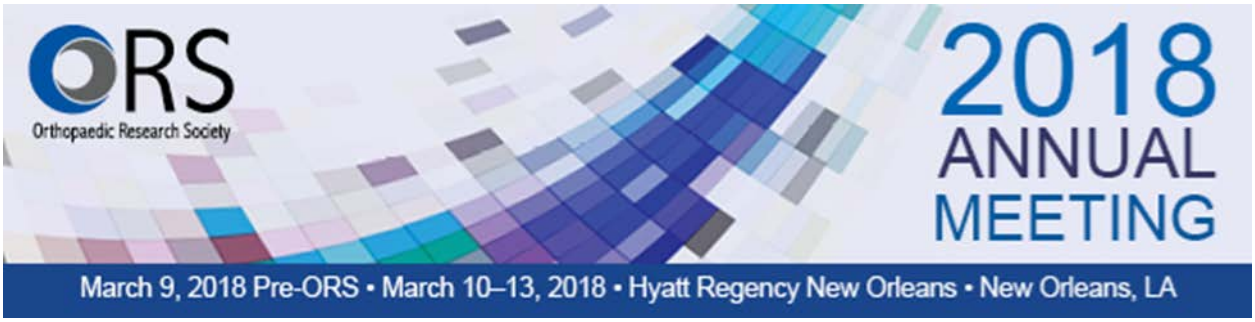


WORKSHOP
In vivo MicroCT Imaging: Longitudinal Assessment of Skeletal Microstructure, Strength, and (Re)modeling Dynamics

Organizers:
Bettina M. Willie, PhD
X. Sherry Liu, PhD

Speakers:
Ralph Müller, PhD
Steven Boyd, PhD
Enrico Dall'Ara, PhD

Preclinical in vivo microCT imaging

Ralph Müller, PhD
Professor of Biomechanics, Institute for Biomechanics, ETH Zurich, Zurich, Switzerland

With recent advances in molecular and regenerative medicine, there is a strong need for longitudinal imaging of skeletal (re)modeling dynamics at the tissue and even cellular level. A number of new in vivo microstructural imaging modalities have been put forward recently allowing quantification with high precision and accuracy of these structures in a time-lapsed fashion in live animals. Although biomedical imaging technology is now readily available, few attempts have been made to expand the capabilities of these systems by adding quantitative analysis tools to assess transient structure-function relationships of bone (re)modeling dynamics in a time-lapsed fashion. In the spirit of 3R, such longitudinal in vivo animal study designs not only allow to refine the methods used to measure biological in vivo function but also to directly reduce the number of animals needed for these studies, avoiding unnecessary cross-sectional studies. Using time-lapsed vivo imaging, each animal can serve as its own reference with respect to the changes observed at each time point. The aim of this contribution is to present recent developments in time-lapsed in vivo computed tomography (CT) of bone (re)modeling dynamics in applications of molecular and regenerative medicine.

X-ray-based CT is an approach to image bone in a hierarchical fashion providing multiscale imaging capabilities with isotropic resolutions ranging from a few millimeters (clinical CT), to a few micrometers (micro-CT) down to one hundred nanometers (nano-CT). A number of groups working in this field have demonstrated over the last two decades that X-ray-based tomographic imaging is a nondestructive and noninvasive procedure that allows precise 3D measurement of bone microstructure on all levels of hierarchy. The technique has been used predominantly in vitro but recently in vivo applications have gained more and more interest due the unprecedented resolutions in the order of 10 µm available in these in vivo systems. Due to the time-lapsed nature of the images, not only static but also dynamic morphometry can be performed to assess bone (re)modeling dynamics. With the recent introduction of computational tools that allow calculation of the mechanical microenvironment in these tissues, links between mechanical cues acting on ensemble of cells or even individual cells and the corresponding tissue adaptation and regeneration can now be monitored non-destructively in individual animals in vivo at relatively moderate cost and great ease of use.

Time-lapsed in vivo microstructural imaging allows longitudinal quantification of bone (re)modeling dynamics, thereby reducing the number of animals needed to show significant results. It is strongly recommended that quantitative imaging is used more often for in vivo animal studies in the area of molecular and regenerative medicine.

References:

- S. K. Boyd, P. Davison, R. Müller and J. A. Gasser. Monitoring individual morphological changes over time in ovariectomized rats by in vivo micro-computed tomography. Bone, 39:854-862, 2006.
- F. A. Schulte, F. M. Lambers, G. Kuhn and R. Müller. In vivo micro-computed tomography allows direct three-dimensional quantification of both bone formation and bone resorption parameters using time-lapsed imaging. Bone, 48:433-442, 2011.
- A. I. Birkhold, H. Razi, R. Weinkamer, G. N. Duda, S. Checa, B. M. Willie. Monitoring in vivo (re)modeling: a computational approach using 4D microCT data to quantify bone surface movements. Bone, 75:210-221, 2015.
- C. M. de Bakker, A. R. Altman, C. Li, M. B. Tribble, C. Lott, W. J. Tseng, X. S. Liu. Minimizing Interpolation Bias and Precision Error in In Vivo µCT-Based Measurements of Bone Structure and Dynamics. Ann. Biomed. Eng., 44(8):2518-2528, 2016.
- P. Christen and R. Müller. In vivo visualisation and quantification of bone resorption and bone formation from time-lapse imaging. Curr. Osteoporos. Rep., 5:311-317, 2017.

Clinical in vivo microCT imaging

Steven Boyd, PhD
Department of Radiology, Cumming School of Medicine, University of Calgary, Canada
McCaig Institute for Bone and Joint Health, *mccaig.ucalgary.ca*

Osteoporosis is a disorder characterized by a decrease in the bone mass and/or quality that causes reduced strength and increases the risk of a fragility fracture. It represents a major public health problem in an aging society, and produces a highly significant financial burden on health care systems. Bone mineral density (BMD) as provided by dual-energy X-ray absorptiometry (DXA) provides important information about bone quantity, but not the underlying microarchitecture. The introduction of high-resolution peripheral quantitative computed tomography (HR-pQCT) allows us to non-invasively explore the relation between human bone microarchitecture, BMD and strength. Since the early 2000's, there has been an exponential increase in the use of HR-pQCT to understand the role of bone microarchitecture in bone strength, or to monitor the changes with disease progression [1].

In conjunction with the assessment of bone microarchitecture, the use of finite element (FE) analysis is frequently employed so that a non-invasive estimation of bone strength can be achieved [2]. The patient-specific strength measurement not only avoids the limitation of DXA that relies on using BMD as a surrogate for strength, but it also provides a practical method to capture the complicated microarchitecture features measured by HR-pQCT into a single outcome. Another important technique is three-dimensional (3D) image registration [3,4], particularly as studies using HR-pQCT are increasingly focused on longitudinal analyses rather than cross-sectional designs. Image registration provides a basis to ensure that the same region of interest (ROI) is assessed at each time point, which is particularly important due to the relatively small sized ROI that is provided by HR-pQCT measurements.

To date, the utilization of HR-pQCT has been primarily for research applications rather than clinical diagnostics. One of the barriers is that a normative reference population is required to provide context to individual patient results. There are a number of population-based cohorts in development, and they will be an important basis to establish clinical reference data that links an individual's measurement of bone microarchitecture to their risk of a fragility fracture. While the field of research using HR-pQCT has advanced considerably since its introduction, and even been extended to assess microarchitecture in diseases other than osteoporosis, such as post-traumatic knee osteoarthritis [5], there is still plenty of opportunity to advance it as a tool for monitoring bone health. With the number of prospective studies employing a longitudinal analyses recently increasing, we are on the cusp of developing a firm understanding how to use HR-pQCT to predict fragility fractures, and a promising opportunity that longitudinal *in vivo* clinical analysis will become a useful approach in the detection and monitoring of bone diseases in the future.

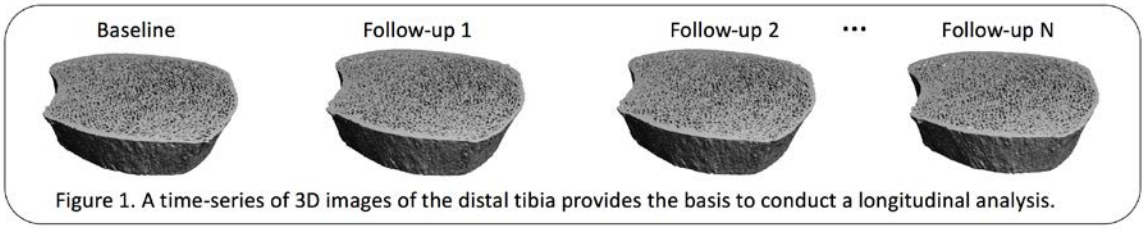


Figure 1. A time-series of 3D images of the distal tibia provides the basis to conduct a longitudinal analysis.

References

1. Cheung AM, Adachi JD, Hanley DA, Kendler DL, Davison KS, Josse R, Brown JP, Ste-Marie LG, Kremer R, Erlandson MC, Dian L, Burghardt AJ, Boyd SK 2013 High-resolution peripheral quantitative computed tomography for the assessment of bone strength and structure: a review by the Canadian Bone Strength Working Group. Curr Osteoporos Rep **11**(2)**:**136-46 doi: 10.1007/s11914-013-0140-9.

2. van Rietbergen B, Ito K 2015 A survey of micro-finite element analysis for clinical assessment of bone strength: the first decade. J Biomech **48**(5)**:**832-41 doi: 10.1016/j.jbiomech.2014.12.024.

3. Boyd SK, Davison P, Müller R, Gasser JA 2006 Monitoring individual morphological changes over time in ovariectomized rats by in vivo micro-computed tomography. Bone **39**(4)**:**854-62 doi.

4. Ellouz R, Chapurlat R, van Rietbergen B, Christen P, Pialat JB, Boutroy S 2014 Challenges in longitudinal measurements with HR-pQCT: evaluation of a 3D registration method to improve bone microarchitecture and strength measurement reproducibility. Bone **63:**147-57 doi: 10.1016/j.bone.2014.03.001.

5. Kroker A, Zhu Y, Manske SL, Barber R, Mohtadi N, Boyd SK 2017 Quantitative in vivo assessment of bone microarchitecture in the human knee using HR-pQCT. Bone **97:**43-48 doi: 10.1016/j.bone.2016.12.015.

Biomechanical in vivo microCT imaging

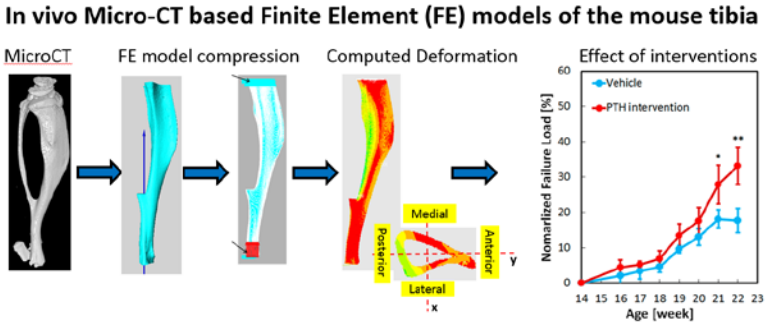
Enrico Dall'Ara, PhD
Department of Oncology and Metabolism and Insigneo institute for in silico medicine, University of Sheffield, United Kingdom

Musculoskeletal diseases such as osteoporosis dramatically affect the morbidity and mortality of our ageing society and degrade the bone density and quality.

In vivo X-ray-based micro Computed Tomography (micro-CT) or High Resolution peripheral Computed Tomography (HR-pQCT) can be used to measure the changes of bone properties over time in peripheral sites of small rodents or of patients, respectively. The finite element (FE) approach applied to these images is an elegant way of estimating non-invasively the effect of ageing, mechanical stimuli, diseases and/or treatments on the structural (stiffness and strength) and local (displacements, strains and stresses) bone mechanical properties. Furthermore, the spatial co-registration of the local predictions of the FE models and of the longitudinal quantification of bone (re)modelling dynamics offers a unique framework to test pre-clinically or clinically the different mechano-regulation hypotheses.

Nevertheless, the outputs of the FE models should be comprehensively validated against accurate and reliable experiments in the laboratory in order to optimize the modelling approaches and increase its applications in the designing and testing of novel treatments. In this contest the application of digital image (or volume) correlation techniques are used to test the credibility of the models for prediction of local mechanical properties, which trigger bone remodelling.

The objective of the talk is to provide an overview of current methods for the non-invasive assessment of local and structural mechanical properties of bone from micro-CT or HR-pQCT images and to stimulate the discussion about their potential and the challenges in using them.



References

Lu Y, Boudiffa M, Dall'Ara E, Liu Y, Bellantuono I, Viceconti M. Longitudinal effects of Parathyroid Hormone treatment on morphological, densitometric and mechanical properties of mouse tibia. JMBBM. 2017; 75:244-251.

Costa MC, Tozzi G, Cristofolini L, Danesi V, Viceconti M, Dall'Ara E. Micro Finite Element models of the vertebral body: Validation of local displacement predictions. PLoS One. 2017; 11;12(7):e0180151.

Levchuk A, Zwahlen A, Weigt C, Lambers FM, Badilatti SD, Schulte FA, Kuhn G, Müller R. The Clinical Biomechanics Award 2012 - presented by the European Society of Biomechanics: large scale simulations of trabecular bone adaptation to loading and treatment. Clin Biomech (Bristol, Avon). 2014; 29(4):355-62.

Razi H, Birkhold AI, Weinkamer R, Duda GN, Willie BM, Checa S. Aging Leads to a Dysregulation in Mechanically Driven Bone Formation and Resorption. JBMR. 2015; 30(10):1864-73.

Burt LA, Manske SL, Hanley DA, Boyd SK. Lower Bone Density, Impaired Microarchitecture, and Strength Predict Future Fragility Fracture in Postmenopausal Women: 5-Year Follow-up of the Calgary CaMos Cohort. JBMR. 2018