Soft X-ray tomography and structured illumination fluorescence microscopy as an integrated high-throughput pipeline for drug discovery, production and post-market surveillance

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The problem & scientific needs

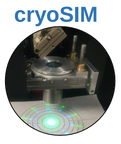
- The development of pharmaceuticals sees significant emphases placed on public safety.
- Conventionally, at the exploratory and pre-clinical stages, methods employed for drug development are based on physicochemical parameters.
- Traditional drug profiling approaches lack high resolution 3D imaging data on native-state biological carriers or their effects on host cells.
- To better understand the action of pharmaceuticals *in cellulo*, we have developed a high-throughput correlative 3D imaging platform and pipeline for biomaterials and cells under near-physiological conditions at beamline B24, Diamond Light Source.
- This can be used to validate drug suitability during R&D through a robust sample preparation protocol which was developed using model cell lines to ensure consistent imaging within cell populations.

Diamond is the largest scientific facility built in the UK in recent times. It is a joint venture between STFC and the Wellcome Trust.

The technology

CryoSIM specifications

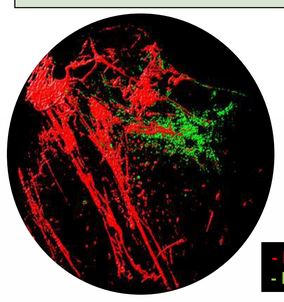
cryoSIM



Cryo-preservation	High Resolution	Multi Channel 3D Imaging
• No chemical fixation required	• Doubles the resolution (240-320nm depending on **λ**)	• Up to 4 colours imaging of structures in samples >10 µm thickness

Cryogenic structured illumination microscopy (cryoSIM)

- Cryopreservation is the gold standard for fixation of biological samples
- Can vitrify samples evenly up to 12 microns



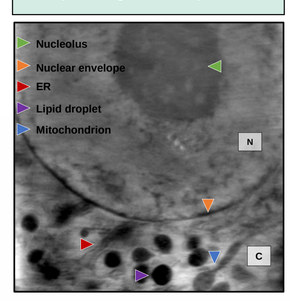
CryoSXT specifications

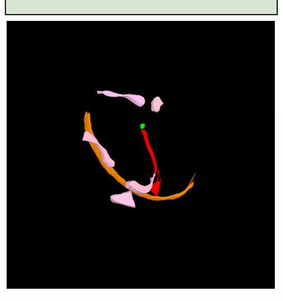
cryoSXT

Cryo-preservation	Super Resolution	3D Data Acquisition
• No chemical fixation required	• Achieves resolutions between 25-40 nm (zoneplate-dependent). • 3D Field of view of 10-16 µm (depending on zone plate)	• Can be used to visualise samples >10 µm thickness. • Capable of tuneable energy spectroscopy to mine elemental information

Cryogenic soft X-ray tomography (cryoSXT)

- Immobilises samples & prevents drifting
- Retains cellular ultrastructure
- Protects samples from radiation damage

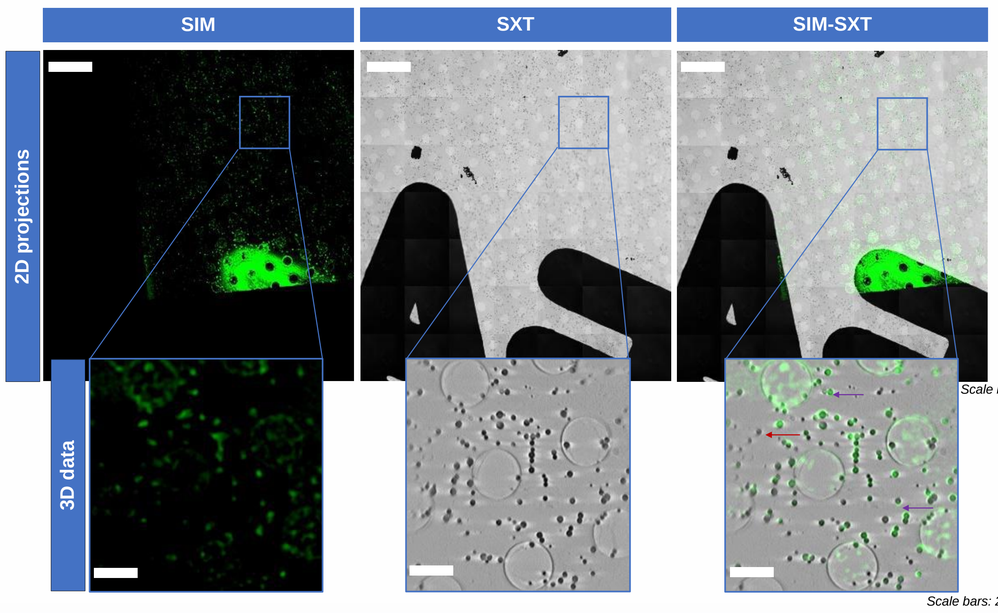




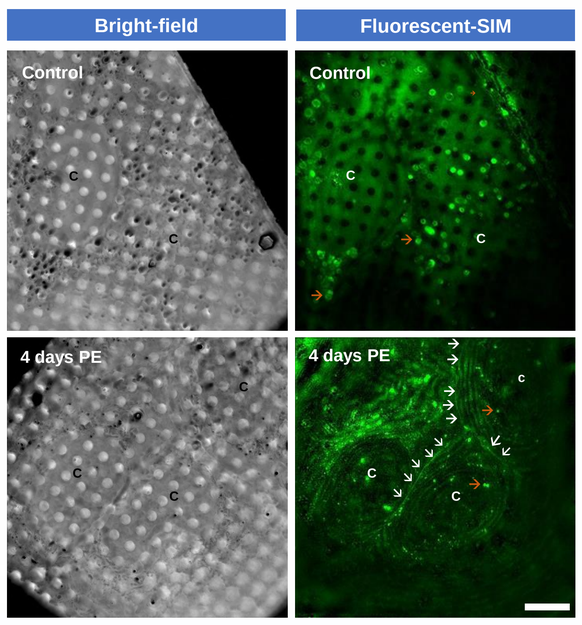
- Primary cilia
- Centriole
- Nuclear envelope
- Mitochondria

A case study

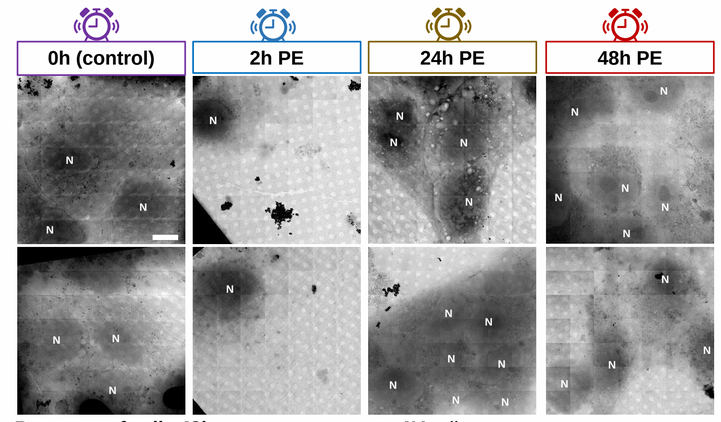
Zika and Chikungunya Active Virosomes as vaccine candidates



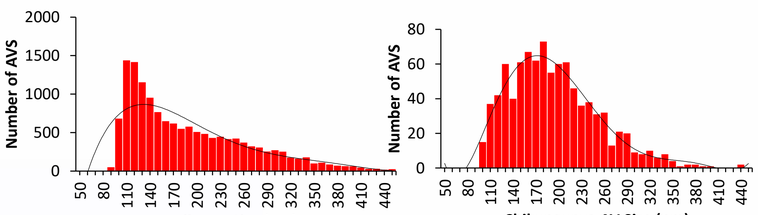
Qualitative correlation of SIM and X-ray datasets- *arrows are pointing to variably-loaded AVs*



CryoSIM confirms that AVs can trigger a second "wave" of immune response- White arrows denote cells antigens expression on the cell surfaces 4 days PE, green arrows show autofluorescence. *Scale bar: 10 µm, "C" = cell*



Recovery of cells 48hrs post-exposure to AVs- "*N" denotes nucleus, Scale bar: 10 µm*



Size distribution of ZIKA and Chikungunya AVs

The potential and benefits

Batch parameters	Values required	Possible implications
Population density	Number per area (#/µm²)	-Reproducibility, -Production standardisation
	# of objects in a size range per area (#/µm²)	
Vesicle structure	Size Range	-Production specificity, -Product purity
	Mean/Modal size	
	Skew/Kurtosis of size distribution	
Antigen loading	Mean pixel intensity	-Variability in protein decoration
	Standard deviation of pixel intensity	
Size clustering	Nearest neighbour distance	-Cell-based restrictions on product architecture
	Nearest neighbour index	

Template for quality assurance

Additional deliverables:

- Cell size and gross morphology
- Polarisation of cytoskeleton & endosomal factors
- Change in size and distribution of organelles
- Nuclear membrane remodeling
- Exosome production

Summary

- We deliver a package of refined protocols
- We offer sample preparation, data collection, processing & a catalogue of cellular features
- Drug-induced side-effects can be scored

Acknowledgements

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