ITA application

Background:
This Inter-Trabecular Angle (ITA) calculation program was developed for the topological analysis of trabecular bone structure (see [Reznikov et al. 2016 Acta Biomaterialia] for full explanation and use).
The input file for the ITA is generated by ImageJ's "Skeleton" plug-in (Plugins>Skeleton), using first the Skeletonize algorithm (http://imagej.net/Skeletonize3D) and then Analyze Skeleton (http://imagej.net/AnalyzeSkeleton).
You must save the output file of Analyze Skeleton, which is called "Branch Information," as a .csv file (manually type ".csv" at the end of the file name). Please save the file(s) you would like to analyze with the end "_BI" (for "Branch Information"). Your file(s) should thus read "samplename_BI.csv". Alternatively, the Branch Information output can be exported as Excel files.
The ITA program processes and prepares the data for the ITA calculation (further information below), and then calculates the angle between each pair of connected trabecular strut (here, struts have been thinned and idealized into straight "edges").
This generates a list of all ITA's, separated into Node-Types. "Node-Type" refers to the number of edges that join at a given node, and thus the convention "3-N," "4-N," "5-N," and so on is used to denote the Node-Type of a given list of angles.
2-N types are not measured because they are generated by curved trabecular struts during skeletonization, and not reflect actual junctions between struts.

Processing:
3D digital tomography data from a bone (i.e. microCT, synchrotron, etc) should be carefully processed, and any thick "non-trabecular" regions (like cortical bone or fusing growth plates) should be excluded from the volume by labelling of the trabecular interior only and then thresholding within the first label. This results in a binary image where trabeculae are white and the background is black. Smoothing or despeckling (Process>Noise>Despeckle) prior to running Skeletonize has been found to reduce error in cases where the thresholded image contains single white pixels not related to the foreground (as a problem of thresholding), but it may disrupt the fabric – the use of denoising functions is thus up to the operator.
Due to inherent computation errors that arise during the Skeletonization algorithm, the following processing is done on the input data before angles are calculated:
- *Self-neighbors* (loops) are deleted
- *Duplicates* of existing edges are deleted
Other forms of processing are done via parameters that the user can input according to their particular data.

Parameters:
When you first run the ITA, you have the option to choose a file or a folder. If you want to run multiple samples as a batch, make sure all of your "_BI.csv" files are within one folder (they can be in subfolders within that one folder). Be aware that too many (>10) Branch Information files may lead to a failure to create a single output file.
(Note: Most of these parameters require a familiarity with your data to use. Please take note of how many voxels long and/or wide, on average, your trabeculae are, and note the dimensions of your sample).

Merge Threshold – This parameter allows you to merge nodes together if the edge between them is small. This is done because the Skeletonization algorithm can place multiple nodes within the same actual node (in the original bone volume). It is best to know the average thickness of the trabeculae in your sample to judge what length an edge can be that is too small to represent a distinct trabecular strut. Any edge with a length below this value will be merged into one node. For example, if at the resolution of your tomogram an average trabecula is 4-5 voxels thick, then we recommend merging the pairs of nodes that are separated by less than 4 voxels. If an average trabecula is 6-7 voxels thick, then set the Merge Threshold to 6. To disable this option make it 0.

Margins – This parameter allows you to choose a 3D margin around your sample that will be ignored in the ITA calculation. Due to cropping at the edges of any sample of continuous trabecular bone, the data there (within the margins) can be problematic. For example, if your average branch length, as calculated by "Analyze Skeleton" is 10 pixels, set your margin to 20.This is useful only if your sample volume is of a regular shape (parallelepiped). If it is of an irregular shape on a black background the Margins parameter doesn't play a role. To disable this option make it 0.

Dead-Ends Threshold – Choose the maximum length for dead-end edges that get deleted (edges with no connections at one end). If a dead end is positioned by the Skeletonize function in the middle of a broad plate-shaped trabecula at an angle to the trabecular axis, that will result in a false node – such a dead end should be deleted. However, if there are real disconnected trabeculae within the sample, you don't want to exclude them as dead ends and thus artificially change the category of the node to which they were connected (removing one edge in a 5-N node will generate a 4-N node, but the remaining angles will be of a 5-N node; that might contaminate the results). As a rule of thumb, do not set the Dead-End Threshold to more than the average branch length in pixels (calculated by Analyze Skeleton). To disable this option make it 0.
Skeleton Size Threshold – This parameter allows you to use more than one skeleton from the ImageJ output, by choosing the minimum number of edges a skeleton must have, in order to be

included in the ITA. The Analyze Skeleton algorithm will count as many skeletons as there are discontinuous voxels in the original volume. An ideally thresholded and skeletonized sample contains a single continuous skeleton. However, this could be not the case if the sample is of irregular shape or some inner areas were digitally excluded. If your volume has two or more separate volumes, there will be multiple skeletons that you want to combine in the ITA calculation. Simply check the Results array (which ImageJ produces along with the Branch Information after Analyze Skeleton is run) for the size of those skeletons (in branches), and set the “skeleton size threshold” low enough to include them. Single branch or single node skeletons should be, of course, excluded, but if there are more than one skeletons of comparable size, use them all based on the size of the smallest one.

The ITA data will be written into an Excel file, and appear in the same folder as your input, with “_ITAdata” at the end. After selecting the output directory type in the output file name that will contain the results.
If you wish to process and look at the results from several samples mark the "*Combine all scans in one xls file*" option. In that case the ITA parameters will be averaged for all the input files.

Bin Width: refers to bin width of the histogram (in degrees), recommended at 4.

Node-Type: node-type for which to make the histograms. We recommend to look at the nodes of 3, 4, 5 and, rarely, 6 edges. The nodes of higher complexity are too sparse in a good sample. They could be abundant in a problematic sample, but then the tomogram processing should be revised. For instance, a node of 40 edges is an unrealistic finding and likely results from an accidentally included fragment of cortical bone, fusing growth plate, orthopaedic screw or a mounting wire.

If using this program, please cite:
Inter-trabecular angle: a new topological parameter of trabecular bone architecture in the human proximal femur. Reznikov N, Chase H, Ben-Zvi Y, Tarle V, Singer S, Brumfeld V, Shahar R, Weiner S. Acta Biomaterialia 2016 http://dx.doi.org/10.1016/j.actbio.2016.08.040