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Computer-Assisted 3D Reconstruction of Serial Sections of Cortical Bone to Determine the 3D Structure of Osteons

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Abstract. The objective of this study was to create three-dimensional (3D) images for the histomorphological study of osteons. Medical imaging technology was used to register digitized 2D images of serial decalcified histological sections of bone, to segment the tissues of interest from the surrounding tissues, and to create 3D reconstructions from the segmented structures. Examination of the 3D reconstructions did not support suggestions in the literature that osteons have a spiraling organization. In contrast, the 3D reconstructions indicated that osteons have a complex pattern of organization that is dominated by branching. Examination of the reconstructions also suggested that osteons described in the literature as being dumbbell shaped are actually artifacts of the plane of sectioning. This study demonstrated the applicability of imaging and visualization technology developed for the 3D reconstruction of medical images to the reconstruction of digitized 2D images of serial sections of bone and additionally demonstrated the feasibility of using 3D reconstructions for the histomorphological study of osteons.

The major microstructural feature of the compact bone of humans and most other large mammals and other vertebrates is the osteon (haversian system). It is generally understood that osteons are produced by basic multicellular units (BMUs) of intracortical bone remodeling [1] and, although their two-dimensional structure has been well described, their three-dimensional (3D) structure remains uncertain. As with any biological structure, osteons exist in three dimensions, and to truly understand their nature, it is essential that we understand their three-dimensional morphology; but the mineralized composition of bone has made this task difficult.

Tappen [2] describes a 3D study of resorption spaces and developing osteons using stained, decalcified, transverse serial sections of bone from several species (dogs, baboons, and humans). His findings, based upon tediously following histomorphological structures through contiguous sections, question some of the assumptions concerning the 3D structure of osteons based upon the earlier work of Cohen and Harris [3] who also used serial decalcified sections of bone.

A major problem in interpreting serial sections is one similar to that encountered with interpreting computed tomography (CT) scan slices, i.e., being able to reconstruct individual two-dimensional cross-sections into a three-dimensional whole. Imaging and visualization technology developed for the 3D reconstruction of medical images [4–10] can be adapted to the reconstruction of digitized 2D images of serial histological sections of bone. This image processing methodology allows one to observe osteons three dimensionally, and store their images so that they can be analyzed, manipulated, and quantified. In this paper we report the results of a study for which medical imaging technology was applied to a series of decalcified serial sections of dog bone to demonstrate the feasibility of performing image processing and 3D reconstruction of osteons.

Methods and Materials

A series of black and white 35-mm photomicrographs of 34 sequential contiguous transverse 30-μm thick sections from a dog's femur[1] from Tappen's original research [2] were digitized at 16-bits and 1024 × 700 pixel resolution (Fig. 1). The original sections were stained with silver nitrate, and we optimized contrast by scanning from black and white 35-mm camera negatives, using a slide digitizer (Polaroid, Sprint Scan 35, CS-2700, Cambridge, MA).

A SUN SPARC workstation (Sun Microsystems, Inc., Mountain View, CA) and ANALYZE™ software (Biomedical Imaging Resource of the Mayo Clinic, Rochester, MN) were used for all image processing and image generation. ANALYZE™ software is specifically designed for multidimensional imaging and processing and contains the most complete set of processing tools currently available for these tasks [6, 8].

Histogram matching was employed to correct for variations in exposure, processing, and digitization among images (Fig. 2a). The resolution of the images was then reduced to 6-μm pixels from 3-μm pixels and the gray scale changed from 16 bits to 8 bits. All 34 slices (sections) were registered (Fig. 2c). The area of interest was reduced to a common field of view. An adaptive histogram process was used to reduce the effect of nonuniform illumination and to enhance the bone structures (Fig. 2e). Additional processing was performed with spatial filters, edge detection, and morphological operators to segment and create schematics of specific structures (Fig. 2f). We were able to view a 3D block of bone from various angles and as a transparent object.

Results

3D volume rendering and sectioning at a number of differ-

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[1] The sections were part of a series of 180, 30-μm thick, decalcified serial sections from an earlier study by Dr. N. Tappen (1977).

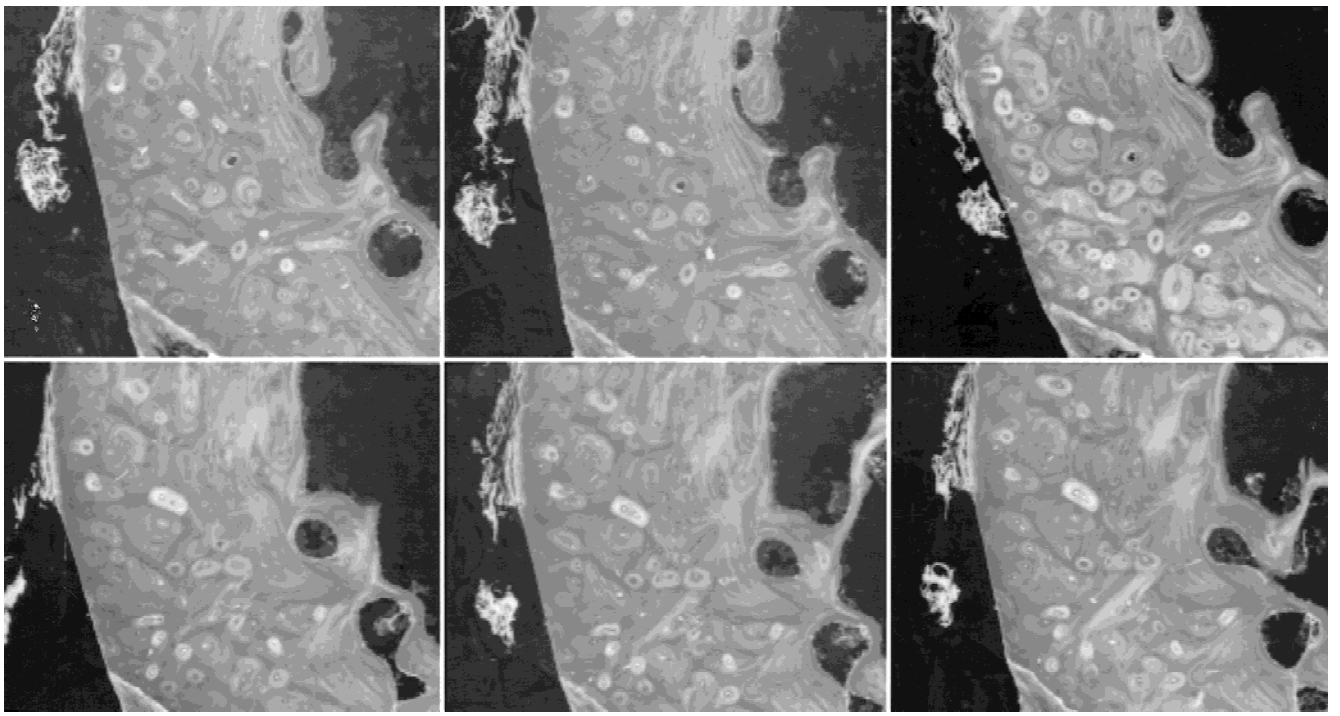

Fig. 1. Six of the dog's 34 cortical bone sections (40×) show how the exposure, field of view, and orientation differed from image to image. The top and bottom rows each contain three contiguous slices.

ing angles allowed us to determine both general and specific structural details. The 34 section outlines were interpolated to 170, to create an isotropic volume consisting of 6-μm voxels (Fig. 3). The interpolation of the volumetric data was necessary to enable uniform 3D visualization of the volume with cubic voxels in all three axes. In Figure 4, the volume is shown sliced into three thick sections of approximately 56 slices each, revealing a number of structures with very complex diverse types of branchings and interconnections. At this level of processing, a large proportion of the channels seem to be interconnected, and multiple branches occur within the 1-cm length of bone.

To illustrate interconnections, a few of the branches that were depicted in one of longitudinal sections of the 3D volume (Fig. 4) were converted to a schematic representation of the canals in a small section of the volume, which aided in visualizing the branches (Fig. 5). Several different types of branchings are observed. For the left branching system, tracings are made of a number of adjacent canals and their branchings. The right branching system illustrates a different type of branching pattern. All of the branches shown are interconnected.

Discussion

Cohen and Harris [3] attempted to study the anatomy of osteons by creating 3D models of the mid-diaphyses of dog femurs. This was accomplished by stacking cardboard models of enlargements of individual serial transverse decalcified sections, and connecting contiguous canals between adjacent sections with color-coded wires. The main concern of their study was to visualize the spatial orientation and extent of osteons. Based upon their models, it was reported that osteons (1) travel distally, and in a periosteal to endosteal orientation; (2) spiral clockwise around the axis of the bone; (3) Individual osteons and their descendants spiral on their own axes and (4) are varied in the cross-sectional areas in different parts of the same osteon, and enlarge as they approach the endosteum; (5) anastomose with transverse Volkmann's canals; and (6) sometimes do not terminate in another system ("blind osteons").

Cohen and Harris [3] only looked at mature osteons, and because they decalcified the bone and did not stain their sections, they were not able to differentiate among various histomorphological stages in the development of osteons. It is clear from their descriptions and measurements, however, that they were unable to distinguish among primary osteons, secondary osteons, vascular canals, and resorptive bays. They additionally speculate that the spiral pattern they observed may be species specific (the dog), bone specific (the femur), or even unique to a specific region of a bone (midshaft), and that other patterns may exist.

A helical nature to the three-dimensional morphology of osteons was also reported by Hert et al. [11]. Their work suggests that osteons exist in two helical systems running in opposite directions and lying on opposite sides of the diaphysis. It also indicates that the orientation of the osteons falls into a fairly consistent 5–15° inclination. They suggest that the different orientations of osteons correspond with the direction of the maximum principal stress and/or strain in the opposing walls of bones. Hert et al.'s sample consisted of humeri, radii, ulnae, femora, and tibiae from a Southern Bohemian ossuary, and they employed a macroscopic method which allowed them to study the entire diaphysis of a bone. The method is actually a modification of the India ink method of Benninghoff [12], and does not permit distinguishing among various types of canals and lumens in bone, e.g., cutting cones, Volkmann's canals, primary vascular canals, primary osteons, haversian canals. A number

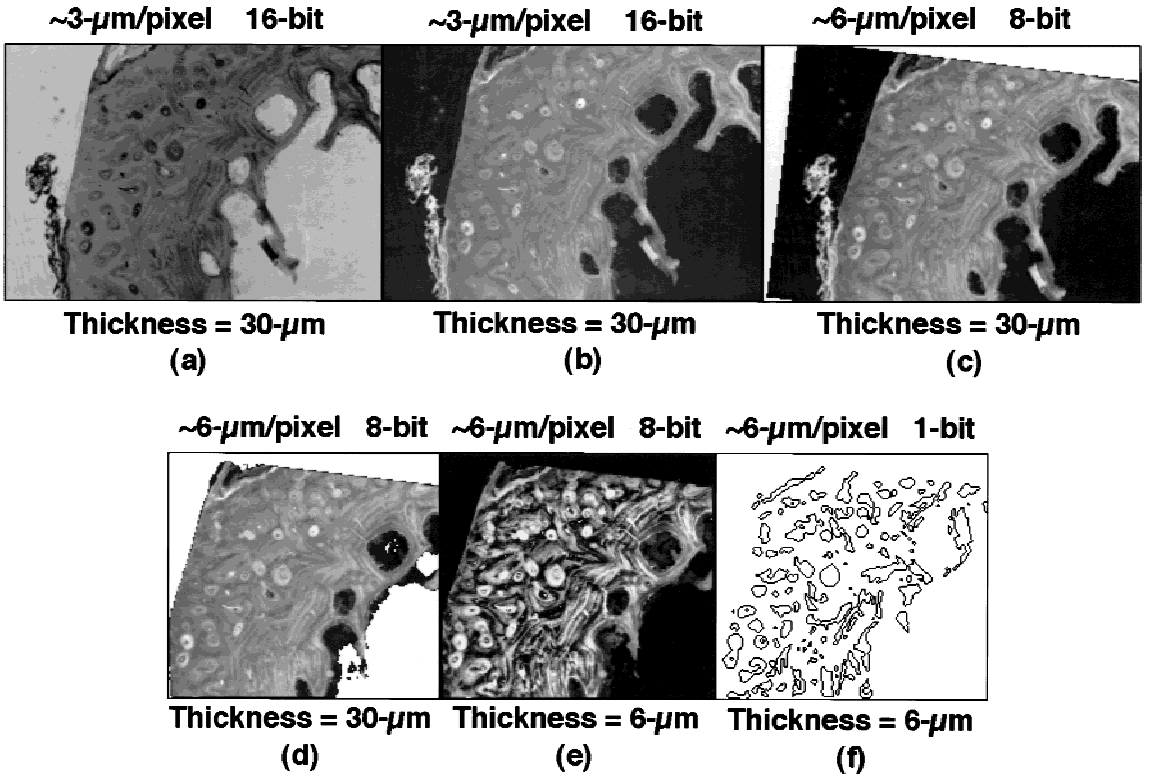

Fig. 2. (a) Original 35-mm black and white digitized slide. **(b)** Exposure was corrected by histogram matching all 34 sections. **(c)** The image resolution was reduced to 6 μm per pixel from 3 μm and grayscale was reduced to 8 bits from 16 bits. All 34 slices were registered. **(d)** The area of all the slices was reduced to a common field of view. **(e)** An adaptive histogram was utilized to both decrease effect of nonuniform illumination and increase contrast. **(f)** Edge detection was used to produce simple outlines of the osteons.

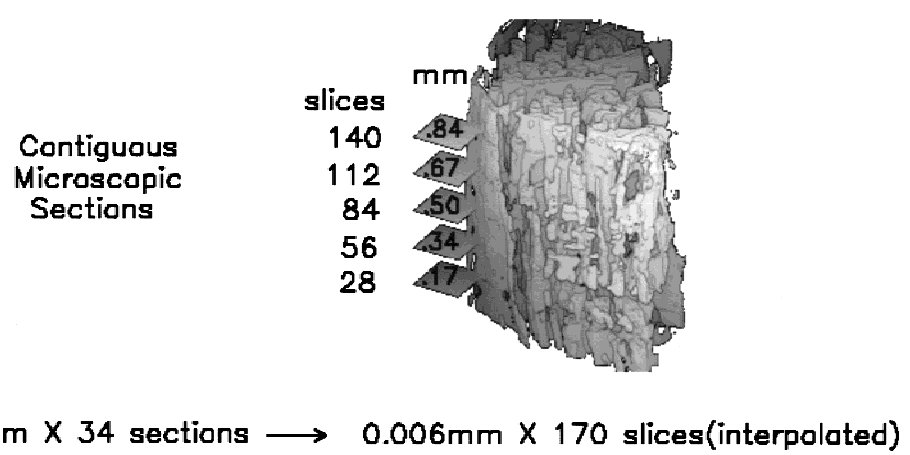

Fig. 3. This isometric view of the 170 interpolated slices shows the thickness of the volume in both millimeters and by interpolated slice number.

of other studies have also reported a helical structure to osteons [13, 14] and different oblique orientations in different locations within the diaphysis [15].

Tappen [2] undertook an elegant and tedious three-dimensional study of resorption spaces and developing osteons using stained decalcified transverse serial sections. His sample included dogs, baboons, and humans. Silver nitrate staining allowed differentiation of the developmental stages of osteons, e.g., cutting cone (resorption), forming osteons, and mature osteons. His results, which did not confirm those of Cohen and Harris, include (1) cutting cones are continuous with the canals of developing osteons; (2) developing osteons may be formed by cutting cones that tunnel either both proximally and distally simultaneously, or in only one direction; (3) in no instances was there evidence that a newly generated osteon was created by resorp-

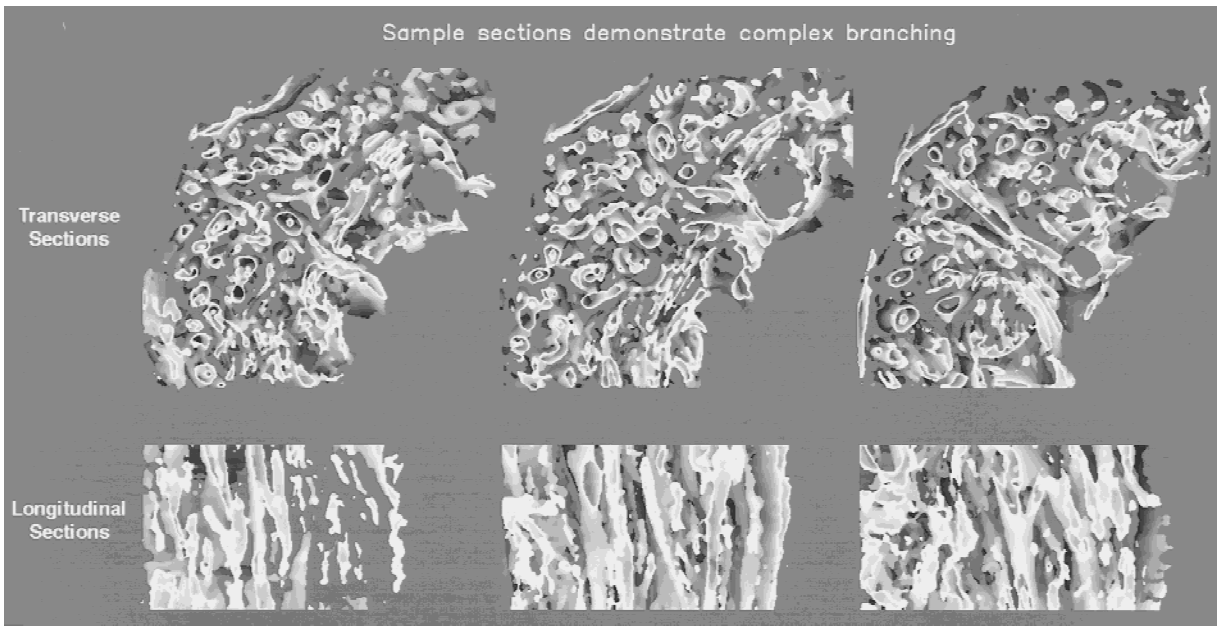

Fig. 4. The three approximately 0.336 mm-thick slice sections depict a number of branchings in both the transverse and longitudinal directions in this 3D volume.

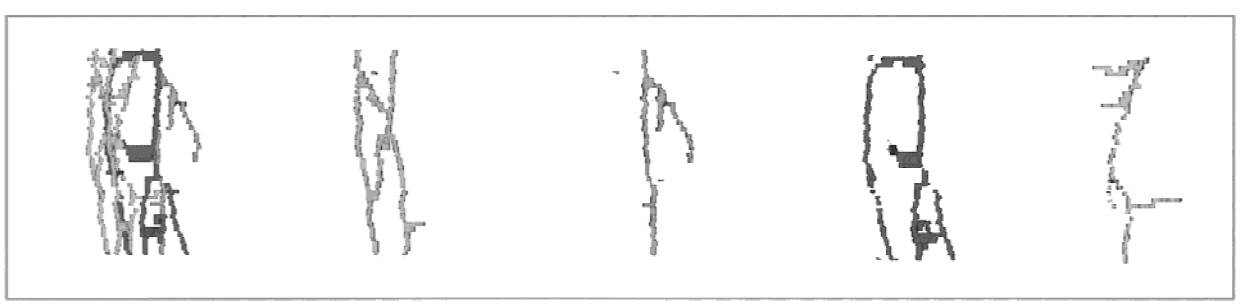

Fig. 5. A schematic of the haversian canals are shown from a small portion of the 3D volume. The far left tracing shows a number of adjacent canals and their branches. The right four tracings depict the different types of branches dissected from the far left tracing. All the branches shown were interconnected.

tion within an already existing canal, i.e., resorption spaces tunnel directly into bone. (Some nonlongitudinal studies have described intraosteonal remodeling in which resorption enlarges a preexisting canal, producing a form of osteon called type II [16].)

Based on our interpretations of the 3D renderings of the dog bone, we cannot support the suggestions of other investigators who report a spiraling organization of osteons. This is in agreement with the findings of Tappen [2] and others who did not find the haversian systems to have a helical nature [17, 18]. Instead, we found a more complex pattern, dominated by branching. We point out, however, that the sections we used were prepared in an earlier study and do not have fiduciary points (fixed points of reference) with which the images can be registered; thus actual topographical relationships may be imprecise. In addition, the decalcified sections were stained, using silver nitrate. This preparation seems to have obscured the contrast between areas of interest (e.g., osteons) and other areas (e.g., primary bone, canals, etc.) and limits our ability to accurately render a clear representation of specific structures, especially for volume rendering.

Our results also suggest that some previously defined morphological “types” of osteons may not exist, but rather are artifacts of the relative orientation of the plane of sectioning, or where along the length of a haversian system the section was made. An example of this is the description of a dumbbell-shaped system in a discussion of the effects of geometry and collagen orientation on the biomechanical properties of osteons [19]. We have determined that this dumbbell-shaped system is produced by sectioning through an osteon branch.

In conclusion, we were able to use advanced medical imaging software to create 3D reconstructions of cortical bone from histological slices of a dog’s femur. Based on our examination of these 3D reconstructions, we cannot support earlier suggestions by investigators who report a spiraling organization of osteons. In contrast, we found that osteons have a complex pattern of organization that is dominated by branching. Our examination of the 3D reconstructions also suggests that osteons described in the literature as being dumbbell shaped are actually artifacts of the plane of sectioning.

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