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Published in:
Journal of Biomechanics

DOI:
10.1016/j.jbiomech.2008.04.016

Citation for published version (APA):

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Spatial orientation in bone samples and Young’s modulus.

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Abstract.

Bone mass is the most important determinant of the mechanical strength of bones and spatial structure is the second. In general the spatial structure and mechanical properties of bones such as the breaking strength are directional dependent. The Mean Intercept Length (MIL) and Line Frequency Deviation (LFD) are two methods for quantifying directional aspects of the spatial structure of bone. The Young's modulus is commonly used to describe the stiffness of bone which is also a directional dependent mechanical property. The aim of this article is to investigate the relation between MIL and LFD on one hand and the Young's modulus on the other.

From 11 human mandibular condyles 44 samples were taken and scanned with high resolution computer tomography equipment (micro-CT). For each sample the MIL and LFD were determined in 72602 directions distributed evenly in 3D space. In the same directions the Young's modulus was determined by means of the stiffness tensor that had been determined for each sample by finite element analysis. To investigate the relation between the MIL and LFD on one hand and the Young's modulus on the other multiple regression was used.

On average the MIL accounted for 69% of the variance in the Young's modulus in the 44 samples and the LFD accounted for 72%. The average percentage of variance accounted for increased to 80% when the MIL was combined with the LFD to predict the Young's modulus. Obviously MIL and LFD to some extent are complementary with respect to predicting the Young's modulus. It is known that directional plots of the MIL tend to be ellipses or ellipsoids. It is speculated that ellipsoids are not always sufficient to describe the Young's modulus of a bone sample and that the LFD partly compensates for this.

Keywords.

Bone, Anisotropy, Young's modulus, Mean Intersect Length (MIL), Line Frequency Deviation (LFD)
Introduction.

Bone mass is the most important determinant of the mechanical strength of bones and the second most important determinant is spatial structure [Allolio 1999] [Cummings 1993] [Gomberg 2003] [Snyder 1993] [Veenland 1997]. In general the spatial structure and mechanical properties of bones such as the breaking strength are directional dependent. For example horizontal and sagittal sections of the femoral neck display different views and different mechanical stiffness, a phenomenon known as anisotropy [Whitehouse 1974] [Homminga 2004]. Whitehouse was the first to describe the anisotropy of bone sections by means of measurements of the Mean Intercept Length (MIL) [Whitehouse 1974]. The MIL plotted as a function of the measuring direction yielded an ellipse in every sample. Harrigan and Man recognized that this property enabled the description of the MIL with a tensor [Harrigan 1984]. Cowin classified the inverse of the MIL tensor as a measure of anisotropy for the spatial structure of bone and by the mid 1990s this method had become standard [Cowin 1985] [Keaveny 2001]. Using the MIL to compare osteoporotic and healthy vertebrae it has been shown that the trabeculae in osteoporotic vertebrae are oriented predominantly in longitudinal direction thus providing sufficient stiffness to endure common loading forces but offering less resistance to any load in an off axial direction [Homminga 2004]. This shows the relevance of directional aspects of bone structure with respect to osteoporotic fractures.

The Young's modulus is defined as the force per unit area divided by the fractional length change. The Young's modulus of bone strongly depends on the spatial structure. When a sample of trabecular bone has an anisotropic spatial structure the Young's modulus is anisotropic as well [Ashby 1983]. Several studies have investigated the relation between structural and mechanical anisotropy. One of those studies was by Van Lenthe and Huiskes [Van Lenthe 2002]. With a generic 2D model various trabecular bone structures were simulated and the corresponding Young's modulus was calculated with finite element analysis. Five of those samples and the corresponding Young's modulus are shown in figure 1 columns 1 and 2. Columns 3 and 4 show the corresponding MIL and the Line Frequency Deviation (LFD) calculated with methods developed for the analysis of 2D images [Geraets 1998]. The MIL plots in column 3 tend to be elliptical however they are a poor approximation of the Young's modulus especially for structures A and C. On the
other hand, the resemblance of columns 2 and 4 is striking, especially for structure C. This visual similarity suggests that the LFD might be closer related to the Young's modulus than the MIL, at least in 2D models of trabecular bone. The present study quantitatively investigates the relation between MIL and LFD on one hand and the Young's modulus on the other using 3D bone samples of the human condyle.
Methods.

Measuring MIL and LFD

Sets of parallel lines are often used for measuring orientation [Chetverikov 1981], [Harrigan and Mann 1984], [Cheal et al. 1987], [Turner 1992]. When an observer studies porous materials through a microscope, grids are projected over the microscopic image to facilitating counting. In the past strategies were worked out to reduce the amount of time and effort needed for the tedious process of counting, but if the counting is done by a computer then a more robust analysis is feasible. Therefore, sets of densely packed parallel test lines are used to measure MIL and LFD values. The measuring procedure is based upon the idea that a test line consists of pixels (or voxels) queued at regular distance of 1 and that a set of parallel test lines of equal length fills up a square (or cube), that can rotate around its centre. Figure 2 shows a grid of 12 x 12 pixels making measurements along one of the diagonal directions in a 2D sample of 18 x 18 pixels. In order to prevent the grid sticking out of the sample, for example when measuring along a diagonal, its size is taken to be the minimum size of the sample divided by $\sqrt{2}$. Likewise in 3D the edge of the cube equals the minimum size of the sample divided by $\sqrt{3}$. For calculations on pixels (or voxels) of the test lines it is convenient to use the square (or cube) as a frame of reference because then the pixels (or voxels) can easily be addressed by two (or three) integer coordinates. Rotation of the square (or cube) is simulated by converting these coordinates to the frame of reference connected with the sample. For example in Figure 2, if the procedure requires the value of the first pixel on the second test line then a rotation of $-45^\circ$ is used to calculate the coordinates of the corresponding image pixel before it is read.

When the test lines in the square (or cube) align with the chosen direction they may cross borders between black and white pixels (or voxels). These crossings divide the test lines into black and white intercepts. Considering the intercepts from all test lines the MIL is computed as the combined length of the intercepts divided by the number of intercepts [Cheal et al. 1987], [Odgaard 1997], [Saltikov 1958], [Turner 1992]. For measuring the LFD, both in two dimensions as well as in three dimensions, first the fraction of bright pixels is calculated for each test line separately and then the standard deviation of these fractions yields the LFD value along the chosen direction [Geraets et al. 1997], [Geraets et al. 1998], [Geraets 1998]. Figure 2 shows the fractions per line and the resulting standard deviation 0.07 which is the LFD value of the sample in the
Bone samples.

In a study on the cancellous bone architecture of the human mandibular condyle 8 female and 3 male cadavers were analysed [Giesen 2000], [Van Eijden 2006]. From 7 left and 4 right condyles 4 cubical samples were extracted resulting in a total of 44 bone samples. The samples comprised bony plates that were approximately parallel to the sagittal plane. The bone samples were scanned with a micro-computed tomograph ($\mu$CT 20, Scanco Medical AG, Bassersdorf Switzerland). Bone and marrow were separated with a fixed threshold. On average the dimensions of the samples were 3.5 x 3.5 x 3.4 mm$^3$ or 105 x 104 x 101 voxels with a voxel size of 34 x 34 x 34 $\mu$m$^3$ [Giesen 2000], [Van Eijden 2006]. The X-axis pointed in the anterior - posterior direction, the Y-axis in the superior direction, and the Z-axis in the medial direction. To visualize the bone samples projection views were created in the XY, YZ and the XZ planes as shown in the top left quarters of figures 3 and 4. While the actual voxels were either bone (value 255) or background (value 0), the shading suggests the distance of bone voxels to the plane of view.

Plotting MIL, LFD, and Young's modulus.

For purpose of visualisation it was decided to measure MIL, LFD, and Young's modulus along a large number of directions. To avoid any directional bias these directions should be distributed evenly in space which can be difficult in 3D. As a compromise between homogeneity and ease of programming the directions were generated by considering a cube of 111 x 111 x 111 voxels having 72602 voxels on the surface. Connecting the center of the cube with one voxel on the surface provided one direction along which MIL, LFD and Young's modulus were measured. For each bone sample the elastic stiffness tensor had been determined previously by means of finite element analysis [Van Eijden 2006], [Van Rietbergen 1995]. To obtain the Young's modulus in any specific direction the coordinate system was rotated in such a way that the Z-axis coincided with the specified direction and then the corresponding element of the rotated tensor was taken [Kaajakari 2002], [Wortman 1965]. The measurements were collected in 3D polar plots that were visualised with the same projection views as the bone samples.
Statistics.

For each sample multiple regression was performed with the Young's modulus as the criterion variable and the MIL and LFD as the predictors. In each multiple regression the value of the Young's modulus, the MIL and the LFD in all 72602 directions were used to establish the relation between the Young's modulus on one hand and the MIL and LFD on the other. For the statistical tests a significance level $\alpha = 0.05$ was used. Because the correlations were calculated per sample there was no need to take the effect of the apparent density of the bone samples into consideration [Keaveny 2001].
Results.

Table 1 shows the mean and standard deviation of Young's modulus, MIL and LFD calculated over all the directions of all samples. The values found for Young's modulus and MIL correspond with values published previously [Giesen 2000], [Van Eijden 2006].

The correlations between Young's modulus on one hand and the MIL and/or the LFD on the other were computed for the 44 samples and then averaged. It was found that on average the MIL accounted for 69% of the variance in the Young's modulus and the LFD accounted for 72%. Both predictors used together accounted for 80% of the variance in the Young's modulus. For both MIL and LFD and their combination the relation with the Young's modulus was statistically significant at $\alpha = 0.05$. The percentages of variance accounted for demonstrate that using the MIL and the LFD combined leads to a better prediction of young's modulus than using either of them separately.

Figures 3 and 4 illustrate bone samples and the corresponding polar plots of Young's modulus, MIL and LFD. For most of the samples the plot of the Young's modulus clearly differed from an ellipsoid which is most striking in figure 3. The sample has maximum Young's modulus in direction (-0.70, 0.56, 0.45) close to the plane $Y=X$ and in direction (0.64, 0.72, 0.29), close to the plane $Y=-X$, the Young's modulus has a secondary maximum with 93% of the maximum value. The angle between the directions of the primary and secondary maximum is 85°.
Discussion.

The paper of Whitehouse [Whitehouse 1974] describing for the first time in literature the elliptical shape of the MIL of bone samples also describes the high degree of anisotropy of some of the bone samples. Planar sections of bone taken close to each other but in different directions had much different appearance. It is justified to wonder why a material with such a chaotic spatial structure yields MIL plots that are nearly perfect ellipses. The tendency of the MIL to produce nearly perfect ellipses and ellipsoids is due to insensitivity of the MIL to orientation [Geraets 1998] [Geraets 2006]. Ellipses and ellipsoids are not always sufficient to describe the Young's modulus or other mechanical properties [Gomberg 2003] [Hoffmeister 2000] [Pidakarti 1997]. This is illustrated by the 2D models A and C in figure 1 and the 3D bone sample in figures 3 and 4. Ellipsoid fitting assumes orthotropic symmetry which may exist in the spine but perhaps not in more complicated skeletal locations as the proximal femur and calcaneus [Gomberg 2003]. It is widely assumed that trabecular bone has orthotropic mechanical symmetry and this may be true in general but it is improbable that each and every individual sample of trabecular bone has three orthogonal planes of mechanical symmetry. This is illustrated by the sample in figure 3 where the angle between the directions of the first and secondary maximum of the Young's modulus is 85°.

The improvement in the prediction of Young's modulus that is obtained by combining the LFD with the MIL might be explained by the lesser tendency of the LFD to produce ellipsoids as illustrated by figures 1, 3 and 4. Further improvement can be obtained by avoiding the partial volume effect which is caused by the need for segmented input [Kuo 1991]. Unlike the MIL method the LFD method can easily be adapted to work with multi level gray values instead of binary data [Geraets 2006]. Therefore, when the spatial structure of bone is to be analyzed it is an interesting option to use the MIL and the LFD combined.
References.


Kaajakari V. Silicon as an anisotropic mechanical material - a tutorial.


Table 1: descriptive statistics Young’s Modulus, MIL and LFD

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus</td>
<td>331 ± 247 MPa</td>
</tr>
<tr>
<td>MIL</td>
<td>0.505 ± 0.117 mm</td>
</tr>
<tr>
<td>LFD</td>
<td>0.123 ± 0.032</td>
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Figures.

**Figure 1:**

Figure 2

Measuring Line Frequency Deviation (LFD) along 135° in an image of 18 x 18 pixels. For each test line the fraction of bright pixels is calculated and then the standard deviation of the fractions yields the LFD value.
Figure 3:
Bone sample, Young's modulus, MIL index of orientation, and LFD index of orientation.

Maximum Young's modulus in direction (-0.70, 0.56, 0.45) and a secondary maximum in direction (0.64, 0.72, 0.29).
Figure 4

Bone sample, Young's modulus, MIL index of orientation, and LFD index of orientation.

Maximum Young's modulus in direction (0.06, 0.95, 0.29).