

Quantification of Connectivity in Cancellous Bone, with Special Emphasis on 3-D Reconstructions

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Abstract

The connectivity of cancellous bone attracts increasing attention, as it has been hypothesized that a primary reason for decreasing strength and stiffness in osteoporosis is caused by a loss of trabecular elements and consequently a loss in connectivity.

The Euler characteristic has in a few previous articles been used to express cancellous bone connectivity, but there are severe problems in using the Euler characteristic uncritically. The Euler characteristic of a three-dimensional structure is a topological invariant, which reports the number of particles of a structure plus the number of enclosed cavities minus the connectivity. As such, one must know the number of components and the number of enclosed cavities in order to use the Euler characteristic as an expression of connectivity. Another difficulty of using the Euler characteristic is that due to edge effects the Euler characteristic of an excised specimen provides a biased estimate of the Euler characteristic of the region from which the specimen was taken.

In this article the intuitive concept of connectivity is given a precise mathematical definition, and a basic topological method for quantifying the connectivity of cancellous bone is presented. The method uses the Euler characteristic, but the above-mentioned problems are controlled. The development of the method and the practical implementation is based on a set of topological notes. It must be stressed that the method is free from assumptions concerning trabecular architecture, and that the method is unbiased. This is in contrast to previously presented methods.

The unbiased and model-free method is used on a series of 3-D reconstructions of cancellous bone specimens, and it is demonstrated that the connectivity of cancellous bone is not simply related to volume fraction (density), and that biased and model-based 2-D methods aimed at determining connectivity do not have any general relationship to connectivity in cancellous bone.

Key Words: Cancellous bone—Trabecular bone—Connectivity—Topology—Bone structure—Histomorphometry.

Introduction

Cancellous bone is a continuous 3-D network of bars and plates, and in addition to varying densities it possesses varying orien-

tations and degrees of orientation. This was noted in the earliest reports on the architecture of cancellous bone (Meyer 1867; Wolff 1870; Koch 1917), and has been carried on to the biomechanical community. Density (Carter & Hayes 1977; Rice et al. 1988) and anisotropy (Harrigan & Mann 1984; Hodgkinson & Currey 1990; Odgaard et al. 1990b; Snyder & Hayes 1990) are currently being examined using quantitative methods and are being used as independent variables in empirical relations between mechanical behavior of cancellous bone and its structural characteristics.

In contrast to these measures, the connectivity of cancellous bone has not attracted the same attention, which is surprising, because connectivity is a fundamental property of networks. When studied, connectivity has, with very few exceptions, only been described in qualitative terms (Mosekilde 1988, 1990), or has been expressed as parameters in simple, model-based and 2-D approaches to cancellous bone structure (Parfitt et al. 1983). The lack of assumption-free quantitative information cannot be explained by a consensus on how connectivity influences mechanical properties, but rather by theoretical and practical difficulties in quantifying connectivity. There is, however, a growing interest in the connectivity of cancellous bone, as indications have been presented that connectivity may be the parameter mostly affected by osteoporotic changes (Kleerekoper et al. 1985; Parfitt 1987; Mosekilde 1990).

In this article connectivity is given a precise definition, which is based on a review of the relevant topological theory. The principles behind a method for unbiased estimation of connectivity in a region will be described, and the method will be demonstrated on sample specimens. The examples are based on 3-D reconstructions, and it is stated that connectivity cannot be determined from 2-D information alone. In the discussion most of the previously reported methods for obtaining connectivity information will be presented.

Preparatory Topological Notes

The metric and directional properties of a three-dimensional structure, like cancellous bone, will vary with deformations of the structure. One may for instance alter the volume fraction and the surface area of a phase (bone or marrow) by inflating the phase, and the anisotropy of a phase will be changed by stretching the structure. But if a new connection between two parts of the structure is not made, if a branch between two parts is not cut, and if no isolated part of the structure is removed, then the

number of parts and the number of connections in the structure will remain constant. Bending, stretching, and other rubber-like deformations are typical examples of such transformations. The properties, which do not change under any of these transformations, are called *topological properties*. A *topological invariant* is a parameter which expresses a topological property. Two structures are *topologically equivalent* if they have the same topological properties (i.e., one structure can be deformed into the other one by rubber-like deformation).

For 3-D structures, the three classical topological properties are 1) the number of components of the reference phase, 2) the connectivity, and 3) the number of components of the complementary phase(s) fully enclosed inside the reference phase. These are the only topological properties which will be considered in this article.

The following is not meant as a comprehensive introduction to topology but should draw attention to topological notions which are of importance to an examination of connectivity in cancellous bone. For a self-contained introduction, see for instance Barrett and Yust (1970), DeHoff (1968), and Hadwiger (1957, 1959). Standard textbooks in topology provide formal definitions and theorems.

Connectivity

Given a network consisting of nodes and branches (points connected by lines), the network is said to be *simply connected* if only one path from any node to any other node exists (Fig. 1a). Such a network is known in topology as a tree and has by definition a connectivity of zero. If an additional branch is added between two nodes, some nodes are now connected by more than a single path, and the structure is said to be *multiply connected* (Fig. 1b). If a branch in a tree is broken, the structure will fall apart, but in a multiply connected structure some branches may be cut without separating the structure into isolated parts. *Connectivity* is defined as a measure of the degree to which a structure is multiply connected, and hence, for a network, reports the maximal number of branches that can be broken before the structure is separated into two parts. If a bone is viewed as a node-and-branch network, the connectivity of the bone may thus be

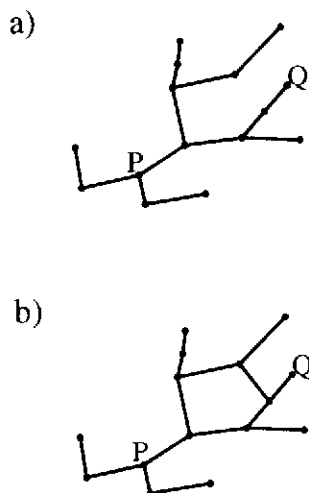


Fig. 1. a) Simply connected node-and-branch network (tree). Only one path exists between any two nodes (e.g., from P to Q). b) Multiply connected network, made from a) by the addition of a single branch. Now, two paths exist (e.g., from node P to node Q).

said to be the number of trabeculae minus one. This defines a trabecula in a topological sense.

One may easily construct a 3-D node-and-branch network of a cancellous bone structure. The process may be imagined by removing most of the trabecular bone tissue and just letting each trabecula be represented by a line. The location of the line within the individual trabecula is of no importance.

When looking at a cancellous bone specimen, it may not be obvious how a trabecula should be defined, but the example shown in Fig. 2 should clarify this and give an understanding of "the number of trabeculae." Connectivity does not, and should not, make any distinction between "thick" and "thin" trabeculae or between "plates" and "rods," and connectivity is, as such, a measure of the degree to which a structure is multiply connected, no matter the shape of the connections.

Even though the above definition of connectivity seems simple, determinations of connectivity will for all practical purposes be based on the Euler characteristic, because it is relatively simple to determine and because it has a known relation to connectivity.

The Euler characteristic

The following introduction to the Euler characteristic will be based on an approach by Hadwiger (1957, 1959), and it will be directed at naturally occurring three-dimensional bodies.

A three-dimensional *convex body* will be defined as a closed set of points which on any 2-D or 1-D section through the body will appear as a single closed set. The definition of a 2-D convex body is analogous. The empty set $\emptyset$ is by definition a convex body.

Once the convex body has been defined, a binary function ϵ can be defined for a convex body P of any dimension as

$$\epsilon(P) = \begin{cases} 1, & \text{if } P \neq \emptyset \\ 0, & \text{if } P = \emptyset. \end{cases} \quad (1)$$

It can be shown that any naturally occurring 3-D body X may be decomposed into convex bodies. Let a particular decomposition

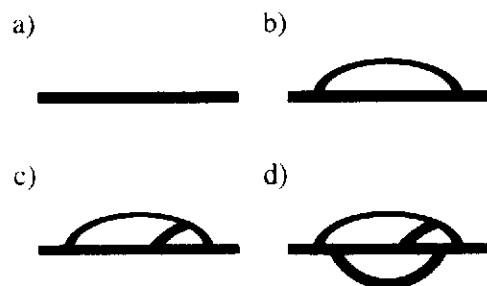


Fig. 2. These figures show a schematic view of cancellous bone. In a) a single trabecula has been created, in b) an extra trabecula has been added, in c) three trabeculae exist, and in d) four trabeculae have been created. But by looking at d), it is not obvious that only four trabeculae exist, because if one counts the number of segments different numbers may be reached, depending on how a segment is defined. The only way to count the number of trabeculae correctly is to reverse the creation of the small universe (i.e., by cutting trabeculae). This can be done three times before the structure is broken into two disconnected parts; by referring to the text, the number of trabeculae then is $3 + 1 = 4$, and indeed, four trabeculae were made in the first place.

of the body X be denoted by (X, P) , then the Euler characteristic of the decomposition, $\chi(X, P)$, may be defined as

$$\chi(X, P) = \sum^1 \epsilon(P_{v1}) - \sum^2 \epsilon(P_{v1} \cap P_{v2}) + \sum^3 \epsilon(P_{v1} \cap P_{v2} \cap P_{v3}) \dots, \quad (2)$$

where $\sum^N$ denotes a summation over all N -permutations of the convex bodies $P_1 \dots P_n$. It may be shown that the Euler characteristic $\chi(X, P)$ is independent of the particular decomposition into convex bodies, and the notion $\chi(X)$ will hence in the following be used for the Euler characteristic of the body X .

Many of the convex body intersections found on the right-hand side of equation 2 will equal the empty set $\emptyset$, and will hence contribute by 0 to the Euler characteristic (cf. definition 1). If these elements are removed, a simpler version of equation 2 may be expressed as

$$\chi(X) = \sum (-1)^i n_i = n_0 - n_1 + n_2 - n_3 \dots, \quad (3)$$

where n_i is the number of convex bodies of dimension i in the decomposition of X into convex bodies.

Equation 3 is particularly suited for 3-D digital images, but counting $n_0 \dots n_3$, of course, depends on the neighborhood relations chosen (Rosenfeld 1970; Kovalevsky 1989; Odgaard 1993). If each voxel is considered to have 26 neighbors (Rosenfeld 1970), n_3 may be seen as the number of voxels, n_2 as the number of voxel faces, n_1 as the number of voxel edges, and n_0 as the number of voxel corners. One should only count a face, edge, or corner once, as illustrated in Fig. 3. It should be noted that the application of a simple 26-neighborhood rule to both bone and marrow phases leads to a violation of the Alexander duality theorem, equations 6 and 7 (Kovalevsky 1989; Odgaard 1993).

The Euler characteristic of the union of two sets A and B is given by

$$\chi(A \cup B) = \chi(A) + \chi(B) - \chi(A \cap B) \quad (4)$$

(i.e., the sum of the Euler characteristics of the two sets minus the Euler characteristic of the intersection of the sets). This is known as the *addition theorem* and has important implications for the estimation of the Euler characteristic of a structure, when only part of the structure is at hand. When the structure in Fig. 4 is divided into four parts, the sum of the Euler characteristics for each of the four parts differ from the Euler characteristic of the entire structure. In consequence, the Euler characteristic of the intersection of two sets (cf. equation 4) must be taken into consideration. Otherwise the Euler characteristic of the union will be overestimated or underestimated depending on the topology of the intersection.

The Euler characteristic is a topological invariant, which can easily be calculated. From the above definition, it is not obvious that the Euler characteristic is of major importance to the study of connectivity, but for individual solid bodies, the Euler characteristic has a simple relation to connectivity. For any solid body which can be deformed into a solid sphere, the Euler characteristic is 1; for any body which can be deformed into a solid torus, the Euler characteristic is 0, and generally

$$\chi(v) = 1 - n \quad (5)$$

for a solid sphere with n handles. Any solid body can be deformed into a solid sphere with n handles. The Euler characteristic, however, does not tell everything of interest for a three-dimensional body, as the Euler characteristic in addition to con-

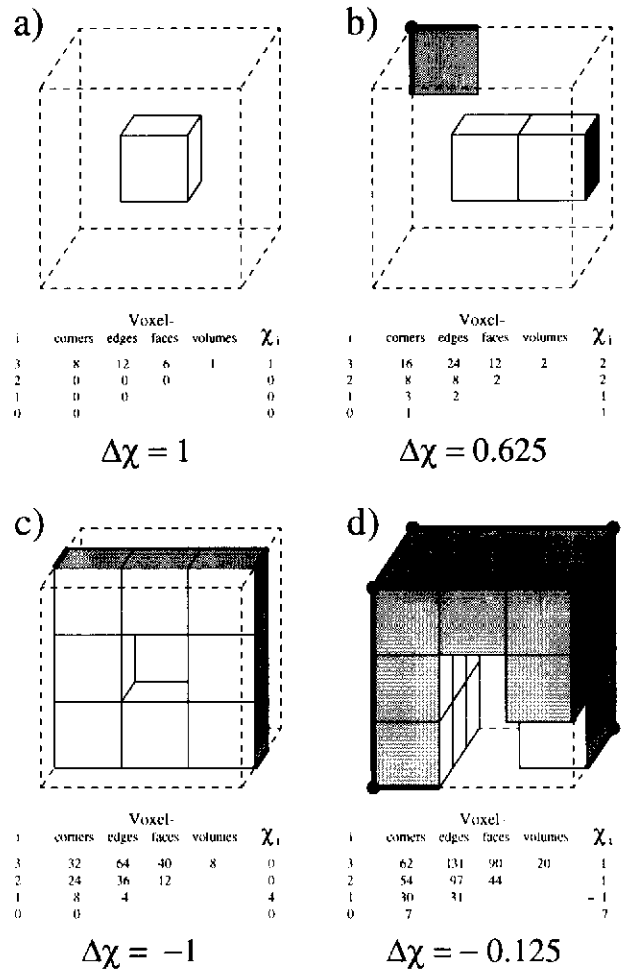


Fig. 3. Examples of determination of the Euler characteristic for an arbitrary examination volume of a $3 \times 3 \times 3$ cube of voxels, illustrating the use of both equation 3 and equation 14 (see relevant text). Voxel surfaces intersecting the artificial 2-D surface of the examination volume are shaded, voxel edges intersecting the artificial 1-D edge of the examination volume are marked with bold lines, and voxel corners intersecting the artificial 0-D corners of the examination volume are emphasized. In the tables, i denotes the dimensional intersection aspect of the examination volume, and by counting the voxel elements which lie in the intersection one reaches χ_i for any i by use of equation 3. The contribution $\Delta\chi$ of the examination volume to the Euler characteristic of the universe from which the examination volume was cut is given by equation 14. The situation in b) emphasizes that any intersection between structure and examination volume should be included, even if the intersection is a face, an edge, or a corner only.

nectivity also depends on the number of objects and the number of cavities in the object(s).

Betti numbers

Betti numbers provide a more detailed numerical description of topological properties than does the Euler characteristic. Using a geometrical interpretation, the zeroth Betti number, β_0 , reports the number of separate particles of the structure. The first Betti number, β_1 , reports the number of handles if the structure is deformed rubber-like into a sphere with handles (i.e., β_1 reports the connectivity, cf. the definition given above). The second Betti number, β_2 , reports the number of cavities enclosed within the structure. For 3-D structures the Betti numbers with a rank higher than 2 will all be zero.

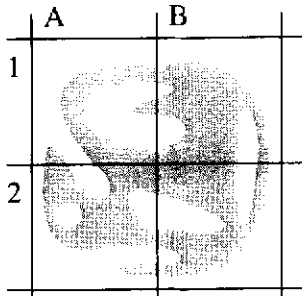


Fig. 4. Simplified 2-D structure separated into quadrants. The Euler characteristic of the entire structure is -3 . The Euler characteristics of the quadrants are 3, 1, 1, and 1 for A1, A2, B1, and B2, respectively. The sum of the Euler characteristics of the quadrants does evidently not equal -3 . The Euler characteristics of the intersections of quadrants are 2, 2, 3, 3, and 1 for $A1 \cap A2$, $B1 \cap B2$, $A1 \cap B1$, $A2 \cap B2$ and $A1 \cap A2 \cap B1 \cap B2$, respectively. By using the addition theorem the Euler characteristic of the entire structure may be calculated correctly as $(3 + 1 + 1 + 1) - (2 + 2 + 3 + 3) + 1$.

The Alexander duality theorem states that for the two phases γ and δ in a two-phase structure.

$$\beta_0(\gamma) = 1 + \beta_2(\delta) \quad (6)$$

and

$$\beta_1(\gamma) = \beta_1(\delta). \quad (7)$$

The equations of the theorem follow intuitively from the geometric interpretation of Betti numbers given above, in that an inclusion of a cavity in the δ -phase will increase the number of disconnected γ -particles by one, and that for each handle in the γ -phase there will be a handle in the δ -phase.

The Euler–Poincaré formula states that for a 3-D structure X ,

$$\chi(X) = \beta_0 - \beta_1 + \beta_2, \quad (8)$$

which should be seen in conjunction with equation 3. This is a surprisingly nice relation considering the definition of the Euler characteristic given above. For 3-D structures with $\beta_2 = 0$, the Euler characteristic is the difference between the number of particles and the connectivity. It follows from equation 8 that the Euler characteristic of a solid sphere (without internal cavities) with four handles is $\beta_0 - \beta_1 + \beta_2 = 1 - 4 + 0 = -3$, and that the Euler characteristic of, for instance, a sphere with seven handles and three internal cavities is $\beta_0 - \beta_1 + \beta_2 = 1 - 7 + 3 = -3$. In consequence, general 3-D structures with the same Euler characteristic are not necessarily topologically equivalent!

A property of the Euler characteristic apparent from equation 8 should be emphasized. If, and only if, a solitary reference phase structure is present ($\beta_0 = 1$), and if no cavities of the complementary phase exists ($\beta_2 = 0$), then χ is simply related to the connectivity (β_1) by $\chi = 1 - \beta_1$. In other words, a multiply connected solid structure consisting of only one particle—for instance, a cancellous bone specimen—must have a negative Euler characteristic.

Cancellous Bone Connectivity

Topological assumptions regarding cancellous bone structure

From numerous observations and qualitative studies of cancellous bone (see for instance, Whitehouse 1971; Singh 1978; Mosekilde 1988, 1990) a consensus has grown that cancellous bone is a multiply connected structure, and that both the marrow and the bone phase are fully connected. It is thus assumed that no

piece of trabecular bone is completely isolated from the main structure, and that no marrow space exists fully isolated, completely encapsulated by bone. It seems that this assumption is valid not only for an excised specimen of cancellous bone, but also for entire bones of the skeleton, as multiple channels exist which allow vessels to enter the bones. In topological terms, the assumptions mean that $\beta_0 = 1$ (only one connected part of bone), and that $\beta_2 = 0$ (no marrow cavities enclosed within bone), and equation 8 may thus be reformulated as

$$\beta_1 = 1 - \chi. \quad (9)$$

There are obvious exceptions to the topological assumption. During bone formation and bone healing isolated islands of bone may exist, but these and related exceptions will not be considered further. The main reason for neglecting these exceptions is that fully isolated bony strands do only contribute very little, if at all, to the mechanical competence of a cancellous bone region. Although no formal demonstration of the assumptions expressed in equation 9 has been published, these assumptions will be used throughout this paper.

Noise in 3-D reconstructions

Throughout this article, it is assumed that a three-dimensional reconstruction of a cancellous bone specimen or even an entire bone is available in voxel representation (Srihari 1981). Three-dimensional images (3-D reconstructions) can be obtained by two principally different approaches. The serial sectioning technique constitutes the classical method for obtaining 3-D images of 3-D structures (Gaunt & Gaunt 1978). In conventional form, the serial sectioning technique is prohibitively time-consuming, but in a highly time efficient and fully automated version this technique allows routine 3-D reconstructions (Odgaard et al. 1990a, 1993). An algorithm for converting 2-D projections directly into a 3-D array has been described by Feldkamp et al. (1984), and this idea has elegantly been implemented in the 3-D micro-CT-scanner (Feldkamp et al. 1989), which constitutes the alternative approach.

In order to obtain a binary 3-D array describing the 3-D structure of cancellous bone, a segmentation technique must be employed on the original grey-level image data (Kuhn et al. 1990; Odgaard et al. 1990a, 1993), and as in any segmentation technique, there is a risk of erroneously marking voxels as bone, even though the voxels should belong to the marrow phase, and vice versa. The threshold techniques used for cancellous bone specimens (Kuhn et al. 1990; Odgaard et al. 1990a, 1993) also have this potential noise problem. Because of the very high number of voxels in 3-D reconstructions, if only a small fraction of voxels is given a wrong phase membership due to noise, this will inadvertently result in a very high number of voxels given a wrong phase membership, and possibly a violation of the assumptions of $\beta_0 = 1$ and $\beta_2 = 0$, and hence of equation 9. In other words, noise may result in apparent bone particles isolated from the main structure and in apparent marrow cavities surrounded by bone.

Voxels which have been assigned an erroneous membership due to noise should, when possible, be assigned the correct membership before determining topological properties. Correcting (binary) digital images for noise and wrong membership assignments is generally a nontrivial problem, but in the present context two sets of voxels may be converted from one phase to the complementary phase with only few considerations. Isolated bone voxels or groups of bone voxels lying sequestered in the marrow phase should be converted to marrow voxels, and isolated marrow voxels or groups of marrow voxels lying fully

surrounded by bone voxels should be converted to bone voxels. The justifications for this purification are the topological assumptions on the cancellous architecture mentioned above. The effect of performing a purification on a binary 3-D representation of a cancellous bone specimen is illustrated in Fig. 5.

Edge problems

If an entire structure with natural borders is available for determination of connectivity (Nyengaard et al. 1988), no theoretical problem exists in determining the Betti numbers for the structure. One may, for instance, unbiasedly determine the total connectivity of the calcaneus. If only a part of the structure is available, a serious edge problem occurs at the artificial edge which

prevents using the connectivity of the specimen as an estimate of the connectivity in the parent region. As such, the connectivity of the isolated specimen, considering it to be the topological space of concern, will only be of limited interest.

Dehoff et al. (1972) have suggested calculating upper and lower bounds of the connectivity, assuming that all or none, respectively, of the trabeculae reaching the edge of the specimen form loops with other parts of the structure outside the examination volume. The correct value of connectivity in the structure from which a specimen has been cut will be located somewhere between the calculated bounds. Depending on the structure, on the location of the examination area, and on the size of the examination area, the relative difference between the upper and lower bound may be too large to make any use of a calculated connectivity value. By choosing a sufficiently large examination volume, the edge problem may be arbitrarily reduced. This method for estimating the connectivity of 3-D structures has been used in a few studies (MacDonald et al. 1986; Scott et al. 1988). The suggestion of calculating upper and lower bounds, however, fails to acknowledge the importance of the addition theorem (equation 4).

Given the two-dimensional structure shown in Fig. 4, and a subdivision of the plane into the squares A1, A2, B1, and B2, the following equation may easily be deduced by repeated use of (4):

$$\chi(T) = \chi(A1) + \chi(A2) + \chi(B1) + \chi(B2) - [\chi(A1 \cap A2) + \chi(B1 \cap B2) + \chi(A1 \cap B1) + \chi(A2 \cap B2)] + \chi(P), \quad (10)$$

where $T = A1 \cup A2 \cup B1 \cup B2$ and $P = A1 \cap A2 \cap B1 \cap B2$. Generalization of the example leads to the following situations for 2-D and 3-D structures.

For any tessellation of rectangular fields in a 2-D image, the Euler characteristic of a structure T in the entire image may be calculated by

$$\chi(T) = \Sigma\chi(F) - \Sigma\chi(E) + \Sigma\chi(P), \quad (11)$$

where F is a two-dimensional face, E is a one-dimensional edge, and P is a zero-dimensional corner. The sum is taken over all faces, all edges bounding two faces, and all corners bounding four faces.

For any tessellation of parallelepipeds in a 3-D image, the Euler characteristic of a structure T in the entire image may also be deduced by using (4):

$$\chi(T) = \Sigma\chi(V) - \Sigma\chi(F) + \Sigma\chi(E) - \Sigma\chi(P), \quad (12)$$

where V is a 3-dimensional volume.

Considering a cubic specimen to be an element of a tessellation of a 3-D space, then the Euler characteristic of the entire 3-D space will not be obtained by simply adding the Euler characteristics of cubic specimens. By doing this, the contribution of the lower dimensional elements (i.e., F , E , and P in equation 12) will not be considered. To calculate the contribution of a cubic specimen to the Euler characteristic of the entire structure, one must consider the Euler characteristic of the lower dimensional elements, and this may be done in one of several ways. The following are two examples:

1. The contribution of the lower dimensional elements are shared equally by the cubes bounded by the elements:

$$\Delta\chi = \chi(V) - \frac{1}{2} \sum_1^6 \chi(F) + \frac{1}{4} \sum_1^{12} \chi(E) - \frac{1}{8} \sum_1^8 \chi(P), \quad (13)$$

where $\Delta\chi$ is the contribution of the cubic specimen to the Euler characteristic of the entire structure. A face bounds two

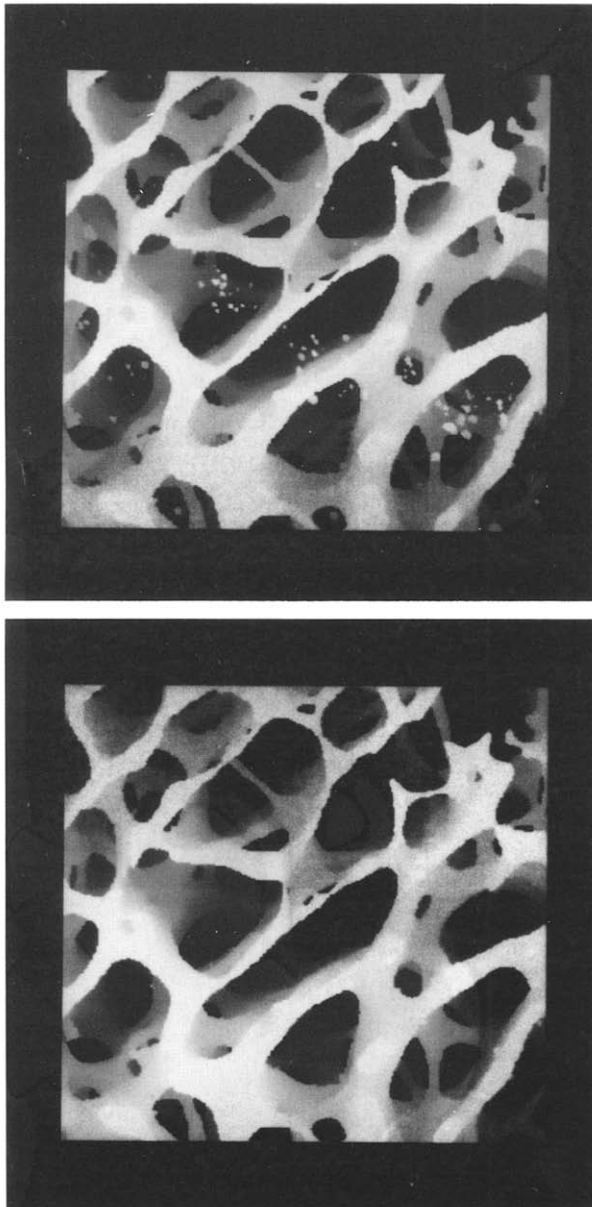


Fig. 5. Top. Three-dimensional reconstruction of cancellous bone specimen (side length 6.5 mm) based on raw data. Bottom. The same specimen as in the top figure after conversion of bone particles (noise) not connected to main structure and of marrow particles (noise) not connected to main marrow space.

cubic specimens, an edge bounds four specimens, and a corner bounds eight specimens; this explains the denominators. If the six faces of the cube are considered to be one surface, and if the 12 edges are considered to be one continuous graph, then equation 13 may easily be rearranged by use of the addition theorem (equation 4):

$$\Delta\chi = \chi_3 - \frac{1}{2}\chi_2 - \frac{1}{4}\chi_1 - \frac{1}{8}\chi_0, \quad (14)$$

where $\chi_3 = \chi(V)$, $\chi_2 = \chi(\cup_1^6 F)$, $\chi_1 = \chi(\cup_1^{12} E)$, $\chi_0 = \sum_1^8 \chi(P)$. This modification of equation 13 may have computational advantages (Odgaard & Gundersen 1991), and is illustrated in Fig. 3.

- Each lower dimensional element is considered to belong to a particular cubic specimen (Bhanu Prasad et al. 1989). Several assignment rules (Kovalevsky 1989) may be used, but the rule should assign the contribution of three faces, three edges, and one corner to each cubic specimen:

$$\Delta\chi = \chi(V) - \sum_1^3 \chi(F) + \sum_1^3 \chi(E) - \chi(P). \quad (15)$$

Whatever method is used, a Euler characteristic of the cubic specimen is obtained which allows correct estimates of the connectivity density, in contrast to an estimate based on $\chi(V)$ alone. The equations 13–15 have been derived under the assumption that all surfaces, all edges, and all corners of the examination

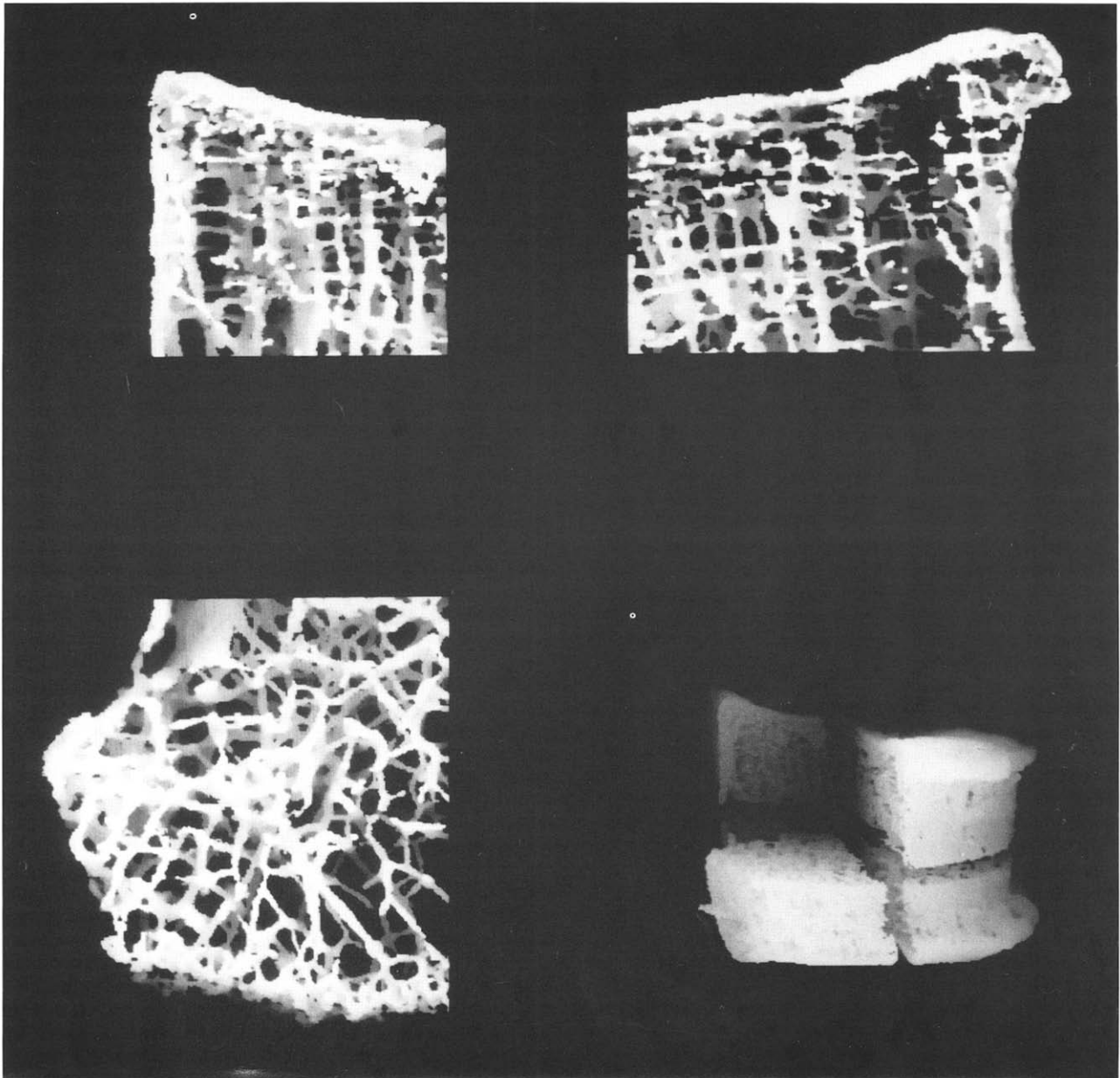


Fig. 6. Three-dimensional reconstruction of entire human vertebral body split into eights (lower right). One part has been removed for exposure of the internal structure. Three sections are shown: sagittal (A, upper left), coronal (B, upper right), and horizontal (C, lower left). The cortical shell of the upper left corner of C bounds the spinal canal, and the lower left corner shows the location where the pedicles were separated from the vertebral body.

Table 1. The influence of examination volume on mean connectivity density

<i>n</i>	Examination volume (mm ³)	Mean connectivity density	
		Correct (mm ⁻³)	Wrong (mm ⁻³)
1	36,325.08	0.63	0.63
2	18,162.54	0.63	0.62
4	9,081.27	0.63	0.62
8	4,540.64	0.63	0.61
16	2,270.32	0.63	0.60
32	1,135.16	0.63	0.58
64	567.58	0.63	0.56
128	283.79	0.63	0.54
256	141.89	0.64	0.52

Correctly calculated (using equation 14) an unbiased value is obtained. By not considering lower dimensional elements in equation 14 the calculated connectivity density depends strongly, but unpredictably, on the examination volume.

volume are artificial. If this is not the case, the summations and unions in the corrective terms should be over the artificial elements only.

A number of different methods have been presented for determination of the Euler characteristic of 2-D and 3-D images (see for instance, Gray 1971; Lobregt et al. 1980; Srihari 1981; Serra 1982; Lee & Rosenfeld 1987; Odgaard 1993), and any of these methods may be used.

In addition to the methods just mentioned for determining the contribution to the Euler characteristic of 3-D reconstructed specimens, it is possible to estimate the connectivity of specimens from projections through relatively thin (from 10 to 40 µm) section pairs (disectors). This method is described by Nyengaard and Marcussen (1993) for sections without artificial edges and by Gundersen et al. (1993) for sections with artificial edges.

Examples

A human thoracic vertebral body was 3-D reconstructed using automated serial sectioning (Odgaard et al. 1993). A graphical representation of the specimen after purification is shown in Fig. 6. The purification assures that there is only one connected bone phase and only one connected marrow phase. The Euler characteristic (using equation 14) was calculated for the entire 3-D reconstruction and for individual parts of the 3-D reconstruction after splitting it into *n* parts (*n* = 2, 4, 8, 16, 32, 64, 128, and 256). In each case the connectivity was calculated using equation

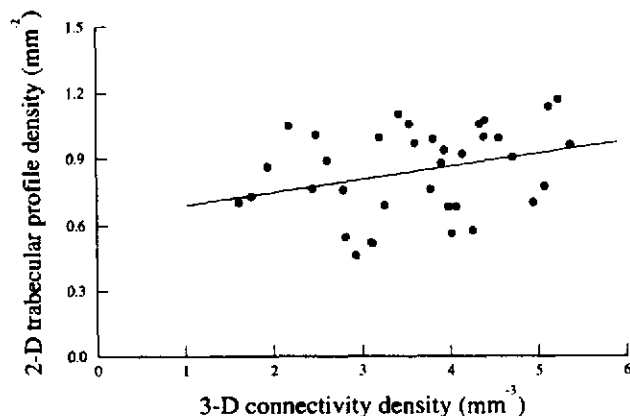


Fig. 7. Number of trabecular profiles per area in random 2-D sections through 36 specimens versus the connectivity density (number of trabeculae per volume) of the specimens.

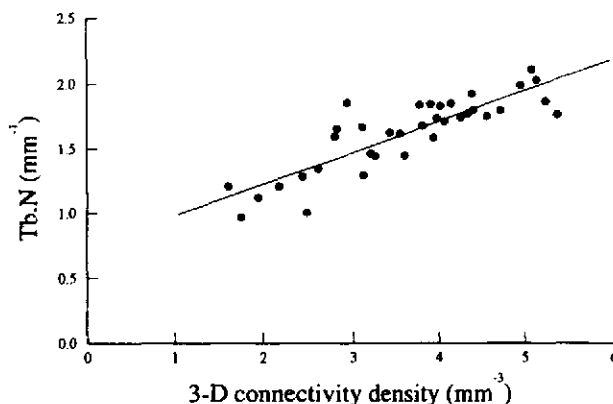


Fig. 8. The histomorphometric quantity "trabecular number," Tb.N, on random sections through the same specimens as in Fig. 7 versus the connectivity density (trabeculae per volume).

9, and the connectivity density was calculated as the connectivity divided by the examination volume. For each examination volume the mean connectivity density was calculated, and these values are shown in Table 1. It should be noted that the correctly calculated mean connectivity density does not change with examination volume, and that the connectivity density calculated without consideration of lower dimensional elements is a biased estimate.

To compare a simple 2-D counting of profiles with the 3-D connectivity, 36 cubic cancellous bone specimens (side length 6.5 mm) from one human proximal tibia were 3-D reconstructed. The Euler characteristic of each specimen was calculated using equation 14, and connectivity calculated using equation 9. Dividing the connectivity by the specimen volume, one obtains the connectivity density (mm⁻³). Random sections were produced from the specimens, and a simple profile counting was performed. The result is seen in Fig. 7, where also the result of performing a linear regression analysis on the relation is shown. Finally, the histomorphometric parameter "trabecular number," Tb.N, was calculated from the random sections through the specimens and also related to the connectivity density (Fig. 8). The relation between connectivity density and volume fraction for the 36 specimens is given in Fig. 9.

Note that none of the relationships in Figs. 7 through 9 are very close relationships (*r* ≤ 0.85), none of them are simple proportional relationships (no regression line includes the origin), and finally, they are all specific for the particular specimens

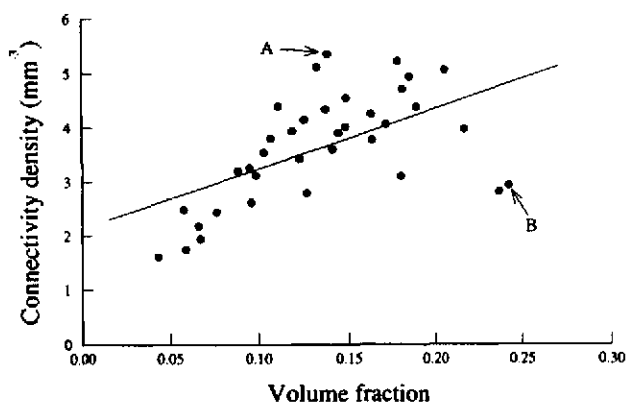


Fig. 9. Connectivity density (trabeculae per volume) versus volume fraction for 36 cancellous bone specimens. The two specimens marked with arrows are shown in Fig. 10.

with their particular size from the particular bone examined. In other words, the relationships cannot be generalized.

Discussion

Qualitative studies of cancellous bone connectivity (Whitehouse 1971; Singh 1978; Mosekilde 1988, 1990) may result in modified or new views of functional anatomy and of disease processes, and may certainly guide intensified research in these fields. The qualitative methods are, however, inferior to quantitative methods when it comes to hypothesis testing and to expressing the relation between connectivity and other parameters. The presented method for quantifying connectivity is an unbiased and basic method which is free of geometric assumptions, and differs as such from methods previously used in cancellous bone research.

The first attempt at quantifying cancellous bone connectivity (Pugh et al. 1974) made use of contiguity ratio, which had been developed for studying particle contacts in multiphase aggregates (Gurland 1968). Using this method on cancellous bone, which hardly is a multiphase aggregate but is instead a continuous two-phase structure, is not straightforward, and requires awkward definitions of interparticle contacts.

In several papers, connectivity has been expressed as the number of "trabeculae" visible in a section through cancellous bone. This number has been determined as the number of separate profiles (Hodgkinson & Currey 1990), or individual trabeculae have been counted distinctively in the profiles (Aaron et al. 1987). With the latter of these approaches there evidently is a problem of defining a trabecula, but for both methods, it is obvious that even when using a correct counting technique (Gun-

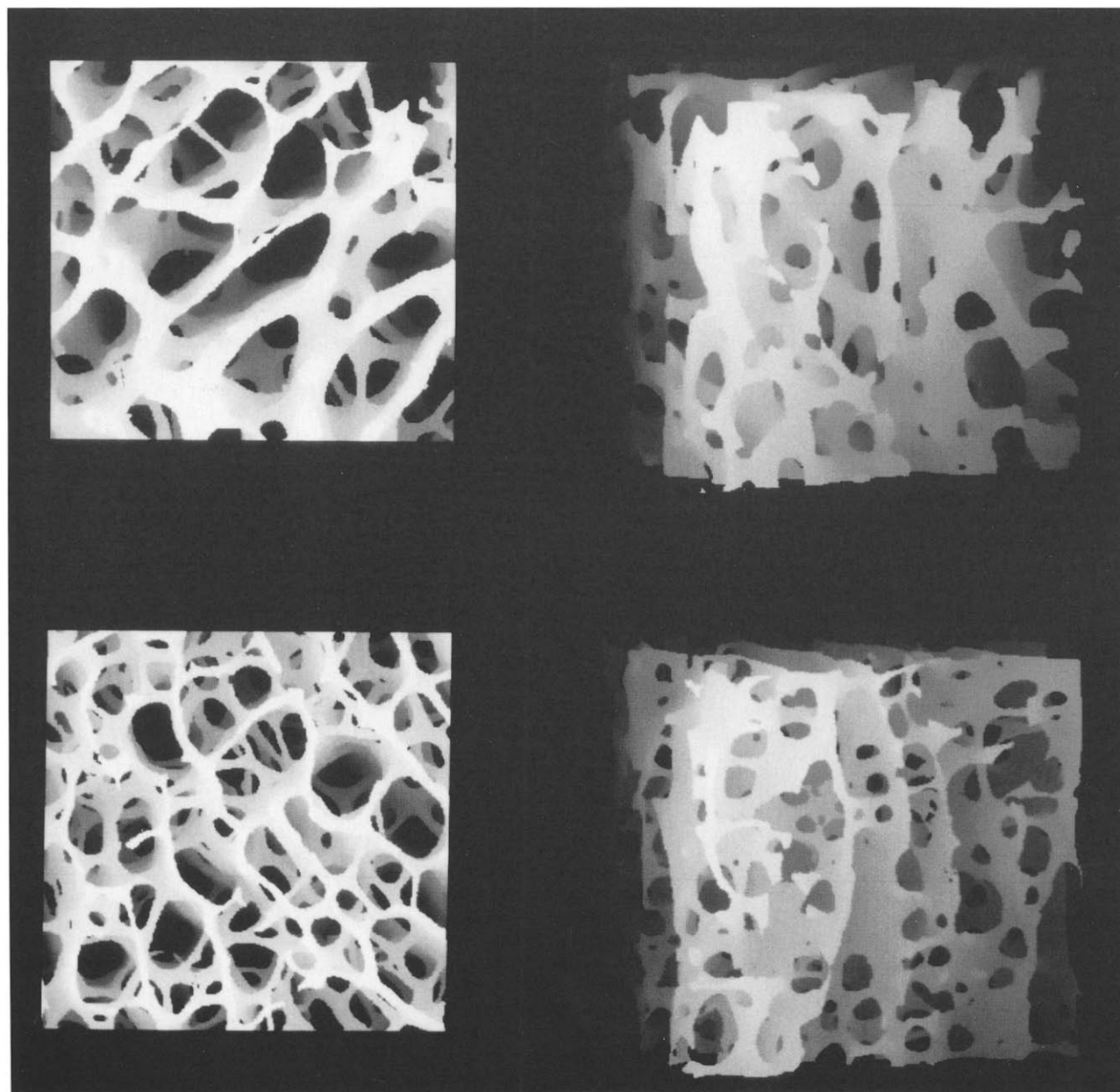


Fig. 10. Graphical presentation of the extremata marked A and B in Fig. 9. A is shown in c (lower left) and d (lower right), whereas B is shown in a (upper left) and b (upper right).

dersen 1977) the count depends upon the orientation of the section and the curvedness of the trabeculae in addition to the dependency upon the number of trabeculae. The same objections are valid for other counts of special features on 2-D sections (Prêtreux et al. 1985; Garrahan et al. 1986; Harrigan et al. 1989). This should indicate that the number of profiles, "trabeculae," or trabecular features in a section has an unknown and extremely debatable relation to the connectivity, which is also illustrated by the poor relation shown in Fig. 7. Admittedly, it may be possible within a certain anatomical region and using a standardized technique to obtain results which show some empirical relationship to connectivity, but the count of profiles should not be considered to be in any way simply related to the connectivity or the number of trabeculae. To get information on the connectivity of a three-dimensional structure, three-dimensional information must be at hand (Giger 1970; DeHoff 1983). One special consequence of considering the 3-D connectivity of cancellous bone to be the number of trabecular profiles found in a 2-D section is that the cancellous bone connectivity may be found to differ significantly from the marrow connectivity, which obviously is an absurd result (cf. equation 7). It has been reported that the number of profiles in a section of cancellous bone is related to the density (Hodgkinson & Currey 1990), but for reasons just mentioned, this has no known relation to the dependency of connectivity upon density.

In conventional histomorphometry a method is claimed for quantification of trabecular architecture. The method is based on only two primary measures (i.e., area fraction of bone and bone perimeter length), assessed in 2-D sections. From these two measures a number of parameters are calculated (Parfitt et al. 1983). The parameters include "trabecular thickness" (Tb.Th), "trabecular number" (Tb.N), and "trabecular separation" (Tb.Sp). It is evident that these in principle independent parameters cannot be derived from two simple 2-D measures without assuming a geometric model, which usually is the plate model. Even though the trabecular number (Tb.N) semantically is very close to the definition of connectivity as the number of trabeculae minus one, the histomorphometric measures do not have any known relation to connectivity. There are two reasons for this. First, as already mentioned, connectivity cannot be measured on individual 2-D sections. Second, the histomorphometric measures are based on the assumption that cancellous bone consists of a set of parallel bony plates, which may be true for some trabecular bone architectures (Figs. 10a,b), whereas the model is close to a misconception for other architectures (Figs. 10c,d) (Singh 1978). Optional modifications of the model, perhaps better to fit experimental data (Birkenhäger-Frenkel et al. 1988) provide no solutions to this problem. For the sample of specimens used in this article, an apparently nice relation was found for Tb.N versus connectivity density (Fig. 8). However, in a recent study (Gundersen et al. 1993) of architectural trabecular changes in an animal model of osteoporosis, a twofold increase in connectivity was found with no change, or even a decrease, in Tb.N. This stresses the fact that no general relationship exists between Tb.N and connectivity, and any use of a relationship shown for one sample of specimens cannot be used on another sample of specimens.

It cannot be stressed too strongly that there is no theoretically conceivable way of estimating 3-D connectivity of cancellous bone or of any other 3-D structure from single 2-D sections. If no theoretically correct method existed, one evidently had to use whatever method was around, if the problems under study were crucial for the understanding of some phenomena. But the risk of using methods which have no theoretical foundation, and the risk of reaching erroneous conclusions, should evidently not be ignored.

Table II. The topological invariants of the specimen shown in Fig. 5

		Nonpurified	Purified
Euler characteristic	χ	-219.75	-650.5
Bone particles	β_0	460	24
Connectivity	β_1	707.75	674.5
Marrow cavities	β_2	28	0

The Euler characteristic (using equation 14) and the number of bone particles and of marrow cavities were determined experimentally, whereas the connectivity was calculated using equation 8.

The connectivity in different tissues has been examined using topological notions, but in several of these articles the edge problem has been ignored (Takahashi 1978; Shimizu et al. 1988). In a pioneering series of papers, the Euler characteristic of cancellous bone has been reported (Feldkamp et al. 1988, 1989; Goldstein et al. 1990) based on the 3-D micro-CT technique. However, in these articles the important topics of the edge problem and the segmentation problem have not been discussed in detail. The segmentation problem is most likely to result in an increase in the zeroth and second Betti numbers and a much smaller increase in the first Betti number, which together may result in a positive Euler characteristic. Neglecting the edge problem may also result in a positive Euler characteristic. In an examination of connectivity versus apparent density, it was found that the variables are linearly related (Goldstein et al. 1990). However, in the presented set of data (Fig. 3 in Goldstein et al. 1990) specimens appear that have positive Euler characteristics, which certainly does not fit with the set of assumptions on the topological properties of cancellous bone, and the reason for this may be the problems just mentioned. The specimen shown in Fig. 5 demonstrates the effect of segmentation on the topological invariants (Table II). It should be noted that the number of bone particles β_0 in the purified specimen is greater than one. The reason for this is that the purification is performed for the entire specimen, whereas the determination of topological invariants is performed in a slightly smaller volume which allows parts of trabeculae in the specimen to enter the examination volume without being connected to other trabeculae in the examination volume. Neglect of the segmentation problem results for the specimen in Fig. 5 in an underestimation of the connectivity by about 66%. This percentage is, however, not general, and no a priori estimation of the error for a given specimen is possible.

In our material we found a poor relationship between connectivity and density (Fig. 9). To further elucidate the postulated relationship, reconstructions of the two extremata marked in Fig. 9 are presented in Fig. 10. One of the specimens has a relatively low density and a relatively high connectivity (Figs. 10c,d), whereas the other specimen has a relatively high density and a relatively low connectivity (Figs. 10a,b). From the graphical reconstructions, it is clear that the basic organization of the two specimens differ. One specimen has a typical platelike structure (Figs. 10a,b) where each plate contributes a lot to density, but relatively little to connectivity, and the other is a typical rod-like structure (Figs. 10c,d) where each trabecula contributes little to the density. This seriously questions the postulated linear relationship between connectivity and density, and suggests that connectivity should be considered an independent parameter in the description of cancellous bone structure.

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