**A Sectioning, Coring, and Image Processing Guide for High-Throughput Cortical Bone Sample Procurement and Analysis for Synchrotron Micro-CT**

***Supplementary Materials***

**CTAnalyser Tasklist**

The following is a CTAnalyser task list created in the Custom Processing menu with the settings used to create the images seen in **Figure 9** and the porosity data shown in **Table 1**.

**To collect data on vascular canals**:

1. Reload the dataset
2. Filter the dataset with a **Gaussian blur** in **3D space** using a **Round** kernel with a radius of 2.
3. Threshold: Apply a **Global** threshold from 0-120.

**NOTE:** This is a guideline only. Threshold values should be adjusted for each experimental set-up.

1. Despeckle: **Remove white speckles** in **3D space** within the range 13 - 2743 voxels. Apply this step to the image.
   1. This removes all osteocyte lacunae from the image. These values are specific to a 0.9 µm voxel image.
2. Despeckle: **Remove black speckles** in **2D space** less than 15,000 pixels. Apply this step to the image.
3. Morphological Operations: **Dilate** in **3D space** with a radius of 10. Apply this step to the image.
4. Repeat step 5 but replace 15,000 pixels with 100,000 pixels.
5. Morphological Operations: **Erode** in **3D space** with a radius of 10. Apply this step to the image (This is the inverse of Step 6).
6. Perform a **3D analysis** to gather the **Basic values** as well **as trabecular separation**, **trabecular thickness**, and **number of objects**.
7. Use **save bitmaps** to save the processed images in a custom subfolder.
8. Perform **Individual object analysis** to gather data specific to each canal.
9. **Bitwise operations:** Clipboard = Region of Interest SUB Image
   1. This reads as “Clipboard equals the region of interest without the currently seen image” for each reconstructed slice of the dataset.
   2. This will allow for easy removal of the vascular canals in the following steps.

**To collect data on osteocyte lacunae**:

1. Repeat steps 1-3.
2. Despeckle: **Remove white speckles** in **3D space** less than 13 voxels. Apply this step to the image.
   1. This removes noise from the scan that may be mistakenly counted as osteocyte lacunae.
3. Despeckle: **Remove white speckles** in **3D space** greater than 2743 voxels. Apply this step to the image.
4. **Bitwise operations:** Image = Clipboard AND Image
   1. This step removes any leftover traces of the vascular canals that weren’t removed in the previous steps, leaving only the osteocyte lacunae.
5. Perform **3D analysis** on the osteocyte lacunae using the same settings as Step 9.
6. Use **save bitmaps** to save the processed images in a custom subfolder.
7. Perform **Individual object analysis** to gather data specific to each lacuna.

**Experiment Settings Employed to Achieve Representative Results**

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| **Table S1. February 2015 Imaging Parameters (Figs. 7A, C, E and 8A-C)** |  |
| Canadian Light Source beamline utilized | BMIT-ID |
| Beam energy | 31 keV |
| Pre-scan flat-field projections acquired | 10 images |
| Pre-scan dark-field projections acquired | 10 images |
| Frame averaging | 4 frames |
| Rotation step | 0.25° |
| Binning | 2x2 |
| Number of projections per scan | 720 images |
| Total scan rotation | 180° |
| Post-alignment scan rotation step | 15° |
| Post-scan flat field projections acquired | 10 images at 18° |
| Scan time | 75 minutes |
| Voxel size | 1.8 µm |

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| **Table S2. June 2018 Imaging Parameters (Figs. 7B, D, F and 8D-F)** |  |
| Canadian Light Source beamline utilized | BMIT-ID |
| Beam energy | 30 keV |
| Pre-scan flat-field projections acquired | 10 images |
| Pre-scan dark-field projections acquired | 10 images |
| Frame averaging | 5 frames |
| Rotation step | 0.20° |
| Binning | N/A |
| Number of projections per scan | 900 images |
| Total scan rotation | 180° |
| Post-alignment scan rotation step | N/A |
| Post-scan flat field projections acquired | N/A |
| Scan time | 85 minutes |
| Voxel size | 0.9 µm |