This data relates to the paper: “**Quantifying intracortical bone microstructure: a critical appraisal of 2D and 3D approaches for assessing vascular canals and osteocyte lacunae”.**

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**Experimental CT datasets:**

1\_T.tif, 2\_T.tif, 3\_T.tif, 1\_H.tif, 2\_H.tif, 3\_H.tif

These are tif files, each of which is a CT stack from a different bone sample. The file names are in the format DUCK\_BONE where DUCK is the ID number for one of the three ducks used in the study and BONE is either H for a sample from the midshaft of the humerus and T is a sample from the midshaft of the tibiotarsus.

**Results files:**

“ExperimentalData.xlsx”

This is an Excel file containing the measured radial, longitudinal, laminar, and oblique indices of canal orientation for each of the 6 experimental CT datasets. Each index is estimated using the 3D (skeletonization) method and the 2D (ellipse fit) method.

“EllipsoidMeasurements.xlsx”

This is an Excel file containing the measurements of the test ellipsoid datasets. Each row contains the measurements for a separate test ellipsoid and there are 100 rows (100 ellipsoids) in each test case. Xymajor and xyminor are the lengths of the major and minor axis of an ellipse fitted to the xy cross section of the ellipsoid. Xzmajor and xzminor are the lengths of the major and minor axis of an ellipse fitted to the xz cross section of the ellipsoid. The 3D major, minor, and int measures are the major, minor, and intermediate axis lengths for an ellipsoid fitted to the test ellipsoid shape. 3D volume is the number of voxels within the test ellipsoid shape. 2D volume one axis is the volume of the ellipsoid calculated using only the xy major and minor axes. 2D volume two axes is the volume calculated using the xy and xz axes as detailed in the paper. Different test cases are in separate sheets within the document, e.g. LIMI0.33 = limited rotation angle case and voxel size 0.33.

“CylMeasurements.xlsx”

This is an Excel file containing the measurements for phi and theta from the test cylinder (vascular canal) datasets, based on 2D ellipse fitting to an xy cross section and 3D skeletonization as detailed in the paper. Each row contains the measurements for a separate test cylinder and there are 100 rows (100 cylinders) in each test case. s is the skew (how much the cylinder was deformed laterally). The results for the different test cases (different skews) are on different sheets in the document, labelled by the value of the skew applied.

**Analysis of experimental datasets:**

“SegmentationCellAnalysis\_experimental.ijm”

This is an ImageJ macro which applies a segmentation and basic analysis script to all image stacks in a directory chosen by the user and saves the resulting segmented dataset, mask used to exclude regions outside the bone area, and analysis results.

“identifyMainBoneAxis.ijm”

This is an ImageJ macro which identifies the main axis of a bone sample in an image stack (assuming it is largely oriented with the transverse section parallel to the xy plane as seen in an image i.e. each image in the stack is an approximate transverse bone section). This is used in the “calculateCylAngles\_experimental” script to correct canal orientations to be relative to this main axis. The macro asks the user to choose an image stack, opens the first and last image in the stack, then asks the user to select points around the edge of a bone surface in order to fit a circle to the bone surface in the first image. Then, the macro moves to the last image and asks the user to move the circle so that it best fits the new bone surface. The properties of the two circles are read into a window called “overlay elements” which the user is then prompted to save manually.

“measureCanalOrientation\_experimental.ijm”

This is an ImageJ macro for application to a segmented bone image (background in black, vascular canals and osteocyte lacunae in white, generated using “SegmentationCell\_Analysis”). It firsts crops the image to the bounds of the data to reduce processing time then runs a particle analysis filtering by minimum size to exclude osteocyte lacunae. A Gaussian blur function is applied to the resulting canal system to smooth the data then a threshold applied to convert back to a binary image. Then, the mid slice of the stack is duplicated and a 2D particle analysis run to fit ellipses to the cross sections of the vascular canals. The results are saved. The 3D stack is then skeletonized, and the skeleton analysed. The results of this analysis (including the end points of each branch of the skeleton) are saved.

“calculateCylAngles\_experimental.ijm”

This is a Python script which reads in the results of the 2D particle analysis and 3D skeleton analysis and calculates an angle of theta and phi for each ellipse or skeleton branch. The angle is adjusted using the midline identified using “identifyMainBoneAxis.ijm”.

“calculateAngleIndices.py”

This is a Python script that reads in the theta and phi values for each shape from “calculateCylAngles\_experimental.ijm” and calculates a longitudinal, radial, and laminar index for the dataset.

**Generation of test datasets**

“createCylMask.py”

This is a Python script which is run in the Python window in Simpleware ScanIP. The script generates a set of n .raw files measuring 500x500x500 voxels, each containing a cylinder of length 400 and diameter 10. Each cylinder has a random value for rotation around the x, y, and z axes. There is also the potential to skew the cross section of the cylinders.

“createSphMask.py”

This is a Python script for generation of ellipsoids which is run in Simpleware ScanIP.

**Analysis of test datasets:**

The following ImageJ macros apply the same processes as their equivalents containing \_experimental but modified to be applied to populations of .RAW files as produced by the shape generation files:

“measureCanalOrientation\_tests.ijm”

“calculateCylAngles\_tests.ijm”

“SegmentationCellAnalysis\_tests.ijm”