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Laboratory Cryo Soft X-ray Tomography Reveals Cellular Ultrastructure at the Nanoscale

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Analysis of three-dimensional biological cell samples is critical for understanding the mechanisms of viral disease and for the development of novel therapeutics. Soft X-ray microscopy (SXM) is the unique technology that can image whole intact cells in 3D under normal and pathological conditions without labelling or fixation, at high throughput and spatial resolution [1-4]. The main challenge of Soft X-ray microscopy is that the photonic illumination required for imaging has heretofore only been available at synchrotron labs [5] and only a very small fraction of the infectious disease research community has been able to access this imaging modality.

SiriusXT has developed a lab-scale soft X-ray microscope for fast and inexpensive three-dimensional imaging of whole cells that can be readily performed in a laboratory. The capabilities of this compact imaging device, combined with complementary light and electron microscopy approaches, is currently being demonstrated through a series of virology use cases to generate new scientific knowledge on the viral life cycle and host cell response to viral infection [6].

Soft X-ray microscopy uses X-rays in the 'water window' that extends from the K-absorption edge of carbon to the K-edge of oxygen, that is from about 282 eV (λ = 4.4 nm) to 533 eV (λ = 2.3 nm). Water is transparent to these X-rays, but organic molecules are absorbing. Therefore, these X-rays can be used as the basis for microscopy of whole cells in their near-native (frozen) state, without need for any contrast enhancing agents. A 3D tomogram with resolution between 25 nm to 60 nm (full pitch) is produced by rotating the cell over a range of angles, with an image acquired at each tilt angle [7]. The concept is equivalent to a medical CT scan applied at the nanoscale and similar to Hounsfield units in medical CT, cellular organelles within the cell can be discernible from each other by their respective x-ray linear absorption coefficient values.

Our studies will allow demonstration of the benefits of the lab-based system relative to both synchrotron based SXM as well as other imaging modalities. However, the most comprehensive view of the complex structure of cells is unlikely to come from a single microscope. Much effort is now invested in combining SXM with other imaging methods, in particular cryogenic fluorescence microscopy. We will facilitate correlation of SXM with light and electron microscopy by integration of a fluorescence microscope, dual modality of sample presenting scheme, including EM grids, and an automatic data analysis pipeline.

Hybrid imaging with lab based SXM microscopy and super-resolution FM enables correlation of fluorescent data with 3D whole-cell images, at up to 20-50nm resolution [8]. This allows identification of virus infection-induced structural changes of the host cell. Based on these findings, the specific regions of interest can be further examined by EM at 1nm resolution. Moreover, cryo correlative workflows could be developed to allow imaging of the same cell by all three modalities SXM, cryo-EM

and Light microscopy. In the first instance, this could be done without any additional sample machining by using cryo-EM to image only the outer 'thin' regions of the sample and SXM to image the much thicker cytoplasmic and perinuclear region. The lab SXM will be commercially available by the end of 2022. In this paper we will present our SXM workflow and tomography data from virus infected cells, along with complementary data from other modalities.

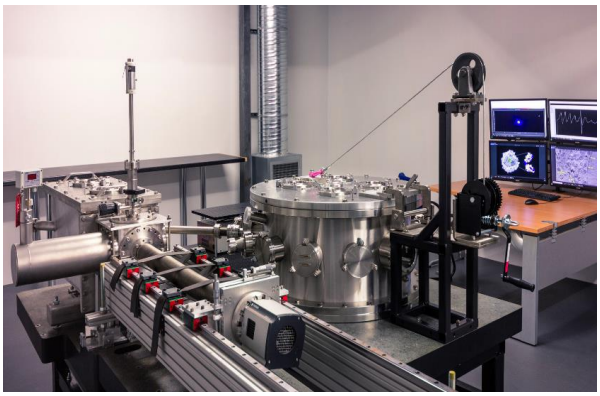

Figure 1. The SiriusXT compact Soft X-ray microscope, with an overall footprint of 3m x 2m.

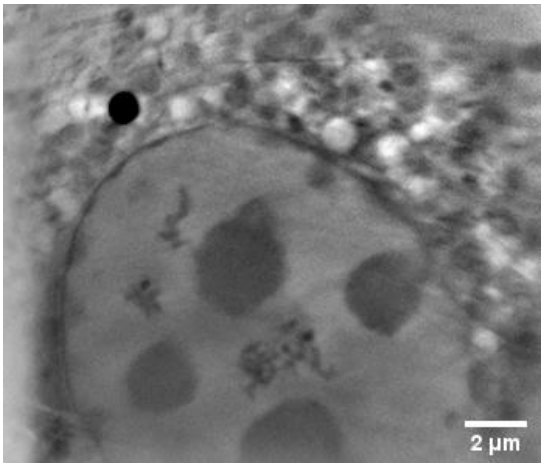

Figure 2. A slice through a reconstructed tomogram of a NIH-3T3 cell acquired on the lab-scale device. The cells are prepared on standard TEM grids, plunge frozen, and imaged over an angular range of ±55° in 1° increments. In this slide nuclear structure is clearly visible as well as lipids and other organelles in the surrounding cytoplasm. The image contrast is entirely natural due to the nature of the 'water window' illumination used.

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