# MULTIMODAL LABEL-FREE NONLINEAR OPTICAL MICROSCOPY ON MURINE CORTICAL BONE TO STUDY SKELETAL DISEASES

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Optical microscopy is an extremely powerful investigation tool in life sciences, thanks to its ability of visualizing morphological details in cells and tissues at the sub-micrometer scale. Nonlinear optical (NLO) microscopy offers additional advantages, such as inherent 3D-sectioning capability and/or label-free modality. In this context, we built an innovative multimodal label-free NLO inverted transmission microscope using off-the-shelf components. It operates in four different modalities: two-photon excitation fluorescence (TPEF), second-harmonic generation (SHG), coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) in the CH-stretching region (2500-3200 $cm^{-1}$) [1]. It displays sub-cellular (<1 μm) spatial resolution with very large (up to 50x50$mm^2$) field of view and high chemical selectivity. The setup is based on a compact multi-branch Erbium-doped amplified fiber laser, guaranteeing turnkey operation, and delivering pump pulses at 780 nm and tunable Stokes pulses in the 930-1060 nm range with ps duration. The microscope's potentialities have been tested by describing the biomolecular content of vacuoles in living human breast cancer cells for the study of iron deficiency [2].

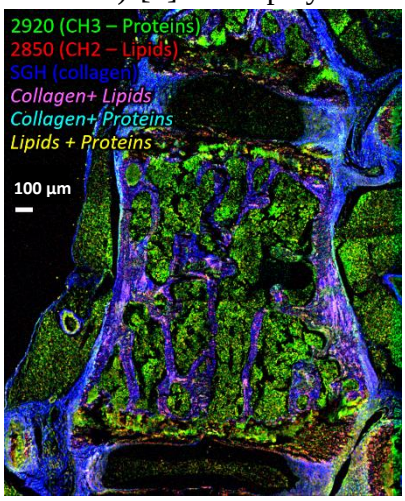


**Figure 1**: Multimodal hyperspectral image of WT murine vertebra section: SHG signal from collagen fibers (blue), SRS signals at 2850 $cm^{-1}$ Raman shift typical of lipids (symmetric $CH_2$ stretch, red) and at 2920 $cm^{-1}$ typical of proteins (symmetric $CH_3$ stretch, green). Image performed at 1 μm/pixel resolution and 5-ms pixel dwell time.

In this work, using a wild-type (WT) murine vertebra, we determined the ensemble of procedures and protocols to be used for the upcoming investigations on a dipeptidyl peptidase 3 knock-out (KO) murine model. Our aim is to further study and deepen the knowledge about the role of this enzyme in bone pathophysiology and maintenance of homeostasis [3]. The merged false-color images allow recovering qualitative and quantitative information on the chemical species present in the sample and their distribution. This technique is a powerful tool to compare the WT and KO models, quantifying related biological changes. The label-free, chemically selective, and noninvasive nature of this NLO technique constitutes a crucial resource for translational and clinical biomedical applications.

[1] Crisafi F. et al. *Spectrochim Acta A Mol Biomol Spectrosc* 2017; 188:135-14
[2] De Bortoli, Maida, et al. *J. Exp. & Clin. Cancer Res.* 37.1 (2018): 75
[3] Menale C. et al. *J. Bone Miner. Res*. 34, 2133-2148 (2019)