1. FINAL PUBLISHABLE SUMMARY REPORT

Bone is a complex heterogeneous, anisotropic and hierarchical material where the separation of scales does not hold, i.e. in order to properly understand how it fails and why it fails, we need to study it at different dimensional scales. For each dimensional scale (e.g. organ level: whole femur, tissue level: portion of trabecular or cortical bone) we can generate computational models for a specific sample, given its geometry, material properties and conditions in which it is loaded are provided. The models can predict non-destructively a number of mechanical properties that would not be measurable in patients.

However, in order to understand how much we can trust the outputs of the models we need first to compare their outcomes with well-controlled experiments in the laboratory (i.e. performing a validation study). Unfortunately, at the tissue level (samples of cortical or trabecular bone a few mm large) the designing of experimental validation tests becomes very challenging due to the complexity of the micro-structures and to the lack of proper techniques to measure the 3D field of such small quantities (e.g. local displacements in the order of some μm and deformations in the order of some hundreds με). The goal of this project was to create an advanced validation framework for evaluating the performances of computational models of bone generated at the tissue level. The challenges were of experimental, imaging and computational nature.

In order to generate and validate computational models of complex material such as bone, we started from a careful preparation of the biological tissues. We decided to define all the procedures on animal bone and in the future the project will be extended to human tissue. Bone samples from a bovine femur were extracted from both cortical (compact) and trabecular (porous) compartments by using state of the art diamond tools. In order to validate the computational models we developed a proper set of experiments for measuring the 3D field of displacements and deformations applied to the sample with a custom made prototype-loading stage.

This jig was designed and manufactured at the University of Sheffield and allowed to properly position the samples within a high resolution microCT system and to perform stepwise compression tests inside the scanner, for both cortical (3mm in diameter) and trabecular (8mm in diameter) bone cylinders (Figure 1). The stepwise loading was possible with a screw-ball joint mechanism and the measurement of the axial load was performed with a 2kN load cell, the output of which was read by an external acquisition system. The jig was also equipped with a liquid cell in order to keep the sample in saline solution during the test, and therefore avoiding dehydration during the scan. A scanning procedure was developed in order to obtain, at each load step, a high-resolution (10μm voxel size) 3D scan of the whole sample in less than 30 minutes (Figure 2) . Proper image processing protocols were developed in order to segment the images and evaluate the morphological and densitometric properties for each sample. The 3D images of the undeformed configuration and of the loaded sample in each step were registered by means of a Digital Volume Correlation (DVC) technique, which allows to find the transformation that maps in every point the undeformed configuration to the deformed one. This novel approach allows to estimate displacement and strain field that the sample undergoes during the testing, providing a great potential for validation of computational models for local properties. This is fundamental if we want to use the models to relate the local deformations of the different bone structural units (e.g. trabeculae and osteons) to the cell activity. During the project we adapted a DVC technique already available at the University of Sheffield (the Sheffield Image Registration Toolkit, ShIRT) originally developed for analyses of soft tissues. We adapted it for the estimation of the displacement for compressed bone samples and we combined it with a commercial Finite Element code in order to convert the displacement field into a strain field. While this method has high potential in a number of engineering applications, we needed to estimate the errors associated with its outputs. Only after that we can use the DVC approach for experimental validation of computational models. In fact, we performed a rigorous study for the determination of its accuracy and precision. Usually in this type of analyses a more accurate and precise method is used in order to estimate the errors of the new approach. However, due to the novelty of the method, we could not compare its outputs with a more precise measurement tool and, therefore, we evaluated the errors related to displacement and strain measurements in a specific case of zero-strain. From repeated scans of cortical and trabecular bone samples we found that there was a power relationship between the accuracy of the method and its spatial resolution (Dall'Ara et al, *Journal of Biomechanics*, 2014). Based on this data we selected the parameters to use in the DVC algorithm and we demonstrated that our method outperform the only commercial software available in the market (Palanca et al., *Journal of Biomechanical Engineering*, 2015), if measurements were performed on the same set of samples. We computed with DVC both local displacement for each load-step and the failed area in the sample.

Figure 1: prototype jig

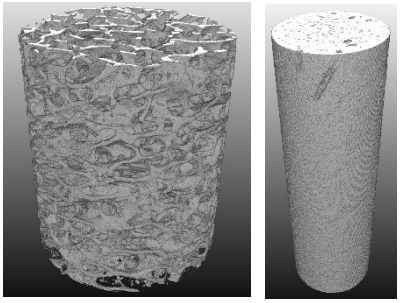

Figure 2: high resolution scans of trabecular and cortical bone samples

Once the accuracy of the method was measured, we focussed on the generation of the computational models for cortical and of trabecular bone. In this study we used linear micro Finite Element (microFE) models, where each voxel of the microCT image is converted into a finite element. This approach can properly represent the bone microarchitecture but,

due to the large number of degrees of freedom (several millions), we needed to solve the models with a parallel computing approach (supercomputer). The best mesh size was obtained from a verification study performed on trabecular (Chen et al., *Journal of Biomechanical Engineering*, 2014) and cortical bone samples. For both microstructures we defined procedures to generate homogeneous (Figure 3) and heterogeneous models.

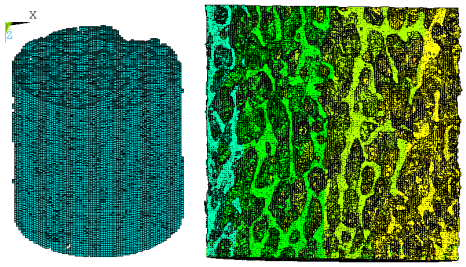

Figure 3: homogeneous microFE model of trabecular bone

The bone elements of the homogeneous models were separated from the background according to their microCT attenuation values and the same material properties were assigned from the literature in case of trabecular bone. We have also performed a sub-study for the investigation of the local material properties of cortical (plexiform) bone tissue by means of micro-indentation tests (Dall'Ara et al., submitted to *Journal of Biomechanics*). For the heterogeneous models we assigned different material properties for each element, by defining a calibration procedure to convert the grey-scale into tissue mineral content that was then converted into mechanical properties based on phenomenological laws from the literature. Finally, we developed a procedure to compare the predicted local properties from the microFE and the measured experimental properties with the DVC experiments. We run the whole procedure on trabecular bone samples for homogeneous models and we showed that the models are able to predict excellently the local displacements only if the proper boundary conditions are assigned (i.e. the ones computed from the experimental DVC results, Figure 4) (Chen et al., talk at *Congress of European Society of Biomechanics*, July2015). The project is actually still running with additional funding from the host organization for the comparison of predicted and measured strain and by extending the validation to heterogeneous models. The procedures will be then replicated for human tissue.

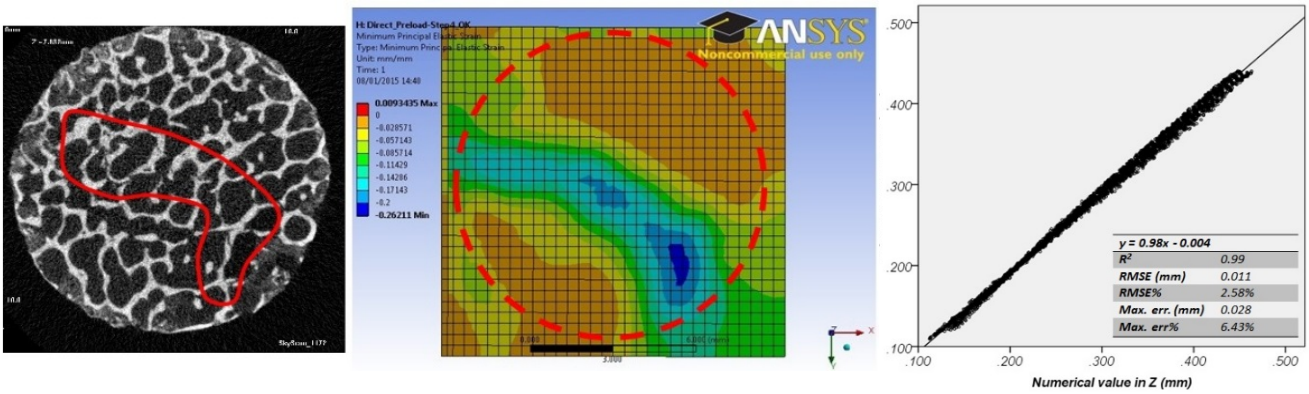

Figure 4: Comparison between failed area of trabeculae from the microCT images (left) and high strain area measured with the DVC approach (middle). On the right the comparison between the displacements measured with the DVC and predicted with the microFE is reported for the first step of load (elastic range).

This project was a fundamental step forward for the definition of a framework of validation experiments for computational models of bone at the tissue level. On the experimental side, we have developed the most accurate and precise method for investigation of local displacements and strain for high-resolution microCT images of trabecular bone. The researcher has already won scanning time at the British Synchrotron facility where he performed super-high resolution images of bone tissue that will allow him to understand the potential of the DVC approaches for optimal images. The experimental approach is currently being adapted for studying the deformation of small rodent bones (mice and rats) for preclinical studies and for human bones at the organ level for clinical oriented questions. Moreover, a pilot study has been started in order to test human trabecular bone tissue extracted from the femoral head of patients who underwent a total hip replacement in order to investigate with this novel approach the failure behaviour of the subchondral bone of osteoarthritic patients. The results will potentially help in the development of novel interventions for osteoarthritis. On the modelling side, we have shown the ability of microFE in predicting the proper local displacements and we will explore the best modelling strategy for predicting the measured local deformations. The best method will be then used in order to study the effect of bone pathologies (such as osteoporosis, osteoarthritis and *osteogenesis imperfecta*) and ageing to the mechanical properties of human bone tissues. The validation study will be extended, with a similar fashion, to the organ level (e. g. whole vertebral body) in order to obtain reliable clinical tools for the prediction of bone local deformations and at the same time to link the organ and tissue scales in the multiscale framework.

To conclude, this project provided a reference procedure and a set of experimental measurements against which every research working worldwide on the computational modelling of bone tissue will have to be measured against. This is the most important step to guarantee the proper credibility to novel models that aim to predict the relationship between bone remodelling and local bone deformations or to estimate how changes in the tissue density, morphology and composition will translate into changes of strength in the bone tissue, and therefore, in the whole bone, and thus an increased risk of bone fracture.