Raman Spectroscopy and AFM for Physico-chemical Imaging of the "en face" Aorta in Liquid and Air

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Raman imaging spectroscopy and Atomic Force Microscopy (AFM) were used to study the physical and chemical properties of the tissue samples. The Raman chemical images were correlated with AFM topographic information taken from the same sample area. The combination of both methods provides the complementary data about the properties of the studied biological system (Fig. 1A).

The research was carried out for the mouse vascular wall in "en face" preparation mode. The presented maps were recorded using WITec alpha 300 system. The main technical issue of the study was the way of the biological samples fixation. The efforts are paid to preserve the physico-chemical features of the tissue. Here, the analysis of the mouse vascular wall was carried out for fixed sample (formalin) in air and for non-fixed sample in the phosphate buffered saline (PBS) solution. Both approaches enable to obtain different information, which can be correlated with the chemical changes in the sample undergoing upon fixation and drying (Fig. 1B). The main differences are observed especially in the amide III band, in skeletal mode of polysaccharides and O-P-O stretching.

Presented here multimodal Raman imaging combined with AFM offers a great potential in biomedical studies to be explored further.
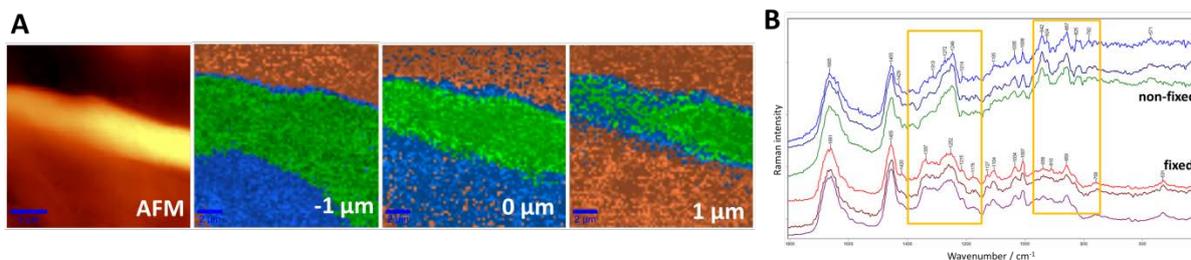

Fig. 1 (A) The AFM topography compared to the Raman depth profiling of the „en face" fragment of the fixed aorta. The chemical images were prepared using K-means cluster analysis. (B) The comparison of the average spectra from the whole maps obtained for fixed and non-fixed samples.