

## §MouseTibia Service

# Evaluation of bone densitometric and mechanical properties for preclinical applications

### Scientific Background

The mouse tibia is a common site for studying bone remodelling and the effect of bone interventions. *In vivo* micro-Computed Tomography (microCT) imaging is considered the gold standard for bone imaging and can be applied to measure bone changes over time (Bouxsein et al., 2010). This approach was found to not affect the measurements of bone properties (Oliviero et al., 2019) and dramatically reduce the number of mice needed for studying the effect of diseases (Viceconti and Dall'Ara, 2019). Standard analyses of morphometric parameters are usually performed in two regions of interest (typically 1 mm in length) located at the proximal tibia (trabecular bone) and at the diaphysis (cortical bone). Trabecular morphometric parameters include trabecular bone volume fraction (Tb.BV/TV, [%]), thickness (Tb.Th, [ $\mu\text{m}$ ]), separation (Tb.Sp, [ $\mu\text{m}$ ]) and number (Tb.N, [ $1/\text{mm}$ ]). Standard cortical morphometric parameters include total cross-sectional area (Tt.Ar, [ $\text{mm}^2$ ]), cortical bone area (Ct.Ar, [ $\text{mm}^2$ ]), cortical area fraction (Ct.Ar/Tt.Ar, [ $\text{mm}^2/\text{mm}^2$ ]) and cortical thickness (Ct.Th, [ $\mu\text{m}$ ]). However, morphometric parameters only provide information about local regions of interest in the tibia.

While these parameters are important to evaluate the effect of interventions on the trabecular and cortical bone, they are not well correlated with the mechanical properties of the mouse tibia (Oliviero et al., 2020). Densitometric properties of the mouse tibia across space and time and mechanical properties of the tibia from longitudinal microCT imaging can provide a complementary assessment of the effect of diseases and treatments in preclinical mouse studies.

### Densitometric analysis

Recently a method has been proposed to analyse the whole tibia volume (Lu et al., 2016, Lu et al., 2017), which provides a comprehensive analysis of the whole bone, complementary to the standard morphometric parameters. This method, combined with longitudinal imaging, enables the assessment of the spatio-temporal distribution of the bone densitometric properties.

After rigid registration of the images acquired from different animals or from the same animal at different time points, the attenuation coefficients acquired in the microCT images are converted into tissue mineral density (TMD) using a calibration law based on weekly scans of a densitometric phantom with five insertions. Bone Mineral Content (BMC) in each voxel is calculated as its TMD multiplied by the volume of the voxel. A volume of interest (VOI) is selected below the growth plate, including 80% of the total length and excluding the fibula. Total BMC, TMD and BV/TV are computed in the VOI. The VOI is also divided into ten longitudinal sections (Section1 located at the proximal tibia, Section10 at the distal tibia). For each longitudinal section, the following parameters are measured: bone mineral content (BMC, [mg]), tissue mineral density (TMD, [mgHA/cc]), total bone volume fraction

(BV/TV, [%], including both trabecular and cortical bone), total cross-sectional area (TA, [mm<sup>2</sup>]), bone area (BA, [mm<sup>2</sup>]), second moment of area in the antero-posterior direction ( $I_{xx}$ , [mm<sup>4</sup>]) and in the medio-lateral direction ( $I_{yy}$ , [mm<sup>4</sup>]), polar moment of inertia ( $I_{zz}$ , [mm<sup>4</sup>]). Lastly, each longitudinal section is divided into four quadrants (anterior, posterior, medial and lateral), defined for each cross-section by two perpendicular lines containing its centroid (40 partitions in total), and BMC, TMD and BV/TV are calculated for each partition.

Measurements of the densitometric properties of the mouse tibia obtained with this approach have been proven more repeatable than standard morphometric parameters (Lu et al., 2016) and have been applied to study the effect of bone anabolic interventions such as ovariectomy (Roberts et al., 2019), parathyroid hormone (Lu et al., 2017, Roberts et al., 2020), and passive mechanical loading (alone or in combination with PTH) (Roberts et al., 2020).

### **microFE analysis**

Structural mechanical properties of the tibia and their changes over time are an important endpoint for clinical translation (Viceconti and Dall'Ara, 2019). Micro-Finite Element (microFE) models based on microCT images can be used to estimate the tibia mechanical properties in compression, after proper validation. MicroFE model predictions have been validated against experimental measurements of local displacements obtained with Digital Volume Correlation ( $R^2 > 0.82$  between experimental and predicted local displacements; mean errors for predicted bone strength below 9%) (Oliviero et al., 2018) and experimental measurements of structural properties (mean errors in absolute or normalised strength below 9%) (Oliviero et al., 2020).

The microCT image of the tibia is segmented using a global threshold, calculated as the average of the grey values corresponding to the bone and background peaks in the histogram of the image. A connectivity filter is applied to remove unconnected voxels. A Cartesian mesh is obtained by converting each bone voxel into an 8-noded hexahedral element with isotropic linear elastic material properties ( $E=14.8$  GPa,  $\nu=0.3$ ). Uniaxial compression is simulated by fully constraining the distal surface of the tibia and applying a uniform displacement at the proximal surface. Stiffness ([N/mm]) is calculated as the sum of the reaction forces at the distal end, divided by the applied displacement. Failure load ([N]) is calculated by assuming that the tibia fails when 10% of the nodes reach a critical strain (third principal strain) of  $-14420 \mu\epsilon$  (Oliviero et al., 2020).

### **The MouseTibia Service**

The MouseTibia Service estimates densitometric and mechanical properties from a microCT image of a mouse tibia.

A job is created by clicking on 'New Job'. This will generate a form in which a microCT image can be selected for the specimen to be analysed. Files can be selected either from Google Drive or your Desktop. A Sheffield University Google account is required to access this service. When you use the Google Drive file picker for the first time this website will ask your permission to add the MouseTibia app to your Google Drive account. You can remove this app at any time in Google Drive by going to:

Settings > Managing Apps > Options > Disconnect from Drive

The user needs to upload a 3D microCT scan as a stack of 2D DICOM images, saved in a compressed .zip folder. Our recommended scan protocol can be found at 'Mouse Tibia Recommended Scan Protocol.docx'. Voxel size and calibration parameters for converting grey levels into TMD are read from the header of the DICOM files. Alternatively, the user can manually input the parameters values in the form, which will overwrite those present in the header. These parameters represent:

- Voxel size [mm]
- Rescale intercept: parameter 0028\_1052 in standard Scanco header
- Rescale slope: parameter 0028\_1053 in standard Scanco header
- Scale factor: parameter 0029\_1000 in standard Scanco header
- Slope of the calibration curve: parameter 0029\_1004 in standard Scanco header
- Intercept of the calibration curve: parameter 0029\_1005 in standard Scanco header

Once the data have been properly uploaded, an expert operator will process them. The operator will align each image to a reference system. The longitudinal axis of the tibia is aligned to the z axis of the global reference system, the antero-posterior direction is aligned to the x axis and the medio-lateral direction to the y axis. This operation is done with a rigid registration operation, using an aligned tibia as reference. The service also offers the opportunity to upload multiple longitudinal scans of the same bone over time. In this case, the first scan will be used as reference for the alignment of the images acquired at subsequent time points. Lastly, the operator selects a volume of interest (VOI) including the 80% of the total length, starting below the proximal growth plate and excluding the fibula.

The user will receive the outputs in a Google Drive folder. For each tibia the following result files are provided:

- **Histogram.tif**: Histogram (frequency plots) of TMD distribution.
- **BMC\_analysis.xlsx**: Excel file with computed densitometric parameters.
- **FE\_analysis.xlsx**: Excel file with estimated structural properties.
- Ten images of binary cross-sections (corresponding to the longitudinal sections, where Section1 corresponds to the proximal tibia, Section10 to the distal tibia).
- For each cross-section, four binary images representing the four sectors (anterior, posterior, medial, lateral).
- Three views of the spatial distribution of first principal strain.
- **Histogram\_first.tif**: Histogram (frequency plots) of first principal strain.
- Three views of the spatial distribution of third principal strain.
- **Histogram\_third.tif**: Histogram (frequency plots) of third principal strain.
- Report in PDF format with a summary of the results (see example in the documents).

## References:

BOUXSEIN, M. L., BOYD, S. K., CHRISTIANSEN, B. A., GULDBERG, R. E., JEPSEN, K. J. & MÜLLER, R. 2010. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *Journal of Bone and Mineral Research*, 25, 1468-1486.

- LU, Y., BOUDIFFA, M., DALL'ARA, E., BELLANTUONO, I. & VICECONTI, M. 2016. Development of a protocol to quantify local bone adaptation over space and time: Quantification of reproducibility. *Journal of Biomechanics*, 2095 - 2099.
- LU, Y., BOUDIFFA, M., DALL'ARA, E., LIU, Y., BELLANTUONO, I. & VICECONTI, M. 2017. Longitudinal effects of Parathyroid Hormone treatment on morphological, densitometric and mechanical properties of mouse tibia. *Journal of the Mechanical Behavior of Biomedical Materials*, 75, 244-251.
- OLIVIERO, S., GIORGI, M. & DALL'ARA, E. 2018. Validation of finite element models of the mouse tibia using digital volume correlation. *Journal of the Mechanical Behavior of Biomedical Materials*, 86, 172-184.
- OLIVIERO, S., OWEN, R., REILLY, G. C., BELLANTUONO, I. & DALL'ARA, E. 2020. Optimization of the failure criterion in micro-Finite Element models of the mouse tibia for the non-invasive prediction of its failure load in preclinical applications. *Journal of the Mechanical Behavior of Biomedical Materials*, 104190.
- ROBERTS, B. C., ARREDONDO CARRERA, H. M., ZANJANI-POUR, S., BOUDIFFA, M., WANG, N., GARTLAND, A. & DALL'ARA, E. 2020. PTH(1-34) treatment and/or mechanical loading have different osteogenic effects on the trabecular and cortical bone in the ovariectomized C57BL/6 mouse. *Scientific Reports*, 10, 8889.
- ROBERTS, B. C., GIORGI, M., OLIVIERO, S., WANG, N., BOUDIFFA, M. & DALL'ARA, E. 2019. The longitudinal effects of ovariectomy on the morphometric, densitometric and mechanical properties in the murine tibia: A comparison between two mouse strains. *Bone*, 127, 260-270.
- VICECONTI, M. & DALL'ARA, E. 2019. From bed to bench: How in silico medicine can help ageing research. *Mechanisms of Ageing and Development*, 177, 103-108.