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JB.11 - Brillouin and Raman microspectroscopy: a new tool for the chemo-mechanical investigation of human bone and cartilage tissue diseases

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Human bone and cartilage are biological tissues characterized by a sophisticated architecture with a strict relationship between their structure, chemical composition, and mechanical performance. As a consequence, an impairment in even one of the sub-constituents at the micrometre level can lead to loss of function of the entire organ, causing the eruption of severe orthopaedic diseases. Osteoarthritis (OA) is a degenerative disease characterized by the progressive erosion of articular cartilage, which covers the surfaces of the long bones in the joints. It is caused by the establishment of inflammatory processes that affects all the joint constituents and subchondral bone, causing in the patient acute pain. Brillouin and Raman micro-spectroscopy is a correlative technique contact-less and not destructive, that allows the simultaneous investigation of both the mechanical and the chemical properties of samples, thanks to endogenous mechanisms, namely the propagation of thermally activated acoustic waves and the macromolecular vibrations. In recent years, this technique has approached applications in the biomedical field, successfully analysing the properties of single cells, complex biomaterials, and tissues affected by oncological and neurodegenerative diseases. Here, we present the results of mapping and imaging performed on both the cortical and trabecular tissue obtained from resections of a human femoral head and diaphysis. The results of the analysis of tissues in healthy conditions will be used to demonstrate the ability of the technique to recognize the major manifestations of osteoarthritic pathology on the cartilage surface and subchondral bone. This proof-of-concept not only constitutes a first step towards the application of the technique in the diagnosis of osteoarthritis but provides an approach that can be extended to other important issues in biomedical research on bone, such as bone infections and cancer.
Keywords: Brillouin spectroscopy, Raman spectroscopy, bone disease

JB-12. *In vivo* metabolic imaging and micromanipulation of individual filamentous fungus cells using different nonlinear laser scanning microscopy modalities

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Nonlinear laser scanning microscopy (NLSM), is an advanced optical technique that utilizes ultrashort laser pulses for structural and functional imaging, as well as laser manipulation of live organisms and cells. Two modalities of NLSM, two photon excitation fluorescence (TPEF) and third harmonic generation (THG) were applied for in vivo and label-free study of oxidative and lipid metabolism of individual cells of filamentous fungus Phycomyces blakesleeanus. Cell membranes and lipid droplets (LDs) are major sources of THG signal. TPEF allows us to determine the redox ratio (reflecting metabolic activity of cells) of the metabolic cofactors FAD and NAD(P)H autofluorescence. In addition, slight modifications of the experimental setup, mostly on software, enabled utilization of femtosecond laser pulses for precise cell microsurgery of hyphal cell wall. The optimized microsurgery procedure we than utilized to obtain protoplasts suitable for patch-clamp electrophysiological recording. Cell surgery of filamentous fungus Phycomyces blakesleeanus, were performed by ultrafast Ti:Sa laser (160 fs pulses). The same laser was used for in vivo autoTPEF imaging of NAD(P)H and FAD at different wavelengths. For in vivo THG imaging of label-free hyphae, we used 1040 nm, 200 fs pulses from Yb KGW laser. In vivo and label-free application of THG imaging enabled, accurately and reliably, detection of changes in distribution, total number, and size of LDs in control and treatment group of cells. Two-photon microscopy made it possible to obtain a redox ratio using autofluorescences of NAD(P)H and FAD in the same regions of live hyphae. The cell microsurgery procedure has been optimized and developed, which enabled the subsequent registration of currents on otherwise unaccessible membrane.
Keywords: nonlinear imaging, cell surgery, cell surgery. **Supported by:** Project HEMMAGINERO, No. 6066079 / Program PROMIS, Science Fund of the Republic of Serbia