Correlative cryo-soft X-ray tomography of cells

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Structural cell biology demands detailed structural and functional descriptions of the different cellular components which must be correlated with a topological 3D map of these components at the whole cellular level. Among the imaging techniques available, visible light microscopy has a privileged position due to its availability at laboratories worldwide and the possibility to image live. However, only the tagged features are being visualized, therefore producing a limited picture of the system. On the other hand, transmission electron microscopy (TEM) allows cell visualization at a higher spatial resolution, thus revealing ultrastructural cellular details. But it requires cellular sectioning as the multiple scattering of electrons imposes a restrictive sample thickness limitation. Complex protocols are therefore required for sample preparation; and overall, whole-cell visualization by TEM is complicated as stacked 2D sections imply difficult and time-consuming experiments. Even though the abovementioned visible light and electron microscopy techniques are broadly used, the correlation of information they provide is hindered firstly by the existing resolution gap in between them and secondly by the different sample thickness range each of them can handle. In this frame, an emerging technique such as cryo soft X-ray tomography (cryo-SXT) (Schneider et al., 2010) can provide structural information at the level of a whole cell without further sample preparation except for the cryo-fixation required to prevent radiation damage while collecting the data. The penetration power of soft X-rays in the so-called *water window* spectral range, between the inner-shell absorption edges of carbon and oxygen (from 284 to 543 eV), allows penetrating water layers of up to 10 μm thickness while carbon-rich structures are

visualized with good absorption contrast (Wolter, 1952). Thus, frozen-hydrated specimens can be imaged close to their native state providing significant complementary and quantitative information to existing biological imaging techniques at a spatial resolution of 40 nm.

After introducing cryo-SXT with respect to the other biological imaging techniques, the standard correlative workflow used at synchrotron beamlines was explained as follows. The cells of interest are usually grown or deposited on to holey carbon EM grids. Once satisfied with the cell confluency and the probability of finding the interesting feature within the cells, the grids are plunge-frozen in liquid ethane. Finally, all vitrified grids are screened with an epifluorescence microscope offline and at cryo-temperatures prior to the X-ray imaging to select the best grids. Only the selected grids will be loaded into the MISTRAL transmission soft X-ray microscope (TXM) (Sorrentino et al., 2015) at the ALBA synchrotron light source (Spain), keeping always the grid in vitrified conditions. During the screening, regions of interest (ROI) are localized using visible light fluorescence for correlative purposes. Indeed, the relevant cellular event, molecule or structure tagged with a fluorescent dye will help in locating again the same ROI with the X-rays. Although, the standard correlative approach using visible light microscopy, mainly epifluorescence or confocal (the last one, prior to vitrification), has a poor spatial resolution, new high-resolution correlative approaches have started to be exploited. For instance, combining super resolution visible light techniques such as stochastic optical reconstruction microscopy (STORM) or cryo-structured illumination microscopy (SIM), or even hard X-ray fluorescence allows correlative information in *X*, *Y*, and *Z* at similar spatial resolution between volumes.

After presenting the correlative workflow at the beamline, a number of different correlative studies were presented, dealing with cell infections or the localization of particular structures within the cellular volume. A first example focused on hepatitis C virus-infected hepatocytes (Pérez-Berná et al., 2016) was shown to illustrate the potential of cryo-SXT to visualize structural modifications at the level of whole cells during the viral

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replication. An example on *Plasmodium falciparum*-infected red blood cells (Varsano et al., 2016) was also presented illustrating the capability of combining cryo-SXT and hard X-ray fluorescence to shed light on the possible crystallization pathway of heme. Studies on the early cholesterol crystal formation in macrophage-like cells (Varsano et al., 2016; Varsano et al., 2018) bringing together STORM and cryo-SXT was also explained showing the coexistence of two different crystal structures depending on the nucleation site within the cell. Another example on nanoparticle-cell interactions, concretely of superparamagnetic iron nanoparticles uptake by MCF7 cells (Chiappi et al., 2016; Conesa et al., 2016), was presented to demonstrate the utility of the spectroscopic X-ray capabilities of SXT which allowed quantifying the nanoparticle uptake per cell with a sensitivity corresponding to one nanoparticle. Finally, a last and promising example combining cryo hard X-ray fluorescence tomography at 70 nm with cryo-SXT was presented showing that it was possible to localize precisely a particular Ir metallodrug within MCF7-treated cells only in specific cellular organelles (Conesa et al., submitted).

Finally, the last part of the lecture focused on how to improve the data collected in cryo-SXT. First, correcting for the point spread function of the optical system (Otón et al., 2016); secondly by reducing the missing wedge inherent to flat samples (such as in electron tomography) by performing dual axis tomography, and finally by doing multifocal data collection to extend the depth of field (Otón et al., 2017) limited by the objective lens used (the Fresnel diffractive lens) in soft X-ray microscopes. Overall, cryo-SXT was unknown to the students that attended the summer school prior to the lecture, and I hope that they have had a flavor of the possibilities it brings to cellular imaging, as well as its complementarity to well-known techniques such as electron microscopy and visible light microscopy.

Compliance with ethical standards

Conflict of Interest Eva Pereiro declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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