

Correlating X-ray Techniques for Cellular Imaging to Model the Uptake and Distribution of Drug-like Molecules

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Project Location: The Diamond Light Source, Oxfordshire

Lay Summary-

Understanding how drugs and other molecules behave inside human cells is a major challenge in biomedical research. We need to image not just the shape and structure of cells, but also detect and map the chemical elements within them. This is particularly challenging when studying tiny nanoparticles or drug-like compounds at a nanoscale level, as well as when combining different imaging methods, where these samples must be transferred between specialised instruments operating under very different conditions.

My project aimed to develop a workflow that bridges two advanced X-ray imaging techniques at the Diamond Light Source Synchrotron: Cryogenic Structured Illumination Microscopy (CryoSIM), Cryogenic Soft X-ray Tomography (Cryo-SXT), as well as hard X-ray nanoprobe imaging, X-Ray Fluorescence and X-ray Absorption Near Edge Structure (XRF and XANES). At beamline B24, I used CryoSIM to image drug-treated Beas-2B (human lung epithelial) cells, revealing subcellular features while preserving the sample's cellular ultrastructure. At beamline I14, I assisted in collecting high-resolution elemental maps using X-ray fluorescence (XRF). I also assisted with interpreting data using XANES and Sparse XANES techniques, which detect the presence and chemical state of elements, such as gold, inside cells. This correlative imaging approach helps us understand how drug molecules are taken up and transformed in different cell compartments. It also teaches us how preparation methods may alter what we see, a vital insight when combining structural and chemical data from different microscopes.

Aims and Objectives-

In this project, we aimed to develop and test a workflow that bridges cryogenic soft X-ray microscopy and hard X-ray nanoprobe imaging from two different beamlines to study how drug-like molecules, specifically fiducials (gold nanoparticles), are taken up and distributed inside human lung cells.

Imaging techniques and Experimental workflow-

My project combined practical work on beamlines B24 and I14, utilising techniques like Cryogenic Structured Illumination Microscopy (CryoSIM), Cryogenic Soft X-ray Tomography (CryoSXT) and hard X-ray spectroscopy.

Beas-2B human lung epithelial cells were cultured in DMEM media. To prepare complete media, we added penicillin and streptomycin, and incubated the cells at 37 °C with 5% CO₂ to maintain optimal growth conditions. Once the cells reached ~60% confluency, trypsin was used to detach them from the surface, and a trypsin inhibitor was added to stop enzymatic activity. Carbon-coated gold TEM grids were prepared inside a sterile biosafety cabinet and placed into sterile petri dishes. These grids were treated with foetal bovine serum to promote cell adhesion before plating the cells. After cell attachment, gold fiducial markers were added to the grids to assist with image alignment across CryoSIM and X-ray datasets. Once prepared, samples were plunge-frozen in liquid nitrogen to vitrify the cells, preserving ultrastructure without ice crystal formation. This step ensured compatibility with cryogenic imaging workflows.

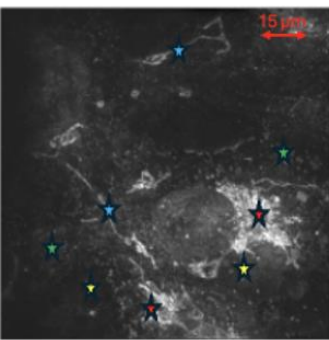
At beamline B24, I was trained to independently operate CryoSIM. I imaged whole vitrified cells labelled with MitoTracker DeepRed and CellMask™ Orange to visualise mitochondria and actin. I acquired multi-channel Z-stacks and aligned channels, gaining hands-on experience in cryogenic sample handling and imaging. CryoSXT provided complementary 3D

ultrastructural data of the same cells, aiding interpretation of intracellular organisation. To prepare for X-ray imaging on beamline I14, the frozen grids were thawed in 4% paraformaldehyde (PFA) for 30 minutes, washed with water, and left to air dry at room temperature.

At Beamline I14, I shadowed my supervisor to perform X-Ray Fluorescence (XRF) and X-ray Absorption Near-Edge Structure (XANES) spectroscopy. I also aligned samples using DAWN software and observed sparse XANES acquisition, where data are collected only at key energy points to increase acquisition efficiency. This part of the workflow helped evaluate how different sample treatments affect the distribution and oxidation state of gold nanoparticles in cells.

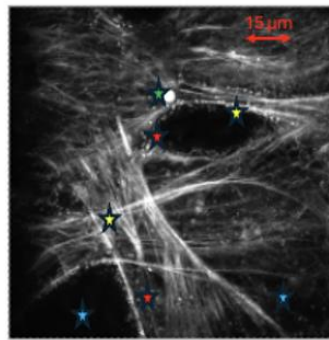


Data Results –



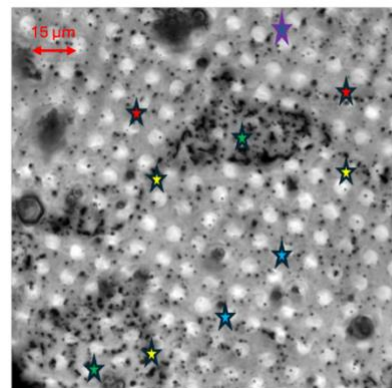
- ★ Densley Conc. Mito
- ★ Elongated Mito
- ★ Nuclear membrane
- ★ Fiducial markers

Figure 1a: Cryo-Structured Illumination Microscopy (CryoSXT) Processed Image of mitochondria in vitrified Beas 2B cells - Elongated (light blue stars) and densely concentrated (red stars) mitochondria are clearly resolved within the cytoplasm. The nuclear membrane (yellow stars) outlines the nucleus, and fiducial markers (green stars) appear as bright puncta, which likely suggests overuse of fiducials. Contrast arises from fluorescence signal intensity, allowing visualisation of mitochondrial morphology and intracellular organisation at high resolution. A scale bar of 15 μm is used for measurement.



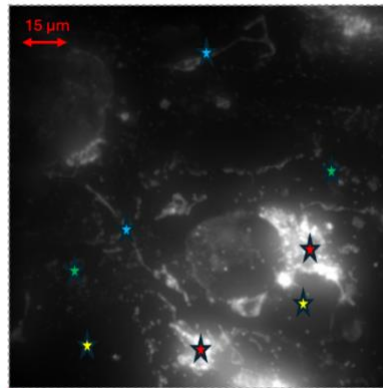
- ★ Actin Filaments/fibres
- ★ Fiducial markers
- ★ Nuclear membrane
- ★ Actin Puncta

Figure 1b: Cryo-Structured Illumination Microscopy (CryoSXT) Processed Image of actin in vitrified Beas-2B Cells Plated on Carbon-Coated Gold TEM Grid - Actin filaments (yellow stars) are well defined and form a dense cytoskeletal network, with many actin puncta (green stars) observed throughout the image. The nuclear membranes (blue stars) are visible and have supported evidence from previous images. Fiducial markers (red stars) appear as bright, discrete points all over the image, which likely suggests overuse of fiducials as it does in Figure 2A.



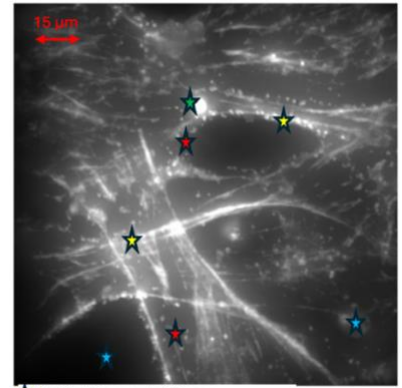
- ★ Fiducial Markers
- ★ Fiducial markers (above cells)
- ★ Outline of nuclear membrane
- ★ Clumped fiducial markers
- ★ Carbon-coated gold TEM grid background

Figure 2a: Cryo-Structured Illumination Microscopy (CryoSIM) Raw Image of mitochondria in vitrified Beas 2B cells - This raw brightfield image reveals vitrified Beas-2B cells plated onto a carbon-coated gold TEM grid. The circular repeating pattern in the background (purple star) corresponds to holes in the carbon support. Fiducial gold markers (red stars) appear as dark puncta and were added to aid tomographic alignment. Some markers lie above the cells (light blue stars), creating a slightly defocused appearance. Yellow stars trace the nuclear membrane, while green stars highlight clumped fiducials likely due to aggregation or settling. Darker zones marked by teal stars indicate cellular regions, providing contrast due to mass thickness variation. The image is unprocessed and reflects the raw optical capture without contrast enhancement or correction. Scale bar = 15 μm.



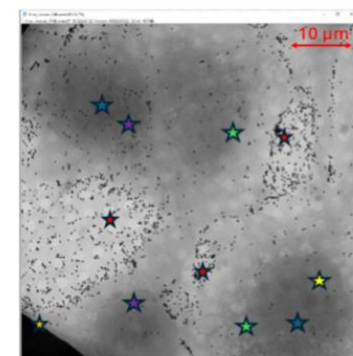
- ★ Densley Conc. Mito
- ★ Elongated Mito
- ★ Nuclear membrane
- ★ Fiducial markers

Figure 2b: Cryo-Structured Illumination Microscopy (CryoSIM) Raw Image of mitochondria in vitrified Beas 2B cells - This raw image displays less contrast and definition compared to the processed dataset, but mitochondrial structures can still be identified. Elongated mitochondria (light blue stars) are observed throughout the cytoplasm, while densely concentrated mitochondrial clusters (red stars) appear as intense, unresolved regions. The nuclear membrane (yellow stars) partially outlines cellular boundaries, and fiducial markers (green stars) appear as scattered bright puncta, some overlapping with cellular regions. The unprocessed data retains background fluorescence and signal variability, similar to the fiducial markers, reflecting the unenhanced image before alignment/processing. A scale bar of 15 μm is used for reference.



- ★ Actin Filaments/fibres
- ★ Fiducial markers
- ★ Nuclear membrane
- ★ Actin Puncta

Figure 2c: Cryo-Structured Illumination Microscopy (CryoSXT) Raw Image of actin in vitrified Beas 2B cells - This raw CryoSIM image shows early-stage visualisation of actin structures. Yellow stars indicate visible actin filaments beginning to resolve across the cytoplasm, while green stars highlight regions of actin puncta. Red stars identify fiducial markers, which appear as bright spots scattered across the field. Blue stars mark the approximate nuclear membrane boundaries. The overall contrast is lower compared to the processed image, with more background noise and less clarity in cytoskeletal features. A 15 μm scale bar is shown for reference.



- ★ Fiducial markers
- ★ Fiducial markers (above cells)
- ★ Carbon-coated gold TEM grid
- ★ Vitrified Beas-2B cells
- ★ Nuclear membrane
- ★ Cytoplasm

Figure 3: Soft X-ray Tomography (CryoSXT) Image of Vitrified Beas-2B Cells Plated on Carbon-Coated Gold TEM Grid - Vitrified Beas-2B lung epithelial cells (dark blue stars) are shown on a carbon-coated gold TEM grid (orange star). Fiducial markers used for alignment appear as dense black dots, located either above cells (purple stars) or within the cellular plane (red stars), likely due to limited uptake time. Lighter grey areas represent cytoplasm (light yellow star), while darker regions correspond to nuclear membranes (green stars). Contrast is driven by differential soft X-ray absorption, allowing near-native structural visualisation. A scale bar of 10 μm is included for measurement.

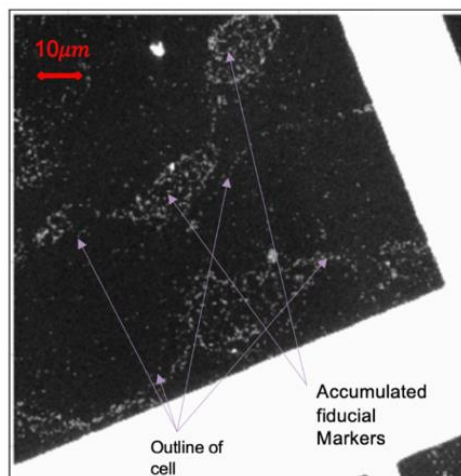


Figure 4: XRF map of Beas-2B Cells on Gold-Coated Grids with Gold Fiducial Markers - X-ray Fluorescence (XRF) data acquired at beamline I14 on vitrified Beas-2B human lung epithelial cells plated on carbon-coated gold TEM grids containing gold fiducial markers. The image shows cellular outlines faintly visible due to signal attenuation, while regions of intense fluorescence correspond to accumulated gold fiducial particles. The strong gold signal likely overwhelms or masks trace element detection from cellular regions. This highlights a limitation of using gold-based supports and markers in correlative imaging workflows, where alternative substrates such as silicon grids may improve contrast and elemental signal from cells. A 10 μm scale bar is shown for reference.

What did I learn from this experience?

This project introduced me to advanced, high-resolution imaging and gave me first-hand experience working with synchrotron facilities. I learned how to handle cryogenic samples, optimise imaging conditions, and operate high-end microscopes at both beamlines B24 and I14. I especially enjoyed learning how to work with the CryoSIM independently, handling vitrified samples with liquid nitrogen and transferring grids between instruments with care and precision; this niche experience is one I will never forget. It also taught me how crucial sample preparation is and how even small inconsistencies can lead to misleading results. Overall, this experience helped me build both technical skills and confidence in working with complex imaging systems and collaborative work environments.

Changes in long-term aspirations?

This project has had a strong impact on my career goals. My interest in biophysics, bioimaging and nanomedicine has grown significantly as I've gained experience with advanced techniques. I am more confident in pursuing a PhD after completing my master's degree. Before this Studentship, I was more interested in pursuing a career that focused on pharmacy or oncology; however, my focus has shifted more towards bioimaging and data-driven structural biology. I enjoyed working in a facility like Diamond Light Source, and I would hope to one day join a research team that applies advanced imaging techniques such as XANES and Cryo-microscopy. I would like to thank RMS and Diamond Light Source Team for giving me this opportunity and experience. I am very grateful and enjoyed my time here.