.4

SD = Standard deviation; CAP = computer-assisted planimetry; PC = point counting; CALA = computer-assisted lineal analysis.

evaluation. Moreover, the set was evaluated 3 times by 1 method before going to another method. With all 3 methods, the time required to evaluate 1 implant progressively decreased. The time required for setup was the same for all methods and amounted to a total of 3 to 5 minutes for the 4 sites on 1 implant. Computer-assisted lineal analysis, using Ribbon software, and point-counting methods required on average 15 minutes to analyze each implant, including setup time. Both of these methods required 10 to 12 decisions per groove to evaluate osseointegration and 15 to 25 decisions per groove to estimate bone density. The use of the image-processing software, as used in the computer-assisted planimetry, was a very different process and required different kinds of decisions, some of which can be very time-consuming—for example, selecting the threshold that gives the best segmentation of the various regions. The best performance achieved using that method routinely required 20 minutes to analyze all 4 sites on 1 implant.

Discussion

Unbiased histologic evaluation of tissues following various clinical therapies is crucial to validate or refute clinical impressions. This is particularly true when the clinical outcome is dependent on a measurable anatomic structure—in the case of dental implants, the attachment (osseointegration) and the nature of the contiguous alveolar bone (bone density). The actual values for the estimated parameters are unknown; therefore,

Table 3 Coefficient of Variation Data for Bone Density and Osseointegration

Method	n		Mean	Median
Bone density, pooled				
CAP	24	*	8.3	6.0
PC	24	† *	4.9	4.6
CALA	24		4.6	3.7
Osseointegration, pooled				
CAP	24		9.5	7.5
PC	24		9.0	8.1
CALA	24		13.0	9.9
Bone density, apical				
CAP	12		4.7	5.7
PC	12		4.0	3.3
CALA	12		3.7	3.3
Bone density, coronal				
CAP	12	*	12.0	8.1
PC	12	† *	5.8	5.9
CALA	12		5.5	4.1
Osseointegration, apical				
CAP	12		7.0	4.5
PC	12		7.0	7.4
CALA	12		8.5	7.9
Osseointegration, coronal				
CAP	12		12.0	9.9
PC	12		11.0	9.0
CALA	12		17.6	15.0

*$P \leq 0.05$; † $P \leq 0.01$.
CAP = computer-assisted planimetry; PC = point counting; CALA = computer-assisted lineal analysis.

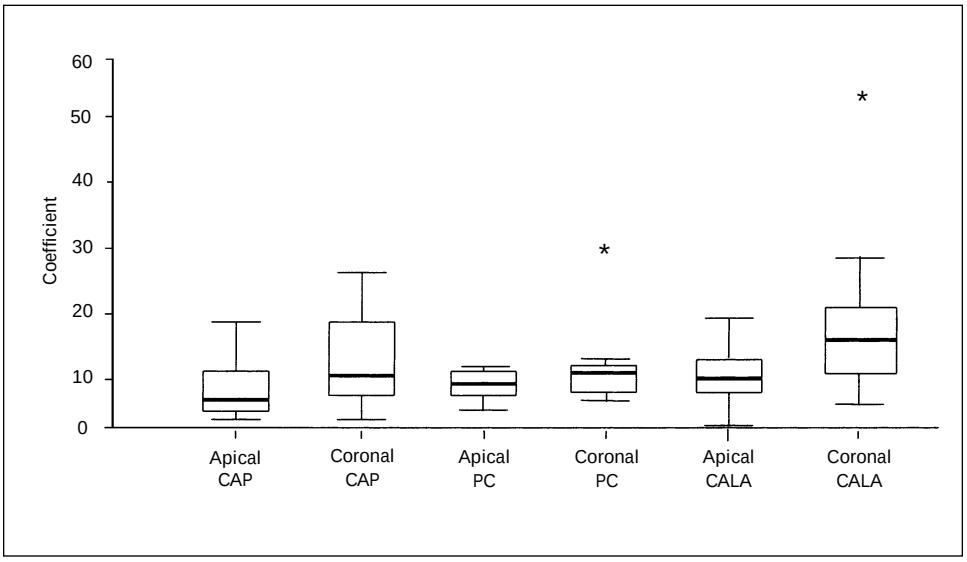

Fig 4a Interquartile range of coefficient of variation for osseointegration. The horizontal bar in each box is the median of the data, and 50% of the coefficient of variation (CV) values are within the box. The top of the box is at the 75th percentile and the bottom is at the 25th percentile of the observed CVs. The lines extending from the bottom and the top of the box describe the smallest and largest observed values that are not outliers. * = Outlier points of more than 1.5 box lengths above the 75th percentile. CAP = computer-assisted planimetry; CALA = computer-assisted lineal analysis; PC = point counting.

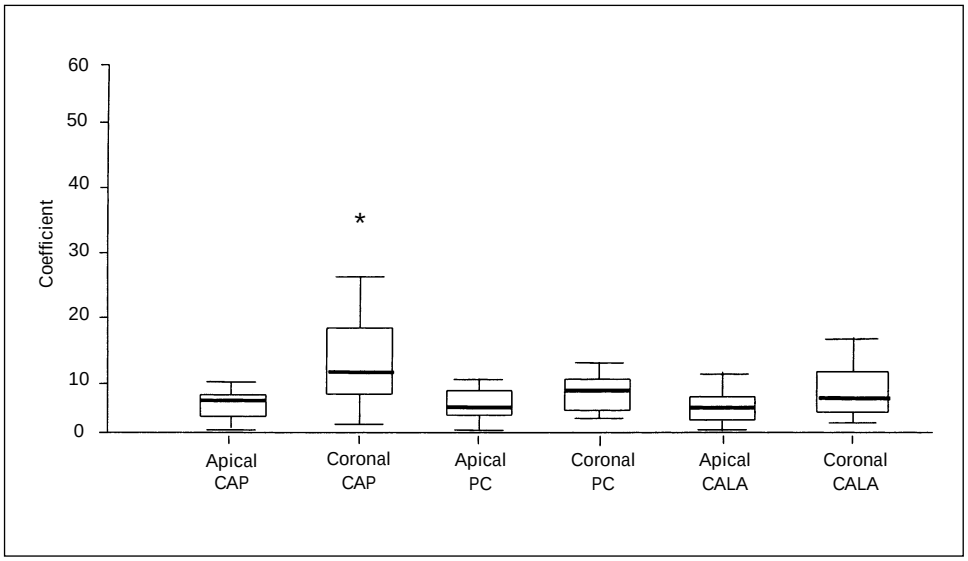

Fig 4b Box plot showing interquartile range of coefficient of variation for bone density (see Fig 4a for explanation of box plot design). * = Outlier points of more than 1.5 box lengths above the 75th percentile. CAP = computer-assisted planimetry; CALA = computer-assisted lineal analysis; PC = point counting.

only the precision (reproducibility) and not the accuracy of each of the 3 methods was evaluated. As precision may vary with the value of the estimated parameter (small versus large), specimens with a wide range of histologic outcomes were used (untreated apical bone and variably regenerated coronal bone).

The results for assessing bone density suggest that the computer-assisted planimetry method of analysis is modestly but significantly ($P < .05$) more variant than the computer-assisted lineal analysis method. This is explained by the variability of the bone stain, so that in some specimens, portions of bone had the same luminance as the implant itself, and in others, the bone had the same luminance as the surrounding soft tissue. This contributed to the difficulty in choosing a discerning threshold in the histogram (segmentation). The greater variance is especially critical in the analysis of implants, because measurements are usually limited to a single section.

For osseointegration, the variances were not significantly different. However, with the planimetry method, there was some difficulty in identifying the interface between the implant and the bone. In those cases, fragments of bone were identified as implant, resulting in an irregular interface. These images required manual correction, increasing the time for analysis. Inasmuch as computer-assisted lineal analysis produced less variation than computer-assisted planimetry, the number of lines required to sustain that advantage was examined. By analyzing the raw data files line by line, it has been shown that in the computer-assisted lineal analysis, a degradation of the measurement estimate occurred when the number of lines was cut in half. This indicates that the number of lines used (65-µm spacing) should not be decreased.

Once the learning curve was completed, the time required for analysis using computer-assisted lineal analysis and point counting was routinely less than that required for computer-assisted planimetry. This difference may be more important when analysis of additional areas of the supporting alveolar bed is required. Computer-assisted planimetry requires separate analysis of each compartment; however, data from computer-assisted lineal analysis can be segmented using software analysis of raw data files. The lengths and locations of individual line segments in the raw data file provided the basis for these calculations, as well as for the evaluation of the size of tissue spaces in the bone.

With the introduction of Ribbon, a computer-assisted lineal analysis program created at Loma

Table 4 Coefficients of Variation for Apical and Coronal Sites for Full vs Partial Analysis

	Mean	Median
Bone density		
Full		
Apical	5.0	4.1
Coronal	5.5	4.1
One half		
Apical	9.6	3.7
Coronal	11.0	6.0
One third		
Apical	12.3	6.3
Coronal	13.3	6.8
One fourth		
Apical	10.1	3.9
Coronal	10.1	3.9
One eighth		
Apical	12.5	5.6
Coronal	37.6	20.5
Osseointegration		
Full		
Apical	13.9	7.5
Coronal	13.2	13.0
One half		
Apical	22.6	14.0
Coronal	23.3	14.6
One third		
Apical	22.3	15.3
Coronal	35.9	28.0
One fourth		
Apical	21.3	17.3
Coronal	31.3	34.9
One eighth		
Apical	20.0	35.4
Coronal	17.3	29.0

Table 5 Coefficient of Variation of Combined Sites for Full vs Partial Analysis

	n	Mean	Median
Bone density			
Full	24	5.3	4.1
One half	24	* 10.3	4.9
One third	24	12.8	6.8
One fourth	24	12.4 *	8.4
One eighth	24	25.0	11.1
Osseointegration			
Full	24	13.6	9.9
One half	24	* 22.9	14.3
One third	24	29.1	20.0
One fourth	24	18.0	17.3
One eighth	24	24.0	14.0

*$P < .05$.

Linda University, the use of line probes for stereologic analysis is especially efficient for complex analyses. Boundary lengths and area fractions can be calculated from a single raw data file. Estimation of tissue parameters at different distances from a reference edge can also be computed. It was concluded that, of the 3 methods tested, computer-assisted lineal analysis is the method of choice.

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