

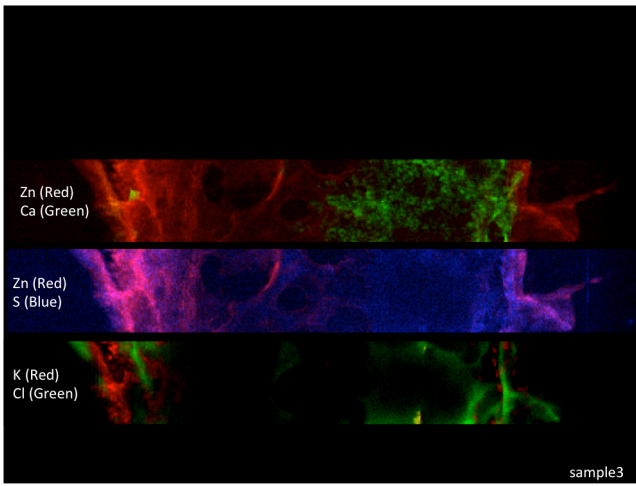


## Experiment Report Form

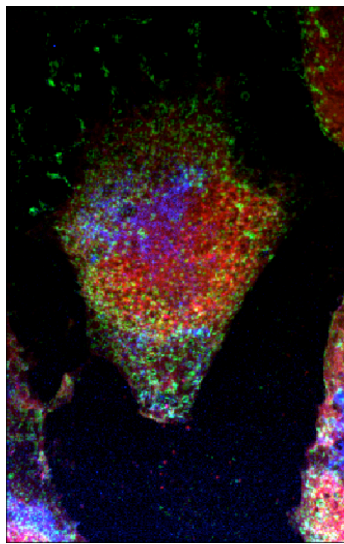
	<b>Experiment title:</b> Role of the TRP (Transient Receptor Potential) family channels in tumorigenesis: tissue metal distribution as a biomarker in defining therapeutic target.	<b>Experiment number:</b> LS-2630
<b>Beamline:</b> ID-16B NA	<b>Date of experiment:</b> from: 13/04/2017 to: 18/04/2017	<b>Date of report:</b>
<b>Shifts:</b>	<b>Local contact(s):</b> Remi Tucoulou, Vanessa Isabel TARDILLO SUAREZ	<i>Received at ESRF:</i>
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**Report:** The project (LS-2630) was designed to evaluate metal distribution in breast cancer cells with a different degree of aggressiveness. We previously established that the cationic channel TRPM7 is overexpressed in breast cancer cells, and it is further overactivated in the cells that acquired resistance to therapy. As a result, intracellular free Zn becomes elevated in specific micro-domains affecting ratios between free and bound ions, and we suggested preferential presence for Zn in subcellular compartments following our preliminary confocal imaging experiments. This imbalance would impact on the activation of growth signaling pathway in cancer cells and thus potential outcome of the disease. Furthermore, the tumor hypoxic microenvironment affects Zn homeostasis. As such, hypoxia during episodes of sleep apnea in aging population would further serve as a risk factor of cancer progression.

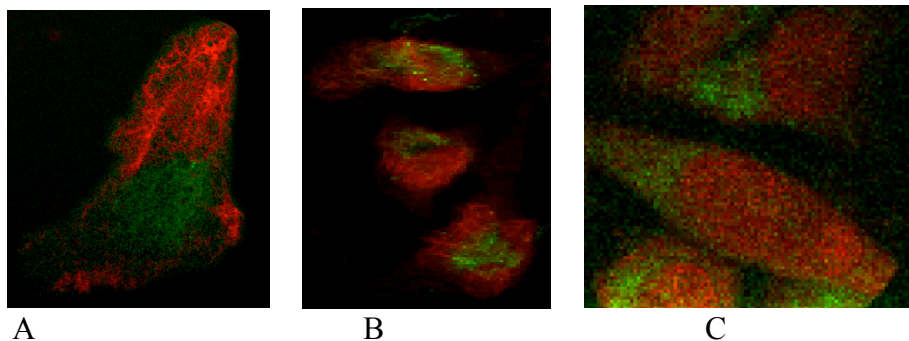
To test our hypothesis we prepared by freeze-dry technique the samples of breast cancer cells MDA-MB-231 expressing different variants of TRPM7 channel that appear to reflect changes of the channel during cancer development. In addition, the cells were subjected to cycles of intermittent hypoxia according to the protocol developed at HP2 laboratory at the Universite Grenoble Alpes. At these conditions, Zn is released from the cells and overall Zn homeostasis is affected. We subjected 5 samples per each condition to x-ray irradiation to image metal distribution at 400 nm resolution. In addition, a few samples were scanned at 100 nm resolution to obtain fine details of metal localization, and two scans were performed on slices taken out of pre-scanned cells at 50 nm resolution.



**Fig. 1.** Element distribution (as indicated) shown for cell area from plasma membrane toward cell nuclear at 50 nm resolution.



**Fig 2.** Whole cell 100 nm resolution image of Zn (red) Fe (blue) and K (green) in migrating MDA-MB-231 cell.



**Fig 3.** Synchrotron X-ray imaging of the cell expressing wild type TRPM7 (A), cells expressing mutated channels in the kinase domain (B), and the cells A subjected to IH (C). Zn – red, Fe – green.

not allow to estimate change in element concentration. However, these analysis are very helpful in determination of relative element presence, e.g. the ratios of distribution within the same zone. The elements like S or K will be most relevant as references. On the other hand, given interplay between Zn and Cu their ratios may provide interesting insights. The changes in the ratios can serve as indication of underlying biological processes related to channel activity.

**Conclusion :** the experiment delived valuable data that is being analyzed in comparison with biochemical data and will be prepedated for publication. Certain information could not be obtained in the setting of this experiment. More studies are needed to overcome the limitations related to sample tickness and preservation of native state of the cells. We would like to enhance the study by using the ID 16A beam line to reconstruct

Despite our report is of preliminary nature, and quantification of the data is on the way, there are several novel and original finding that are highlighted below. 1) First, the high resolution images clearly show heterogeneous distribution of elementss in subcompartments and microdomains (Fig.1) Cleraly, Zn and Ca localization is complimentary while undertsandibly Zn and S overlap. Cl and K are very distinct showing no clear co-localization but proximity.

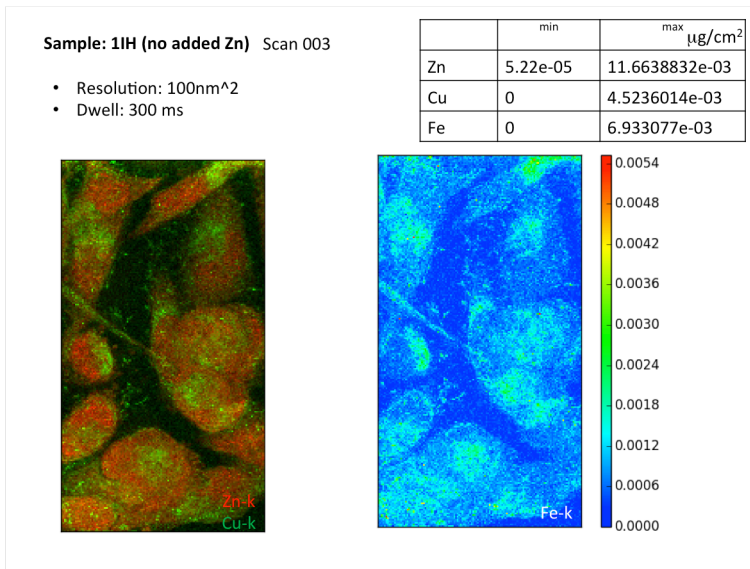
2) The cell culture was represented by both proliferating and migrating cells specific for MDA-MB-231 cancer cell line. Interestingly, Zn localized mostly to the nuclear compartment in both cell types was also detected in the tail of the migrating cell (Fig. 2). This result serves a

direct visual proof of specific physiological function of Zn in cell migration.

3) Our preliminary biochemical data demonstrate that the kinase portion of TRPM7 can be modified and even cleaved, particularly in the condition of oxidative stress. Fig. 3 demonstrates that both mutation in the kinase domain of the channel protein and IH treatment likely produce similar effect on metal distribution within cancer cells.

Two elements, Zn and Fe are compared demonstrating more overlap between in the cells with mutated kinase or subjected to IH as compared to control non-treated cells expressing wild type protein. It should be noted that while a high resolution image (100 nm) shows fine details, certain damage occurred during the sample preparation involving a freeze drying procedure. We thus believe that the quality of the images will be improved using intact frozen samples which can be analysed using the ID16A beam. In addition, it is not evident where the nucleus is localized in this cell. Thus the 3D scan will help to answer this question.

4) Quantification of the scanned samples was performed using AXO standard. Since the thickness of the samples was unknown, the concentrations are expressed in areal density ( $\mu\text{g}/\text{cm}^2$ ). Fig. 4 shows the example of calculated images with corresponding scale bars. While calculations performed on the 2D images provide absolute quantity data, they do



**Fig. 4** Quantitative images of Zn Cu and Fe 2D distribution in MDA-MB-231 cells.

3D image in order to determine the change in element concentration following oxidative stress or mutation in the TRPM7 protein. Combined with obtained results the new data will allow to obtain clear and full information on the quantitative and spatial distribution of elements, in particular Zn, Ca, Fe, Cu, and S. On the protein level, we will determine the functional significance of these changes by overlaying signature metal maps and the intracellular distribution of TRPM7.