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P71 - Elemental distribution and sample integrity comparison of freeze-dried and frozen-hydrated biological tissue samples with nuclear microprobe

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The analysis of biological samples in frozen-hydrated state with micro-PIXE technique at Jožef Stefan Institute (JSI) nuclear microprobe has matured to a point that enables us to measure and examine frozen tissue samples routinely as a standard research method.

Cryotome-cut slice of frozen-hydrated biological sample is mounted between two thin foils and positioned on the sample holder. In first experiments, silicon nitride windows were used [1] and later replaced by polymer foils stretched over aluminium frames. In this way, we avoid the Si background peak from the acquired PIXE spectrum, which now enables us to detect trace elements in the low energy range from F to Cl. In addition, the use of polymer windows results in less demanding sample mounting procedure on the sample holder. The temperature of the cold stage in the measuring chamber is kept below 130 K throughout the insertion of the samples and the proton beam exposure. Matrix composition of frozen-hydrated tissue is consisted mostly of ice. Sample deterioration during proton beam exposure is monitored during the experiment.

The aim of this experiment was to determine differences and similarities between two kinds of sample preparation for micro-PIXE analysis, namely freeze-dried and frozen-hydrated sample preparation.

In the presented work, a standard micro-PIXE configuration for tissue mapping at JSI was used with five detection systems operating in parallel, with proton beam cross section of $1.0 \times 1.0 \mu m2$ and a beam current of 100 pA. The comparison of the resulting elemental distributions measured at the biological tissue prepared in the frozen-hydrated and in the freeze-dried state revealed significant differences in elemental distribution of particular elements at the cellular level due to the morphology alteration in particular tissue compartments induced by water removal in the lyophilization process.

[1] Vavpetic et al., Micro-PIXE on thin plant tissue samples in frozen hydrated state: A novel addition to JSI nuclear microprobe, NIM B, Beam interactions with materials and atoms, [Print ed.], 2013, DOI: 10.1016/j.nimb.2012.12.035

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