ESRF	**Experiment title:** *Correlative microscopy of the intracellular calcium compartments in stress condition of Hepatitis C viral infection*	**Experiment number**: LS 2693
Beamline: ID16ANI	**Date of experiment**: from: 31/01/2018 (8:00 am) to: 05/02/2018 (8:00 am)	**Date of report**: 28/02/2018
Shifts: 15	**Local contact(s)**: Sylvain Bohic	*Received at ESRF:*

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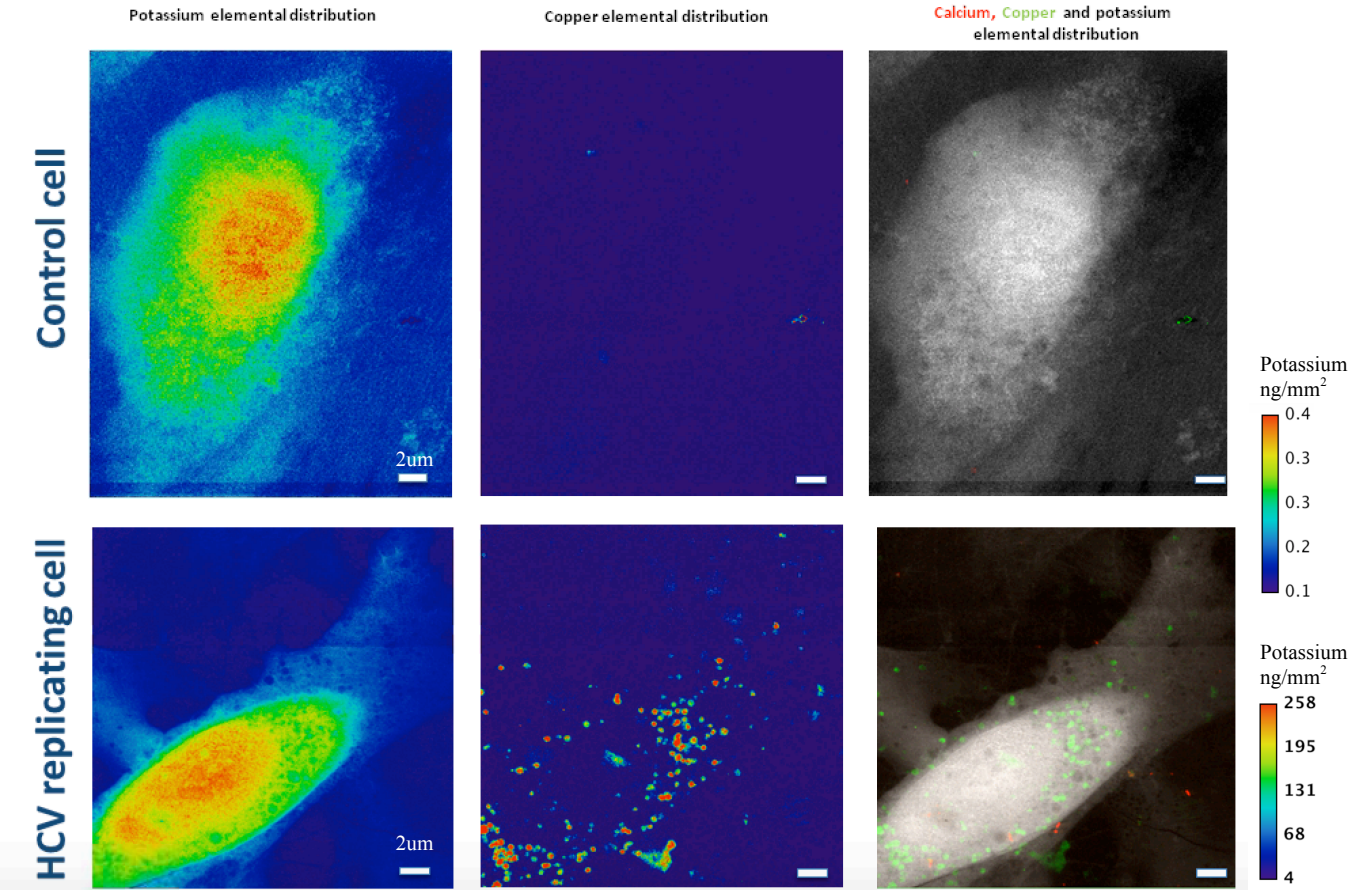
Report:

The experiments focused on the investigation by correlative microscopy of intracellular calcium compartments in stress condition of Hepatitis C viral infection by soft X-ray cryo tomography (cryo-SXT) and nanoscopic X-ray fluorescence imaging (nano XRF). For this, 4 SiN round windows of 3 mm diameter and 50nm thickness (one control sample and 3 samples of HCV infected cells) were first measured at MISTRAL beamline (ALBA) to obtain 3D cellular maps at 50 nm resolution. Unfortunately, only one of those windows corresponding to control hepatocytes could be loaded at ID16a succesfully without breaking. As we knew that the manipulation of those thin windows was technically difficult and no appropriate holder was available at ID16a at that time, we also prepared samples on the usual 5mm × 5mm thicker membranes (200 nm) that are commonly used at ID16a although this prevents the correlative approach with soft X-rays. All windows were measured by cryo-epifluorescence at Alba prior to the ESRF beamtime, so we do have correlative cryo-epifluorescence and XRF measurements.

The collected measurements are summarized in the following table:

Samples	Cryo Soft X-Ray Measured	Cryo-Epifluorescence Measured	Number of 2D maps of cells by nano XRF
HCV replicon-harbouring cells	No	Yes	10
HCV replicon-harbouring cells	No	Yes	4
Control cells	No	Yes	9
Control cells	Yes	Yes	5

We have collected 5 2D maps of control hepatocytes and 4 2D maps of HCV replicating cell at low resolution (200nm) and 9 and 10 respectively at high resolution (70nm). Our cryo-SXT results of infected HCV cells have revealed the presence of aberrant mitochondria, lacking visible cristae, displaying matrix condensation as well as budding of mitochondria-derived vesicles and local ruffling of mitochondrial membrane and accumulation of electron-dense material at the interphase[1]. HCV protein expression is sufficient to cause mitochondrial dysfunction by the induction of ER stress. Unfortunately, little is known about the presence during the infection of specialized structures in the ER or mitochondria responsible of Ca^{2+} influx. The goal of correlating cryo-SXT with cryo-XRF was to allow understanding the role of Ca^{2+} in the mitochondria through mitochondria-associated ER membranes (MAMs). Unfortunately, without the 3D cellular maps, it is not possible to associate specific Ca signal to any cellular organelle. We expected to detect Ca^{2+} intracellular signal in the HCV infected cell during the viral replication, but we did not expect to have Cu signal as seen in the figure. Cu signal was not present in control cells, but only in HCV replicating cells. We are now trying to understand biochemically this surprising result.



In future experiments we plan to successfully correlate cryo-SXT and cryo-XRF by designing a specific holder for SiN windows that can be used at both beamlines. This correlative approach is mandatory to understand the Cu and Ca localization within specific cellular organelles in infected cells.

1. Perez-Berna, A. J.; Rodriguez, M. J.; Chichon, F. J.; Friesland, M. F.; Sorrentino, A.; Carrascosa, J. L.; Pereiro, E.; Gastaminza, P., Structural Changes In Cells Imaged by Soft X-ray Cryo-Tomography During Hepatitis C Virus Infection. *ACS nano* 2016, *10* (7), 6597-611.