

SHG, CONFOCAL AND TPE MICROSCOPY TO STUDY BONE FRAGMENTS.

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Goal of this communication is to report about a recent study on bone fragments obtained from biopsies of human patients of different ages and sex by means of three-dimensional microscopy. Samples were selected from 8 patients and classified by age into subgroups. Confocal and two-photon excitation microscopes allow getting three-dimensional information from thick samples like the ones we are dealing with bone fragments. Second harmonic generation (SHG) microscopy is coming into use as a tool for studying the distribution of collagen within the 700-1100 nm range. It is promising in characterizing collagen - both in distinguishing different collagen types and their packing and in identifying degradation of collagen in pathologic conditions. Even if many aspects of image formation in SHG microscopy of collagen remain not completely understood, we decided to couple SHG with confocal and two-photon excitation imaging^{1,2}. Morphological and functional properties of such bone fragments can be studied by exploiting autofluorescence mainly due to collagen content. For our studies it was mandatory the utilization of a scanning head endowed of spectral capability. Moreover, for both two-photon excitation and SHG imaging we needed a tunable ultrafast laser source. The spectral ability was crucial for following the SHG signal and to track changes of the autofluorescence due to composition of the samples themselves. We used a Leica SP2 AOBS spectral system and a Chameleon XR laser source. Two-photon excitation fluorescence was collected in a de-scanned mode while SHG was captured both in the forward and in the backscattering direction³. Even if collagen of different type is the major source of our signal, we are also considering the role of advanced glycation end-products (AGEs) formed in long-lived matrix proteins by a non-enzymatic reaction with sugar. It was demonstrated that AGEs have an influence on the activity of different organs, such as brain (Alzheimer's disease, dementia), vessels (arteriosclerosis), bone resorption (osteoporosis). We observed an increase of autofluorescence in those bone fragments belonging to the old subgroup compared to the autofluorescence of young patients. So far, we speculated that this effect could be due to the increasing concentration of the AGEs products that must be higher in the osteoporitic subject.

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2) Cox G., Kable E., Jones, A., Fraser, I., Manconi, F., Gorrell M.D. (2003) 3-Dimensional imaging of collagen using second harmonic generation. Journal of Structural Biology, 141, 53-62.

3) Diaspro A., et al. (2002) Functional Imaging of Living Paramecium by means of Confocal and Two-Photon Excitation Fluorescence Microscopy. Proc. of SPIE, 4622, 47-53.