

Dear Editor,

Please find enclosed our manuscript entitled " **Biological samples preparation for speciation at cryogenic temperature using high-resolution X-ray absorption spectroscopy** " that we would like to be considered for publication in Journal of Visualized Experiments. This paper highlights a detailed protocol to prepare cryo-samples (here, cancer cells and planktons) for synchrotron-based X-ray absorption spectroscopy (XAS) experiments. This a comprehensive detailed protocol that aim researchers to study a cryo-preserved cellular preparation kept under anoxic conditions for the presented cases for selenium and iron speciation in cancer cells and phytoplankton respectively using High Energy Resolution Fluorescence Detected X-ray absorption spectroscopy. This protocol can be extended to other biological samples and other X-ray techniques for which radiation damages can be induced under irradiation.

We consider of value publishing these data in Journal of Visualized Experiments, as cellular preparation for synchrotron X-ray absorption spectroscopy studies are quite specific and is a key step to get robust and reliable results particularly when studying the fate of some compounds in biologic samples.

The techniques presented in this paper and demonstrated in video format will be highly useful for researchers working in the field of synchrotron but more generally to all using high-resolution analytical techniques for elemental speciation for which critical care should be taken to avoid any chemical changes under intense X-ray photons flux.

S. Bohic, O Proux, C Bissardon and MP Isaure designed the procedures described in the manuscript. *S Bohic, Bissardon, O. Proux, E. Lesuisse, E Lesuisse, M Rovezzi, and E Lahéra* performed the experiments and analyzed the data. Finally, *S. Bohic, O. Proux , MP Isaure and C. Bissardon* wrote the manuscript and all authors discussed the results, assisted in the preparation and contributed to the manuscript. All authors approved the final version of the manuscript.

During the preparation and submission of this manuscript, we have been kindly assisted by *Benjamin Werth*.

Thank you for your consideration of this manuscript. We look forward to hearing from you.

Sincerely yours,

Sylvain BOHIC