# Co-registration of Synchrotron Radiation-microCT and micro-MRI images: a new method for the complete characterization of newly-formed bone

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**Purpose** Each year, the number of people undergoing maxillofacial surgery for dental implants is constantly increasing. The use of biomaterials scaffolds for bone regeneration/augmentation represents one of the most used strategies in dentistry to support prosthetic restorations. Bone substitute biomaterials (BSBs) should have specific biological and clinical peculiarities[1]. Often implant failure can be associated to the trabecular bone quality of the jaw, a very highly load-bearing organ [2, 3]. The trabecular bone quality is traditionally evaluated in-vitro with histology and 2D-optical microscopy [1,2]. Histological characterization remains the gold standard for the identification of different bone-phases, blood vessels and other biological microstructures [4], but traditional microscopy doesn't allow obtaining a 3D imaging of samples and, as recently demonstrated, it can also underestimate the size of biological microstructure[5]. Nowadays, 3D trabecular structures are often investigated by means of micro computed tomography. Nonetheless quantitative information about bone mineralization is still hardly obtained without the use of advanced and costly holo-tomography. We present here a 3D characterization of extracted jawbone cores based on co-registration of X-ray Synchtron Radiation-microCT (SRμCT) and micro-MRI (μMRI) techniques with new custom software [6, 7]. The combination of the two techniques enhances the possibility to obtain quantitative 3D characterization of extracted jawbone cores and to distinguish between different biological tissues.

**Methods** A bone sample was extracted to prepare the bone to host dental implant, from a site of human jawbone healed by biomaterial augmentation using Bio-Oss bioceramic (Geistlich, Bio-Oss®). SRμCT acquisitions were made at the SYRMEP@ELETTRA beam-line[1,8] using energy of 23KeV, with a pixel size of 9 μm. The same sample was investigated with $T_2$-weighted μMRI, because $T_2$ in heterogeneous systems [10,11] is affected by the magnetic gradients fields at the bone-liquid interfaces [9]. MRI measurements (voxel dimensions 18x18x200 $\mu m^3$) were carried out on Bruker Avance spectrometer operating at 9.4T , and by using a multislice multiecho sequence with four different echo times $T_E$ (4,8 ms;9,5 ms; 14,3 ms; 19,1 ms). A T2-map of the sample was obtained by a fitting of the intra-voxel signal with the function $S(T_E)=S0(\exp(-T_E/T_2^{APP}))$. $T_2^{APP}$ is defined by[11]: $1/T_2^{APP}=1/T_2+ACD\cdot(\gamma\cdot IMFG\cdot T_E)^2/12$, where $\gamma$ is the proton-gyromagnetic ratio, ADC is the apparent diffusion coefficient and IMFG is the effective internal magnetic field gradient. Despite the very different spatial resolution of the SRμCT and μMRI we obtained, with a new software[12,13], the superposition of the two imaged volumes. An histological evaluation of sample was also performed.

**Results and discussion** The rescaled SRμCT images show the presence of a blood vessel network inside the marrow spaces (pictured in light grey in figure 1.a). Newly microvasculature is expected as part of the healing process in both implant insertion and biomaterial application. Nevertheless, the contrast between bovine bone biomaterial and human bone is very low (white and light grey). $T_2$-weighted μMRI detects different phases in the 3D-bone core (figure 1.b): marrow, native-bone and newly formed bone, as assessed by quantitative analyses (from $T_2$ weighted and $T_2^{APP}$ maps) performed on Regions of Interest guided by the histological analysis (figure 1.c-d).

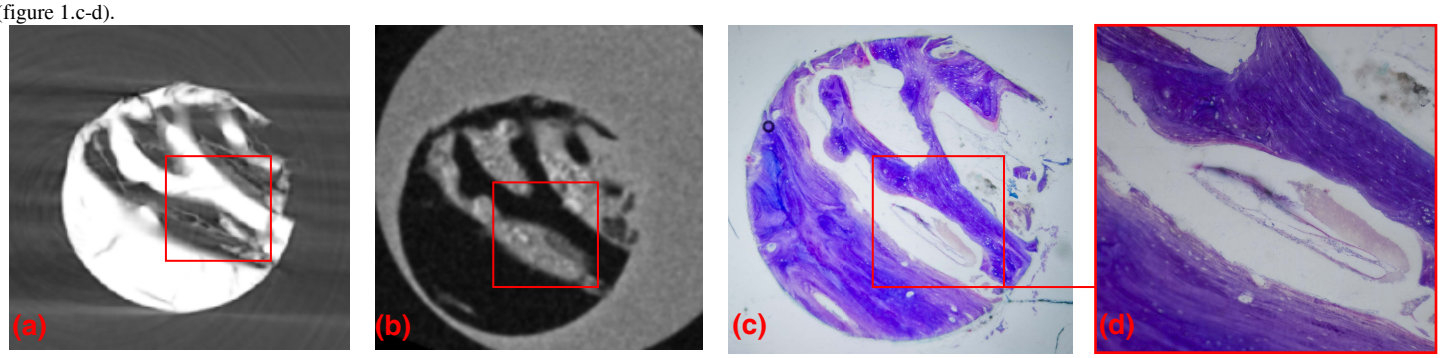


**Figure 1**: Slice of a bone core extracted from an augmented site, obtained with SRμCT (a) and with μMRI (b). Slice (a) has been resized to the μMRI resolution. An histological evaluation of the same slice (c) unambiguously revealed newly formed bone, pictured in light pink (d).

The ability of μMRI to distinguish between different bone phases is related to the interplay between NMR spin spin relaxation process and magnetic susceptibility differences at the interface between different tissues. This phenomenon depends on the degree of restraint tested by water molecules during their motion and hence on the degree of bone mineralization. Thus, μMRI revelead soft tissues and tissues with a high micro-porosity (low electron density not detected by SRμCT) in which the relaxation of partially confined water is highly influenced by magnetic susceptibility gradients. Also the interaction between water and biological macromolecules affects the $T_2$ relaxation time allowing detection of volumes possibly including microvasculature and multinucleate cells. However, the distinction between blood vessels and nucleate cells can be obtained from co-registered SRμCT. By this co-registration, conformation and thickness of bone are accurately defined by SRμCT and the degree of calcification is better recognizable in the μMRI structure allowing investigation of progressively more mature bone[14].

**Conclusions** Quality and contrast of SRμCT images are sufficiently good to reveal 3D-bone structure and blood vessel network inside the marrow spaces but doesn't allow distinguishing unambiguously between different degrees of calcification to quantify the human newly formed bone. μMRI data results to be very sensitive to different bone microstructure allowing investigation of progressively more mature bone[12]. Moreover μMRI confirms the presence of blood vessels in the bone marrow, microvasculature and multinucleate cells. Histological evaluation of this sample corroborates our results. In this context we propose to exploit μMRI investigation for the evaluation of goodness of biomaterial bone augmentation and as a method to assess local bone quality with a sub-millimeter resolution.

**References** [1] Komlev V S, Mastrogiacomo M, Pereira R C, et al. eCM Journal. 2010;19:134-146. [2] Petrie T A, Raynor J E, Dumbauld D W, et al Transl. Med. 2010;2(45):45-60. [3] David V, Martin A, Lafage-Proust M H , et al. Endocrinolgy 2006;148:2553-2562 [4] Traini T, Degidi M, Sammons R, et al.. J. Periodontol. 2008;79:1232-1240. [5] Deyhle H, Schmidli F, Krastl G, et al. Proc. of SPIE. 2010; 780417:1-9. [6] Sinibaldi R, Pecci R, Somma F, et al. Ann Ist Super Sanita. 2012; 48(1):42-48. [7] Sinibaldi R, Conti A, Pecci R, et al. JBGC. 2013; 3 (4): 85-91.[8] Sell C A, Masi J N, Burghardt A, et al. Calcif Tissue Int. 2005;76 (5):355-364. [9] Tenner M S, Spiller M, Koenig S, et al. Invest. Radiol. 1995; 30 (6): 345-353. [10] De Santis S, Rebuzzi M, Di Pietro G, et al. 2010; 55: 5767-5785. [11] Rebuzzi M, Vinicola V, Taggi F, et al. Bone. 2013; 57(1): 155-163.[12] Jung M , Lommel D, Klimek J.. Int Endod J. 2005; 38(9):617-626. [13] Edberg E L, Kroese-Deutman H C, Shih C K, et al. Methods [14] Potter K, Leapman R D , Basser P J, et al..J. Bone. Miner. Res. 2002;17(4):652-660.