Showcasing report for the SXT-100 soft X-ray microscope

Facility name: SiriusXT
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Technology offered: Soft X-ray Tomography
Technology description: Analysis of three-dimensional biological cell samples is critical for understanding the mechanisms of viral disease and for developing novel therapeutics. Cryo soft X-ray tomography (cryo-SXT) 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. The main challenge of cryo-SXT is that the photonic illumination required for imaging has heretofore only been available at synchrotron labs.

SiriusXT was founded as a company to develop and commercialise a laboratory-scale Soft X-ray Microscope (the SXT-100) that is to be used by scientists working in disease research and drug discovery to produce high resolution 3D images of the whole internal structure of biological cells. The core IP in the microscope, which allows it to be located in a laboratory, is a compact soft X-ray illumination source that was developed by SiriusXT's co-founders prior to the formation of the company. The microscope is now routinely imaging cryo prepared biological cells for external partners, and these studies are showcasing how the SXT-100 adds value to the research work being carried out by these organisations.

The SXT-100 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

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(frozen) state, without need for any contrast enhancing agents. A 3D tomogram with resolution of ~50 nm (full pitch) is produced by rotating the cell over a range of angles, with an image acquired at each tilt angle. The concept is equivalent to a medical CT scan applied at the nanoscale, revealing the morphology, interactions and complexities of subcellular organelles. The SXT-100 will greatly benefit research projects that need the following information;

- Whole cell information
- Complex 3D networks
- Volumetric information
- Compositional information
- Rare events

Cells are prepared on standard 3.05mm TEM grids, with roll-out of a dual capability to accept in-suspension cells in ultra-thin-walled glass capillaries expected by the end of Q1 2023. FEI AutoGrids can also be accepted as a sample carrier, thus facilitating the establishment of integrated multiscale hybrid microscopy methods that could benefit by combining cryo-SXT with light and electron microscopy techniques. Integration of cryo-SXT into CLEM imaging workflows offers a powerful new method for 3D imaging of whole cells and tissue. While cryo-SXT can quantitatively image fully hydrated, intact cells at about 50 nm resolution, TEM generates high-resolution (~5 nm) data from small regions of interest of cells. Combining these two powerful imaging techniques with data from fluorescence microscopy will provide a more informative picture e.g. by placing the functional imaging into the 3D context of a whole cell structure mapped at different resolutions.

The SXT-100 has been routinely imaging partner samples since early 2022 while located at SiriusXT's own lab facilities. By the end of Q1 2023 a 'pilot' microscope will be commissioned at the local Conway Institute of Biomedical and Biomolecular Sciences, University College Dublin, from where the company will continue to operate an imaging service. In parallel the company has built and fully certified its first commercial product, for launch in 2023. The SXT-100 full specification can be found in Appendix I.

Access protocol and data formats: SiriusXT has developed a means (equipment and procedures) for inspecting the quality of cryo-prepared grids that have been received from customers who have the ability to cry-prepare cells themselves. When receiving cryo-grids direct from customers, the grids are shipped in 5-litre 'Dry Shippers' as shown in figure 1. These containers will hold grids at Liquid Nitrogen temperatures (-195 °C) for typically 12 to 15 days, which is sufficient time to ship the container from one location to another. The Dry Shippers are approved for air transportation and are shipped in an external box to protect them from damage.

Figure 1. Sample dry shipper

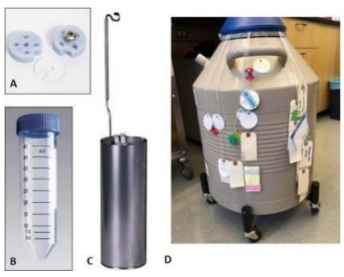

Figure2. Grid storage.

Once the Dry Shipper arrives at SiriusXT, the cryo-grids are transferred to a larger Liquid Nitrogen storage tank (50 litre capacity in our case), as shown in figure 2. Here the grids can be stored for long periods of time once any evaporated Liquid Nitrogen is replenished. The grids are held in small cartridges that fit into falcon tubes for safe keeping during transit, but which are then stored in cryoEM pucks to allow assignment of a unique location ID to each cartridge. Up to 24 cartridges can be stored in each puck, with pucks stored vertically in sample-holding

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canisters. Puck location and sample information is then stored in SiriusXT's sample database. Before soft x-ray imaging the cryo-grids are typically inspected with a Fluorescence Microscope to identify the best cells for imaging.

The location of targeted cells are noted by their grid reference on special 'Finder Grids' (see Fig 3) so that when the grid is inserted into the soft x-ray microscope, the targeted position can be directly accessed.

A customer will typically ship between 1 and 4 grid boxes (4 and 16 grids) to SiriusXT. In some cases they will have been able to inspect the quality and note the position of suitable cells before shipment. Whether they do or don't, SiriusXT typically will inspect all incoming grids to ensure that the grids are still vitrified and nothing has gone awry during transportation. To date, SiriusXT has inspected over 100 of such customer-supplied grids on a cryo-fluorescence microscope. SiriusXT has also cell culture and sample vitrification tools available at its facility.

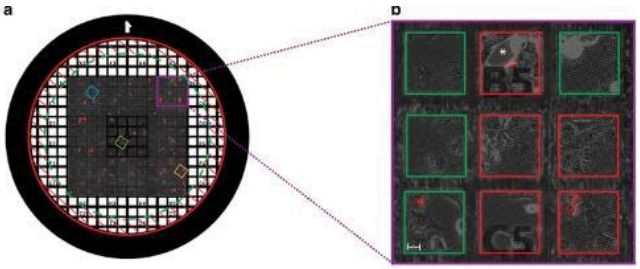

Figure3. Identification of cell locations using fluorescence (Carzaniga et al, Protoplasma (2014) 251:449–458).

Data pipeline: A data pipeline has been fully established for grid-based datasets, and this has been integrated into the overall SiriusXT control and data management architecture and UI (Fig. 4). From image acquisition to final 3D construction many of the steps are now fully automated, and we are continuing to build on these developments with the ultimate goal of a single 'push button' workflow. Currently, data acquired by the SXT-100 is automatically saved to the SiriusXT local server datastore, and also transferred (incl. metadata) to a virtual machine responsible for data analysis. Using a web interface, the specific dataset to be analysed is then user selected, and the processing activated (a fully automated alignment and reconstruction routine). The analysed data (e.g. 3D image reconstruction) is then automatically saved to the local datastore as well as to a DropBox directory shared with

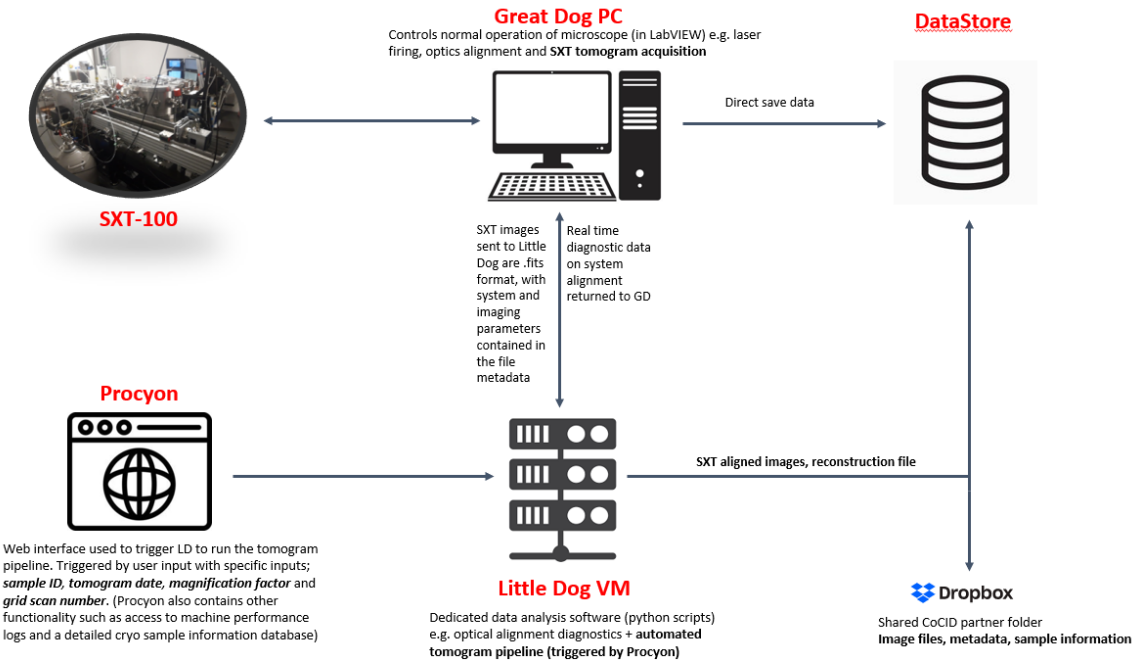

Figure 4. SiriusXT data workflow, highlighting the integration of the automatic data pipeline for SXT image analysis.

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project partners. Appropriate metadata and sample information/directory is included alongside the image data, so that users can easily link the data to the specific sample provided (Fig. 5).

Data sharing: Dropbox is used to share imaging data with partners, with the overall structure, tomogram list file and metadata outlined below.

```
/Partner Restricted
    CoCID
        CoCID_WP2_UHEI
            SXTSAM_123456
                area01
                    sxtsam123456_partnerGridID_areapos1_metadata.txt
                    sxtsam123456_partnerGridID_areapos1_tlt.mrc
                    sxtsam123456_partnerGridID_areapos1_FF.mrc
                    sxtsam123456_partnerGridID_areapos1_ang.rawtlt
                    sxtsam123456_partnerGridID_areapos1_tlt_ali.mrc
                    sxtsam123456_partnerGridID_areapos1_SIRT30.mrc
                    sxtsam123456_partnerGridID_areapos1_SIRT30_bin2.mrc
                area02
                    sxtsam123456_partnerGridID_areapos2_metadata.txt
                    sxtsam123456_partnerGridID_areapos2_tlt.mrc
                    sxtsam123456_partnerGridID_areapos2_FF.mrc
                    sxtsam123456_partnerGridID_areapos2_ang.rawtlt
                    sxtsam123456_partnerGridID_areapos2_tlt_ali.mrc
                    sxtsam123456_partnerGridID_areapos2_SIRT30.mrc
                    sxtsam123456_partnerGridID_areapos2_SIRT30_bin2.mrc
            SXTSAM_123470
            SXTSAM_123510
            tomogram_list.csv
        CoCID_WP6_CSIC
        CoCID_WP7_UCD
        CoCID_WP8_UKHD
        CoCID_WP9_JYU
    CZI
    MSCA-xxxx
```

areapos – imaged area position on a finder grid, e.g. "H51_01", "H51_02", etc.
if non-finder grid: "area01", "area02", etc.

Contents of **tomogram_list.csv**

```
SiriusXT sample ID , Partner sample ID , tomo location , partner-specific data
SXTSAM_123456      , Omicron sample 1  ,   H51_01      ,   A549, 4% PFA 1.25% GA , Omicron, …
SXTSAM_123456      , Omicron sample 1  ,   H51_02      ,   A549, 4% PFA 1.25% GA , Omicron, …
SXTSAM_123470      , Control, fixed    ,   B34_01      ,   A549, 4% PFA 1.25% GA , … , …
SXTSAM_123510      , Control, unfixed  ,   I22_01      ,   A549, … , …
```

Contents of **sxtsam123456_partnerGridID_areapos1_metadata.txt**

```
Image pixel size:               20 nm
Tomogram exposure:              2.48 hr
Detector pixel size:            13.5 um
Detector binning:               1
Magnification:                  675

Zone plate:                 35 nm ANT
MLM S/N:                    ABC001
Laser S/N:                  DEF002
Target run-time (tomo start, tomo end):    (2.02, 5.30)

Alignment:                  automatic
Reconstruction:                 SIRT with 30 iterations

Exposure per frame:             15 s

Frames per tilt:
10 11 11 11 10 10 10 10 9 9 9 8 8 8 8 8 8 8 8 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
8 8 8 8 8 8 8 8 8 9 9 9 9 9 10 10 10 10 11 11 12 12
```

Figure 5. Metadata and file listings shared with customer.

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User projects: To date SiriusXT has had visits from ten different disease research organisations to view its imaging facilities. Twelve different cell types, provided by seven different imaging groups, have been imaged with very positive feedback being received as to the quality of the images produced. In particular, the capabilities of the SXT-100, 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 in an EU H2020 project called CoCID https://cocid.eu/. Open access to data and published papers from this project is provided through the CoCID community on ZENODO https://zenodo.org/communities/cocid/?page=1&size=20.
An overview of cells provided by CoCID partners is shown below (Fig. 6), followed by a more detailed description of each of the four Scientific Use Cases that utilise the SXT-100 to help understand how the structure and function of cellular organelles are affected by viral infection and by anti-viral drugs. CoCID is a 4-year project which commenced in January 2021 and will run until 31 December 2024.

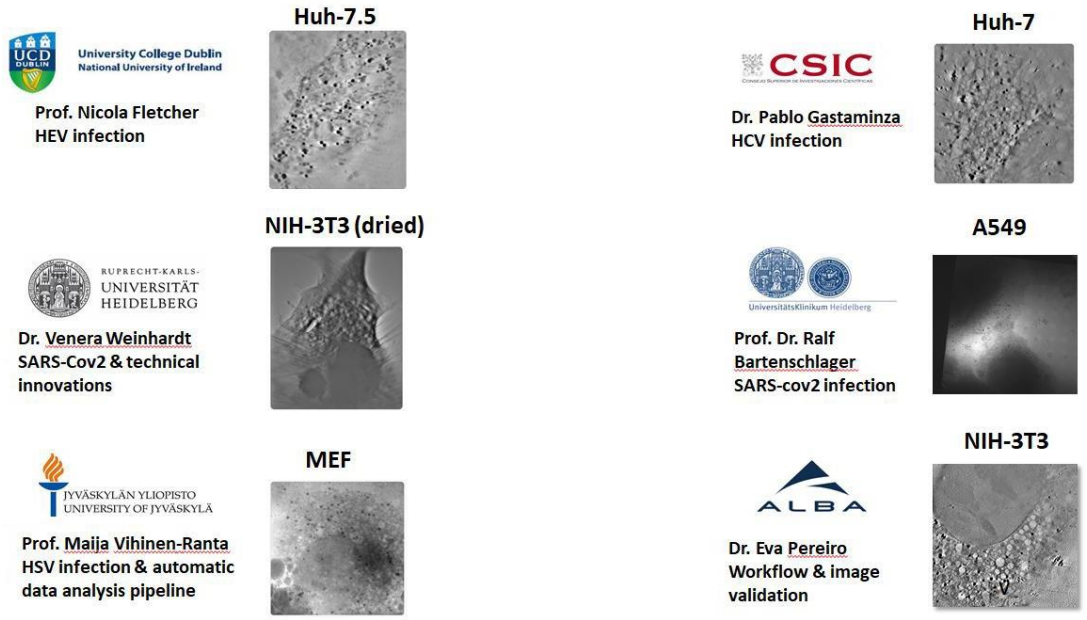

Figure 6. An overview of the samples imaged in the SXT-100 provided by CoCID partners, with representative 2D projection images also shown.

Scientific Case #1

Dr. Pablo Gastaminza, Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC), Madrid, SPAIN

Description: Chronic HCV infections induce structural alterations of the mitochondria and endoplasmic reticulum. These changes, caused by active HCV replication, can be reversed by a prolonged treatment with clinically approved direct-acting HCV antivirals, for example, a combination of sofosbuvir and daclatasvir. Our goal is to understand how virus-induced changes in cellular structures are reversed after pharmacological elimination of viral

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machinery from the host cell. SXM imaging at the ALBA synchrotron has shown that various antiviral drugs lead to differential efficiency in the reversion of the structural alterations in HCV-infected cells. To gain further insights into the mechanisms of cellular processes that govern the recovery of cellular components, we will use lab-scale SXM and advanced data analysis of HCV-replicating cells in the presence of different inhibitors of cellular proteostasis pathways during antiviral treatment.

Latest results: Both control and Hepatitis-C infected Huh-7 cells were cryo prepared at CSIC and shipped by dry shipper to SiriusXT for imaging. In particular the partner is using the SXT-100 as a platform to investigate the structural alterations to the mitochondria and ER in HCV infection. Preliminary analysis has revealed many structural alterations to the infected cell cytosol compared with non-infected control cells (Fig. 7). These changes are consistent with those observed in a similar study performed at the ALBA synchrotron soft X-ray microscope in the recent past. In the next phase of this study, cells exposed to direct acting antivirals will be shipped to Dublin to investigate the potential reversion of these structural changes.

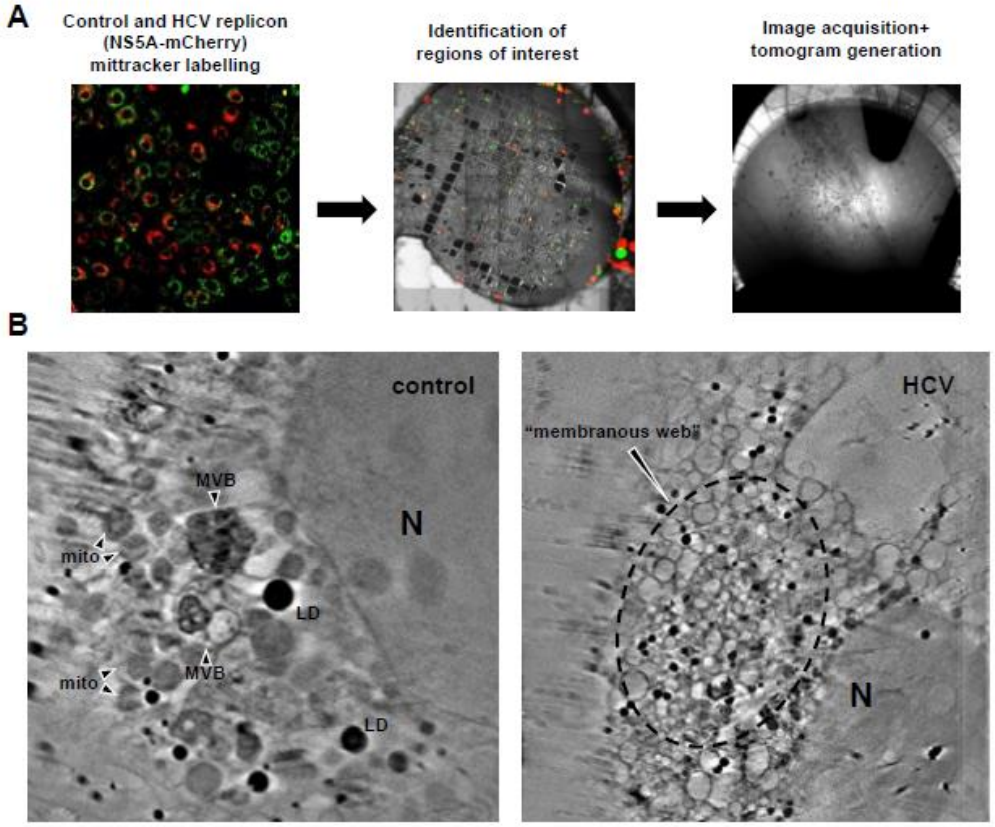

Figure 7: The figure above shows SXT-100 images of HCV replicon cells that were shipped to SiriusXT for imaging. A panel: The HCV replicons were prepared with a fluorescent reporter for viral replication (red channel) and mitochondrial staining (green channel) in order to help identify cells of interest. Cells of interest were identified in the SXT-100 integrated fluorescent microscope immediately prior to cryo-SXT imaging.
On the B-Left panel tomogram section of control cells where nucleus (N), mitochondria (mito), lipid drop-lets (LD) and multivesicular bodies (MVB) were segmented and observed. On the B-Right panel tomogram section showing a characteristic "membranous web" colonizing most of the cytoplasmic area, next to the nucleus (N) were segmented and observed.

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Project related publications:

- Garriga, D.; Chichón, F.J.;Calisto, B.M.; Ferrero, D.S.; Gastaminza, P.; Pereiro, E.; Pérez-Berna, A.J. Imaging of Virus-Infected Cells with Soft X-ray Tomography. Viruses **2021**, 13, 2109. https://doi.org/10.3390/v13112109
- Ana J. Perez-Berna et al. _ Reversion of hepatitis C virus-induced cellular alterations Acta Cryst. (2021). D77, 1365–1377

Expected publications: *Time-resolved ultrastructural assessment by TEM and cryo-SXT of the reversion of morphological alterations during and after virus elimination*. A full description of the ultrastructural events occurring during elimination machinery will include functional studies of the underlying mechanisms and will showcase the benefits of using the SXT-100 in this research.

Scientific Case #2

Prof. Nicola Fletcher, School of Veterinary Sciences, UCD Dublin, IRELAND

Description: Due to the lack of in vitro model systems that support robust HEV infection, few studies have attempted to elucidate the infection steps, i.e., viral entry, replication, and assembly. Here, we will use recently developed model systems for HEV, such as pig liver tissue and 3D organoids generated from human cell lines, to recapitulate the uniquely polarized nature of hepatocytes. We will visualize sites of HEV replication and assembly in pig and human polarized and non-polarised cell lines, and confirm our findings using HEV-infected liver slices by lab-scale SXM combined with other imaging techniques, such as electron and light microscopy. Imaging with SXM will bridge the gap between previously applied microscopy techniques, and will serve as an interface for correlative studies. These correlative studies, in combination with human and pig hepatocyte systems, will allow us to perform a full analysis of the HEV lifecycle and the effect of antiviral therapeutics and inhibitors on HEV infection.

Latest results: Collection of Huh 7.5 SXM data has been performed by SiriusXT. Suitable cells identified during live fluorescence and cryo-fluorescence screens guide the imaging process, being prioritised for SXM projection screening (*Fig. 8*) followed by tomogram collection of suitable cells and subcellular areas.

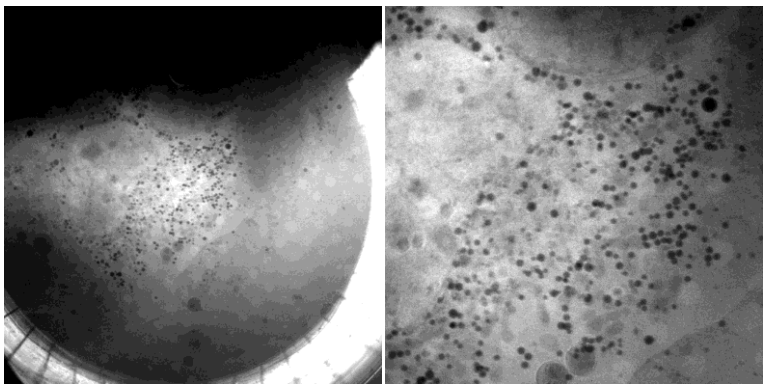

Figure 8 .SXT-100 projections of control Huh 7.5 cells, (left) low magnification, (right) high magnification.

Tomograms of control Huh 7.5 cells have been aligned, reconstructed, and analysed in collaboration with SiriusXT. Pixel classification was used for segmentation of all organelles apart from the nucleus which used carving (Ilastik). Segmentations of nucleus and prediction maps of other organelles were exported for further analysis and smoothed and volume thresholded for display as models in

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volume renders. Analysis of Huh 7.5 data, led by SiriusXT, has identified several organelles including mitochondria, lipid drops, endoplasmic reticulum, vesicles and putative lysosomes and endosomes. Internal structure including cristae can be seen in several mitochondrial examples (*Fig. 9.*).

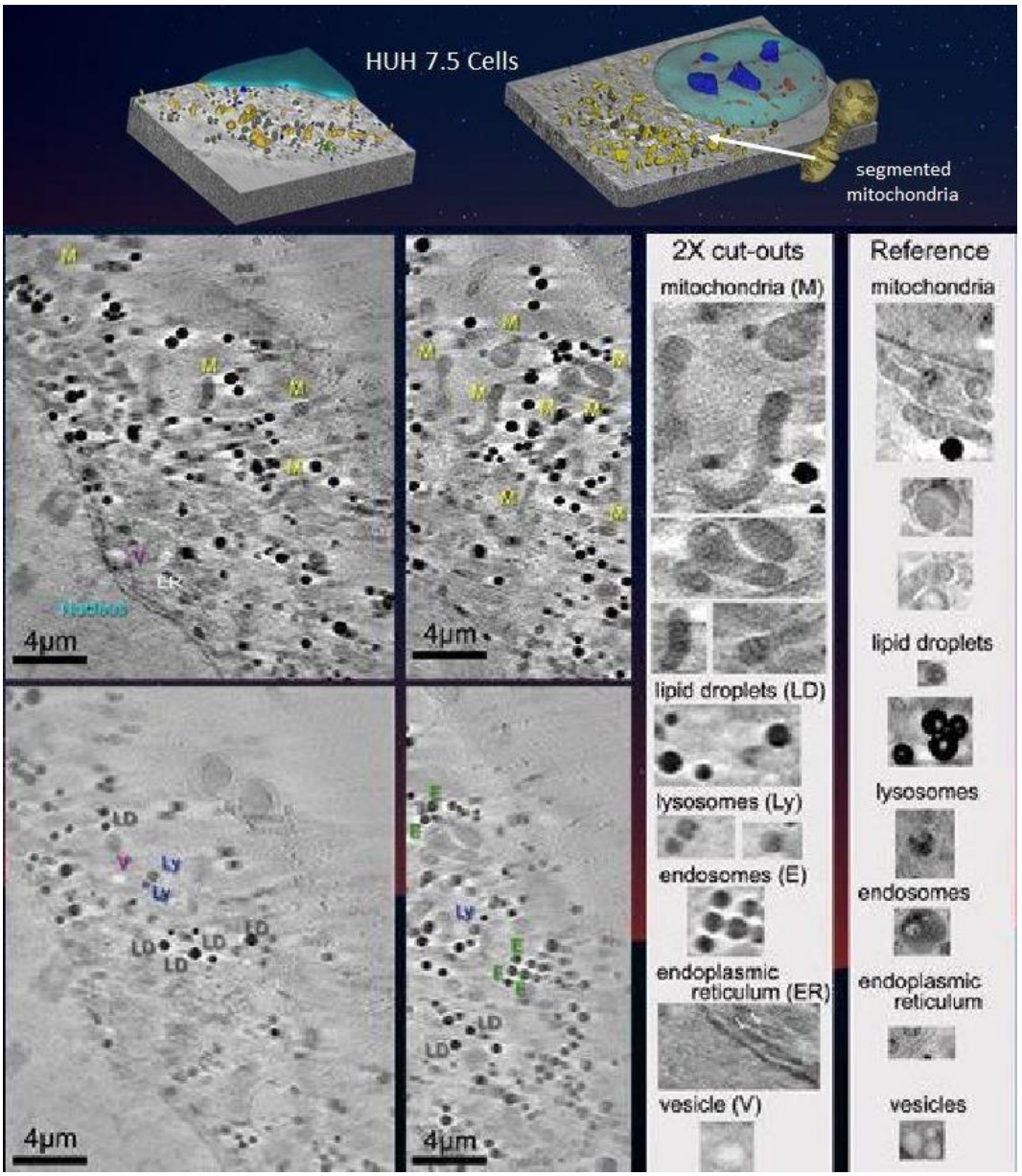

Figure 9. SXT-100 tomogram of control Huh 7.5 cells adapted from SiriusXT analysis. Reference images are from A.J. Pérez-Berná et al., ACS Nano 2016, 10, 7, 6597–6611 & J. Groen et al., Biophysical Reviews (2019) 11:611–619.

Current ongoing work includes optimising the electroporation of full-length, HEV Genotype 1 and 3 viral RNA sequences into Huh 7.5 cells. Once full-length HEV virus infection in human cell lines is confirmed and characterised, these transfected cells will be fixed at various time points during the viral lifecycle and investigated with correlated immunofluorescence and (immuno-) scanning transmission electron microscopy. These

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fixed samples will be correlatively imaged using the SXT-100 along with light and electron microscopy.

In vitro transcribed, selectable, HEV viral RNA replicons have been prepared and will be electroporated into hepatocytes to increase the percentage of transfected cells within a population. This will give a greater yield and number of options in downstream experiments. Genotype 1 and 3 replicon transfected cells will also be imaged with the SXT-100. Structural changes will again be investigated and correlated with light and electron microscopy data.

Expected publications: Scientific data obtained using the complementary imaging techniques (light, electron and soft X-ray microscopy) will be published covering the following key objectives:

- Structural differences in HEV-infected pig and human cell lines.
- Imaging of HEV-infected ex vivo porcine precision cut liver slices.
- Structural changes in HEV-infected cells treated with antivirals.

Scientific Case #3

Prof. Dr. Ralf Bartenschlager, Dept. of Molecular Biology, University Hospital Heidelberg, GERMANY

Currently, little is known about how SARS-CoV-2 exploits cellular resources to achieve high-level virus propagation. Moreover, it is also unclear to what extent disease severity is linked to the direct cytopathogenity of the virus versus an inflammation-dominated immune response. First insights into these questions have mostly been gained using cell lines, but the physiological relevance of the results obtained remains to be determined. Moreover, it is unclear whether host cell perturbations induced by SARS-CoV-2 differ between cell types, which calls for validation studies in physiologically relevant (ideally 3D cell culture) models. This set of questions calls for a multi-scale and correlative imaging approach, from the whole cell to the subcellular and even molecular levels. We will combine recently established integrative imaging approaches with advanced lab-based SXM and automated segmentation based on machine learning to establish a 3D model for the intracellular modification of SARS-CoV-2 infected cells. Quantitative and spatiotemporally resolved analyses of the virus-induced morphological changes of infected cells will reveal new principles of cellular homeostasis and its perturbation by SARS-CoV-2 infection. These studies will provide important information about viral and cellular targets that might be suitable for the development of antiviral therapies.
Latest results: Preliminary cryo-SXT imaging with the SXT-100 has revealed major cell changes caused by Omicron and Wuhan strains of SARS-cov2 in A549 cell lines. This work is continuing with regard to unambiguously identifying all structures present in the cytosol, such as potential sites of viral replication, and comparing this data to previous cryo-SXT work done at NCXT Berkeley as well as room temperature FIB-SEM. Ongoing work will continue to leverage the power of cryo-SXT in following the progression of other viruses such as dengue virus (DENV) in Huh7 cells. These cells are highly permissive for DENV and have a cell morphology that is well suited to both capillary and grid-based cryo-SXT. Based on immunofluorescence data, samples are being prepared for SXT imaging at 24 and 48 hours post infection.

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Project related publications:

- Cortese, M., Lee, J. Y., Cerikan, B., Neufeldt, C. J., Oorschot, V. M. J., Köhrer, S., Hennies, J., Schieber, N. L., Ronchi, P., Mizzon, G., Romero-Brey, I., Santarella-Mellwig, R., Schorb, M., Boermel, M., Mocaer, K., Beckwith, M. S., Templin, R. M., Gross, V., Pape, C., … Bartenschlager, R. (2020). Integrative Imaging Reveals SARS-CoV-2-Induced Reshaping of Subcellular Morphologies. *Cell Host and Microbe*, *28*(6), 853-866.e5. https://doi.org/10.1016/j.chom.2020.11.003
- Loconte et al., Using soft X-ray tomography for rapid whole-cell quantitative imaging of SARS-CoV-2-infected cells , 2021, Cell Reports Methods 1, 100117 November 22, 2021 https://doi.org/10.1016/j.crmeth.2021.100117

Expected publications: Scientific data obtained using the complementary imaging techniques (light, electron and soft X-ray microscopy) will be published covering the following areas:

- SARS-cov2 virus-induced changes in cytosol structure using lab-based soft X-ray tomography.
- Cryo Correlative Light, Electron and Soft X-ray Imaging of virus infected cells.

Scientific Case #4

Dr Maija Vihinen-Ranta, Dept. of Biological and Environmental Sciences, University of Jyväskylä, FINLAND

Since intranuclear chromatin reorganization is potentially crucial for the nuclear egress of capsids it is essential to understand how the infection induces changes in chromatin. This is important from the perspective of basic research into virus–cell interactions, and also for the improvement of oncolytic HSV-1-mediated virotherapy, which requires effective cellular release and the transduction of newly formed viruses to neighbouring cells. Little is currently known about the mechanisms of virus-induced changes in the chromatin architecture and the mobility of viral capsids in chromatin. Here, we will employ a strategy integrating 3D soft x-ray imaging of chromatin remodelling, fluorescent microscopy, and advanced data analysis and modelling in order to study the HSV-1-induced changes in the molecular organization of host chromatin in more detail.

Latest results: The sample preparation was performed in Jyväskylä (Finland). Mouse embryonic fibroblast (MEF) cells were grown and infected with herpes simplex virus type 1 (HSV-1) containing fluorescent fusion protein (HSV-1 ICP4-EYFP) on the carbon quantifoil finder grid. The grids were frozen using Leica EM GP2 Cryo-Plunge. The cells were then monitored using a microscope with the INSTEC cryo-stage coupled with a long working distance objective lens for fluorescence imaging. After this preliminary microscopy screening grids were sent in the dry-shipper to ALBA (Synchrotron MISTRAL beamline, Barcelona, Spain) or Sirius XT Ltd (Dublin, Ireland) for SXM imaging. Host chromatin distribution, compaction, and the presence of low-density regions or channels in noninfected and infected were investigated by soft X-ray microscopy (SXM) both in ALBA (3 times) and in Sirius XT (several times). The images show significant changes in intranuclear localization of chromatin in infected cells.

Furthermore, the role of the mitochondria during viral replication is being investigated, and a range of complementary techniques, including cryo-SXT, are being used to reveal how HSV infection alters the structure and function of mitochondria. SXT imaging combined with other imaging techniques and advanced data analysis indicated that both structure and function of mitochondria are significantly changed in HSV-1 infected cells. Specifically, SXT and

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other imaging analyses showed the mitochondria become elongated in infected cells. The analysis of the SXT data is in process.

Project related publications:

- Aho V, Salminen S, Mattola S, Gupta A, Flomm F, Sodeik B, et al. (2021) Infection-induced chromatin modifications facilitate translocation of herpes simplex virus capsids to the inner nuclear membrane. PLoS Pathog 17(12): e1010132. https://doi.org/10.1371/journal.ppat.1010132
- Salminen S, Ruokolainen V, Aho V, Gupta A, Leclerc S, Shav-Tal Y, Garini Y, Vihinen-Ranta M. Impact of lamin A/C induced chromatin organization or chromatin targeting drugs on herpesviral capsid mobility in late infection (manuscript in preparation)
- Aho V, Mattola S, Vihinen-Ranta M. The physical nature of chromatin and viral capsid mobility in the chromatin landscape. Frontiers in Cell and Developmental Biology (Invited manuscript, submission in November 2022).

Expected publications:

- Leclerc S, Gupta A, Weinhardt V, Ruokolainen V, Ekman A, Kunnas K, Chen JH, Perez-Berna AJ, Pereiro E, Kapishnikov S, Fahy K, Vihinen H, Jokitalo E, Larabell C, Aho V, Vihinen-Ranta M. Herpesvirus induced changes in structure and function of mitochondria. (Manuscript in process, to be submitted in early 2023).

Additional SXT-100 example images

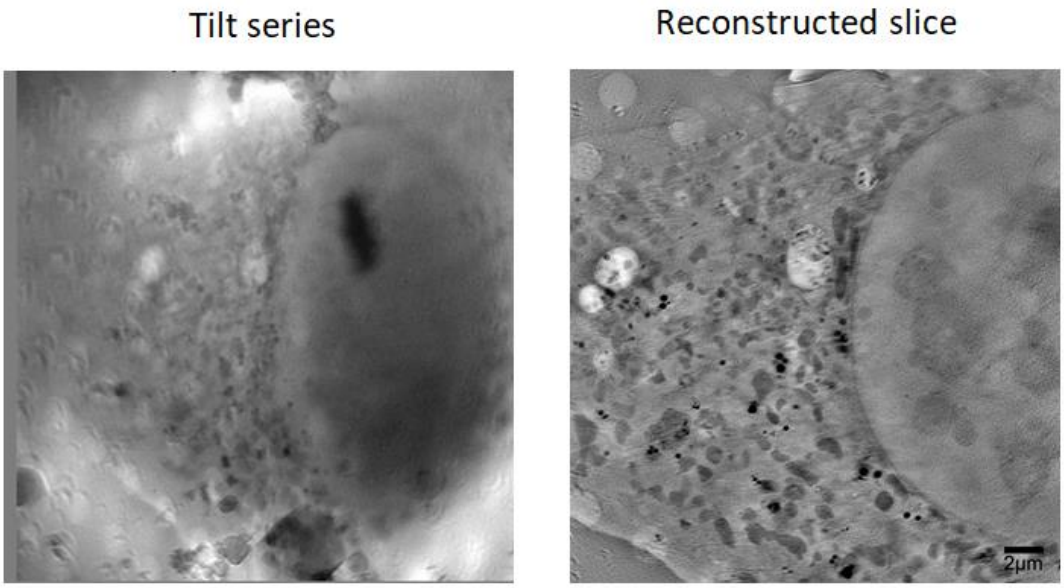

Figure 10: SXT-100 tomogram of a NIH-3T3 cell, showing a raw 2D projection image (left) and a slice through the volume reconstruction (right). A critical step to having good tomogram reconstruction is tilt series alignment. This step often relies on tracking fiducial markers such as gold nanobeads introduced to the sample before tomogram collection. In the absence of fiducial markers, tilt series alignment becomes challenging. It often involves hundreds of clicks manually tracking sample features to find the tilt series rotation axis. SiriusXT now employs a fully automated fiducial-less tomogram alignment program abandoning the need to click. The fully automated fiducial-less alignment program was developed by Dr. Axel Ekman, University of Jyväskylä, in the framework of the CoCID consortium.

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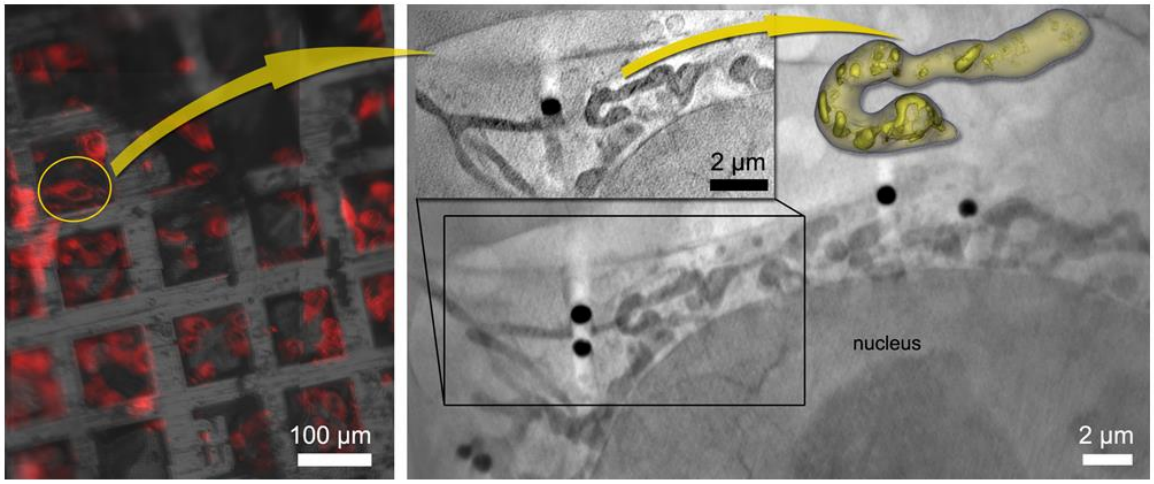

Figure 11: Left panel: integrated cryo-light fluorescence microscope image of HeLa cells grown on a standard TEM grid. Right panel: a 28x18 µm sub-field of view cryo-soft X-ray transmission image of a HeLa cell. Inset: a 20 nm thick virtual slice cropped from a tomographic reconstruction of the cell. Yellow rendering of a cell mitochondrion is shown to the right of the inset. Dark spheres are carbon-rich lipid droplets illustrating the native contrast mechanism of soft X-ray tomography imaging.

Other relevant research publications associated with the technology

Kenneth Fahy *et al* Compact Cell Imaging Device (CoCID) provides insights into the cellular origins of viral infections, 2021 *J. Phys. Photonics* **3** 031002

Recent synchrotron SXT papers

2023 papers

Loconte, V., Chen, J.-H., Vanslembrouck, B., Ekman, A.A. McDermott, G., Le Gros, M.A., Larabell, C.A. (2023) **Soft X-ray tomograms provide a structural basis for whole-cell modeling**. *The FASEB Journal*, 37:e22681. doi:10.1096/fj.202200253R

2022 papers

Synchrotron radiation based *operando* characterization of battery materials

A. P. Black, A. Sorrentino, F. Fauth, I. Yousef, L. Simonelli, C. Frontera, A. Ponrouch, D. Tonti, M.R. Palacín

Chemical Science 2022: https://doi.org/10.1039/D2SC04397A

Cryo soft X-ray tomography to explore *Escherichia coli* nucleoid remodeling by Hfq master regulator

A. Cossa, S. Trépout, F. Wien, J. Groen, E. Le Brun, F. Turbant, L. Besse, E. Pereiro, V. Arluison

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J. of Structural Biology 214, 107912 (2022)

Hydroxyapatite nanoparticles-cell interaction: new approaches to disclose the fate of membrane-bound and internalised nanoparticles
M. Bonany, AJ. Pérez-Berná, T. Ducic, E. Pereiro, H. Martín-Gómez, C. Mas-Moruno, S. van Rijt, Z. Zhao, M. Español, MP. Ginebra
Biomaterials Advances 142, 213148 (2022)

Spatial magnetic imaging of non-axially symmetric vortex domains in cylindrical nanowires by Transmission X-ray Microscopy
J.A. Fernández-Roldán, C. Bran, A. Asenjo, M. Vázquez, A. Sorrentino, S. Ferrer, O. Chubykalo-Fesenko, R. P. del Real
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Glycerol amendment enhances biosulfidogeneses in acid mine drainage-affected areas: an incubation column experiment
A.M. Ilin, C.M. van del Graaf, I. Yusta, A. Sorrentino, I. Sánchez-Andrea, J. Sánchez-España
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Micromagnetics of magnetic chemical modulations in soft-magnetic cylindrical nanowires
L. Alvaro-Gómez, S. Ruiz-Gómez, C. Fernández-González, M. Schöbitz, N. Mille, J. Hurst, D. Tiwari, A. De Riz, M. Andersen, J. Bachmann, L. Cagnon, M. Foerster, L. Aballe, R. Belkhou, J-C. Toussaint, C. Thirion, A. Masseboeuf, D. Gusakova, L. Pérez, O. Fruchart
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Lethal interactions of atomically precise gold nanoclusters and Pseudomonas aeruginosa and Staphylococcus aureus bacterial cells
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P. Zamora-Perez, C. Xiao, M. Sanles-Sobrido, M. Rovira-Esteva, J.J. Conesa, V. Mulens-Arias, D. Jaque, P. Rivera-Gil
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Available resources at facility

The microscope will be available at the UCD Conway Institute of Biomedical and Biomolecular Sciences from the beginning of Q2 2023 https://www.ucd.ie/conway/.
The SXT-100 will be integrated with the existing infrastructure available there.

- Cell culturing
- Cryo sample prep
- Cryo fluorescence grid mapping
- Super resolution STED
- STED with conventional TEM or SEM
- Correlative CLEM & SXT
- Data processing and analysis
- Cryo sample storage

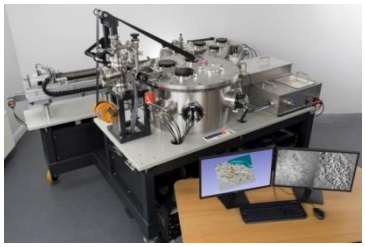

Figure 12. UCD Conway imaging infrastructure including the SXT-100.

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Other evidence of need for SXT-100

The only alternative soft X-ray microscopes are available at 6 synchrotrons worldwide. Applications for beam time must be made months in advance, beam time is limited, and the productivity of which is reliant on good quality samples which can sometimes go wrong. The number of synchrotron users is growing as evidenced by the growth in published SXT papers over the past few years. Furthermore, as the industry moves evermore towards a correlative and multimodal multiscale approach to imaging, the availability of a lab scale SXT microscope will allow much greater flexibility for development and integration of SXT into novel pipelines.

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Appendix I

Technical specification of the SXT-100

SXT-100 Soft X-ray Microscope

SXT-100™ Product Specification

Feature	Performance
SXM	
Operating wavelength	2.73 nm (455 eV)
Polarisation state	Unpolarised
Image field of view	~ 20 µm (at ~900X magnification)
Magnification	Adjustable (depends on objective lens)
Zone plate objective[1]	dr=35 nm (matching condenser NA = 0.039)
Image resolution (3D)	~35 nm (half pitch)
Image acquisition time	<3 hours (per 100 2D projections)
Detector	Back-illuminated X-ray CCD, 2k x 2k pixels, 13.5 µm pixels, 16 bit
Raw data format	*.fits
In-line fluorescence microscope	
Configuration	Epi-fluorescence @90° to X-ray axis
Objective[2]	User specified, long work distance
Channels	4-channels: Hoechst, GFP, RFP, Alexa Fluor 647
Sample presentation	
Sample tilt range	+60° to -60°
Sample support[3]	Standard 3mm carbon coated TEM grid
Sample type[4]	Typ. adherent cells, 3 µm to 8 µm thick
Sample state[5]	Typ. vitrified by plunge freezing
Laboratory requirements	
System dimensions	3 m x 2 m x 1 m (L x W x H)
Power	10 kW
Cooling water	<16°C @ 20 L/min
Lab temperature	21 +/- 1 °C
Lab humidity	< 50%
Gas supply	Ar or N2 (N6.0 Certified)

[1]Partial coherence can be obtained with the use of a different objective NA.
[2]Objective is on a xz stage and can be retracted away from the cold sample when not in use. Typically we have used 10X and 50X objectives for grid screening, but higher magnification shorter working distance objectives can also be accommodated.
[3]Other support types can be accommodated for correlative work, such as FEI autogrids and cryo-FIB lift-out grids.
[4]Non adherent cells have also been prepared and imaged on TEM grids with the SXT-100.
[5]Samples are typically vitrified, but critical point dried and chemically fixed samples have also been imaged with high contrast on the SXT-100.

Contact info@SiriusXT.com or visit www.SiriusXT.com for more information

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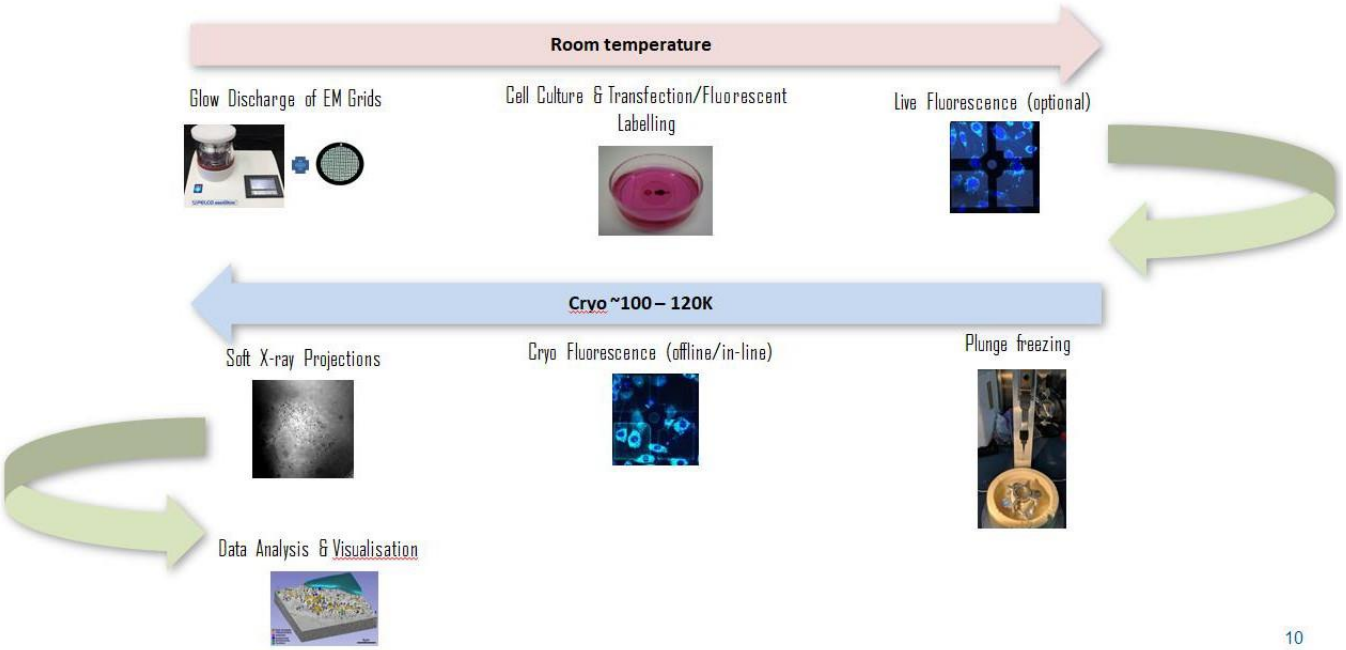
Appendix II

SXT-100 cryo-sample preparation workflow



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