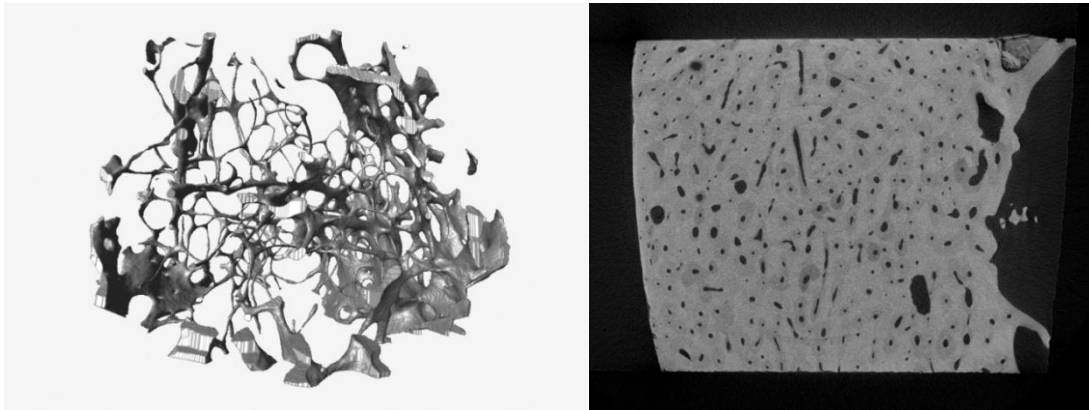


SKYSCAN

X-RAY MICROTOMOGRAPHY  
X-RAY NANOTOMOGRAPHY  
IN-VIVO SMALL ANIMAL IMAGING



## Structural parameters measured by Skyscan™ CT- analyzer software.

Morphometric parameters are calculated by CT-analyser either in direct 3D based on a surface-rendered volume model, or in 2D from individual binarised cross-section images. Further, for both 3D and 2D measurements, there are two types of analysis. Firstly, all objects in the selected region can be analysed together, and the integrated results calculated, such as total volume or surface of all objects, or mean thickness of all objects, etc. Secondly, individual “discreet” objects can be categorised, on the basis of being entirely surrounded by space either in 2D (on a single cross-section only) or in 3D (for a selected volume comprising many cross-sectional levels). Morphometric parameters in 3D and 2D can be carried out on all these individual objects.

Therefore four categories of analysis can be performed, comprising both integrated analysis of all selected objects (with a single result output for all objects collectively), and individual analysis of all discreet objects (with a separate result output for each object); and both of these are calculated in both 2D and 3D.

All calculations are performed over a selected region. Consistent and accurate selection of the regions or volumes of interest is fundamentally important to obtaining accurate and meaningful data. Some clarification of terminology for this is useful. The term “region of interest”, or ROI, will refer to a selected region of a single cross-sectional image. 2D analysis is performed within a ROI. The “volume of interest” refers to the collective sum of all ROIs over a contiguous set

of cross-sectional image slices, representing a selected 3D volume. Morphometric analysis in 3D is performed in a VOI. Note however that 2D type analysis can also be performed on a VOI, by integrating or summing the results of 2D analysis over many slices. This is sometimes called “slice-by-slice” analysis. It can yield calculations (or estimates) of 3D parameters such as surfaces and volumes and thicknesses of objects, but it should be understood that these results are based on integration of many 2D analyses of separate cross-sections, and this is not the same as true direct 3D analysis, which is performed on a surface-rendered 3D volumetric model. So, within a 3D volume of interest, both true 3D analysis, and 2D “slice-by-slice” analysis, can be performed in CT-analyser. (Note further – you do not see this 3D surface rendered model of objects during 3D analysis – it is created “in the background” but not displayed. Creation and display of 3D models is also provided in the CT-analyser and CT-volume programs, but these are separate procedures not connected to morphometric analysis.)

Parameter names follow two alternative nomenclatures, “General Scientific” or “Bone ASBMR”, the latter based on Parfitt et al. (1987). Parfitt’s paper proposed 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. In this document, both the General Scientific and the Bone ASBMR name will be given at the title of each parameter. In the contents pages, however, only the General Scientific names are given, for conciseness.

Within Skyscan CT-analyser four alternative dimensional units are selectable: mm,  $\mu\text{m}$ , inch or pixel. For clarity in this document, all dimension units are given as mm.

All measurements of morphometric parameters in 3D and 2D are performed on segmented or binarised images. Segmentation or “thresholding” must be done prior to morphometric analysis.

A final note – in the CT-analyser preferences (general tab) you can also specify numerical parameter reporting in scientific notation (e.g. 1.2345E-001) or non-scientific (e.g. 0.12345). Scientific notation is useful for preserving the same decimal point accuracy level for parameter values over different orders of magnitude.

**SUMMARY: Morphometric analysis in 3D.**

3D integrated analysis of all objects in VOI (all selected image levels)			3D individual analysis of all objects in the VOI (over all selected image levels)		
<i>Parameter</i>	<i>Symbol</i>	<i>Unit</i>	<i>Parameter</i>	<i>Symbol</i>	<i>Unit</i>
VOI volume	TV	mm <sup>3</sup>	Object volume	Obj.V	mm <sup>3</sup>
Object volume	Obj.V	mm <sup>3</sup>	Object surface	Obj.S	mm <sup>3</sup>
Percent object volume	Obj.V/TV	%	Volume of pores	Po.V	mm <sup>3</sup>
VOI surface	TS	mm <sup>2</sup>	Surface of pores	Po.S	mm <sup>2</sup>
Object surface	Obj.S	mm <sup>2</sup>	Porosity	Po	%
Intersection surface	i.S	mm <sup>2</sup>	Number of pores	N.Po	
Object surface / volume ratio	Obj.S/Obj.V	mm <sup>-1</sup>	Centroid x	Crd.X	mm
Object surface density	Obj.S/TV	mm <sup>-1</sup>	Centroid y	Crd.Y	mm
Fragmentation index	Fr.I	mm <sup>-1</sup>	Centroid z	Crd.Z	mm
Centroid (x)	Crd.X	mm			
Centroid (y)	Crd.Y	mm			
Centroid (z)	Crd.Z	mm			
Structure model index	SMI				
Structure thickness	St.Th	mm			
Structure linear density	St.Li.Dn	mm			
Structure separation	St.Sp	mm <sup>-1</sup>			
Degree of anisotropy	DA				
Eigenvalue 1					
Eigenvalue 2					
Eigenvalue 3					
Fractal dimension	FD				
Number of objects	Obj.N				
Number of closed pores	Po.N(cl)				
Volume of closed pores	Po.V(cl)	mm <sup>3</sup>			
Surface of closed pores	Po.S(cl)	mm <sup>2</sup>			
Closed porosity (percent)	Po(cl)	%			
Volume of open pores	Po.V(op)	mm <sup>3</sup>			
Open porosity (percent)	Po(op)	%			
Total volume of pores	Po.V(tot)	mm <sup>3</sup>			
Total porosity (percent)	Po(tot)	%			

Default results filename	[prefix]_3D.txt/csv	Default results filename	[prefix]_i3D.txt/csv
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## SUMMARY: Morphometric analysis in 2D

2D integrated analysis of all objects in VOI (all selected image levels, line-by-line)			2D individual analysis of all objects in the ROI (from the single current image level only)		
Parameter	Symbol	Unit	Parameter	Symbol	Unit
VOI volume	TV	mm <sup>3</sup>	Area	Ar	mm <sup>2</sup>
Object volume	Obj.V	mm <sup>3</sup>	Perimeter	Pm	mm
Percent object volume	Obj.V/TV	%	Form factor	FF	
VOI surface	TS	mm <sup>2</sup>	Equivalent circle diameter	ECD	mm
Object surface	Obj.S	mm <sup>2</sup>	Roundness	Rd	
Object surface / volume ratio	Obj.S/Obj.V	mm <sup>-1</sup>	Euler number	EN	
Mean total crosssectional ROI area	T.Ar	mm <sup>2</sup>	Porosity	Po	%
Mean total crosssectional ROI perimeter	T.Pm	mm	Extent	Ext	
Mean total crosssectional object area	Obj.Ar	mm <sup>2</sup>	Orientation	Or	°
Mean total crosssectional object perimeter	Obj.Pm	mm	Eccentricity	Ecc	
Mean number of objects per slice	Obj.N		Centroid x		mm
Average object area per slice	Av.Obj.Ar	mm <sup>2</sup>	Centroid y		mm
Average object equivalent circle diameter per slice	Av.Obj.ECD	mm	Moments of inertia x	MMIx	mm <sup>4</sup>
Mean polar moment of inertia	MMI (polar)	mm <sup>4</sup>	Moments of inertia y	MMIy	mm <sup>4</sup>
Mean eccentricity	Ecc		Polar Moment of inertia	MMI <sub>p</sub>	mm <sup>4</sup>
Crosssectional thickness	Cs.Th	mm	Product of inertia	pl	mm <sup>4</sup>
Structure thickness (plate model)	St.Th(pl)	mm	Minimum principal moment of inertia	MMI(min)	mm <sup>4</sup>
Structure separation (plate model)	St.Sp(pl)	mm	Maximum principal moment of inertia	MMI(max)	mm <sup>4</sup>
Structure linear density (plate model)	St.Li.Dn(pl)	mm <sup>-1</sup>	Major axis	Ma	mm
Structure diameter (rod model)	Tb.Dm(rd)	mm	Minor axis	Mi	mm
Structure separation (rod model)	Tb.Sp(rd)	mm	Major diameter	d(max)	mm
Structure linear density (rod model)	St.Li.Dn(rd)	mm <sup>-1</sup>	Minor diameter	d(min)	mm
Mean fragmentation index	Fr.I	mm <sup>-1</sup>	Aspect ratio	AR	
Closed porosity (%)	Po(cl)	%	Mean Thickness	Av.Th	mm
Centroid (x)	Crd.X	mm			
Centroid (y)	Crd.Y	mm			
Centroid (z)	Crd.Z	mm			
Mean fractal dimension	FD				
Total intersection surface	i.S	mm <sup>2</sup>			

Default results filename	[prefix]_2D.txt	Default results filename	[prefix]_3D.txt/csv
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## ***PART A: 3D MORPHOMETRIC PARAMETERS***

### **A1: 3D morphometric parameters integrated for the whole volume of interest (VOI)**

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Total VOI volume	Tissue volume
Parameter symbol	TV	TV
Unit	mm <sup>3</sup>	mm <sup>3</sup>

Total volume of the volume-of-interest (VOI). The 3D volume measurement is based on the 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 <sup>3</sup>	mm <sup>3</sup>

Total volume of binarised objects within the VOI. The 3D volume measurement is based on the marching cubes volume model of the binarised objects within the VOI.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Percent object 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 well-defined biphasic region of solid and space, such as a trabecular bone region, and does not for example extend into or beyond the boundary of the object – such as the cortical boundary of a bone sample. The meaningfulness of measured percent volume depends on the criteria applied in selecting the volume of interest. Where the ROI / VOI boundaries are loosely drawn in the surrounding space around an object for instance, then % object volume has no meaning.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	VOI surface	Tissue surface
Parameter symbol	TS	TS
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The surface area of the volume of interest, measured in 3D (Marching cubes method).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object surface	Bone surface
Parameter symbol	Obj.S	BS
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The surface area of all the solid objects within the VOI, measured in 3D (Marching cubes method).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Intersection surface	Intersection surface
Parameter symbol	i.S	i.S
Unit	mm <sup>2</sup>	mm <sup>2</sup>

Intersection surface is the surface of the VOI intersected by solid binarised objects, that is, the part of the VOI boundary surface that runs through solid objects. This parameter is useful, for example, 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	Object surface / volume ratio	Bone surface / volume ratio
Parameter symbol	Obj.S/Obj.V	BS/BV
Unit	mm <sup>-1</sup>	mm <sup>-1</sup>

The ratio of solid surface to volume measured in 3D within the VOI. Surface to volume ratio or “specific surface” is a useful basic parameter for characterising the thickness and complexity of structures.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object surface density	Bone surface density
Parameter symbol	Obj.S/TV	BS/TV
Unit	mm <sup>-1</sup>	mm <sup>-1</sup>

The ratio of surface area to total volume measured as described above in 3D, within the VOI.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Fragmentation index	Trabecular bone pattern factor
Parameter symbol	Fr.I	Tb.Pf
Unit	mm <sup>-1</sup>	mm <sup>-1</sup>

This is an inverse index of connectivity, which was developed and defined by Hahn *et al.* (1992) for application to trabecular bone. It was applied by these authors originally 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 3D, by comparing volume and surface of binarised solid before and after a single voxel image dilation. It is defined:

$$Tb.Pf = \left( \frac{S_1 - S_2}{V_1 - V_2} \right) \quad (1.)$$

Where S and V are solid surface and volume, 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 surface. By contrast, open ends or nodes will have their surface 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. This parameter Tb.Pf or fragmentation index is best considered as a relative index for comparing different scanned objects; its absolute value does not have much meaning.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Centroids X, Y, Z	Centroids X, Y, Z
Parameter symbol	Crd.X, CrdY, CrdZ	Crd.X, CrdY, CrdZ
Unit	mm	mm

The centroid is the 3D XYZ coordinate of the average Cartesian vectorial position of all voxels within the VOI.

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 osteoporotic degradation 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:

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

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	Structure thickness	Trabecular thickness
Parameter symbol	St.Th	Tb.Th
Unit	mm	mm

With 3D image analysis by micro-CT a true 3D thickness can be measured which is model-independent. Local thickness for a point in solid is defined by Hildebrand and Ruegsegger (1997a) as the diameter of the largest sphere which fulfils two conditions:

- the sphere encloses the point (but the point is not necessarily the centre of the sphere);
- the sphere is entirely bounded within the solid surfaces.

The key advantage of the local thickness measurement is that the bias from the 3D orientation of the structure is kept to a minimum (Ulrich et al. 1999).

Distance transform methods described by Remy and Thiel (2002) are the basis for the implementation by CT-analyser of local thickness measurement. The method starts with a “skeletonisation” identifying the medial axes of all structures. Then the “sphere-fitting” local thickness measurement is made for all the voxels lying along this axis.

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 thickness (and separation also) with an interval of two pixels. Thickness distribution is a powerful method for characterising the shape of a complex structure.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Structure linear density	Trabecular number
Parameter symbol	St.Li.Dn	Tb.N
Unit	mm <sup>-1</sup>	mm <sup>-1</sup>

Structure linear density or trabecular number implies the number of traversals across a trabecular or solid structure made per unit length on a random linear path through the VOI.

Again the complexities of model dependence associated with 2D measurements are eliminated by true 3D calculation of St.Li.Dn / Tb.N from 3D micro-CT images. This parameter is measured in CT-analyser in 3D by application of the equation for the parallel plate model (fractional volume/thickness), 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.

Furthermore, another alternative definition of trabecular number, based on 3D measurements of the spacing of trabeculae, is:

$$Tb.N = \frac{1}{(Tb.Th + Tb.Sp)} \quad (3.)$$

Since both Tb.Th and Tb.Sp are measured in 3D by sphere-fitting, this latter equation could be considered a calculation directly from a 3D-measured trabecular spacing.

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

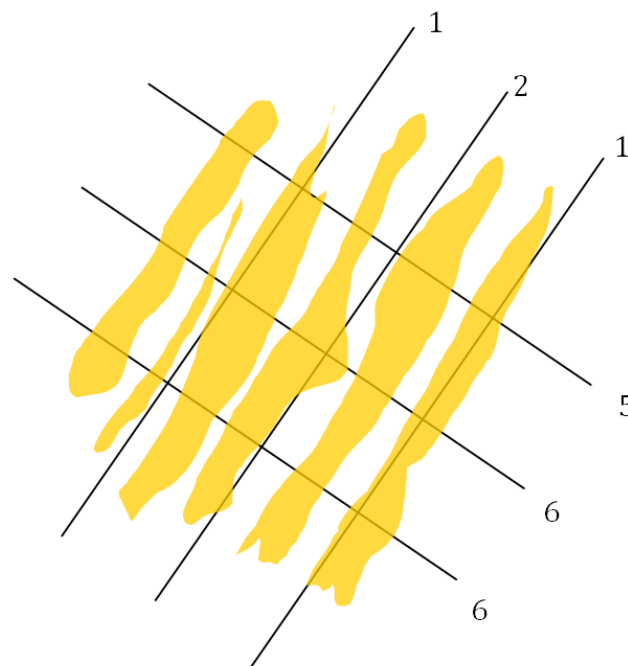
Trabecular separation is essentially the thickness of the spaces as defined by binarisation within the VOI. Skyscan CT-analyser software measures Tb.Sp directly and model-independently in 3D by the same method used to measure trabecular thickness (see above), just applied to the space rather than the solid voxels.

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.

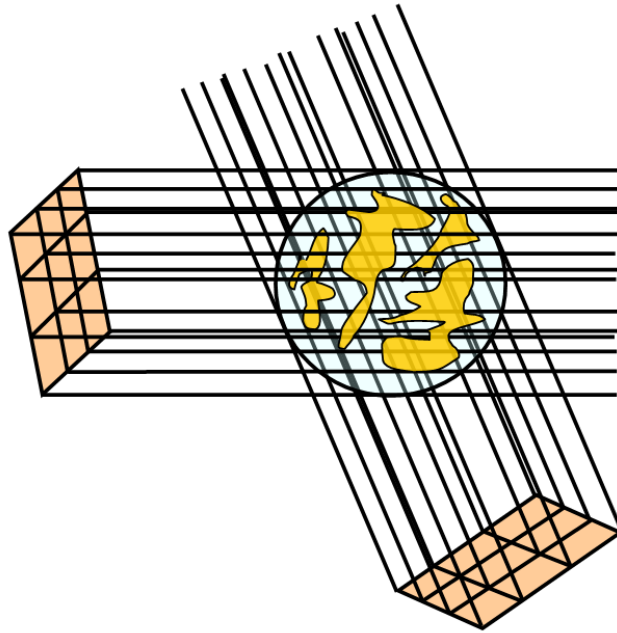
Consider a region or volume containing two phases (solid and space), both having complex architecture, such as a region of trabecular bone. We can study this volume to determine isotropy. If the volume is isotropic, then a line passing through the volume at any 3D orientation will make a similar number 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 test 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 CT-analyser preferences (the “advanced” tab).



**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).

This requires that a spherical region is first defined within which the MIL analysis will be done and anisotropy 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 CT-analyser 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.

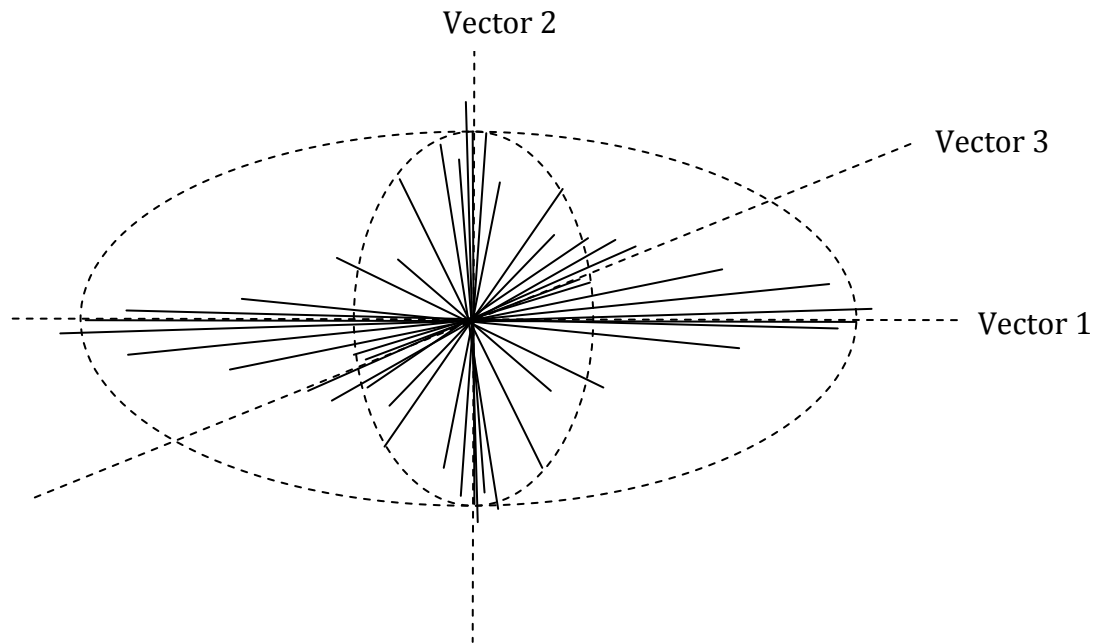


**Figure 2.** For the MIL analysis, a grid of lines is sent through the volume over a large number of 3D angles (just two are illustrated here). The MIL for each angle is calculated as the average for all the lines of the grid.

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 also outputs 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 MIL values (distance between 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:

$$DA = \left( 1 - \left[ \frac{\min \text{ eigenvalue}}{\max \text{ eigenvalue}} \right] \right) \quad (4.)$$

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	Eigenvalues 1, 2, 3	Eigenvalues 1, 2, 3
Parameter symbol	none	none
Unit	none	none

The three eigenvalues are each an index of the relative MIL values (distance between intercepts) in each of the three directions of the three MIL analyses. These three directions are also expressed as the eigenvectors, and are orthogonal to each other.

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 true 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 CT-analyser. For the 3D calculation of FD, the volume is divided into an array of equal cubes, and the number of cubes containing part of the object surface is counted. This is repeated over a range of cube sizes such as 2-100 pixels. The number of cubes containing surface is plotted against cube 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).

For more details on fractal dimension please refer to:

[http://en.wikipedia.org/wiki/Fractal\\_dimension](http://en.wikipedia.org/wiki/Fractal_dimension)

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Number of objects	Number of objects
Parameter symbol	N. Obj.	N. Obj.
Unit	none	none

The total number of discreet binarised objects within the VOI is reported. A discreet 3D object is a connected assemblage of solid (white) voxels fully surrounded on all sides in 3D by space (black) voxels.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Number of Closed Pores	Number of Closed Pores
Parameter symbol	Po.N(cl)	Po.N(cl)
Unit	none	none

The total number of discreet binarised closed pores within the VOI is reported. A closed pore in 3D is a connected assemblage of space (black) voxels that is fully surrounded on all sides in 3D by solid (white) voxels.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Volume of Closed Pores	Volume of Closed Pores
Parameter symbol	Po.V(cl)	Po.V(cl)
Unit	mm <sup>3</sup>	mm <sup>3</sup>

The total volume of all closed pores within the VOI, as defined above (under “number of closed pores”) is reported.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Surface of Closed Pores	Surface of Closed Pores
Parameter symbol	Po.S(cl)	Po.S(cl)
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The total surface area of all closed pores within the VOI, as defined above (under “number of closed pores”) is reported.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Closed Porosity (percent)	Closed Porosity (percent)
Parameter symbol	Po	Po
Unit	%	%

Percent closed porosity is the volume of closed pores (as defined above) as a percent of the total of solid plus closed pore volume, within the VOI. (Please note

– this is a “material porosity”, and is calculated differently from of open porosity and total porosity, where the denominator is total VOI volume.)

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Volume of Open Pore Space	Volume of Open Pore Space
Parameter symbol	Po.V(op)	Po.V(op)
Unit	mm <sup>3</sup>	mm <sup>3</sup>

The total volume of all open pores within the VOI, is reported. An open pore is defined as any space located within a solid object or between solid objects, which has any connection in 3D to the space outside the object or objects.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Open Porosity (percent)	Open Porosity (percent)
Parameter symbol	Po(op)	Po(op)
Unit	%	%

Percent open porosity is the volume of open pores (as defined above) as a percent of the total VOI volume.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Total Volume of Pore Space	Total Volume of Pore Space
Parameter symbol	Po.V(tot)	Po.V(tot)
Unit	mm <sup>3</sup>	mm <sup>3</sup>

The total volume of all open and closed pores within the VOI, is reported.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Total Porosity (percent)	Total Porosity (percent)
Parameter symbol	Po(tot)	Po(tot)
Unit	%	%

Total porosity is the volume of all open plus closed pores (as defined above) as a percent of the total VOI volume.



Nomenclature	General Scientific	Bone ASBMR
Parameter name	Euler number	Euler number
Parameter symbol	EN	EN
Unit	none	none

The calculation of Euler number in 3D is based on software code kindly provided by Erasmus University, Rotterdam, The Netherlands (Dr Erwin Waarsing) using the Conneulor method from Aarhus University, Denmark (Dr. Anders Odgaard; see Gunderson *et al.* 1993).

Note also that up to CTAn version 1.9.3.2 Euler number was calculated in the 2D analysis only (both in the integrated and individual object 2D analyses). From CTAn version 1.9.3.3 onwards Euler connectivity and number are also calculated in 3D, and included in the standard (integrated) 3D analysis.

The Euler-Poincare number –abbreviated to “Euler number” – is 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 or distortion 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:  $\beta_0$  is the number of objects,  $\beta_1$  the connectivity, and  $\beta_2$  the number of enclosed cavities. The Euler-Poincare formula for a 3D object X is given:

$$\chi(X) = \beta_0 - \beta_1 + \beta_2 \quad (5.)$$

Euler analysis provides a measure of connectivity density, indicating the number of redundant connections between trabecular structures per unit volume. Trabecular connectivity can contribute significantly to structure strength (Odgaard 1997). One useful and fast algorithm for calculating the Euler connectivity in 3D is the “Conneulor” (Gunderson *et al.* 1993).

## A2: 3D morphometric parameters calculated for all individual binarised 3D object within the volume of interest (VOI)

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object volume	Bone volume
Parameter symbol	Obj.V	BV
Unit	mm <sup>3</sup>	mm <sup>3</sup>

Total volume of each binarised 3D object within the VOI. The 3D volume measurement is based on the marching cubes volume model.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object surface	Bone surface
Parameter symbol	Obj.S	BS
Unit	mm <sup>2</sup>	mm <sup>2</sup>

Total surface of each binarised 3D object within the VOI. The 3D surface measurement is based on the marching cubes model.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Volume of Pores	Volume of Pores
Parameter symbol	Po.V	Po.V
Unit	mm <sup>3</sup>	mm <sup>3</sup>

The total volume of all pores within each discreet 3D object.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Surface of Pores	Surface of Pores
Parameter symbol	Po.S	Po.S
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The total surface area of all pores within each discreet 3D object.

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

Percent porosity is the volume of closed pores within each discreet 3D object as a percent of the total volume of that object (including any closed pore volume).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Number of Pores	Number of Pores
Parameter symbol	Po.N	Po.N
Unit	none	none

The number of pores within each discreet 3D object.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Centroids X, Y, Z	Centroids X, Y, Z
Parameter symbol	Crd.X, CrdY, CrdZ	Crd.X, CrdY, CrdZ
Unit	mm	mm

The centroid is the XYZ coordinate of the average Cartesian vectorial position of each discreet 3D object, within the VOI.

## **PART B: 2D MORPHOMETRIC PARAMETERS**

### **B1: 2D morphometric parameters calculated “slice-by-slice”, integrated for the whole volume of interest (VOI)**

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Total VOI volume	Tissue volume
Parameter symbol	TV	TV
Unit	mm <sup>3</sup>	mm <sup>3</sup>

Total volume of the volume-of-interest (VOI). The 2D measurement is the total number of voxels of (solid and space) in the VOI times the voxel volume. Please note that in the case of Bone ASBMR nomenclature, the word “tissue” 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 <sup>3</sup>	mm <sup>3</sup>

Total volume of binarised objects within the VOI. The 2D measurement is equal to the number of voxels of binarised solid objects within the VOI times the voxel volume.

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

The percentage of the VOI occupied by binarised solid objects. This parameter is only relevant if the studied volume is fully contained within a well-defined biphasic region of solid and space, such as a trabecular bone region, and does not for example extend into or beyond the boundary of the object – such as the cortical boundary of a bone sample. The meaningfulness of measured percent volume depends on the criteria applied in selecting the volume of interest. Where the ROI / VOI boundaries are loosely drawn in the surrounding space around an object for instance, then % object volume has no meaning.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	VOI surface	Tissue surface
Parameter symbol	TS	TS
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The surface area of the volume of interest, measured in 2D, using the Pratt algorithm (Pratt 1991). Note that there are two components to surface measured in 2D for a 3D multilayer dataset; first the perimeters of the binarised objects on each crosssectional level, and second, the vertical surfaces exposed by pixel differences between adjacent crosssections.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object surface	Bone surface
Parameter symbol	Obj.S	BS
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The surface area of all the solid objects within the VOI, measured in 2D, using the Pratt algorithm (Pratt 1991). Note that there are two components to surface measured in 2D for a 3D multilayer dataset; first the perimeters of the binarised objects on each crosssectional level, and second, the vertical surfaces exposed by pixel differences between adjacent crosssections.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Object surface / volume ratio	Bone surface / volume ratio
Parameter symbol	Obj.S/Obj.V	BS/BV
Unit	mm <sup>-1</sup>	mm <sup>-1</sup>

The ratio of solid surface to volume (both as defined above) measured in 2D within the VOI. Surface to volume ratio or “specific surface” is a useful basic parameter in characterising the complexity of structures.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Mean total crosssectional ROI area	Mean total crosssectional tissue area
Parameter symbol	T.Ar	T.Ar
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The mean of the crosssectional ROI area for all slices in the selected range of the VOI. Note that by using the “shrink-wrap” function, the ROI boundary can become effectively the outer boundary of the object, while not including any internal spaces or structure in the measurement. This allows for example measurement of inner and outer surfaces of porous or hollow objects, such as the periosteal (outer) and endosteal (inner) cortical bone surfaces.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Mean total crosssectional ROI perimeter	Mean total crosssectional tissue perimeter
Parameter symbol	T.Pm	T.Pm
Unit	mm	mm

The mean of the crosssectional ROI perimeter for all slices in the selected range of the VOI. Note that by using the “shrink-wrap” function, the ROI perimeter can become effectively the outer perimeter of the object, while not including any internal spaces or structure in the measurement. This allows for example measurement of inner and outer surfaces of porous or hollow objects.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Mean total crosssectional object area	Mean total crosssectional bone area
Parameter symbol	Obj.Ar	B.Ar
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The mean of the crosssectional object or bone area for all slices in the selected range of the VOI.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Mean total crosssectional object perimeter	Mean total crosssectional bone perimeter
Parameter symbol	Obj.Pm	B.Pm
Unit	mm	mm

The mean of the crosssectional object or bone perimeter for all slices in the selected range of the VOI.

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

The mean number of discreet 2D objects in a single crosssectional image slice. This parameter can be an indicator of structural connectivity – in interconnected structures (e.g. trabecular bone or other networks, scaffolds or foams) high connectivity results in highly interconnected objects which are few in number in a single crosssection – by contrast fragmentation results in larger numbers of smaller objects (e.g. Vermeirsch *et al.* 2004, 2007).

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 <sup>2</sup>	mm <sup>2</sup>

The mean area of discrete 2D objects in a single cross-sectional image slice. This parameter can be an indicator of structural connectivity – in interconnected structures (e.g. trabecular bone or other networks, scaffolds or foams) high connectivity results in highly interconnected objects which are few in number in a single cross-section – by contrast fragmentation results in larger numbers of smaller objects (for application of this parameter to bone morphometry see for example Vermeirsch *et al.* 2004, 2007).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Average object equivalent circle diameter per slice	Average object equivalent circle diameter per slice
Parameter symbol	Av.Obj.ECD	Av.Obj.ECD
Unit	mm	mm

The mean equivalent circle diameter of all discrete 2D objects in a single cross-sectional image slice, defined as the diameter of the equivalent circle having the same area as the measured object.

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 <sup>4</sup>	mm <sup>4</sup>

This 2D cross-sectional function is a basic strength index and indicates the resistance to rotation of a cross-section about a chosen axis (assuming uniform material stress-strain strength properties).

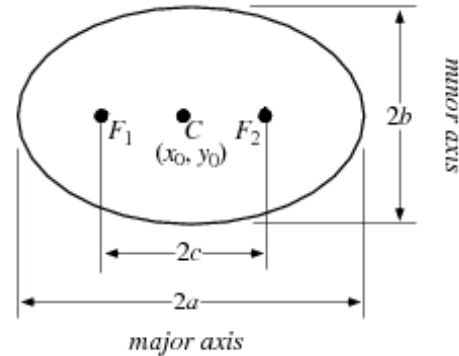
Moment of inertia is the rotational analogue of mass for linear motion and must be specified with respect to a chosen axis of rotation. For a point mass (represented by an image pixel) the moment of inertia (I) is simply the mass (m) times the square of perpendicular distance (r) to the rotation axis,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

For more detail on moment of inertia please refer to:

[http://en.wikipedia.org/wiki/Moment\\_of\\_inertia](http://en.wikipedia.org/wiki/Moment_of_inertia)

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

Eccentricity is a 2D shape analysis of discrete binarised objects. Objects are approximated as ellipses, and eccentricity is an elliptic parameter indicating departure from circular shape by lengthening (a circle is an ellipse with 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 "semi major axis"; likewise, 2b is the minor axis. Eccentricity *e* is a function of the (semi)major and minor axes such that:



$$e = \sqrt{1 - \frac{b^2}{a^2}} \quad (6.)$$

Higher eccentricity means generally elongated objects, while decreased values means an approach toward circular shape.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Crosssectional thickness	Crosssectional thickness
Parameter symbol	Cs.Th	Cs.Th
Unit	mm	mm

Crosssectional thickness is calculated in 2D on the basis of the plate model where  $Th = 2 / (\text{surface}/\text{volume})$ . Surface in 2D integrated "slice-by-slice" analysis is calculated as described above (Obj.S) from both horizontal and vertical pixel boundaries. Crosssectional thickness is a calculation of thickness in which the vertical surfaces, between pixels of adjacent crosssections, are excluded; only the perimeters of crosssections are used to calculate surface. This definition of surface excludes error caused by large artificially cut surfaces that sometimes occur at the top and bottom of VOIs, such as a VOI representing a short section of cortical bone with large exposed crosssections at each end. (By contrast, the "structure thickness" or "trabecular thickness" 2D parameters – below – include surface measured both vertically and horizontally: thus this latter thickness calculation yields smaller thickness values.)



Nomenclature	General Scientific	Bone ASBMR
Parameter name	Structure thickness (plate and rod models)	Trabecular thickness (plate and rod models)
Parameter symbol	St.Th (pl) or (rd)	Tb.Th(pl) or (rd)
Unit	mm	mm

Calculation – or estimation – of St.Th or Tb.Th from 2D measurements requires an assumption about the nature of the structure (a “structural model”). 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:

$$Tb.Th = \frac{2}{(BS / BV)} \quad (7.)$$

Cylinder rod model:

$$Tb.Th = \frac{4}{(BS / BV)} \quad (8.)$$

Sphere model:

$$Tb.Th = \frac{6}{(BS / BV)} \quad (9.)$$

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

Note that for the above equations, if the surface measurements are made from one or a few 2D crosssectional slices (such as in histological analysis), then the numerator (2, 4 or 6) should be divided by a correction factor of 1.199. This approximates a correction from 2D to 3D. However as mentioned above (object surface, Obj.S) the “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.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Structure separation (plate and rod models)	Trabecular separation (plate and rod models)
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 from 2D images with model assumptions. Applying the structural models as for thickness, structure separation is calculated:

Parallel plate model:

$$Tb.Sp = \left( \frac{1}{Tb.N} \right) - Tb.Th \quad (10.)$$

Cylinder rod model:

$$Tb.Sp = Tb.Dm \times \left( \sqrt{\left( \frac{\pi}{4} \right) \times \left( \frac{TV}{BV} \right)} - 1 \right) \quad (11.)$$

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 (or Tb.Dm) value derived from the corresponding plate or rod model.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Structure linear density (plate and rod models)	Trabecular number (plate and rod models)
Parameter symbol	St.Li.Dn(pl)	Tb.N
Unit	mm <sup>-1</sup>	mm <sup>-1</sup>

Structure linear density or trabecular number implies the number of traversals across a solid structure, such as a bone trabecula, made per unit length by a random linear path through the volume of interest (VOI).

Plate and rod model 2D-based definitions of Tb.N again take the corresponding Tb.Th values:

Parallel plate model:

$$Tb.N = \frac{(BV / TV)}{Tb.Th} \quad (12.)$$

Cylinder rod model:

$$Tb.N = \frac{\sqrt{\left(\frac{4}{\pi}\right) \times \left(\frac{BV}{TV}\right)}}{Tb.Dm} \quad (13.)$$

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Fragmentation index	Trabecular bone pattern factor
Parameter symbol	Fr.I	Tb.Pf
Unit	mm <sup>-1</sup>	mm <sup>-1</sup>

This is an inverse index of connectivity, which was developed and defined by Hahn *et al.* (1992) for application to trabecular bone. It was applied by these authors originally to 2D images of trabecular bone, and it 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 by comparing area and perimeter of binarised solid before and after a single voxel image dilation. It is defined:

$$Tb.Pf = \left( \frac{P_1 - P_2}{A_1 - A_2} \right) \quad (14.)$$

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

Structural / trabecular connectedness results in enclosed spaces and concave perimeters, and dilation of such perimeters will contract them, resulting in reduced perimeter. By contrast, open ends or nodes which have convex perimeters will have their surface expanded by surface dilation, thus causing increased perimeter. 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. This parameter is best considered as a relative index for comparing different scanned objects; its absolute value does not have much meaning.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Closed Porosity (percent)	Closed Porosity (percent)
Parameter symbol	Po(cl)	Po(cl)
Unit	%	%

Closed porosity is measured in the 2D slice-by-slice analysis in CT-analyser. Binarised objects are identified containing fully enclosed spaces, and closed 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 closed pores. Closed porosity measurement ignores space which is not fully surrounded by solid. Note – closed porosity in 2D is usually much larger than the equivalent parameter measured in 3D; a space region is more likely to be surrounded by solid in a single crosssectional plane in 2D than in all directions in 3D.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Centroids X, Y, Z	Centroids X, Y, Z
Parameter symbol	CrD.X, CrdY, CrdZ	CrD.X, CrdY, CrdZ
Unit	mm	mm

The centroid is the XYZ coordinate of the average Cartesian vectorial position of all voxels within the VOI.

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 true 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 CT-analyser. For 2D calculation of FD, the crosssection image is divided into an array of equal squares, and the number of squares containing part of the object surface is counted. This is repeated over a range of square sizes such as 2-100 pixels. The number of

squares containing surface is plotted against square 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). Fractal dimension is a useful indicator of complexity in structures.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Intersection surface	Intersection surface
Parameter symbol	i.S	i.S
Unit	mm <sup>2</sup>	mm <sup>2</sup>

Intersection surface is the surface of the ROI intersected by solid binarised objects, that is, the part of the ROI boundary surface that runs through solid objects. This parameter is useful, for example, in evaluating bone growth at a fixed distance away from an orthopaedic bone implant. The intersection surface is calculated for each separate cross-section level, but is also integrated over all VOI levels in the data summary at the head of the 2D analysis report. Note that the intersection surface calculated in 2D does not include vertical (between-cross-section) surfaces, only perimeters of objects within cross-sections. This can make the 2D calculation of i.S. useful for excluding artificial cut surfaces at the top and bottom of the VOI, as was discussed for the parameter cross-sectional thickness.

## B2: 2D morphometric parameters calculated for each individual discreet binarised 2D object within the region of interest (ROI) for the single selected image level only

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Area	Area
Parameter symbol	Ar	Ar
Unit	mm <sup>2</sup>	mm <sup>2</sup>

The area of the individual 2D object, calculated using the Pratt algorithm (Pratt 1991).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Perimeter	Perimeter
Parameter symbol	Pm	Pm
Unit	mm	mm

The perimeter of the individual 2D object, calculated using the Pratt algorithm (Pratt 1991).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Form factor	Form factor
Parameter symbol	FF	FF
Unit	none	none

The form factor of the individual 2D object. FF is calculated by the equation below:

$$FF = \left( \frac{4 \times \pi \times Ar}{Pm^2} \right)$$

Where Ar and Pm are object area and perimeter respectively. Elongation of individual objects results in smaller values of form factor.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Equivalent Circle Diameter	Equivalent Circle Diameter
Parameter symbol	ECD	ECD
Unit	mm	mm

The equivalent circle diameter of a discreet 2D object is defined as the diameter of the circle having the same area as the measured object.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Roundness	Roundness
Parameter symbol	R	R
Unit	none	none

Roundness of a discreet 2D object is defined as:

$$R = \frac{4A}{(\pi \cdot (d_{max})^2)}$$

The value of R ranges from 0 to 1, with 1 signifying a circle.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Euler number	Euler number
Parameter symbol	EN	EN
Unit	none	none

The Euler-Poincare – or the abbreviated “Euler number” – is also an indicator of connectedness of a complex object. The Euler number 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:  $\beta_0$  is the number of objects,  $\beta_1$  the connectivity, and  $\beta_2$  the number of enclosed cavities. The Euler-Poincare formula for a 3D object X is given:

$$\chi(X) = \beta_0 - \beta_1 + \beta_2 \quad (15.)$$

In calculating the Euler number of an individual object,  $\beta_0$  will always be 1. The values of  $\beta_1$  and  $\beta_2$  will therefore determine the single object’s Euler number.

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

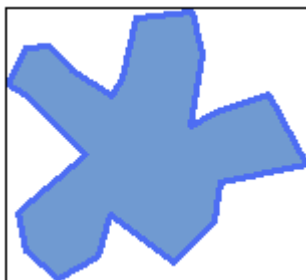
Porosity for individual objects in 2D is defined as closed porosity as described elsewhere – the volume of any spaces fully surrounded in the given crosssectional plane by solid, as a percent of the volume of solid plus closed pores.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Extent	Extent
Parameter symbol	Ext	Ext
Unit	none	none

The parameter “extent” for an object is calculated as:

$$Ext = \frac{area}{width \times height} \quad (16.)$$

Extent is the area of the object as a fraction of the area of the horizontally aligned rectangle bounding the object (see the image below).



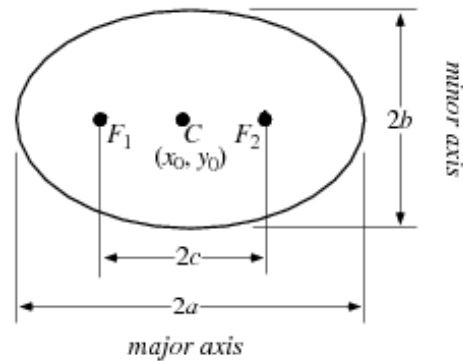
Nomenclature	General Scientific	Bone ASBMR
Parameter name	Orientation	Orientation
Parameter symbol	Or	Or
Unit	degree	degree

The orientation of the major axis of the object, in degrees. Orientation is defined in the upper hemisphere only, with values from 0-180 degrees.



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

Eccentricity is a 2D shape analysis of discrete binarised objects. Objects are approximated as ellipses, and eccentricity is an elliptic parameter indicating departure from circular shape by lengthening (a circle is an ellipse with 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 "semi major axis"; likewise, 2b is the minor axis. Eccentricity *e* is a function of the (semi)major and minor axes such that:



$$e = \sqrt{1 - \frac{b^2}{a^2}} \quad (17.)$$

Higher eccentricity means generally elongated objects, while decreased values means an approach toward circular shape.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Centroids X, Y	Centroids X, Y
Parameter symbol	Crd.X, CrdY	Crd.X, CrdY
Unit	mm	mm

The centroid of an individual object in 2D is the XY coordinate of the average Cartesian vectorial position of all the pixels within the object.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Moment of inertia parameters	Moment of inertia parameters
Parameter symbol	MMI	MMI
Unit	mm <sup>4</sup>	mm <sup>4</sup>

Moment of inertia is a 2D cross-sectional function indicating mechanical strength in terms of the resistance to rotation of a cross-section about a chosen axis (assuming uniform material stress-strain strength properties). For individual 2D objects, the X, Y, polar, product of inertia, minimum and maximum moments of inertia are calculated.

Moment of inertia is the rotational analogue 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 cross-section – for example of cortical bone – is the integral of all the solid (bone) pixels.

Note that the maximum and minimum MMI values are the two orthogonally opposed MMI values for which the product of inertia equals zero.

For more detail on moment of inertia please refer to:

[http://en.wikipedia.org/wiki/Moment\\_of\\_inertia](http://en.wikipedia.org/wiki/Moment_of_inertia)

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Major axis	Major axis
Parameter symbol	Mj	Mj
Unit	mm	mm

The length of the major axis of an approximation of the object as an ellipse. The major axis is two times the elliptical parameter a, the “semi-major axis” already described under eccentricity. For more details on the major axis and the ellipse, please refer to:

[http://en.wikipedia.org/wiki/Major\\_axis](http://en.wikipedia.org/wiki/Major_axis)

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Minor axis	Minor axis
Parameter symbol	Mi	Mi
Unit	mm	mm

The length of the minor axis of an approximation of the object as an ellipse. The minor axis is two times the elliptical parameter b, the “semi-minor axis” already described under eccentricity. For more details on the minor axis and the ellipse, please refer to:

[http://en.wikipedia.org/wiki/Minor\\_axis](http://en.wikipedia.org/wiki/Minor_axis)

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Major diameter	Major diameter
Parameter symbol	d(max)	d(max)
Unit	mm	mm

The major diameter is defined for an individual 2D object as the distance between the two most distant pixels in that object.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Minor diameter	Minor diameter
Parameter symbol	d(min)	d(min)
Unit	mm	mm

The minor diameter is defined for an individual 2D object as longest chord through the object that can be drawn in the direction orthogonal to that of the major diameter (see above).

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Aspect Ratio	Aspect Ratio
Parameter symbol	AR	AR
Unit	none	none

The aspect ratio of a 2D object is defined as the ratio of the major to the minor diameter,  $d(\max)/d(\min)$ , these two parameters defined as stated above.

Nomenclature	General Scientific	Bone ASBMR
Parameter name	Mean Thickness	Mean Thickness
Parameter symbol	Av.Th	Av.Th
Unit	mm	mm

Mean thickness is calculated for an individual 2D object by a 2D implementation of the sphere-fitting local thickness method used for 3D thickness measurement, using circles not spheres.

Please note that several of these definitions of individual 2D objects conform to definitions established by the ASTM (American Society for Testing and Measurement) in the document Designation: F 1877 – 98 (Reapproved 2003) entitled: *“Standard Practice for Characterization of Particles”*.

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