Structural parameters measured by the Skyscan™ CT-analyser software.

Morphometric parameters are calculated by CT-analyser either in 3d based on a volume model, or in 2d from crossection images (individually or integrated over a volume-of-interest). Some parameters are measured in both 2d and 3d. Parameter names follow two alternative nomenclatures, general scientific or bone ASBMR, the latter based on Parfitt *et al.* (1987). Parfitt's paper proposes a system of symbols for bone histomorphometry, and the principles of Parfitt's system are applied here to both the bone (ASBMR) and the general scientific parameter names. Within Skyscan CT-analyser four alternative dimensional units are selectable: mm, µm, inch or pixel. For clarity in this document all dimension units are given as mm.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Total VOI volume	Tissue volume
Parameter symbol	TV	TV
Unit	mm^3	mm^3

Total volume of the volume-of-interest (VOI). Measured in both 2d and 3d. The 2d measurement is simply the total number of voxels of (solid and space) in the VOI times the voxel volume. The 3d volume measurement is very similar but based on the hexahedral marching cubes volume model of the VOI. Please note that in the case of Bone ASBMR nomenclature, the word "tissue" simply refers to the volume of interest. It does not mean any kind of recognition of any particular density range as biological tissue, soft, hard or otherwise.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object volume	Bone volume
Parameter symbol	Obj.V	BV
Unit	mm^3	mm^3

Total volume of binarised objects within the VOI. Measured in both 2d and 3d. The 2d measurement is simply the number of voxels of binarised solid objects in the VOI times the voxel volume. The 3d volume measurement is very similar but based on the hexahedral marching cubes volume model of the binarised objects within the VOI.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Percent volume	Percent bone volume
Parameter symbol	Obj.V/TV	BV/TV
Unit	%	%

The proportion of the VOI occupied by binarised solid objects. This parameter is only relevant if the studied volume is fully contained within a biphasic region of solid and space such as a trabecular bone region, and does not for example extend into or beyond the bounding cortical wall of bone.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object surface	Bone surface
Parameter symbol	Obj.S	BS
Unit	mm^2	mm^2

In 2d the binarised object surface includes both the crossection slice perimeter measurements plus also the vertical surfaces between solid and space, so in fact is an essentially 3d measurement based on a simple cubic voxel. This means for example that in 2d based thickness estimates the correction factor of 1.199 for converting perimeters to a surface estimate should not be used (see object thickness, below). The 3d measured surface is based on the faceted surface of the marching cubes volume model.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object specific surface	Bone specific surface
Parameter symbol	Obj.S/Obj.V	BS/BV
Unit	mm^{-1}	mm^{-1}

The ratio of binarised solid surface to volume measured as described above in both 2d and 3d within the VOI. Surface to volume ratio or “specific surface” is a useful basic parameter in characterising the complexity of structures and is the basis of model-dependent estimates of thickness.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object surface density	Bone surface density
Parameter symbol	Obj.S/TV	BS/TV
Unit	mm^{-1}	mm^{-1}

The ratio of surface area to total volume measured as described above in both 2d and 3d within the VOI.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Interception surface	Interception surface
Parameter symbol	i.S	i.S
Unit	mm^2	mm^2

Interception surface is the surface of the VOI intercepted by solid binarised objects, that is, the part of the VOI boundary surface that runs through solid objects. This parameter is useful in evaluating bone growth at a defined boundary – for example at a fixed distance away from an orthopaedic bone implant.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Structure thickness	Trabecular thickness
Parameter symbol	Sr.Th	Tb.Th
Unit	mm	mm

Calculation – or estimation – of Tb.Th from 2D measurements requires an assumption about the nature of the structure. Three simple structure models, the parallel plate, the cylinder rod and the sphere model, provide the range of values within which a hypothetical “true” thickness will be located. Thickness defined by these models (Parfitt *et al.* 1987) is given:

Parallel plate model:

$$Tb.Th = \frac{2}{(BS/BV)} \qquad (1.)$$

Cylinder rod model:

$$Tb.Th = \frac{4}{(BS/BV)} \qquad (2.)$$

Sphere model:

$$Tb.Th = \frac{6}{(BS/BV)} \qquad (3.)$$

Where *BS/BV* is the surface to volume ratio, mm^{-1}.

Note that for the above equations, if the surface measurements are 2d and based only on crossectional perimeters of binarised objects, then the numerator (2, 4 or 6) should be divided by a correction factor of 1.199. However as mentioned above (object surface) 2d surface measurement in CT-analyser includes vertical surfaces between solid and space voxels in adjacent image slices. It is thus in fact an essentially 3d measurement based on a simple cubic voxel model. Therefore the thickness model based estimated should not here include the 1.199 correction factor.

With 3D image analysis by micro-CT a true 3D thickness can be measured which is model-independent. This is determined as an average of the local thickness at each voxel representing solid (or bone) (Ulrich *et al.* 1999). Local thickness for a point in solid is defined by Hildebrand and Ruegsegger (1997a) as the diameter of a sphere which fulfils two conditions:

(a) the sphere encloses the point (but the point is not necessarily the centre of the sphere);

(b) the sphere is entirely bounded within the solid surfaces.

Histomorphometrists typically measure a single mean value of bone Tb.Th from a trabecular bone site. However a trabecular bone volume – or any complex biphasic object region – can also be characterised by a distribution of thicknesses. CT-analyser outputs a histogram of thicknesses with an interval of two pixels.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Structure separation	Trabecular separation
Parameter symbol	Sr.Sp	Tb.Sp
Unit	mm	mm

Trabecular separation is essentially the thickness of the spaces as defined by binarisation within the VOI. It can also be calculated either from 2D images with model assumptions or directly in 3D. Applying the surface area-based models as for thickness, structure separation is calculated:

Parallel plate model:

$$Tb.Sp = \left(\frac{1}{Tb.N}\right) - Tb.Th \qquad (4.)$$

Cylinder rod model:

$$Tb.Sp = Tb.Th \times \left(\left[\left(\frac{4}{\pi}\right) \times \left(\frac{TV}{BV}\right)\right] - 1\right) \qquad (5.)$$

where TV is total volume of VOI and BV is bone (or solid) volume (Parfitt *et al.* 1987). Note that each of the above definitions takes the Tb.Th value derived from the corresponding plate or rod model.

In practice in trabecular bone analysis it is unsafe to employ 2D model assumptions, especially because the "plate-like" or "rod-like" character of trabecular bone can change both between bone samples and from one end of a studied bone volume to the other (see "structure model index", below). Therefore Skyscan analysis software can measure Tb.Sp directly and model-independently in 3D from micro-CT images by the same method used to measure trabecular thickness (see above).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Structure linear density	Trabecular number
Parameter symbol	Sr.L.D	Tb.N
Unit	mm^{-1}	mm^{-1}

Structure linear density or trabecular number implies the number of traversals across a trabecular or solid structure made per unit length on a linear path through a trabecular bone region. Plate and rod model 2D-based definitions of Tb.N again take the corresponding Tb.Th values:

Parallel plate model:

$$Tb.N = \frac{(BV/TV)}{Tb.Th} \qquad (6.)$$

Cylinder rod model:

$$Tb.N = \frac{\sqrt{\left(\left(\frac{4}{\pi}\right) \times \left(\frac{BV}{TV}\right)\right)}}{Tb.Th} \qquad (7.)$$

Again the complexities of model dependence are eliminated by true 3D calculation of Sr.L.D / Tb.N from micro-CT images. This parameter is measured in CT-analyser in 3d by application of equation (6) for the parallel plate model, but using a direct 3D measurement of thickness. Note that the optional stereology analysis (not included in this report) includes measurements of thickness, separation and number/linear density based on the mean intercept length (MIL) analysis which represents an alternative basis for these architectural measurements.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Fragmentation index	Trabecular bone pattern factor
Parameter symbol	Fr.I	Tb.Pf
Unit	mm^{-1}	mm^{-1}

This is an index of connectivity of trabecular bone, which was developed and defined by Hahn *et al.* (1992). It was applied by these authors to 2D images of trabecular bone, and calculates an index of relative convexity or concavity of the total bone surface, on the principle that concavity indicates connectivity (and the presence of “nodes”), and convexity indicates isolated disconnected structures (struts). Tb.Pf is calculated in 2D (slice-by-slice) and in 3D, by comparing area and perimeter (or volume and surface, respectively) of binarised solid before and after an image dilation. It is defined:

$$Tb.Pf = \left(\frac{P_1 - P_2}{A_1 - A_2} \right) \qquad (8.)$$

Where P and A are solid area and perimeter, and the subscript numbers 1 and 2 indicate before and after image dilation.

Where structural / trabecular connectedness results in enclosed marrow spaces, then dilation of trabecular surfaces will contract the perimeter. By contrast, open ends or nodes will have their perimeter expanded by surface dilation. As a result, lower Tb.Pf signifies better connected trabecular lattices while higher Tb.Pf means a more disconnected trabecular structure. A prevalence of enclosed cavities and concave surfaces can push Tb.Pf to negative values – as with the structure model index (SMI) – see below.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Euler number	Euler number
Parameter symbol	Eu.N	Eu.N
Unit	mm^{-1}	mm^{-1}

The Euler-Poincare – or the abbreviated “Euler number” – is also an indicator of connectedness of a 3D complex structure. The Euler number is characteristic of a three-dimensional structure which is topologically invariant (it is unchanged by inflation or compression of the structure). It measures what might be called “redundant connectivity” – the degree to which parts of the object are multiply connected (Odgaard *et al.* 1993). It is a measure of how many connections in a structure can be severed before the structure falls into two separate pieces. The components of the Euler number are the three Betti numbers: β_0 is the number of objects, β_1 the connectivity, and β_2 the number of enclosed cavities. The Euler-Poincare formula for a 3d object X is given:

$$\chi(X) = \beta_0 - \beta_1 + \beta_2 \qquad (9.)$$

Euler analysis provides a measure of connectivity density (Eu.Conn.D, mm^{-3}), indicating the number of redundant connections between trabecular structures per unit volume. To calculate this, divide the connectivity β_1 (a unitless number) by the analysed volume (VOI, mm^{-3}). Trabecular connectivity may contribute significantly to structure strength although this relation is a question requiring further research (Odgaard 1997).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Degree of anisotropy	Degree of anisotropy
Parameter symbol	DA	DA
Unit	(none)	(none)

Isotropy is a measure of 3d symmetry or the presence or absence of preferential alignment of structures along a particular directional axis. Apart from percent volume, DA and the general stereology parameters of trabecular bone are probably the most important determinants of mechanical strength (Odgaard 1997). Mean intercept length (MIL) and Eigen analysis are used to calculate DA, and these involve some quite advanced engineering mathematics. However the essentials of the MIL eigen analysis can be summarised in normal English.

Consider a region or volume containing two phases (solid and space), both having complex architecture, such as a region of trabecular bone. We can study this volume to determine isotropy. If the volume is isotropic, then a line passing through the volume at any 3d orientation will make a similar number of intercepts through the solid phase. A bag of marbles would be isotropic. However a packet of spaghetti would be non-isotropic, or anisotropic, since lines going along the direction of the spaghetti would make few intercepts along the spaghetti rods while lines crossing at right-angles would make many intercepts. Figure 1 illustrates the difference in the number of intercepts for lines from different directions through an anisotropic, aligned group of structures.

Mean intercept length (MIL) analysis measures isotropy (it is usual to talk of measurement of the negative quantity anisotropy). Mean intercept length is found by sending a line through a 3D image volume containing binarised objects, and dividing the length of the test line through the analysed volume by the number of times that the line passes through or intercepts part of the solid phase. Note that in this MIL calculation the intercept length may correlate with object thickness in a given orientation but does not measure it directly. Therefore it will give an accurate result if analysing a volume containing a sufficiently large number of objects, but is not suitable for analysis of single or small numbers of objects. For the MIL analysis, a grid of lines is sent through the volume over a large number of 3d angles. The MIL for each angle is calculated as the average for all the lines of the grid. The spacing of this grid can be selected in CTan preferences (the “advanced” tab). This requires that a spherical region is first defined within which the MIL analysis will be done and isotropy measured, since the test lines must all cross the sphere centre and have an equal distribution of lengths, covering all 3d angles but distributed at random. In CTan you can actually set a spherical volume of interest (VOI). However if a non-spherical VOI is set, the MIL analysis fits a sphere enclosing the VOI.

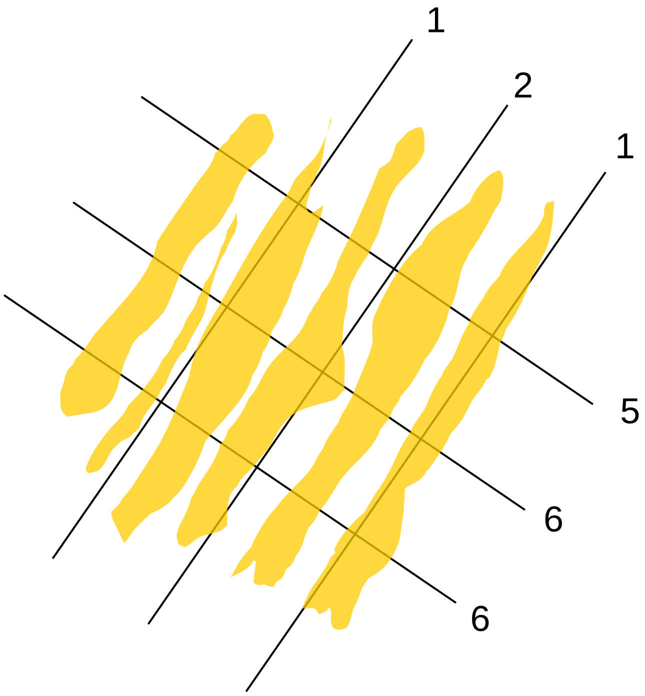


Figure 1. A group of aligned long structures has a high anisotropy: test lines make few intercepts through the solid objects in the direction of the long axis of the structures, but perpendicular to the structures the lines make many more intercepts (numbers of intercepts are shown for each line).

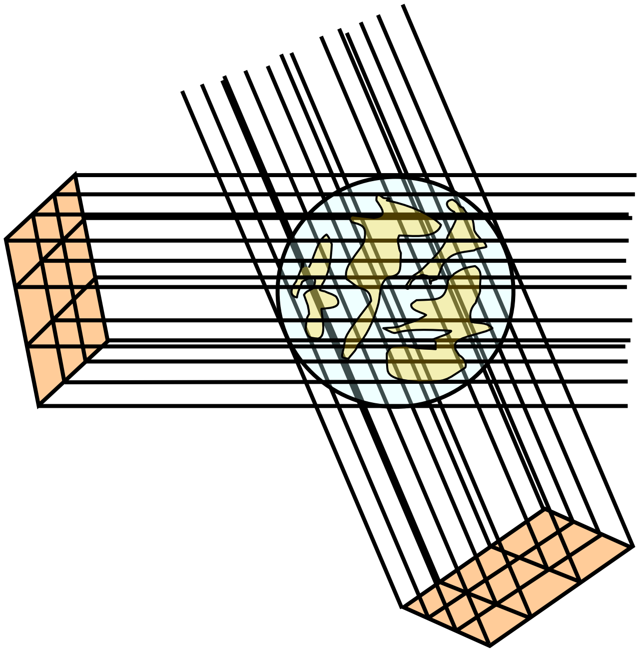


Figure 2. For the MIL analysis, a grid of lines is sent through the volume over a large number of 3d angles (just two are illustrated here). The MIL for each angle is calculated as the average for all the lines of the grid.

The next step involves visualisation of the 3d distribution of MIL lengths as an ellipsoid. All the MIL lines are drawn passing through one point, and the length of each line is the bone phase MIL for that line. This process is called a polar plot of MIL. In 3d this creates a dense pin-cushion like effect with lines in all directions at different lengths.

Figure 3 shows in a simple diagram the appearance of an MIL distribution in 3d. Any asymmetry in the MILs with respect to 3d angle - which will represent the anisotropy of the bone in the spherical region - will make the line distribution depart from an overall spherical shape and become elongated in the direction where the solid structures have the longest MIL (such as the axis of the spaghetti packet).

Clearly the MIL "pin-cushion" is a complex object, and a method is needed to extract some summary numerical parameters defining the orientation and isotropy / anisotropy of the MIL distribution. This is where the anisotropy tensor analysis steps in. (Tensor means matrix.) This method is probably best attributed to Harrigan and Mann (1984) and describes the MIL distribution as an ellipsoid. An ellipsoid is a 3d ellipse. As shown in figure 1, an ellipsoid has three axes. These describe the longest orientation, and the length and width (major and minor axes) of the ellipse section at right-angles to the longest orientation. The ellipsoid can be asymmetric in one axis only, like a rugby ball, or in two axes, like a bar of soap.

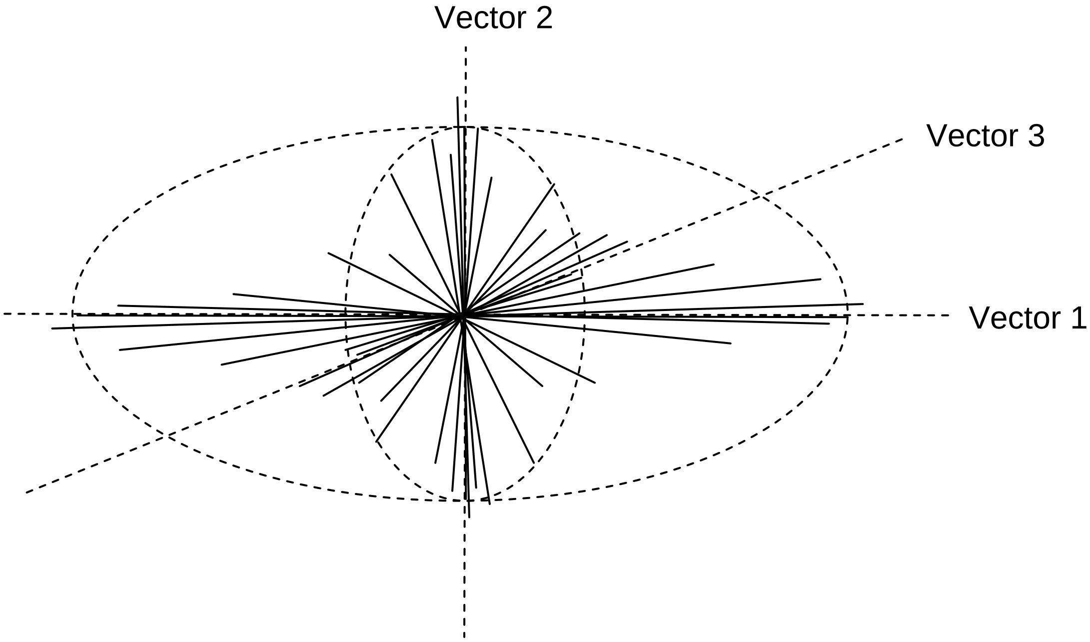


Figure 3. An ellipsoid (3D ellipse) is fitted to the 3D distribution of MILs (mean intercept lengths) measured over a full range of 3D stereo-angles. This ellipsoid is fitted statistically and has 3 vectors which are orthogonal (at right-angles to each other). A tensor (matrix) of 9 (3x3) eigenvectors describes the directions of the three vectors.

An ellipsoid is fitted to the MIL "pin-cushion" 3d polar plot. This is a statistical fit, finding the ellipsoid which most closely describes the 3d shape of the MIL distribution. MIL analysis therefore should also output values indicating the strength of fit of the ellipsoid and associated error, such as the correlation coefficients.

A tensor or matrix is a way of describing an ellipse by a 3x3 matrix of numbers. Technically this is a second order tensor. The tensor describing the anisotropy ellipsoid is an orthogonal tensor, since it describes the ellipsoid axes which are orthogonal (at right angles) to each other. The end result of the anisotropy tensor analysis is the eigen analysis, eigen meaning characteristic. This comes in two parts. You have the 3x3 matrix of eigenvectors which describe the 3d angles of the three axes of the ellipsoid as described above - one column of 3 numbers for each vector. And the three eigenvalues are each an index of the relative length of bone intercepts in each of the three axes described by the eigenvectors.

Finally, you can derive from the tensor eigen analysis a single parameter measuring anisotropy: this is the degree of anisotropy (DA), and is traditionally expressed as the maximum eigenvalue divided by the minimum eigenvalue. Values for DA calculated in this way vary from 1 (fully isotropic) to infinity (fully anisotropic). Mathematically this is a cumbersome scale. A more convenient mathematical index of anisotropy is calculated as:

$$DA = \left(1 - \left[\frac{\min eigenvalue}{\max eigenvalue}\right]\right) \qquad (10.)$$

Here DA is 0 for total isotropy and 1 for total anisotropy. (Both values are reported by CT-analyser).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Structure model index	Structure model index
Parameter symbol	SMI	SMI
Unit	(none)	(none)

Structure model index indicates the relative prevalence of rods and plates in a 3d structure such as trabecular bone. SMI involves a measurement of surface convexity. This parameter is of importance in osteoporosis of trabecular bone which is characterised by a transition from plate-like to rod-like architecture. An ideal plate, cylinder and sphere have SMI values of 0, 3 and 4 respectively.

The calculation of SMI is based on dilation of the 3d voxel model, that is, artificially adding one voxel thickness to all binarised object surfaces (Hildebrand *et al.* 1997b). This is also the basis of the Tb.Pf parameter (see above) which explains why changes in both parameters correlate very closely with each other. SMI is derived as follows:

$$SMI = 6 \times \left(\frac{S' \times V}{S^2}\right) \qquad (11.)$$

where S is the object surface area before dilation and S’ is the change in surface area caused by dilation. V is the initial, undilated object volume.

It should be noted that concave surfaces of enclosed cavities represent negative convexity to the SMI parameter, since dilation of an enclosed space will reduce surface area causing S’ to be negative. Therefore regions of bone containing a prevalence of enclosed cavities – such as regions with relative volume above 50% – can have negative SMI values. As a consequence, the SMI parameter is sensitive to relative volume, and this can accentuate differences between experimental groups in the measured SMI value.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Fractal dimension	Fractal dimension
Parameter symbol	FD	FD
Unit	none	none

Fractal dimension is an indicator of surface complexity of an object, which quantifies how that object's surface fills space. For examples of fractal objects, "fractal art" is abundant on the internet. True fractal objects have surface shapes which are repeated over many spatial scales. So the closer you look (i.e. the higher the magnification or "zoom in") the more self-similar structure you see. A typical example is a fern leaf in which each side-branch is very similar to the whole fern leaf, and likewise each side-finger of each side branch also looks the same as the whole fern leaf, and so on. A fractal object essentially has fractional, non-integer dimension, i.e. a line "trying" to fill a plane, or a plane trying to fill a 3d space, having dimension somewhere between 2 and 3.

Fractal dimension is calculated using the Kolmogorov or "box counting" method. It is calculated in both 2d and 3d in Skyscan CTAn. The surface or volume is divided into an array of equal squares or cubes, and the number of squares containing part of the object surface is counted. This is repeated over a range of box sizes such as 3-100 pixels. The number of boxes containing surface is plotted against box length in a log-log plot, and the fractal dimension is obtained from the slope of the log-log regression. Fractal characteristics of trabecular bone, and methods for measurement of fractal dimension, are discussed by Chappard *et al.* (2001).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Average object area per slice	Average object area per slice
Parameter symbol	Av.Obj.Ar	Av.Obj.Ar
Unit	mm^2	mm^2

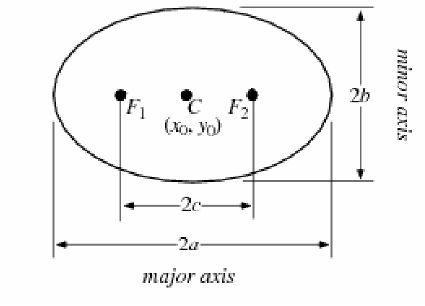
This is a parameter output from the slice-by-slice 2d analysis in CT-analyser. It is a useful indicator of structural connectivity – in interconnected structures (e.g. trabecular bone) high connectivity results in few and large discreet binarised objects in crossection – by contrast fragmentation results in large numbers of smaller objects. This parameter has proved powerful experimentally as an indicator of bone tumour damage (Vermeirsch *et al.* 2004).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Average number of objects per slice	Average number of objects per slice
Parameter symbol	Av.Obj.N	Av.Obj.N
Unit	(none)	(none)

This is a parameter output from the slice-by-slice 2d analysis in CT-analyser. It is also a useful indicator of structural connectivity – in interconnected structures (e.g. trabecular bone) high connectivity results in few and large discreet binarised objects in crossection – by contrast fragmentation results in large numbers of smaller objects. This parameter has proved powerful experimentally as an indicator of bone tumour damage (Vermeirsch *et al.* 2004).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Eccentricity	Eccentricity
Parameter symbol	Ecc	Ecc
Unit	(none)	(none)

A 2d shape analysis of discreet binarised objects. Objects are approximated as ellipses, and eccentricity is an elliptic parameter indicating departure from circular shape by lengthening (a circle has an eccentricity of zero). An ellipse is defined as having two focal points (F1 and F2), a centre (C) and major and minor axes. The major axis is defined as 2a where a is the "semimajor axis"; likewise, 2b is the minor axis. Eccentricity *e* is a function of the (semi)major and minor axes such that:



$$e = \sqrt{1 - \frac{b^2}{a^2}} \quad (12.)$$

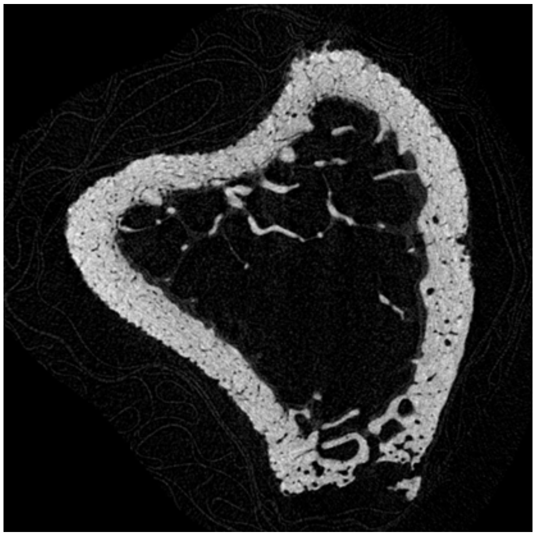
Nomenclature	General Scientific	Bone ASBMR
Parameter name	Mean polar moment of inertia	Mean polar moment of inertia
Parameter symbol	MMI(polar)	MMI(polar)
Unit	mm^4	mm^4

This 2d crossectional function is a basic strength index and indicates the resistance to rotation of a crossection about a chosen axis (assuming uniform material stress-strain strength properties). It is useful in application to cortical bones scanned with crossections at right-angles to the bones long axis.

Moment of inertia is the rotational analog of mass for linear motion and must be specified with respect to a chosen axis of rotation. For a point mass (represented by an image pixel) the moment of inertia (I) is simply the mass (m) times the square of perpendicular distance (r) to the rotation axis, $I = mr^2$. Moment of inertia for a crossection – for example of cortical bone – is the integral of all the solid (bone) pixels.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Porosity	Porosity
Parameter symbol	Po	Po
Unit	%	%

Porosity is measured in the 2d slice-by-slice analysis in CT-analyser. Binarised objects are identified containing fully enclosed spaces, and porosity is the area of those spaces as a percent of the total area of binarised objects. Note that the denominator of total object area includes the enclosed spaces. Porosity measurement ignores space which is not surrounded by solid. Although it is measured in 2d, since it is based on voxel counting an accurate 3d porosity can be obtained by integrating porosity measured over all the image levels of the VOI – this integrated measure of porosity is reported in the summary values of the 2d slice-by-slice analysis in CT-analyser.



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