Report ESRF LTP MD-431 – January 2011
"Multi-scale analysis of bone tissue using Synchrotron Radiation micro-CT"
Starting : 2009/II to 2011/I

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A) Introduction

Purpose of the Long Term Proposal:

Synchrotron Radiation (SR) microtomography (micro-CT) is a privileged technique to get experimental data on bone tissue characteristics at different scales, which are mandatory to develop a model of bone fragility. The goal of the present LTP was the multi-scale analysis of bone tissue using Synchrotron Radiation micro-CT and the research actions were decomposed in the following topics :

1. 3D assessment of the lacuno-canalicular system
2. 3D assessment of micro-damage: evaluation of nonlinear ultrasonic technique
3. Analysis of bone micro-vascularisation in a mice model
4. Analysis of bone formation in scaffolds and of the effect of gravity
5. Multi-scale analysis of cortical bone : morphometry and bone material properties

Teams involved in the LTP :

1. Inserm U1044, CNRS 5220 (CREATIS), INSA Lyon, Université de Lyon, Dr. F. Peyrin (coordinator), C. Olivier, Dr. M. Langer
2. Inserm U1059, Laboratoire de Biologie du Tissu Osseux et contraintes mécaniques (LBTO), Saint Etienne, Dr L Vico, Pr. MH Lafage-Proust, Dr. A. Rattner
3. CNRS UMR 7623, Laboratoire d'Imagerie Paramétrique (LIP), Paris, Dr. P. Laugier, Dr A. Saied, Dr. M. Talmant, Dr Q. Grimal
4. CNRS UMR 7052, Laboratoire de Biomécanique et Biomatériaux Ostéo-Articulaires, Paris, Dr. V. Bousson, Pr. JD Larédo, Dr. C. Bergot, Dr G Haiat, Dr. C. Chappard
5. Inserm U658, Orléans, Dr C. L. Benhamou
6. CNRS, Laboratoire MAP 5, Paris, Dr. Sylvie Sevestre
7. Charité University, Berlin, Julius-Wolff- Institute & Berlin-Brandenburg Graduate School for Regenerative Therapies, Germany, Dr. K. Raum
8. Darmstadt Technical University, Mathematics, Dr. A. Gerisch
9. Technical University Wien, Biomechanics, Austria, Pr. P. Zysset
10. Magnetic Resonance Science Center, San Fransisco, USA, Pr. S. Majumdar, A. Burghardt, Dr. G Kazakia
11. Istituto Nazionale per la Ricerca sul Cancro (Unige/IST), Genova, Italy, Pr. R. Cancedda, Dr. M. Mastrogiacomo

Beamtime allocation

- The LTP was allocated from 2009/II To 2011/I a total of 48 shifts on ID19 and 6 shifts on ID22.
- The year I has the following allocation: 9 + 9 shifts on ID19 and 0 + 3 shifts on ID22
- The year II has the following allocation: 15 + 15 shifts on ID19 and 3 + 0 shifts on ID22

The experiments were performed according to the following schedule :

Exp.	Dates	Shifts scheduled	Shifts Done	Participants	Projects
MD-431	18-19/11/2009	6, ID19	6	1,2,9	1,3,5
MD-431	20/01/2010	3, ID19	3	1,11	4
MD-431	07/04/2010	3, ID22	3	1	1
MD-431	28-30/04/2010	6, ID19	6	1,3,7	2,5
MD-431	14-15/06/2010	3, ID19	3	1,11	4
MD-431	27-28/10/2010	3, ID22	3	1	1
MD-431	25-26/11/2010	6, ID19	3	1	1
MD-431	18-19/02/2011	9, ID19	6	1,2,3	2,3
MD-431	10-11/04/2011	3, ID19	3	1,4	5
MD-431	29-30/09/2011	6, ID19	6	1,10	5
MD-431	16-17/11/2011	6, ID19	5	1,3	1,2,5

Due to technical problems on beamline ID19 and date constrain problems, 4 shifts were postponed to 2012 I.
A request for a 3rd year extension (15 shifts in 2012 II and 18 shifts in 2013I) is proposed (see page 19).

B) Description of work

Experiments were mainly carried out on beam line ID19 where we used the synchrotron radiation (SR) micro-CT setups but we also had the opportunity to use the new nano-CT setup on beam line ID22. The beam-time allocated in the Long Term Project was shared between the sub-projects.

1. 3D assessment of the lacuno-canalicular system (partners 1,2)

At the cellular level, the osteocytes play a fundamental role as they have been demonstrated to act as orchestrators of bone remodeling process. The osteocytes are located in small ellipsoidal cavities (a few µm long), called lacunae and they communicate through slender channels (diameter of a few hundreds of nm) called canaliculi. Today, a major concern is the limited methods currently available to assess the lacuno-canalicular network (LCN) that would ideally require a very high resolution 3D technique providing a sufficiently large field of view. Ptychography based on synchrotron measurements has recently been proposed but the constraints in field of view, sample preparation and acquisition time are limiting. Our aim was to develop a method based on 3D SR micro-CT to quantify the LCN.
In LTP-239, we observed canaliculi from 3D SR micro-CT images at 280nm for the first time. However acquisition was difficult to reproduce due to the very small diameter of canaliculi. With a voxel size of 280nm, canaliculi represent at the best one voxel in the image, thus each source of degradation in image formation must be avoided. LTP MD-431 gave us the opportunity to optimize imaging conditions and to acquire data on the osteocyte system both on the ID19 3D micro-CT setup and the new ID22 nano-CT setup.

Experiments on ID19
First acquisitions were performed in Nov 2009 (3 shifts) using different combinations of parameters to test the best conditions to image canaliculi in terms of sample preparation, sample size, number of projections, exposure time, optimal distance to detector. Cortical bone samples were taken from diaphysis in aged human femurs and cut in subsamples of different sizes (thickness between 500µm and 3mm). We used the 2K Frelon camera with a pixel size of 280nm and energy between 16 and 24 keV. It was difficult to make an exhaustive study of acquisition parameters due to the large number of variables impacting image quality and the limited beamtime. However the experiments highlighted the crucial role of the detector. The visibility of canaliculi depended of the type and thickness of the scintillator (YaG, LaG, GGG_6.2, GGG_6.6) fixing the final spatial resolution in the image. Canaliculi could only be observed in the images acquired with the GGG 6µm. In addition, some images were corrupted by motion artifacts related to the effect of dose deposition within the samples. Improving both spatial resolution and efficiency is somehow incompatible, thus a compromise had to be found.

Second acquisitions were performed in Nov 2010 (3 shifts) using the same pixel size of 280 nm with the GGG-7µm scintillator and energy of 17.6 keV. The aim was to evaluate in 3D the LCN structure in normal, osteoporotic and arthritic. Six cortical samples from human normal, osteoporotic and arthritic femoral heads provided by partner 5 were prepared. We used pink beam in multibunch mode. Unfortunately, due to the very high flux, the samples were damaged during scanning and image quality was poor because of sample motion as result of dose induced sample shrinkage (Fig. 1a).

New acquisitions were performed in order to optimize the compromise between radiation dose and image quality. Since signal to noise ratio (SNR) is proportional to the square root of the

dose, maximizing SNR implies a high level of radiation dose which as seen before causes sample motion. Thus the efficiency of the detector including both the scintillator and the CCD camera is essential to reduce dose, however a high spatial resolution must be preserved. For this reason we tested the combination between a new scintillator (LSO-4.5µm) which offers both efficiency and high resolution and the E2V camera which is more efficient than the 2K camera. This system allowed us to reduce significantly the exposure time and consequently the delivered dose. The reconstructions showed that canaliculi were visible and that image quality was improved (Fig. 2). A quantitative analysis comparing image quality in different conditions versus the estimated dose was performed and allowed us to define a protocol for SR micro-CT imaging the LCN [Pacureanu A. et al., Med Phys 2012].

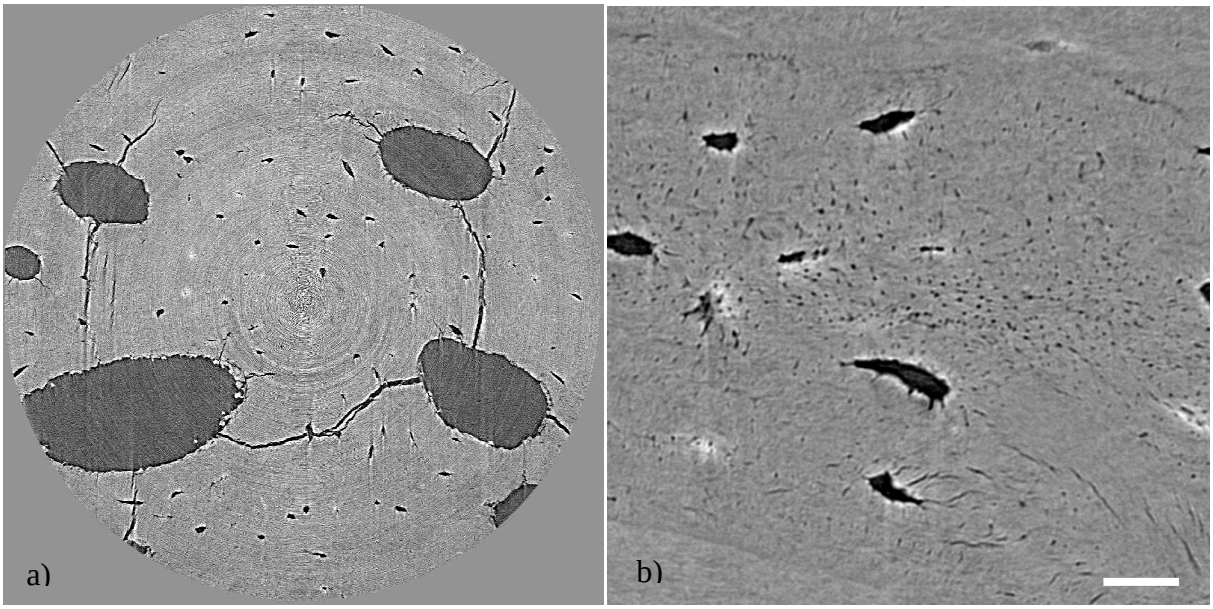


Fig. 1: ID19 SR micro-CT with a voxel size of 280 nm a) Reconstructed slice showing the problems caused by the dose: sample motion and crack propagation, image width: 570µm b) Region in a reconstructed slice from acquisition with reduced dose conditions showing an improved image quality. Scale is 10µm. Canaliculi are visible as dots.

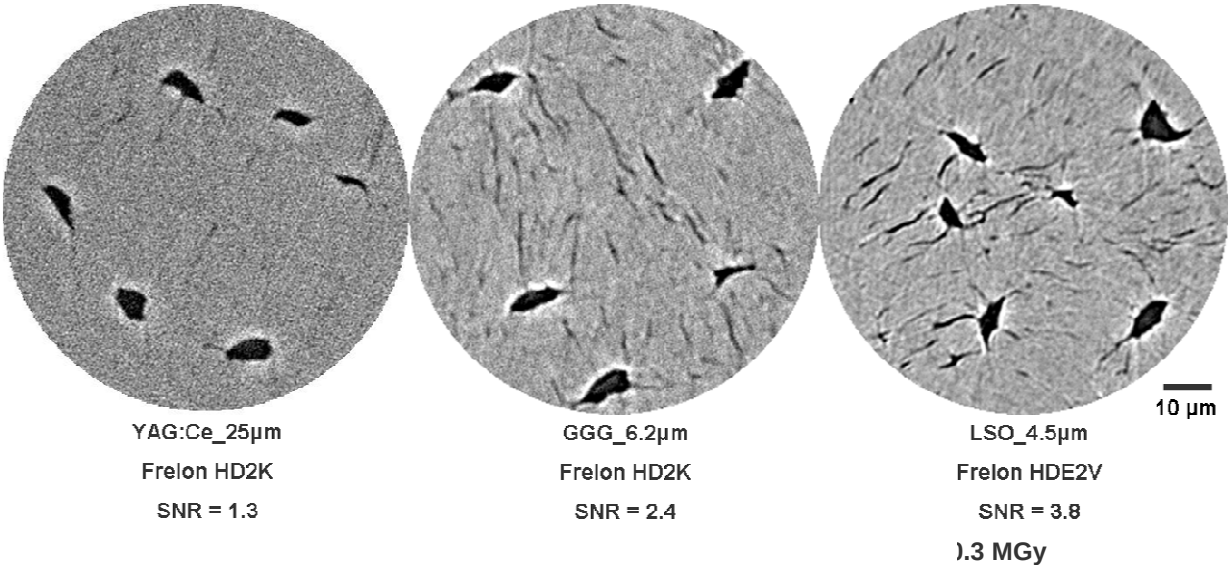


Fig. 2: Image quality obtained with different combinations of scintillators and CCD cameras. The estimated total radiation dose is showed for each scan in Mega Greys (MGy).

Data processing

Image analysis which is required to visualize and quantify the LCN from the SR micro-CT images, is also challenging. Since the structures are at the limit of the resolving power of the imaging system and possibly corrupted by photonic noise and residual motion artifacts, the automatic detection of canaliculi is demanding. Standard thresholding segmentation methods are not usable. Other conventional segmentation approaches based on image gradient are neither not adapted since mathematically gradients are not well defined for 1-voxel thick object. Finally due to the complexity of the structure, manual segmentation is not feasible. To address this problem, we implemented and tested different approaches. We first proposed a non linear filter combined with a line-filter to enhance the contrast of canaliculi by exploiting their tubularity [Pacureanu A. et al., IEEE ISBI 2009]. Then, we designed an original segmentation method based on a variational region growing scheme [Pacureanu A. et al., IEEE ISBI 2010].

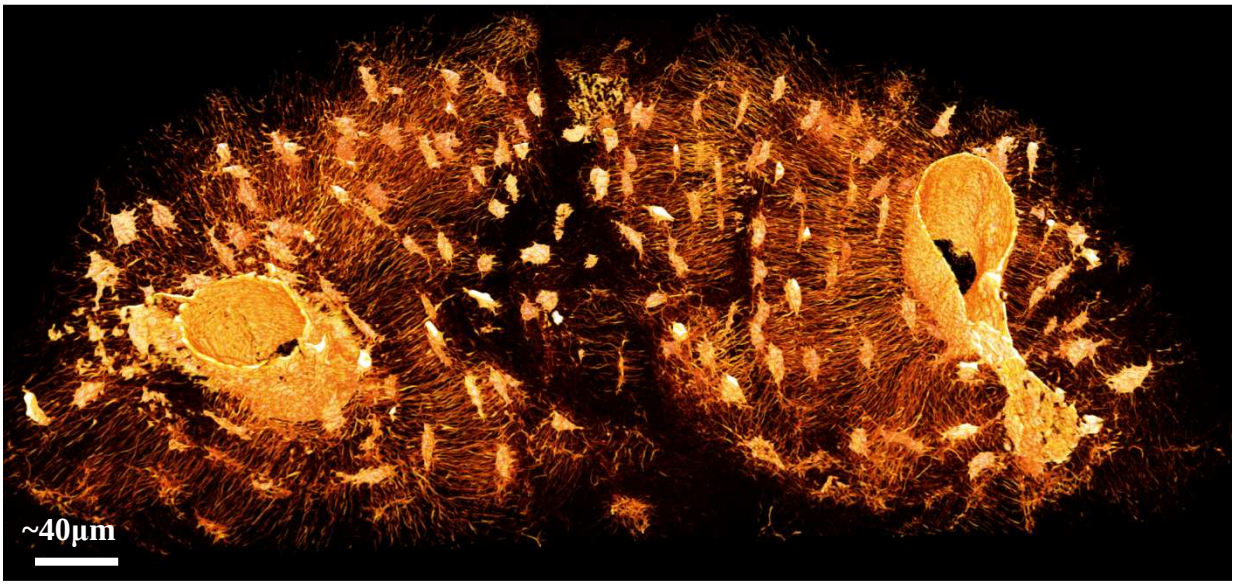


Fig. 3: ID19 SR micro-CT with a voxel size of 280 nm :3D rendering of the lacuno-canalicular network around Haversian canals.

The originality of the method was to derive new energy functionals adapted to the geometry of the cell network (publication to be submitted). We also experimented a level sets method combined with connected component analysis [Pacureanu A. et al., IEEE ISBI 2011]. Thanks to this development, it was possible to achieve 3D visualization of the LCN in a large field of view which is a noticeable achievement (Fig. 3)

Characterization of the LCN in human cortical bone

The application of the method to human femoral cortical bone provides new data at the cellular scale. Osteons are the primary structural and functional units of cortical bone. Due to the possibility to access simultaneously, information on the 3D morphology of the osteocyte network and on the mineralization degree of the bone matrix, the delimitation of the osteons could be done. Three-dimensional renderings of the LCN on a large field of view covering several osteons were obtained. These images reveal the complexity of the LCN in 3D with a large number of canaliculi running radially from the Haversian canal, orientated mainly perpendicular. Canaliculi branch shortly after leaving from the cell and there are junctions between canaliculi coming from two or three lacunae, at a longer distance from the cell. The behavior of the canaliculi in the vicinity of the cement line was also observed. A publication on these results will be submitted to Bone.

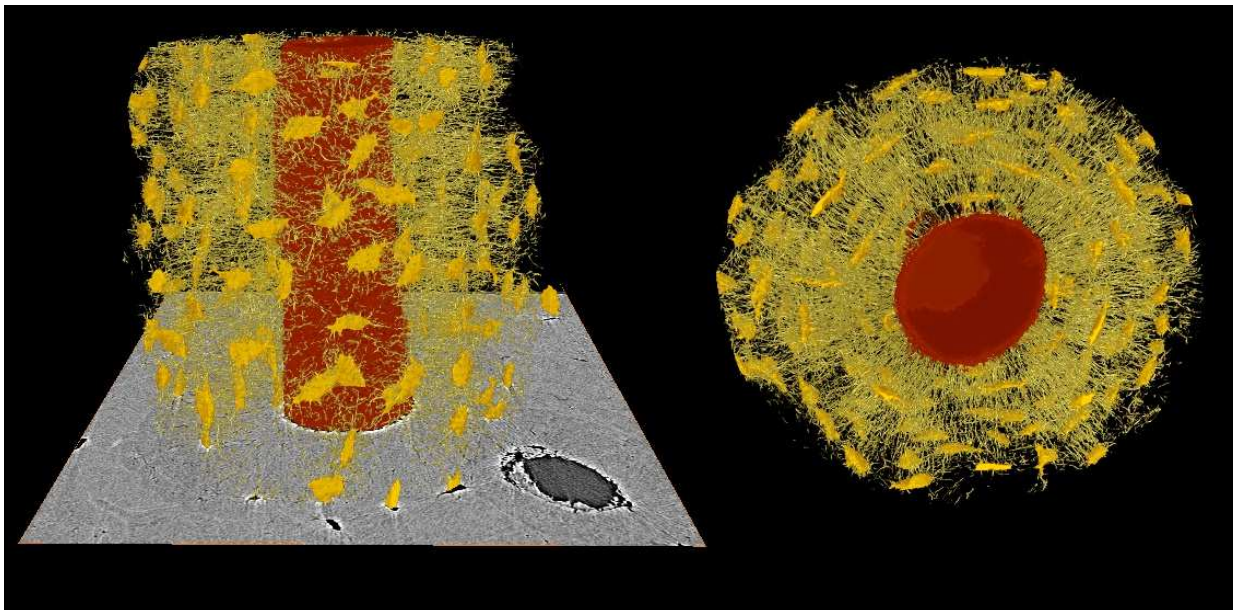


Fig. 4: 3D display of the LCN (yellow) within one osteon, left : side view, right : top view. These are the first 3D visualizations ever provided.

Experiments on ID22

These experiments were respectively performed on the ID22 nano-CT setup were beamtime was allocated in April 2010 (3 shifts) and October 2010 (3 shifts).

April 2010 :

The aim of this experiment was to test the feasibility of phase-based nano-CT on ID22 to image the lacunar canalicular system. The samples were small parallelepipeds (0.4-0.5mm in diameter) from dried human femoral diaphysis (cortical bone). The spatial resolution was 62 nm, the energy was 17.5 keV and the scintillator was a LSO-20µm. The size of the projections was 1500x1500. Data acquisition consisted in scanning the samples at three different distances. The 3D images were reconstructed off-line after phase retrieval. The first results were quite encouraging. The experiment enabled 3D imaging of the lacuno-canalicular structure with higher precision at the expense of a smaller field of view in comparison to ID19 data. In addition, the close inspection of the image lets us think that information on the orientation of the HA crystals and collagen fibers is also available. Thus, this technique offers unique information on the lacuno-canalicular porosity together with details on orientation and density variations in the surrounding mineralized bone matrix (Fig. 5). A publication on these findings was accepted with minor revision in Plos One [Langer, PlosOne 2012].

October 2010 :

The aim of the experiment was to study the architecture of the lacuno-canalicular system and the structure of the mineralized matrix in bone affected by osteoporosis or arthritis comparing to normal bone. This kind of investigation has never been done at this scale in 3D. The spatial resolution was 60 nm, the energy was 17 keV and the scintillator was a GGG-30µm. Three small samples of cortical bone prepared from human normal, osteoporotic and arthritic femoral heads were acquired. However due to calibration problems, the first reconstructions were not satisfying. Data analysis requiring specific preprocessing is still in progress.

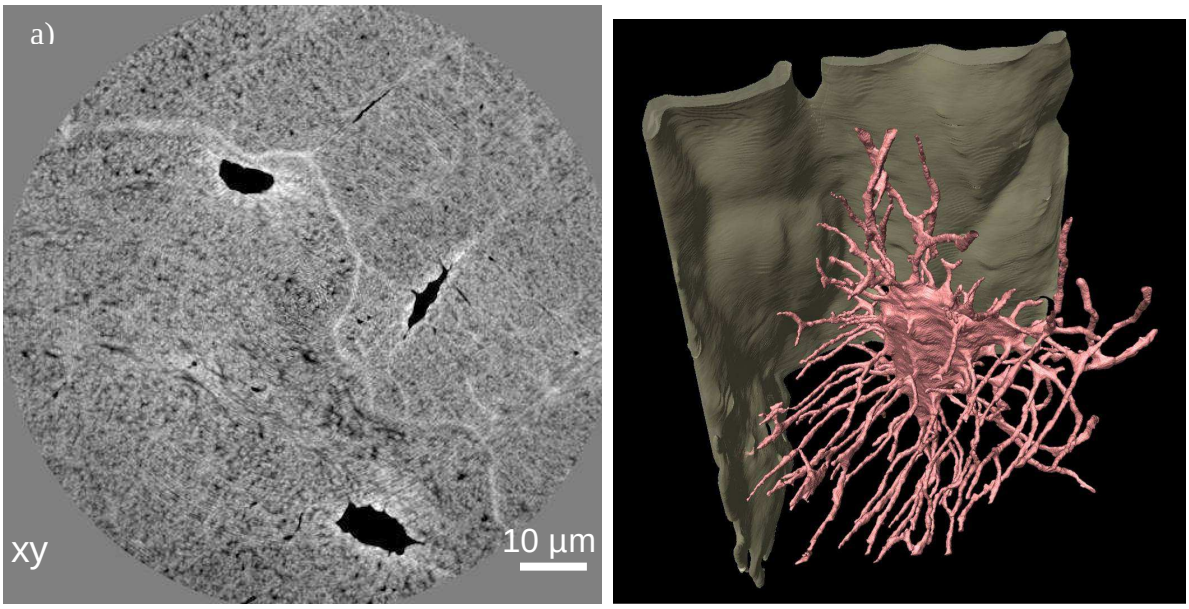


Fig.5. ID22 SR nano-CT with a voxel size of 60 nm : a) Reconstructed slice after phase retrieval; b) 3D renderings of a lacuna and canaliculi in the vicinity of a cement line.

Conclusion

The LTP allowed us to define conditions for 3D imaging of the lacuno-canalicular system which had so far never been achieved on more than a few cells. Both the ID19 SR micro-CT at 280 nm and the ID22 SR nano-CT are promising and allowed us to acquire unique data. The first 3D renderings of the lacuno-canalicular system on large field of views recently presented in some conferences raised a great interest. This work was supported by a FRM (Fondation de la Recherche Medicale) project (2009-2012).

Perspectives include data acquisition on pathological samples both on ID19 and ID22, improvements in the segmentation process by means on anew approach based on minimal path (work of Post Doc M Zuluaga [Zuluaga, IEEE MIC 2011], development of quantification methods of the LCN (work of P Dong, PhD student) and associated software development for the automatic analysis of the LCN. A related experiment has been submitted in Sept 2011 (main proposer : K Raum, partner 7) to study the LCN in jaw osteonecrosis on beamline ID22.

2. 3D assessment of micro-damage: evaluation of nonlinear ultrasonic technique (partners 1,2,3)

Microscopic damage accumulates in bone tissue due to physiological loading and mechanical stress occurring in daily life. Microdamage increases dramatically with age and is often associated to bone fragility fractures. However it is also hypothesized to drive bone remodeling by sending stimuli to osteocytes and to play a major role in the repair process. They are difficult to observe and there is a lack of quantitative data on microdamage. It often appears in the form of linear microcracks as thin planar defects. The most widespread technique for *in vitro* investigation of microdamage consists in observing thin slices of bone by microscopy after staining. The goal of this subproject was to analyze the 3D morphology of micro-cracks from 3D Synchrotron Radiation (SR) micro-CT.

Assessment on micro-damage in trabecular bone

Data acquisition

In the previous LTP-MD239, SR micro-CT images to study micro-damages in trabecular bone were acquired (voxel size 1.4µm). Human trabecular bone samples taken from femoral heads were imaged. The samples were provided by L Vico and A Rattner (partner 2). Imaging at a spatial resolution of 1.4µm was found to be a good compromise to see the micro-cracks while keeping a sufficient filed of view.

Data processing

A new tailored 3D image analysis technique was developed to segment and quantify microcracks during the PhD of Aymeric Larrue. The method allows to segment micro-cracks and osteocyte lacunae automatically in the 3D SR micro-CT images. Then quantitative parameters such as the number of cracks, their width, length, thickness, and orientation are calculated. This is the first time that various types of microcracks in unconstrained human trabecular bone - from the simplest linear crack to more complex cross-hatch cracks - have been examined and quantified by 3D imaging with this level of accuracy. Fig 6 illustrates a trabecular bone surface (white) and the segmented microcrack (blue) and osteocyte lacunae (pink). This work was published in [Peyrin, Ost Int, 2009] and in [Larrue, PlosOne, 2011].

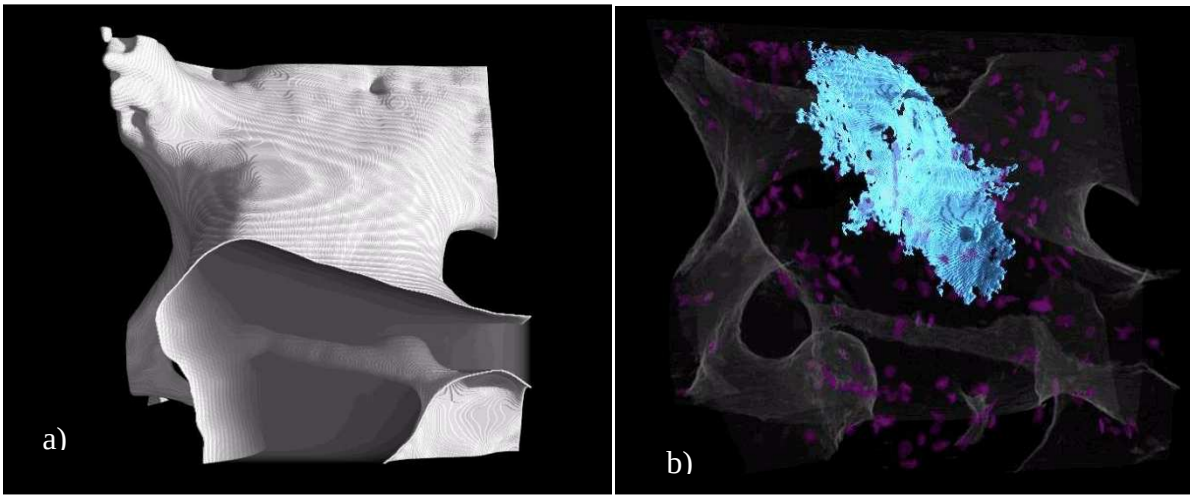


Fig.6: SR micro-CT images, voxel size : 1.4µm : a) trabecular bone surface (white) and b) segmented microcrack (blue) and osteocyte lacunae (pink)

Assessment on micro-damage in cortical bone

Data acquisition

A second study concerned the investigation of micro-cracks in human cortical bone after biomechanical tests with partner 3. The objective of the study was evaluate the potential of Nonlinear Resonant Ultrasound Spectroscopy (NRUS) (partner 3) for measuring micro-damage accumulation in cortical bone.

Human cortical bone specimens were machined as parallelepiped beams and damaged using four-point bending cycling fatigue. Damage progression was controlled by measuring the linear elastic beam theory modulus (ELEBT), known to reflect microdamage accumulation. Before and between each damage step, the nonlinear ultrasonic elastic coefficient was measured by NRUS. At the end of each cycling fatigue, a subset of bone samples was measured by µCT at the European Synchrotron Radiation Facility).

In the experiment of April 2009, a first set of 9 cortical samples were imaged using SR micro-CT after biomechnanical testing and NRUS analyses. Preliminary tests indicated that interesting micro-cracks pattern can be observed in SR micro-CT images at 1.4µm. In each sample, 4 to 5 regions of interest (ROI) were acquired leading to a total data set of 156 Gb.
In the experiment of November 2010, it was planned to acquire new samples with progressive micro damage induced by controlled fatigue and toughness experiments. However, due to a failure on beamline ID19 on Nov 25, the experiment was interrupted. This experiment was then postponed to February 2011. 15 samples with 4 ROIs in each were acquired at 1.4 µm with a monochromatic energy of 25 keV. 2500 projections 1400x2048 were acquired on a total angle of 360°. Reconstruction was performed after correction of distortions due to the optic. The final volumes were 2048x2048x1400.

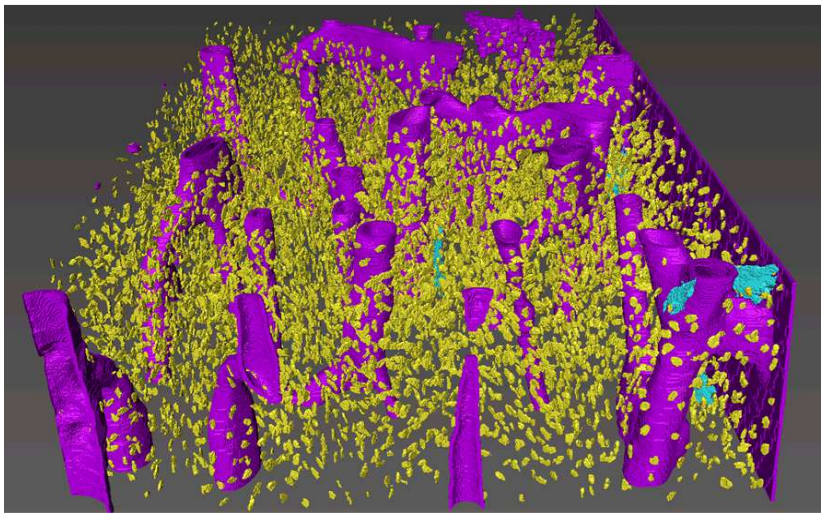


Fig.7: SR micro-CT images of cortical bone sample after 3D analysis : canal network in pink, lacunae in yellow and micro-crack in light blue.

2D data processing
The first analyses of data acquired in 2011 characterized micro-damage on 12 regularly spaced. 2D transverse cross-sections extracted from the 3D reconstructed bone volumes by measuring the density of microcracks. The results showed that the variation of elastic nonlinearity was significantly correlated to the variation of the density of small microcracks which almost doubled in damaged regions. The progressive increase of NRUS coefficient along fatigue cycling suggests that NRUS measurements are sensitive to micro-damage accumulation [Haupert et al., IEEE Ultrasonics Symp 2011].

3D data processing
The automatic 3D image processing method previously developed to segment and quantify micro-cracks in trabecular bone was evaluated on these data. However, since cortical bone is denser than cortical bone, the number of points to be processed was increased by a factor 4 to 5, and computing time became prohibitive. Thus, a large effort was dedicated to the optimization of the program. A first version was developed in C in 2010, and a second version was developed using ITK (Image ToolKit Library) in 2011 to speed up calculation time. Figure 7 illustrates the results of processing providing different colors for the canal network, micro-cracks and lacunae. The software will be further used to analyses the latest data.

3. Analysis of bone micro-vascularisation in a mice model (partners 1,2)

Vascularisation plays a major role in bone formation, bone growth and repair. Up to now it has mainly been assessed from stained histological slices. If contrast-agent micro-CT has been proposed, it generally requires bone decalcification prior to imaging, making it impossible to study bone and vessels simultaneously. The purpose of this project was to develop 3D SR micro-CT imaging method to study bone microvascularisation in animal models.

In LTP-MD239 we showed the advantages of SR micro-CT for studying simultaneously bone micro vascularization and micro structure [Jia, The Anatomical Record, 2010]. Forty samples in control and PHT treated rats were acquired with two regions of interests (diaphysis and metaphysis) resulting in a very large data set (1 Terabyte) that was processed during the present LTP. A new segmentation technique was developed to segment vessels and bone and to identify the cortical and trabebular envelopes, along with associated vessels and porosity inside the cortical and trabecular envelopes. The method was applied to study the effect of a PTH treatment providing significant different between the two groups. Figure 8 illustrates a 3D rendering of micro vascularisation in cortical bone (orange), and in trabecular bone (red).

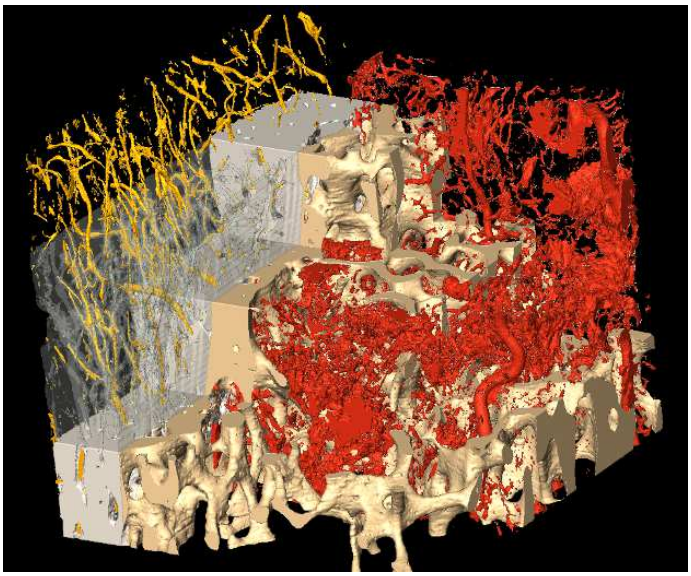


Figure 8: 3D rendering of micro vascularisation in cortical (orange) and trabecular (red) bone. This is the first time that vessels and bone microstructure can be analyzed simultaneously with the advantages of studying the inter-relationships between the two structures.
The development of the data analysis was published in two conference papers [Langer, IEEE MIC 2009] [Langer, IEEE EMBS 2009] and a journal article [Langer, IEEE Trans Nucl Sci 2011]. The report on the biological findings including histology and standard micro-CT was published in JBMR [Prisby, JBMR 2011].

Experiments performed on ID19 in Nov 2009 were designed to adapt the method to mice, the motivation being the larger span of specific phenotypes of mice being available. The purpose of the work was to determine the most reliable technique for imaging bone microvasculature in mice. To this aim, different protocols were tested (nature of the contrast agent: barium sulfate or Microfil, bone sample decalcification or not, embedding technique). Acquisitions were performed in two groups of CD-1/129 mice (7-month vs 17-month old) at 2.8 µm. Additional experiments on BM5 offered the opportunity of testing a spatial resolution of 1.5 µm. The results were compared to histology. It was found that barium sulphate provided the best

imaging and preservation of the bone vascular network. This study allowed assessing the structural organization of bone vessels. Figure 9a) shows a mouse femur slice where bone is seen in gray and vessels are seen in white. Figure 9b) shows a 3D display of a small region of the vessel network after segmentation with the method developed in [Langer, IEEE TNS 2011]. The good preservation of the continuity of the vascular network can be noticed [Roche, Bone 2011]. These results are quite unique and open promising perspectives for the analysis of bone micro-vascularization in mice animal models.

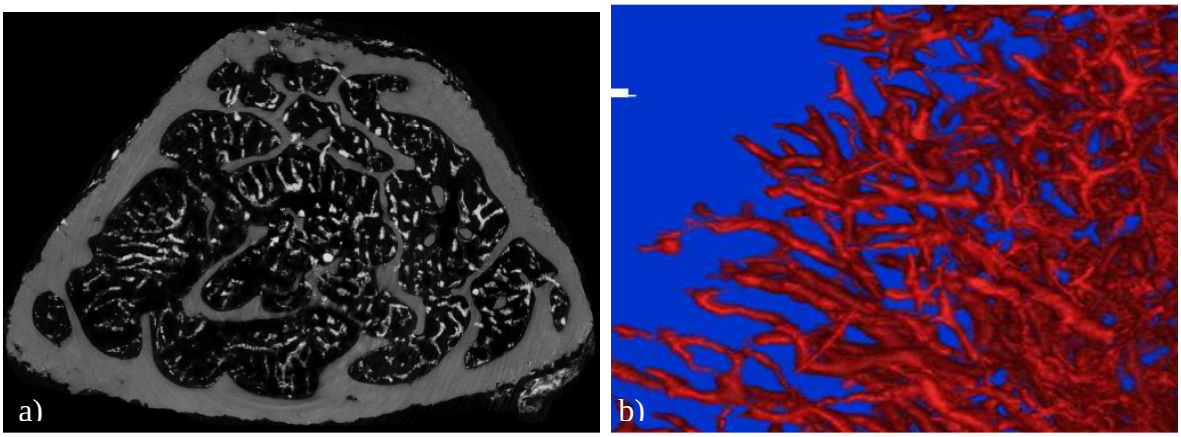


Figure 9: SR micro-CT image in an injected mice femur at 1.5µm: a) raw slice showing bone (gray) and vessels (white), b) 3D rendering of micro vessels showing the preservation of the continuity.

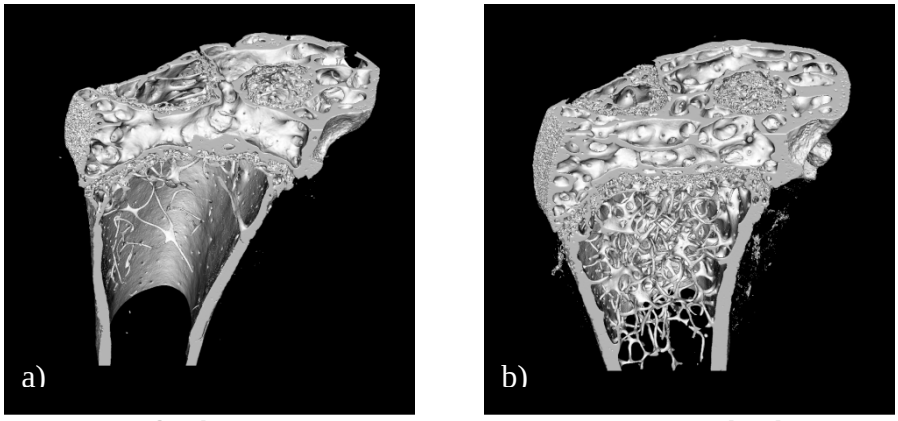
Other data on bone from different sites, such as femur, tibia and vertebra were acquired. These samples came from groups treated in different ways, either with PTH (as described in the original study), full body vibration or placebo. The data will be analyzed using the developed data analysis algorithms. These data form the basis for several studies.
These achievements both in terms of imaging and in terms of image analysis, offers many perspectives since there are little data on the relationships between vascularization and disease. In further work, a new focus will be devoted to the understanding of osteoarthritis. The main application envisaged is the analysis of mineralization and vascularization in spontaneous osteoarthritis. This work has been submitted in an “ANR blanc” project gathering partners 1, 2 and two internationally recognized partners in bone research and osteoarthritis (Univ Pierre Marie Curie, UR4, F Berembaum, X Houard), and INSERM U606, Paris (M Cohen-Solal). 3D imaging of osteoarthritis the vascularisation of the osteochondral junction will be compared in sham and DMM mice. The influence of TSP-1 deficiency will be studied.

4. Analysis of bone formation in scaffolds and effect of gravity (partners 1, 11)

During LTP MD 239, absorption and holotomographic data acquisition were performed on various hydroxyapatite scaffolds provided by partner 11. During this LTP, the analysis of these data has provided new results. SR micro-CT allowed the analysis of bone formation and scaffold resoprtion in porous calcium phosphate scaffolds [Komlev, Euro. Cell. Mater., 2010]. Phase contrast imaging allowed the visualization of soft tissue formation in scaffolds after implantation [Komlev, Tissue Engineering, 2009]. New developments in holotomography [Langer, JOSA 2010] [Langer, IEEE Trans Image Processing 2010] have also provided valuable results for the analysis of pre bone matrix formation in Skelite scaffolds cultured *in vitro* [Langer, J Microscopy 2010].

These works have been pursued with partner 11 in the context of the ERISTO III MAP project managed by the European Space Agency (ESA). The purpose was to study the effects of microgravity at bone cell and tissue levels for applications in bone tissue engineering. Exposure to microgravity results in a bone loss of approximately 1% per month induced by an uncoupling of bone remodeling equilibrium between bone formation and resorption. These changes result in weakened and brittle bones prone to fracture on re-entry and in accelerated osteoporosis, making bone deterioration a major problem obstructing the prospects of long-duration manned space flight. In this context, a major objective is to investigate the effects of microgravity on transgenic or inbred mice as a tool to study genetic mechanisms underlying bone mass pathophysiology.

Experiments were performed in January 2010 (3 shifts) and July 2010 (3 shifts) to image femoral mice bone after a space flight. Partner 11 was involved in an experiment with the Italian Space Agency including 6 mice, 3 C57BL wild type and 3 transgenic which were submitted to a 3 months space flight. In January 2010, it was possible to image the femoral bone of 9 femoral bone samples including 3 flight mice (1 wild type and 2 transgenic) and 6 control mice (3 wild type and 3 transgenic). The images were acquired with a voxel size of 1.9µm at 22 keV. For each sample, two regions of interest respectively in metaphysis and diaphysis were acquired in absorption and phase contrast. In July 2010, images of 3 ground control mice (1 wild type and 2 transgenic mice), 6 lab control mice (3 transgenic and 3 wild), 3 flight mice (2 transgenic and 1 wild) and 2 control mice (1 trangenic and 1 flight) were acquired. Due to time limitation, only one metaphysis scan could be acquired per sample. In addition, due to some problems with the scintillator, some images were blurred and not exploitable. Figure 10 illustrates 3D renderings of a space flight and a control lab mice.



micefw2d2 micelw3d2

Figure 10: 3D renderings of bone surfaces from SR micro-CT image of femoral mice at 1.9µm: left: space flight mice and right: control lab mice.

One difficulty in the analysis is the limited number of mice in each groups since only 3 space mice were available, and the differences in the scanned regions in January and July. Quantitative parameters on bone morphometry such as cortical thickness, porosity and mineralization were extracted from the diaphysis January data. The analysis of the metaphysis January data and the July data is more complex since it first requires selecting a sub region of interest avoiding cartilage growth. This process should be done manually and preferably by a biologist. The results will be confronted to histomorphometry performed by partner 11. The first results in the diaphysis indicate that there were significant differences in normal and gravity conditions after three months of space flight. A decrease of cortical thickness more pronounced for transgenic mice was found due to the lack of gravitational loading.

In the prolongation of this LTP, we intend to continue the imaging of biomaterials to increase the understanding of weightlessness on the bone resorption process. This work will be performed in the ESA ERISTO IV MAP project "Effect of microgravity at bone cell and tissue level: in vitro bone" which has just been approved. This project gather partners 1, 2 and 11 of the LTP. Phase tomography will be used to image in vitro cultivated 3D ceramic bone scaffolds. The aim is to investigate the effects of microgravity on bone deposition, bone resorption and on vessel formation. Further, SR techniques and histology will be used to validate Optical Coherence Tomography (OCT) that will be implemented in a Biotechnology Mammalian Tissue Culture Facility (BMTC) bioreactor. This will provide real-time, online imaging of scaffold/bone-like tissue, adding a 4th dimension to the system. It will be particularly interesting to compare OCT to SR phase tomography since both techniques can provide images that have similar spatial resolution (between 2 and 5 μm) which both are sensitive to soft and hard tissues.

In parallel with this activity, we are also researching reconstruction algorithms for phase tomography. This is based on the still existing limitations in the phase retrieval techniques. Until now, they permit either the retrieval of homogeneous objects, or weakly absorbing objects. We are pursuing development of phase retrieval algorithms on two fronts: continued developments of prior terms towards general objects, mainly to come to terms with low frequency noise often present in the reconstructions, and development of non-linear inversion algorithms to improve the spatial resolution in the reconstructions and take into account the increased non-linear contribution to contrast at high resolution due to the increased relative propagation distance. We have developed a new phase retrieval algorithm based on an a priori for multi-material objects, based on reconstruction, treatment and forward projecting of the attenuation scan. This will allow quantitative reconstruction, in terms of gray level correspondence to local mass density, both in soft and hard tissue in the scaffolds [Langer, Optics Letters submitted 2011]. Further, we have developed a non-linear algorithm based on the Fréchet derivative of the contrast formation functional [Davidoiu, Optics Express 2011] [Sixou, ISBI 2011] [Davidoiu, ISBI 2012]. This has shown promising results on simple objects. We plan on implementing this algorithm in the phase retrieval framework at the ESRF. We have applied for funding to continue this work through the ANR Jeune Chercheuse Jeune Chercheur program 2011 (main proposer: M Langer).

5. Multi-scale analysis of cortical bone: morphometry and bone material properties

Bone is an example of a musculoskeletal mineralized tissue achieving a unique combination and variability of stiffness and strength. The complexity of bone relies on its hierarchical organization at several levels of structural arrangements. The understanding of the structure-function relations in cortical bone requires both experimental assessment of heterogeneous elastic and structural parameters and theoretical modelling of the elastic deformation behaviour.

SR micro-CT is essential to quantify the 3D porous network of cortical bone at different scale together with its properties in terms of mineralization. Experiments conducted in Nov 2009 and in April 2010 allowed to acquire SR micro-CT images in view to model the biomechanical properties of bone. The images were used for the characterization of 3D morphometry and mineralization or as realistic numerical models for biomechanical simulation [Haiat, J Biomech 2009] [Sansalone, J Biomech 2010].

Impact of mineralization on biomechanical properties

At the tissue level, the local material properties of human cancellous bone are heterogeneous due to constant remodeling. Since standard high resolution CT scanning methods are unable to capture this heterogeneity in detail, local differences in mineralization are normally not incorporated in computational models. However, SR micro-CT offers a unique possibility to measure mineralization and exploit it on biomechanical properties. In a first study (partner 4), the spatial variations of bone porosity and of the degree of mineralization of the bone matrix (DMB) obtained from SR micro-CT images were used to derive an homogenization model to estimate the variation of the elastic coefficients across the bone cross-section and along the bone longitudinal axis [Sansalone, J Biomechanics 2010]. This method was further used to analyze 18 cortical bone samples from the femoral neck images by SR micro-CT [Sansalone, Bone 2012]. In another study (partner 10), SR micro-CT images of trabecular bone imaged were used to study the impact of the heterogeneity in mineralization in a simulation study. SR micro-CT based micromechanical finite element models which accounted for mineral heterogeneity were compared with homogeneous models. Evaluation of the apparent stiffness tensor of both model types revealed that homogeneous models led to a minor but significant ($p<0.05$) overestimation of the elastic properties of heterogeneous models of 2.18±1.89% [Gross, CMBBE 2012].

Biomechanical modelisation based on SR micro-CT and SAM

This work was done in the context of the German Research Council project "Biomimetic Materials Research: Functionality by Hierarchical Structuring of Materials" (SPP 1420). The originality of the project is to construct numerical models of stress and strain distribution in bone by coupling quantitative measurements of bone mineralization and bone elasticity. While mineralization is provided by SR micro-CT, elasticity is given by with scanning acoustic microscopy (SAM). The local acoustic impedance of the samples was then measured with SAM by partners 3 and 7 [Rupin, Japanese Journal of Applied Physics, 2009], [Rupin, Ultrasound in Med. & Biol 2010] [Raum, ECCM 2010], [Raum, ASBMR 2010].

Concerning SR micro-CT, since an accurate assessment of mineralization is required, it is important to optimize image quality. Thus, during these experiments, the precise energy was specifically measured and a constructed experimental phantom was imaged to verify the accuracy of the reconstructed values and serve as calibration.

Characterization of elastic properties in human bone samples (partners 3, 1)

Fresh bone specimens were prepared from a collection of 6 left femora of woman cadavers, with age ranging from 78 to 98 years (mean ± std: 84.1 ± 7.6 years) (partner 3). Six transverse cross-sections (one section per femur), 7 mm in thickness, were cut from the mid-diaphysis in a plane perpendicular to the long bone axis. Then, from each one of the cross-sections, parallelepiped samples were extracted from different anatomical quadrants. Finally, we obtained a data collection of 12 samples (dimensions ~ 5x5x7 mm^3). The samples were imaged in SR micro-CT at a voxel size of 5µm and energy of 27 keV (April 2010). The SR-micro-CT gray levels were converted into a degree of mineralization of bone (DMB) in g/cm3 and the Haversian porosity was estimated (cf Figure 11). The same samples were also imaged by 50MHz Scanning Acoustic Microscopy providing the bone tissue acoustic impedance, the porosity (2D porosity). Finally the mesoscopic stiffness coefficients were assessed using a ultrasonic contact method. The objective of this study was to investigate the determinants of human cortical bone elasticity at the mesoscale; and in particular, to determine the relative contributions of Haversian porosity and mineralized matrix stiffness. The first results suggest that, for the elderly population, the elastic properties of the mineralized matrix do not undergo large variations among different samples and the porosity accounts for most for the variations

of mesoscale elasticity [Mouchet, Proc. IEEE Int Ultrasonics Symp 2010] [Mouchet, Proc 3rd Int Conf BME, 2010] [Mouchet, Int Soc of Biomechanics Congress, 2011] [Mouchet, Bone, 2012].

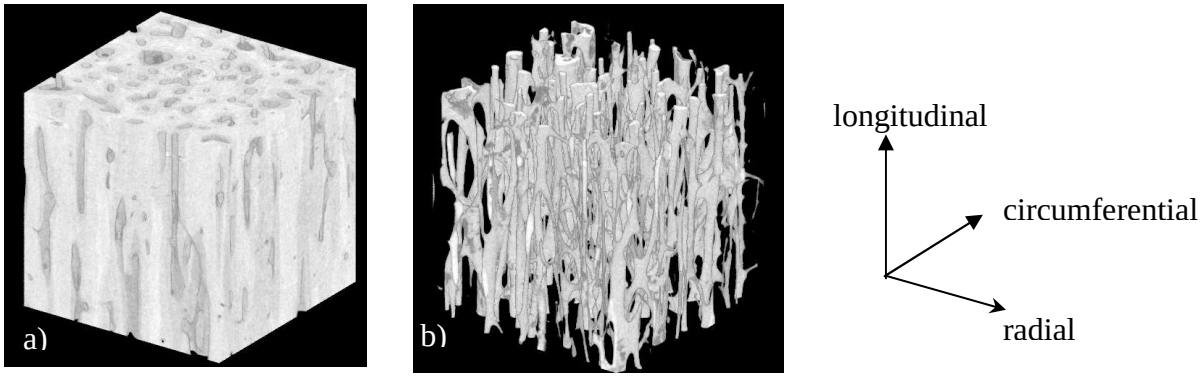


Figure 11 : SR micro-CT images at 5µm a) 3D display of cortical bone, b) 3D display of corresponding porous network

During the same experiment, 7 samples coming from 4 human radiuses were also acquired in the same conditions in view to assess site-matched mineral density, porosity and thickness for comparison with ultrasonic measurement. These data will allow validating ultrasonically determined thickness and elastic properties of bone samples (partner 3).

Characterization of elastic properties in mineralized turkey tendons (partners 7, 1)
The aim of this experiment was to verify the relationship between elasticity and mineralization to stiffness in a wide range of mineralization (partner 7,8). Cortical bone samples from sheep and mineralized turkey tendons were imaged at 5µm (April 2010): Data were also acquired in holotomography at 2 distances to achieve as precise local mineralization and density measurement as possible. The regularized phase retrieval method recently developed was used [Langer, IEEE Trans Image Proc 2010]. The samples were also analyzed by 200-MHz Scanning Acoustic Microscopy (SAM)by partner 7.
The mineralized turkey tendons show a gradient of mineralizations from an unmineralized to a fully mineralized collagen matrix. Site-matched SAM and SR micro-CT were used to assess the acoustic impedance parallel (Z_{33}) and perpendicular (Z_{11}) to fibrils long axis as a function of tissue degree of mineralization (DMB). From these data, mass density and elastic coefficients (c_{33}, c_{11}) were derived (cf Figure 11). A multiscale model of the mineralized fibril bundle based on homogenization was developed and showed a good agreement with experimental data [Molnar, ESCUB 2011] [Tiburtius, SimOrtho 2011] [Gerisch, ICIAM 2011].

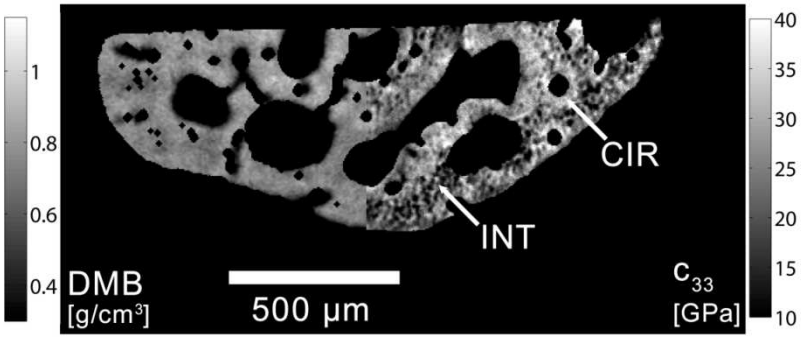


Figure 11: Fused DMB (SR micro-CT, left) and stiffness c33 (SAM, right) map of a section perpendicular to the length axis of a mineralized turkey tendon. CIR and INT denotes circumferential and interstitial tissue, respectively.

Concerning image reconstruction, the phase retrieval algorithm that was used is based on an assumption of homogeneity which is not ideal in this case. In future, we expect to exploit new developments based on iterative algorithms, either based on the introduction of new prior problem [Langer, Optic Letters, 2012] or on the direct resolution of the non linear phase retrieval problem [Davidiou, Optics Express 2011] to process such data.

Characterization of cortical micro-structure (partners 1, 4).
The purpose of this work was to assess the variability of cortical bone at different locations in the human femur undergoing diverse regimens of mechanical loads (partner 4). To this aim, three samples at the supero-part of the femoral neck, the inferior neck and diaphysis were prepared by partner 4 and cut from 20 cadavers (mean age of 78.3±12.4). The samples were imaged with a voxel size of 7.5 µm and energy of 27 keV. Figure 11 illustrates cortical slices at the three locations. The usual morphologic and topologic parameters were measured and were found to be location dependent. The results provided evidence of large variations in canal network structure which exhibited a more homogeneous structure at the diaphysis compared to the femoral neck. A publication reporting the findings in details is in revision [Chappard, Osteoporosis Int 2012, in revision].

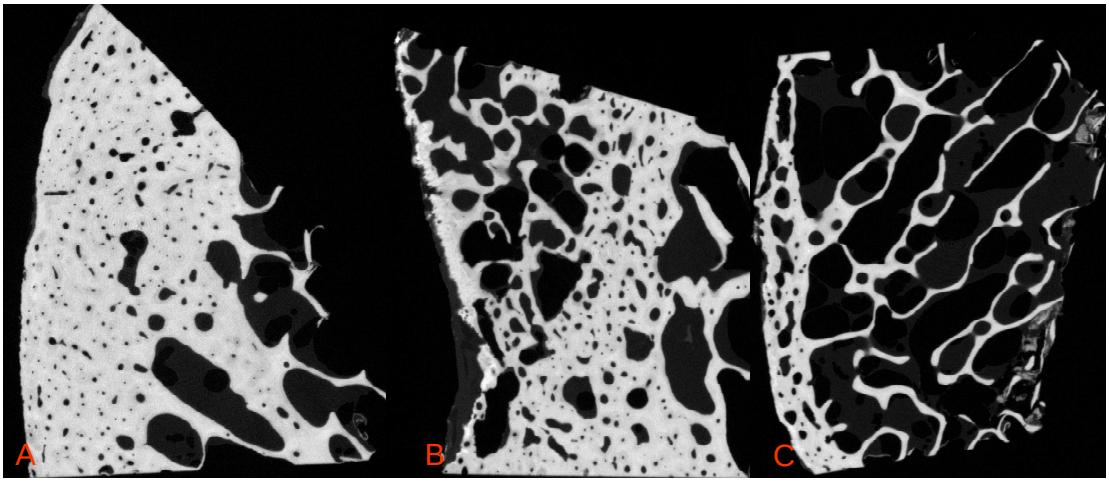


Figure 11: 2D slices of human cortical bone in different locations from the same individual . A: inferior neck, B diaphysis, C: superior neck.

In addition, a new analysis technique was proposed by partner 6 to describe the shape of the canals and their surface regularity. They were used to compare SR micro-CT to standard micro-CT images [Bensalah S, Int Conf on Biomedical Engineering Biomed 2011].
Finally, due to the quantitative nature of the cortical bone image, it was also possible to study in details the structure of osteons extracted by a newly developed segmentation method. A publication has been submitted to Bone [Peter Z, Bone 2011, submitted].

Biomechanical modeling based on SR micro-CT, SAM and SAXS (partners 1, 3)
In 2010, we began collaboration with A Gourrier (LPS, Paris) who is performing SAXS analysis on beamline ID13 at the ESRF. SAXS can provide information of the lamellae orientation in bone ultrastructure. Our aim is to couple SAXS to synchrotron imaging, SAM and to other techniques such as infrared and Raman spectroscopy mastered at INSERM U831 in Lyon.
Experiments were performed in November 2010 (1 shift). Human and sheep bone samples prepared in thin slices (~100 µm) were imaged at ID19 to assess bone mineralization. By

coupling this information to SAXS analysis it is possible to map different properties of lamella. Data analysis is under way.
This work which has been done in the context of a PEPS CNRS project will be pursued in future with partners 3 and 7. The goal is to exploit the properties of bone tissue assessed by these different techniques at different scales for biomechanical modeling. This has also motivated the submission of a new ANR project, called MULTIPS, in January 2012.

Assessment of tissue mineralization in cortical bone (partners 1, 10).
The spatial variation of mineralization in bone is particularly important in assessing bone metabolism and is receiving increased attention in the biomechanical modeling of bone. Accurate methods for the quantification of bone tissue mineralization are therefore required. In this context, the aims of this project were to 1) Evaluate SR micro-CT (and holotomography) to quantify mineralization; and 2) Compare these techniques to a polychromatic micro-CT system in order to provide calibration. The end goal is to validate the more accessible technology of conventional polychromatic micro-CT imaging for studies of bone mineralization. Once established, density-calibrated micro-CT imaging could provide an accessible, relatively inexpensive, and powerful technique in bone quality studies.
Cylinders of cortical bone (4mm x 4mm, n=20) were harvested from human cadavers. Specimens were taken from a variety of sites (tibia, femur, radius) in an effort to include a range of mineral densities. The cylinders were imaged using the Scanco microCT 40 scanner (Scanco Medical AG., Basserrsdorf, Switzerland) at a voxel size of 8 µm. Using a hydroxyapatite phantom, density-calibrated microCT reconstructions of each specimen were created. The samples were then transported to the ESRF and imaged using SR micro-CT and holotomography (6 shifts, Sept 2011). Density-calibrated reconstructions of these data will be created. Each specimen will be processed using established gravimetric protocols to determine ash density, including Archimedes's method to directly determine tissue volume. To make spatially-resolved comparisons, microCT and SR microCT reconstructions will be registered based on morphological landmarks, and compared.
These analyses will be used to elucidate physical phenomena – for example scatter artifacts or beam-hardening effects not corrected by the microCT post-processing algorithms – accounting for disparities between modalities. The analyses described above will permit the evaluation of new beam-hardening correction algorithms for polychromatic micro-CT systems. The end goal is to improve the feasibility of conducting density-calibrated polychromatic micro-CT studies of bone mineralization. This will enable researchers to use the more accessible technology of conventional polychromatic micro-CT imaging and relieve demand for synchrotron facilities. Important applications include: measuring age-, disease-, and drug-related changes in mineralization, determining spatial mineralization patterns, creating accurate finite element models, or exploring relationships between mineralization and tissue damage.

C) Project environment

Human resources at ESRF working on the LTP :

- Permanent:
  - F. Peyrin (DR1 Inserm),
  - M Langer (CR2 CNRS, since Oct 2010),
  - C. Olivier (Engineer Inserm)
- Post Docs :
  - M Langer (Janv 2010-Sept 2010),
  - M Zuluaga (Feb 2011-Janv 2012)
- PhD students:
  - A. Larrue (2009),
  - A Pacureanu (Sept 2009- Janv2012),
  - P Dong (since oct 2010)
  - B Hesse (since oct 2010, collab. Berlin Unviversity (partner 7)
- Trainees :
  - B. Kevin (2009, 3 months),
  - R. Renaudot (2009, 3 months),
  - C. Buis (2010, 6 months),
  - A Duval (2010, 4 months),
  - J. Rollet (2010, 3 months)

Technical resources brought to the LTP

- 2 workstations (Linux computer 84 Gb memory and 96 Gb memory)
- Software for 3D image processing: VGStudioMax and AVIZO
- Software development:
  - 3D line-filter enhancement (PhD A Pacureanu)
  - Segmentation of bone micro-cracks (PhD A Larrue, PJ Gouttenoire)
  - Quantification of osteocyte lacunae (PhD P Dong, PJ Gouttenoire)
  - 3D display with VTK (PJ Gouttenoire)

Associated Projects

The work presented in the present LTP was supported by a number of funded projects :
- The FRM project (partner 1) entitled MICROTOMOS is concerned with the examination of bone tissue at the cellular level (sub project 1). It allowed to employed a post doctoral fellow for two years (M Langer, 1st year and M Zuluaga, 2nd year).
- The German Research Council project "Biomimetic Materials Research: Functionality by Hierarchical Structuring of Materials" (partner 7) leaded by K Raum, has motivated a fruitful collaboration. It lead us to recruit a common PhD student Bernhard Hesse which with co funded by Berlin university and the ESRF an co supervised by Drs K Raum and F Peyrin.
- The BONUS ANR project (partner 3) deals with the examination of bone micro-cracks by non linear ultrasound methods (sub project 2).
- The work done in the framework of this LTP is also part of the LIA (International CNRS - laboratory) leaded by Pascal Laugier (sub project 5).
- The collaboration with A Gourrier from the LPS, Paris has been supported by a French CNRS PEPS project in 2010 entitled "Caractérisation multi-échelle de l'impact de l'organisation des nanoparticules minérales dans les tissus osseux sains et pathologiques ».
- The french CNRS GDR Mecanotransduction, led by T Hoc and P Chabrand (2009-2012).

- The french CNRS GDR Stic Santé led by F. Peyrin (since Jan 2011).

Following the works and the ideas that were developed in the present LTP, different projects were submitted
- project MULTIPS "Multiphysic and multiscale assessment of bone quality", led by P Laugier, and involving partner 1, 3, Inserm U831, Lyon (Dr G Boivin), LIPhy UMR 5588 CNRS – U. Joseph Fourier, Grenoble, (A Gourrier), IFSTTAR (Dr D Mitton), submitted Jan 2012 (ANR Blanc).
- project MULTIMOS "Multimodality Imaging of the Osteocyte Network", leaded by F. Peyrin, involving partners 1 and 5, submitted Jan 2011.
- ERISTO IV project, "Effects of microgravity at bone cell and tissue levels : in vitro Bone", with partners 2 and 11, new European MAP project managed by ESA (European Spatial Agency), 11submitted in Dec 2010.
- European Osteocyte Network : we participated to the "First International Osteocyte Workshop" in Orleans 2011, co-organized by Prof CL Benhamou and Prof N Fazzalari from Adelaide, Australia. Due to the interest in this structure, it was decided to apply for an European Network on this subject at the European level.
- project AngOA "Osteochondral angiogenesis in osteoarthritis», led by X Houard (UPMC, UR4, Paris) involving partner 1 and 2, and Inserm U606, Paris, submitted Jan 2012 (ANR Blanc).
- project MIREOS "Bone adaptation to mechanical loading and aging - Longitudinal study of bone microarchitecture and strength measured in vivo in women and men" led by S Boutroy (Inserm U831, Lyon) involving partner 1 and Dr Van Rietbergen, Netherlands, , submitted Jan 2012 (ANR Blanc).
- project PHASEX "Tomographic phase reconstruction and imaging with X-rays" led by M Langer (partner 1), submitted Jan 2012 (ANR Jeune Chercheur).

In addition, since imaging bone tissue at different scale is a major issue to improve biomedical modelisation, new collaborations on this topic have emerged with different teams (V Sansalone, CNRS, Creteil, Paris) or are in discussion with an Australian team. It is thus expected that the consortium will even be enlarged.

D) Request for a 3rd year extension of the LTP

The committee recommended allocation for 2 years with a report to be submitted after 2 years for a potential 3rd year extension.
The two first years permitted the development of new imaging methods and results opening many scientific perspectives. Thus, we request a 3rd year extension of 15 shifts in 2012 II and 18 shifts in 2013 I on beamline ID19. In continuation with previous works, the following experiments are proposed:

1. 3D assessment of the lacuno-canalicular system – 6 shifts

The two first years of the LTP allowed us to define a protocol for image acquisition at 280 nm and design image processing methods to analyze the LCN (Lacuno-Canalicular Network). During the LTP, the osteocyte system and the LCN became a hot topic in bone research due to its major role on mechanotransduction and in bone remodeling. New imaging methods have been proposed, and in this domain, our work is quite unique and should be continued.

We intend to acquire bone samples likely to exhibit differences in terms of LCN : young versus aged (16 samples), osteoporotic versus osteoarthritic (16 samples), bone on a loaded

(femur) versus unloaded (skull) (4 samples). Samples will be machined in small parallelepiped (side 0.5-1 mm) and imaged on beamline ID19, following the protocol previously established. Our aim is to go further than simple imaging and extract quantitative results from the images in order to study if the differences between the groups are significantly significant. This work will be done in collaboration with Partners 5, 10 and international teams involved in the project of European Osteocyte Network. In particular we shall collaborate with Pr Jenneke Klein-Nullend, (ACTA, Amsterdam, Netherlands) who is a world expert on the osteocyte system in relation to biomechanical constraints.

2. 3D assessment of micro-damage: (partner 9) 6 shifts

The growing incidence of fractures caused by osteoporosis and falls in aging societies constitutes a major socio-medical challenge. The estimation of bone fracture risk by CT-based finite element simulation could be helpful for the management of patients with osteoporosis. The fracture process, however, is dominated by the post-yield behavior of bone tissue. So far little is known about this and the mechanisms leading to irreversible deformations. Often, the post-yield behavior of bone is modeled using the concept of damage and plasticity. However, it is unclear how damage evolves under changing loads and how plastic strains occur in bone.

In the previous work, we have shown the feasibility of imaging bone damage in the form of 3D linear micro-cracks. This project will exploit this technique to investigate the generation of damage and plastic strains in bone in a multi-scale experimental setup.

SR micro-CT at the micrometer scale will be associated to nano-indentation experiments to different indentation depths performed by partner 9 (Pr Zysset) who is an expert on this technique. SR micro-CT will be used to investigate the formation of cracks underneath the indent, and to investigate whether plastic strains can be detected as increasing mineralization due to compaction of material underneath the indenter tip. In addition, different load combinations of compression, tension and shear will be exerted on dumb-bell shaped compact bone specimens prior to SR micro-CT. It is expected to track and compare cracks due to the different macroscopic loading modes. This will allow to investigate the loading mode and direction dependent evolution of micro-cracks in lamellar bone. Especially, mechanisms to hinder crack extension such as crack detection, bifurcation, bre bridging are of interest. Furthermore, the region around the crack tips will be investigated with regard to the possible formation of a plastic zone as it is known to develop in ductile materials.

3. Analysis of bone micro-vascularisation in a mice model (partners 1,2) 6 shifts

During the first two years of this LTP we developed an imaging protocol associated to an image analysis method to assess the micro-vascular network in mice models. This method will be capitalized upon by applying it in new areas of research.

We expect to apply the vascularization imaging method to the study of spontaneous osteoarthritis and the influence of TSP-1 deficiency. For this purpose, samples from 20 wildtype (WT) and 20 TSP-1-deficient (TSP-1) mice that have received destabilization of the medial meniscus (DMM) and mice that have received “sham” surgery, in which the ligament are visualized but not transected (controls), will be prepared according to the previously developed protocol. Mice will be sacrificed at 4 and 6 weeks. Samples will be collected in 4 equal groups with 10 samples each. Samples will be imaged at 2.8 μm pixel size at 22 keV.

This work has been submitted in an “ANR blanc” project gathering partners 1, 2 and two internationally recognized partners in bone research and osteoarthritis (Univ Pierre Marie Curie, UR4, F Berembaum, X Houard), and INSERM U606, Paris (M Cohen-Solal). 3D imaging of osteoarthritis the vascularisation of the osteochondral junction will be compared in sham and DMM mice, as well as the effect of TSP-1 deficiency.

4. Analysis of bone formation in scaffolds and effect of gravity (partners 1, 11) 9 shifts

We intend to continue the imaging of biomaterials to increase the understanding of weightlessness on the bone resorption process. This work will be performed in the ESA ERISTO IV MAP project "Effect of microgravity at bone cell and tissue level: in vitro bone" which has recently received funding. This project gather partners 1, 2 and 11 of the LTP. Phase tomography will be used to image in vitro cultivated 3D ceramic bone scaffolds. The aim is to investigate the effects of microgravity on bone deposition, bone resorption and on vessel formation. Further, SR techniques and histology will be used to validate Optical Coherence Tomography (OCT) that will be implemented in a Biotechnology Mammalian Tissue Culture Facility (BMTC) bioreactor (OCT will be performed by Philipps University Marburg and Ruhr Universität Bochum). 20 samples will be prepared in the BMTC bioreactor, representing different biomaterials and culture conditions, and will be imaged with 5 µm pixel size at 30 keV energy using propagation based phase contrast imaging and holotomographic reconstruction. Four propagation distances will be used: ~0, 300, 450 and 900 mm.

5. Multi-scale analysis of cortical bone: 6 shifts

In previous works SR micro-CT has been successfully coupled to SAM in view of the modeling of bone elastic properties.

The global aim of this new project will be to assess the relationships between human cortical bone measured at the millimetric scale of ultrasound wavelength used to probe bone in vivo, and bone quality factors measured at the micro- and nanometer length scales. It will done in the context of the MULTIPS project (submitted ANR 2012) gathering five partners (partners 1,3 and Inserm U831, Lyon (Dr G Boivin), LIPhy UMR 5588 CNRS – U. Joseph Fourier, Grenoble, (A Gourrier), IFSTTAR (Dr D Mitton). "State-of-the art" technologies to carry out multiple length scale measurements of stiffness, toughness, micro and nano-porosity, mineral and organic structure. We intend to use a multimodal and multiphysic approach including resonant ultrasound spectroscopy (RUS), mechanical tests, SR micro and nano-CT, synchrotron quantitative scanning small-angle X-ray scattering imaging (qsSAXSI), Fourier-transform infrared microspectroscopy (FTIRM) and biochemistry.

Fourty specimen from human cortical femur (20 men and 20 women) will be prepared according to a precise protocol fully detailed in the project to provide samples for each techniques. SR imaging will be performed at two scales. The lower resolution imaging of the Haversian systems and mineralization (5µm voxel size) will be performed using $3x4x5mm^3$ samples subsequently to the RUS measurement. The high resolution imaging of osteocyte lacunae and canaliculi (0.28µm voxel size) will use $3x4x0.4mm^3$ samples cut using a high precision low-speed circular saw. Those multimodal and multiscale developments will provide a unique set of data which will serve as a basis to gain a better understanding on how bone structural properties are related to bone biomechanical competence.The elucidation of the relationships between all measured variables is also ultimately expected to enhance fracture risk prediction in major bone pathologies such as osteoporosis.

E) Conclusion

This project used the unique properties of SR micro-CT in terms of spatial and density resolution to obtain new data on bone tissue eventually coupled to other state of the art techniques. All the different sub projects have been achieved with a great rate of success that is confirmed by publications in per reviewed journals (23 published or in press and 5 submitted), 3 book chapters, 16 invited communications and 46 presentations in international conferences.

This LTP gathered a consortium of 11 partners who successfully worked together. One particularity of this consortium is that partners are working in complementary fields such as ultrasound, biomechanics, image analysis, bone biology and medicine. The limited number of partners allowed an efficient collaboration resulting in many common works.

One difficulty that we met was the perfect reproducibility of experimental conditions when a project had beamtime allocation at different times. Reproducibility requires setting up carefully the experiment, but the ID19 SR micro-CT setup is flexible, evolutive and some components like scintillator, optic lenses or X-ray windows can degrade throughout time. Thus the availability of the same component is not guaranteed leading to problems when merging data from different experiments. This is especially sensitive for projects requiring quantitative images for the assessment of mineralization (projects 4,5) or those requiring images at the limit of the resolving power of the system (projects 1, 2).

The new data or images acquired in the project motivated new research topics in data analysis and modeling like for instance phase retrieval methods, image segmentation of very thin structures like micro-cracks and canaliculi, 3D quantification of the Havers-Volkman system, biomechanical modeling and finite element simulation.

This research domain is still raising increasing interest in the national or international community. So the possibilities of continuation are wide on topics like the investigation of bone tissue at the nanoscale, the investigation of microvascularisation which is an essential process in pathology or reparation, the analysis of bone implants and bone materials, the problems of fracture healing and biomechanical modeling. There is a large demand on these topics from other groups at the European level. During the LTP, contacts were taken with various groups having expertise in bone biology, physics in life science, image analysis or biomechanical modeling, resulting in the proposal of new scientific projects.
The support obtained by the LTP in this area was very valuable since the unique properties of SR micro-CT were essential for the project.

F) **PUBLICATIONS RELATED TO SYNCHROTRON EXPERIMENT FROM THE USERS GROUP – 2009-2011**

PUBLICATIONS IN PEER REFERRED JOURNALS

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2. PEYRIN F., Investigation of bone with synchrotron radiation imaging: from micro to nano, Osteoporos Int, Jun 2009, vol 20, n° 6, pp. 1057-1063
3. HAÏAT G, PADILLA F, SVRCEKOVA M, CHEVALIER Y, PAHR D, PEYRIN F, LAUGIER P, ZYSSET P, Relationship between ultrasonic parameters and apparent trabecular bone elastic modulus: a numerical approach. J Biomech. 2009, vol 42, n° 13, 2033-2039.
4. LANGER M., CLOETENS P., PEYRIN F., Fourier-wavelet regularization of phase retrieval in x-ray in-line phase tomography, JOSA A, 2009, vol 26, n° 8, pp 1876-1881.
5. RUPIN F., SAIED A., DALMAS D., PEYRIN F., HAUPERT S., RAUM K., BARTHEL E., BOIVIN G., LAUGIER P., Assessment of Microelastic Properties of Bone Using Scanning Acoustic Microscopy : a Face-to-Face Comparison with Nanoindentation, Japanese Journal of Applied Physics, 2009, vol 48, p. (07GK01)1-6
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