

Computational Methods for Analyzing the Structure of Cancellous Bone in Planar Sections

Arthur D. Kuo and Dennis R. Carter

Department of Mechanical Engineering, Stanford University, Stanford; and Veterans Affairs Rehabilitation R&D Center, Palo Alto, California, U.S.A.

Summary: Conventional stereologic methods for expressing the orientation of anisotropic materials are limited to materials assumed to possess orthogonal directions of orientation. In many substances, including cancellous bone, this assumption is unsubstantiated. Presented here are two simple methods for characterizing the orientation of any anisotropic material within a plane. By modeling the substance as a series of lines oriented in particular directions, it is possible to arrive at either a "phase distribution" that expresses the degree of orientation distributed over a range of angles or a series of "primary orientations" that express the degree of orientation at a select number of angles, with an additional measure of the degree of isotropy. This characterization of anisotropy is highly dependent on such parameters as feature size, sample size, test line spacing, and test line width. Given the careful selection of these parameters, the new methods provide simple measures of orientation, which may prove useful in testing Wolff's trajectorial theory of the relationship between mechanical stresses and the orientation of cancellous bone. **Key Words:** Cancellous bone—Stereology—Wolff's law—Trajectorial theory.

Considerable attention has been paid to characterizing the orientation of cancellous bone. Much of this interest has been directed toward verifying or refuting the trajectorial theory developed by the German anatomist Wolff (26), which postulates that cancellous bone remodels to adapt to its stress environment: the trabeculae become aligned to the (mutually perpendicular) principal stress trajectories so that bone provides maximum strength with minimal weight (16). This concept evolved from the discovery by the anatomist Meyer (15) that the trabecular architecture of the femoral head resembles the principal stress trajectories in a crane drawn by

the engineer Culmann. Wolff used this observation as evidence of an unspecified mathematical relationship between trabecular trajectories and applied stresses. As Roesler (20) points out in his review paper, this theory has led to a debate, consisting mostly of unsubstantiated arguments, that has remained unresolved for a number of decades. Researchers in the field of stereology have recently made more quantitative analyses of bone architecture. Whitehouse and Dyson (25) used stereologic techniques to characterize the trabeculae of the proximal human femur, including in their study a measure of the trabecular "departure from isotropy." Raux and associates (19) developed rigorous procedures for preparing specimens for stereologic analysis and applied them in a study of the trabecular architecture of the human patella. They identified zones of single or mixed orientation within the patella that suggest a sheet-and-rod

Received December 5, 1989; accepted March 12, 1991.

Address correspondence and reprint requests to Dr. A. D. Kuo at Design Division, Mechanical Engineering Department, Stanford University, Stanford, CA 94305, U.S.A.

model of the trabecular structure. Hayes and Snyder (11) extended these techniques in a rigorous quantitative comparison between trabecular architecture and principal stresses calculated from a finite element model of the patella. Providing the first quantitative support for the trajectorial theory, their findings showed a significant correlation between the orientation of the trabeculae and the computed principal stresses.

Harrigan and Mann (9) used the stereologic measure of anisotropy in orthotropic materials to form a tensor, thus consolidating orientation information in three dimensions. Cowin (4,5) used a variation of this tensor, the fabric tensor, in his mathematical formulation of Wolff's law of trabecular architecture.

The trajectorial theory as stated by Wolff has, however, also been challenged. Oxnard and Yang (17), in their study of Fourier transforms of radiographs of human and primate vertebrae, review several objections to the theory and offer modifications to Wolff's law, suggesting that cancellous bone architecture is not necessarily orthotropic (as principal stresses are). Bacon and colleagues (1) used neutron diffraction techniques to assess the orientation of trabeculae in the bones of the human foot; they showed that in areas of the calcaneus subjected to complex time-varying stresses, the trabeculae may have widely varied and sometimes nonorthotropic orientations. They also offered a revised version of Thompson's (21) diagram of the trabecular pattern in the foot, showing lines that are clearly not orthotropic. Cheal and co-workers (3) conducted a careful experiment to test the remodeling response of cancellous bone around implants in the equine patella. Their control specimens showed a significant correlation between trabecular orientation and principal stress directions calculated using a finite element model. However, they found a poor correlation between the *changes* in these two measures in their experimental patellae. Fyhrie and Carter (7) proposed a quantitative unifying principle relating stresses in bone and trabecular morphology. Applying this principle in a finite element analysis of simple loading conditions from a single direction, trabecular architecture should be orthotropic, coinciding with principal stress trajectories. However, under complex time-varying loading conditions, Carter and collaborators (2) suggest that the trabeculae should be aligned so as to best support stresses from a variety of directions (equiva-

lent stresses), and therefore need not be orthotropic.

Stereologic studies of cancellous bone have in the past been based primarily on polar plots of the mean intercept length (*MIL*), as described below. When characterizing the orientation of a material, the plot of the *MIL* is fit to a single ellipse. Because the principal axes of an ellipse are orthogonal, this technique presupposes that the material being studied is orthotropic. This condition must be verified when applied to cancellous bone, in light of the objections to the trajectorial theory discussed above. This article describes computational methods similar to those cited by others (12,13,18), which can be used in stereologic analysis of a two-dimensional section of a substance to test for orthotropy within the plane, to give a measure of the isotropy or departure from isotropy that is superior to conventional measures, to provide quantitative evidence of the number and degree of orientations, and to test models of the material architecture directly—all of which are readily applicable to developing a better understanding of the relationship between mechanical loading and cancellous bone architecture.

CONVENTIONAL STEREOLOGY

Stereology uses statistical measures gathered from two-dimensional sections of a substance to infer information concerning the structure of the material. Underwood's (22) classic text explains in detail the stereologic technique most often used for analysis of cancellous bone morphology, the directed secant method. This method calls for laying a grid of parallel lines, at an angle Θ about an arbitrary axis, across the section, as in Fig. 1A, and counting the intersections between the test lines and the boundaries of the "trabeculae." This process is repeated with the test lines arranged at a series of angles Θ from 0 to 180°.

The total number of intersections per unit test line length [$I_L(\Theta)$] is recorded for each angle, and its polar plot is often referred to as the "rose of intercepts," as shown in Fig. 1B. Alternatively, the inverse of $I_L(\Theta)$, the mean intercept length *MIL*(Θ) is plotted. Typically, a single ellipse is fit to the polar plot of *MIL*(Θ) (24). However, because an ellipse has orthogonal principal axes, using these axes to characterize the material orientation may be misleading if the substance is not orthotropic, even if the ellipse provides a good fit.

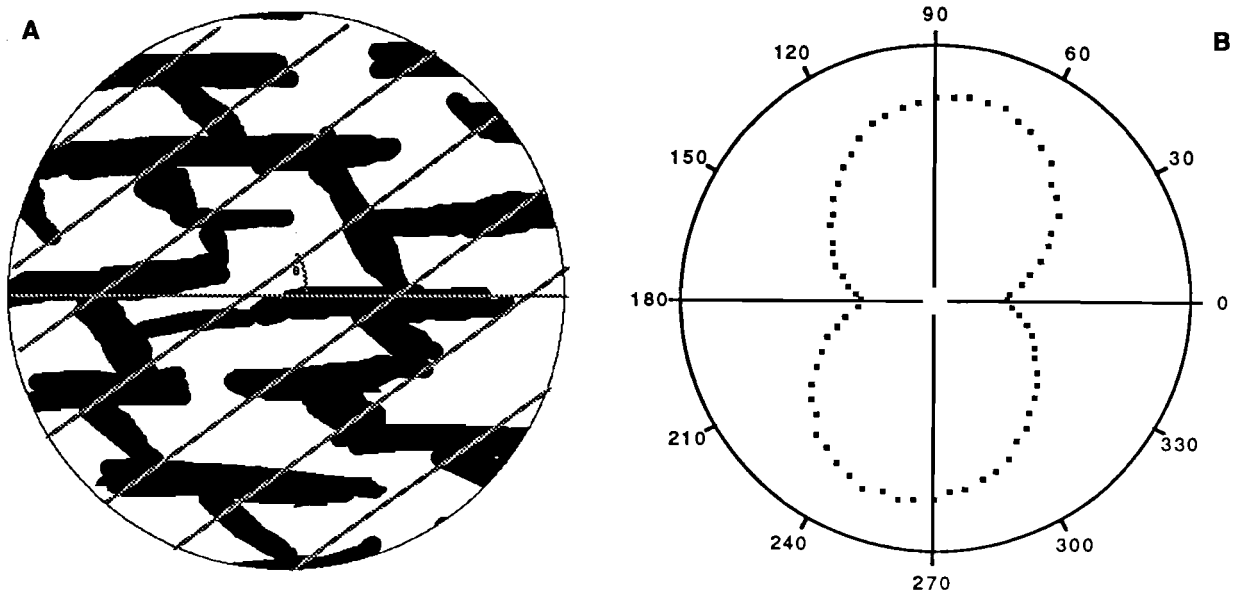


FIG. 1. **A:** Sample pattern exhibiting obvious nonorthotropic characteristics. Test lines at angle Θ shown. **B:** Rose of intercepts for sample pattern.

The degree of orientation is another important stereological measure. It is easily determined based on the stereological measure of the boundary length per unit area B_A (also referred to as perimeter length density). Hayes and Snyder (11) and Whitehouse and Dyson (23) used

$$B_A = \frac{\pi}{2} \overline{I_L(\Theta)} \quad (1)$$

B_A is used to find the degree of orientation

$$\% \text{ orientation} = \frac{100[I_L(\max) - I_L(\min)]}{B_A} \quad (2)$$

This measure is most applicable for materials possessing a single direction of orientation; for materials with multiple directions of orientation, the results suggest little about the actual morphology of the test substance. For example, for the test pattern shown in Fig. 1A, which is highly oriented in two directions. Eq. 2 yields 55% orientation, which is an uninformative description.

As discussed above, trabecular bone is a complex, anisotropic material that may not possess mutually perpendicular directions of orientation. Conventional techniques of fitting an ellipse to $MIL(\Theta)$ are not adequate for describing the orientation of such a material. We will present methods based on

the work of Hilliard (12) that are better suited for planar sections.

METHODS

It has been noted that the directed secant method, when applied to a sample pattern of straight lines with orientation ϕ with respect to the horizontal, will produce a rose of intercepts such as shown in Fig. 2 (22). Mathematically, the rose of intercepts for this sample pattern can be expressed as

$$I_L(\Theta) = c_0 |\sin(\Theta - \phi)| \quad (3)$$

where c_0 is an indicator of the total line length in the direction ϕ . Figure 3 shows that the rose of intercepts for a sample pattern consisting of an array of straight lines in two different directions ϕ_1 and ϕ_2 is of the form

$$I_L(\Theta) = c_1 |\sin(\Theta - \phi_1)| + c_2 |\sin(\Theta - \phi_2)| \quad (4)$$

and c_1 and c_2 describe the relative total line lengths in the two principal directions (22). The corresponding $MIL(\Theta)$ plot is a combination of two ellipses.

Cancellous bone is often considered to be made up of struts and plates arranged in a variety of positions and directions. If we assume that a two-dimensional slice of cancellous bone exposes these structural elements as a series of lines of various lengths arranged at various positions and orienta-

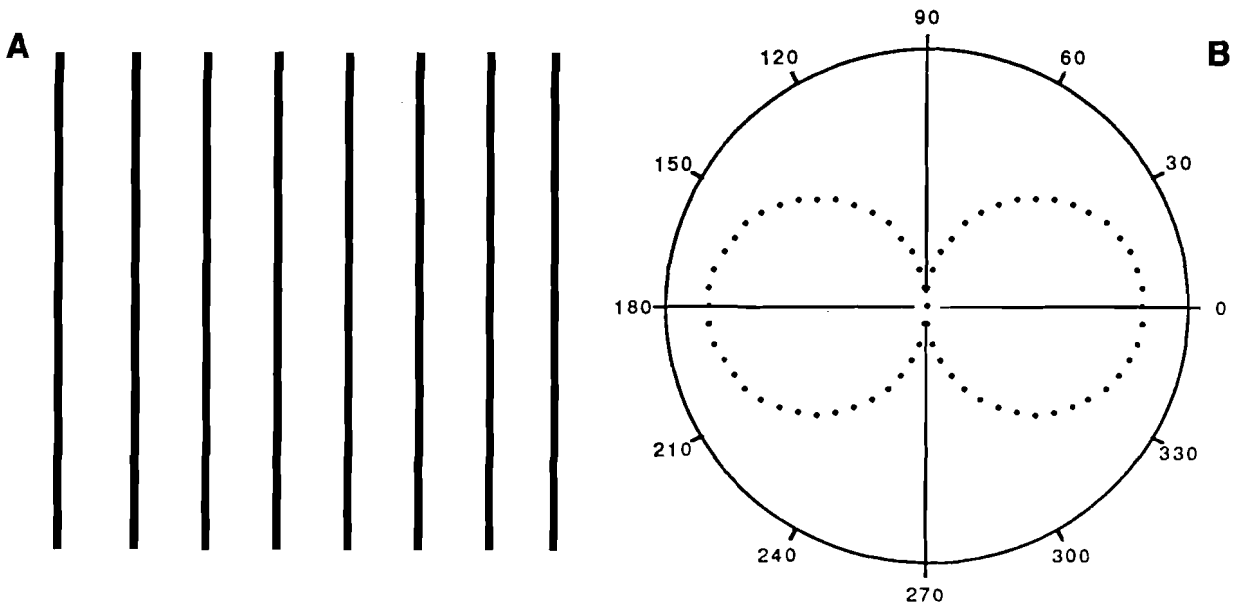


FIG. 2. A: Sample pattern of straight lines with orientation $\phi = 90^\circ$. B: Corresponding rose of intercepts.

tions, we can find the estimated rose of intercepts $\hat{I}_L(\Theta)$ with

$$\hat{I}_L(\Theta) = \sum_{j=1}^n c_j |\sin(\Theta - \phi_j)| \quad (5)$$

where the bone pattern is modeled as a series of lines pointing in n directions, with a weighting (or degree of orientation) c_j corresponding to each direction ϕ_j . Hilliard likened this relation to a convolution between the sine function and c expressed in the continuous domain (12).

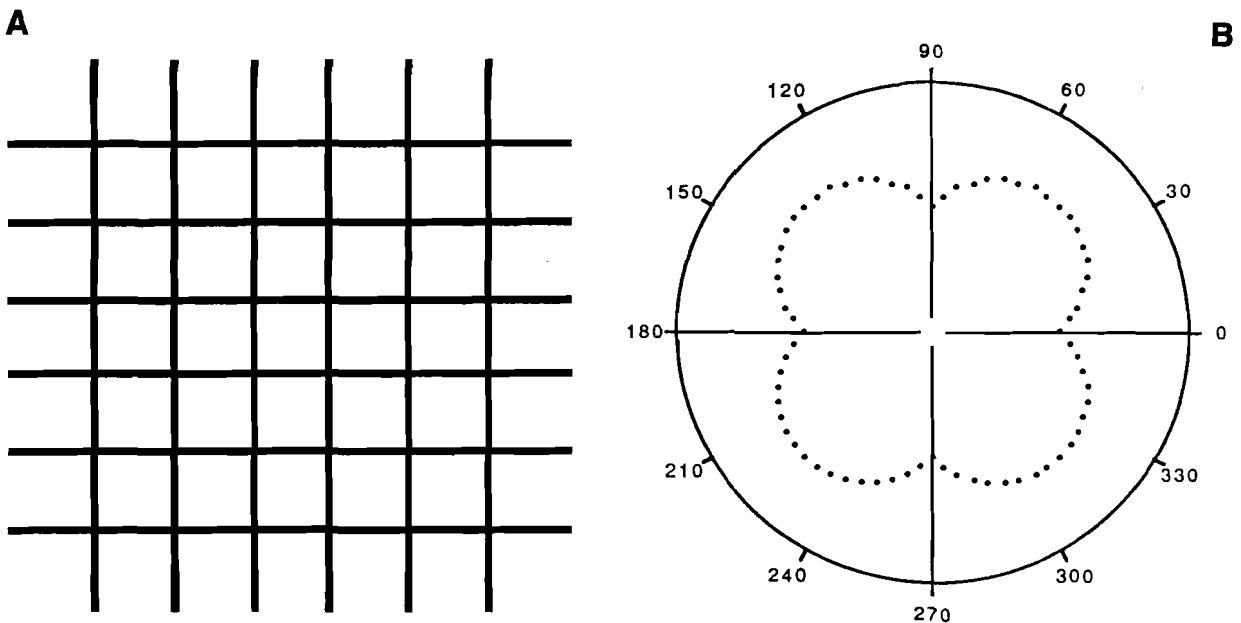


FIG. 3. A: Sample pattern of straight lines in two perpendicular directions. B: Corresponding rose of intercepts.

Phase Distribution Method

From Eq. 5, we see that the rose of intercepts is simply a sum of rectified sine waves of identical frequencies but varying phases—the phases being the angles of orientation. Just as a frequency distribution plot produced from a Fourier transform provides the magnitude of sinusoidal signals over a range of frequencies, the phase distribution plot provides the magnitudes of sine waves over a range of phases.

Plots similar to the phase distribution have proved useful in describing the preferred orientations of cancellous bone, but they typically are vaguely qualitative in nature (17). The rose of intercepts, however, can be used to produce a more quantitative phase distribution computationally. The technique is very flexible and is compatible with many conventional automated stereology systems.

For Hilliard (12) the continuous function c is solved by deconvolving the continuous version of Eq. 5. Unfortunately, this technique does not constrain the magnitudes to be positive, resulting in a different interpretation of c from that given above. Kanatani (13) solved the continuous problem for c [which was denoted the distribution density $f(\Theta)$] constrained positive, with a Fourier series approximation. The results are problematic for complex materials, because the harmonics of the Fourier series confuse the results. For the purposes of studying cancellous bone, we prefer discrete “bins” for the phase distribution.

To produce the phase distribution from the rose of intercepts, the angles ϕ_j ($j = 1, 2, \dots, n$) are typically selected over a range $0^\circ \leq \phi_j < 180^\circ$, distributed uniformly. Least-squares parameter estimation facilitates the calculation of the unknown magnitudes c_j ($j = 1, 2, \dots, n$). If the rose of intercepts is given at m angles Θ_i ($i = 1, 2, \dots, m$), the number of phases that can be solved for is $n \leq m$. Use of $n \geq m$ results in overfitting of the data.

We wish to minimize the difference J between the measured rose of intercepts and that obtained from the model. Using a least-squares measure of this difference, the objective (14) can be written as

$$\begin{aligned} \text{minimize } J &= \sum_{i=1}^m \left(I_L(\Theta_i) - \hat{I}_L(\Theta_i) \right)^2 \\ &= \sum_{i=1}^m \left(I_L(\Theta_i) - \sum_{j=1}^n c_j |\sin(\Theta_i - \phi_j)| \right)^2 \end{aligned} \tag{6}$$

subject to $c_j \geq 0$ for $j = 1, 2, \dots, n$. This can be expanded after substituting

$$\Phi_{ij} = |\sin(\Theta_i - \phi_j)| \tag{7}$$

so that the objective is to minimize

$$\begin{aligned} J &= \sum_{i=1}^m \left(I_L(\Theta_i)^2 + \sum_{j=1}^n (c_j \Phi_{ij})^2 \right. \\ &\quad + 2 \sum_{j=1}^n \sum_{k=1}^n c_j c_k \Phi_{ij} \Phi_{ik} \\ &\quad \left. - 2I_L(\Theta_i) \sum_{j=1}^n c_j \Phi_{ij} \right) \end{aligned} \tag{8}$$

subject to $c_j \geq 0$ for $j = 1, \dots, n$. $I_L(\Theta_i)^2$ is not a function of c_j and therefore may be dropped from the objective function. By also making the following substitutions into the elements of \mathbf{Q} , an n by n matrix, and \mathbf{b} and \mathbf{x} , both n by 1 vectors:

$$Q_{jk} = \sum_{i=1}^m \Phi_{ij} \Phi_{ik} \tag{9}$$

$$b_j = - \sum_{i=1}^m I_L(\Theta_i) \Phi_{ij} \tag{10}$$

$$x_j = c_j \tag{11}$$

for $j = 1, \dots, n$ and $k = 1, \dots, n$, the optimization problem may be written as

$$\begin{aligned} \text{minimize } \bar{J} &= \frac{1}{2} \mathbf{x}^T \mathbf{Q} \mathbf{x} + \mathbf{b}^T \mathbf{x} \\ \text{subject to } \mathbf{x} &\geq 0. \end{aligned} \tag{12}$$

Parameters describing the accuracy of the least-squares fit can be obtained using the following equations (6):

error sum of squares

$$SSE = \sum_{i=1}^m (I_L(\Theta_i) - \hat{I}_L(\Theta_i))^2 \tag{13a}$$

total sum of squares

$$SST = \sum_{i=1}^m (I_L(\Theta_i) - \overline{I_L(\Theta_i)})^2 \tag{13b}$$

estimated variance

