doi:10.1017/S1431927622005463
Microsc. Microanal. 28 (Suppl 1), 2022

Synchrotron Radiation and Laser Light Microscopy Partnership for the Study of Biological Systems: The Case of Soft X-ray Tomography and Structured Illumination Microscopy at Cryogenic Temperatures

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CryoSXT offers us the opportunity to image cells and cell populations in 3D to a resolution of 25 nm (**Figure 1**). This places SXT well within capacity to deliver clear cellular imaging and, given the sizeable fields of view achieved (anywhere between 10 to 20 μm), document ultrastructure through swathes of intracellular and pericellular space in 3D. SXT imaging is done through absorption contrast in the water window of X-ray light where carbon-rich biological structures obstruct light as it passes through them as opposed to the oxygen-rich surrounding medium that does not. As a result, the impression left on a detector when an image is taken is a negative projection of the cell structure exactly like in the case of medical X-ray imaging. In fact, in concept and optical implementation this method is the cellular equivalent of a full body CT scan in a medical setting only adapted for the microworld of cells. Because imaging depends on cellular content alone, there is no requirement for contrast enhancing chemicals to be added and therefore the information captured has not been altered in any way by foreign material. An additional benefit comes from the fact that to help cells endure sustained exposure to X-ray light during imaging we snap freeze them before use, thereby perfectly preserving the exact instant of their lives that is of interest without altering any of the structures that we see.

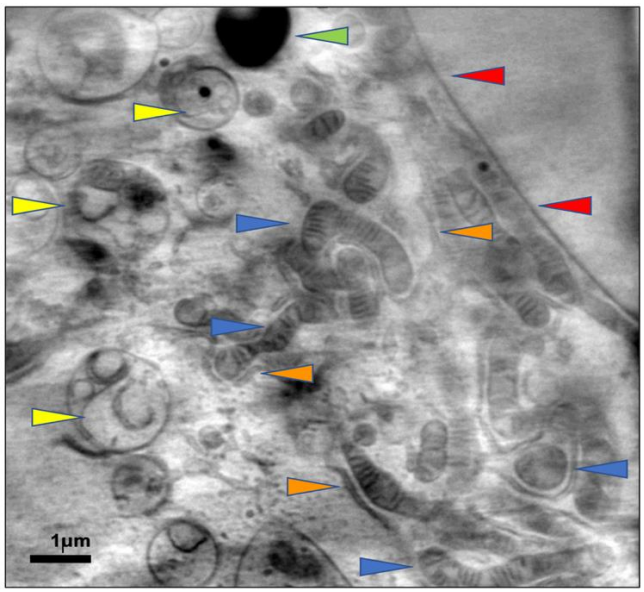

Figure 1. Projection through a human bone osteosarcoma fibroblast; field of view (10x10 microns) captures a cytoplasmic perinuclear area full of organelles and a small part of the nucleus (top right). Projection collected on B24 at 500 eV using a 25 nm objective zone plate. Arrows point to selected organelles; blue – mitochondria; green – lipid droplets; orange–endoplasmic reticulum; yellow–multivesicular bodies; red – nuclear membrane.

Using cryoSXT we capture cellular ultrastructure that is faithful to the native conformation of the fully hydrated intracellular world in 3D. The next challenge we face is decorating this 3D map of life at the cellular level with chemical information pertinent to the distribution of biomolecules of interest such as the proteins within. Therefore, correlative imaging of the same sample across techniques and scales is both a necessary and rewarding proposition but also a challenging one. Collecting data on the same sample at different microscopes while preserving all its delicate structures intact and associating these data to a rich and meaningful correlated volume is a real challenge.

B24 is the correlative cryo-imaging beamline at the UK synchrotron, Diamond Light Source where two high resolution imaging systems have been developed side by side to enable the in-depth interrogation of biological systems at near physiological states in a semi-automated and user accessible platform [1]. The two imaging methods are: cryoSXT which uses the natural absorption contrast of hydrated biological material, such as cells and tissues and cryo-fluorescence structured illumination microscopy (cryoSIM) which records localisation of tagged molecules, organelles and other cellular structures within the cellular map captured through X-ray imaging [2]. The attraction of this combination is that samples that are due to be used for X-ray imaging can be used first to generate 3D fluorescence information at high resolution on identified areas of interest before taken to the transmission X-ray microscope allowing for the accumulation of directly correlated localisation data meaning that the same sample is imaged sequentially avoiding sample to sample variations and therefore enabling the unambiguous interpretation of data.

The imaging pipeline we have described has allowed us to investigate pathogen-host interaction at the cellular level (example shown in Figure 2), drug-induced cellular landscape remodelling, cell-to-cell killing mechanisms, contaminant clearance and many other areas of biomedical importance [3]. A compendium of these results will be presented alongside particulars regarding the use of CLXT for their requirements.

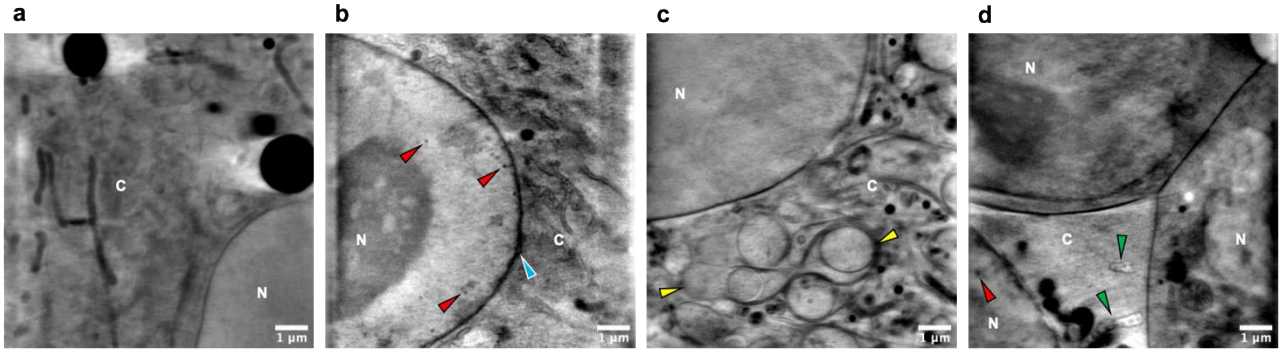

Figure 2: CryoSXT used to study the replication of MDV in primary and T-lymphoma cells. (a), Uninfected chicken embryo fibroblast (CEF) cells isolated from 9-day old chicken embryos. (b), CEF cells infected with the vaccine strain of MDV serotype-1 (CVI988/Rispens) showing the capsids formation (indicated by red arrows) in the nucleus 4-day post-infection and the budding event taking place on the outer nuclear membrane (indicated by the blue arrow). (c), CEF cells infected with the vaccine strain of MDV serotype-3 (HVT) showing remodelling of endoplasmic reticulum (ER) and formation of multivesicular bodies (indicated by yellow arrows) in the cytoplasm 4-day post-infection. (d), T-lymphoma cell line derived from testes of a line P chicken infected with MDV serotype-1 (pRB-

https://doi.org/10.1017/S1431927622005463 Published online by Cambridge University Press

1B5) showing the formation of capsids (indicated by red arrow) in the nucleus and the transport of MDV capsids in the ER lumen (indicated by green arrows) in the cytoplasm. (a-d) The cytoplasm is represented by letter C and nucleus is represented by letter N.

A new and powerful tool for correlative high resolution 3D imaging of cells at near-physiological states will be presented here alongside its many applications through new biological findings that have been facilitated through its application.

References:

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