

Correlative **L**ight and **X**-ray Tomography (CLXT)

Imaging partnerships at a
synchrotron setting

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Correlative Cryo-imaging Beamline B24
Diamond Light Source, UK

Beamline B24 @ Diamond Light Source



The Diamond Project & the correlative cryo-imaging beamline B24



Biological Cryo-Imaging B24 eBIC

B24: 3D Correlative Cryo-Imaging for the Life Sciences

Welcome to B24

B24 is a correlative cryo-imaging beamline offering 3D imaging with soft X-ray tomography (cryoSXT) complemented by super resolution fluorescence structured illumination microscopy (cryoSIM). All associated sample preparation and evaluation as well as post-data collection processing are also available at the beamline (see sections below).

CryoSXT offers us the opportunity to image cells and cell populations in 3D to a resolution of 25 nm. To put this in context, ribosomes are only 20-30 nm across while mitochondria can be anywhere between 500 to 3000 nm. This places SXT well within capacity to deliver clear cellular imaging and, given the sizeable fields of view achieved (anywhere between 10 to 20 μ m), 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 are hoping to see.

B24 Contact

Beamline phone numbers:

- +44 (0) 1235 567594 (ECT - main)
- +44 (0) 1235 567499 (ECT - DECT)
- +44 (0) 1235 567499 (ECT - DECT)
- +44 (0) 1235 777596 (CC3)

Principal Beamline Scientist:

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E-mail: maria.harkiolaki@diamond.ac.uk

First Steps for Users

1. Please take the time to look through our publications to help you make the best use of our techniques.
2. Make sure you can prepare samples correctly - this should be discussed with us before applying.
3. Please get in touch! We are keen to discuss potential projects with you.

What does the B24 beamline at Diamond do?



The B24 beamline areas today

Sample preparation areas @ B24



X-ray Imaging @ B24

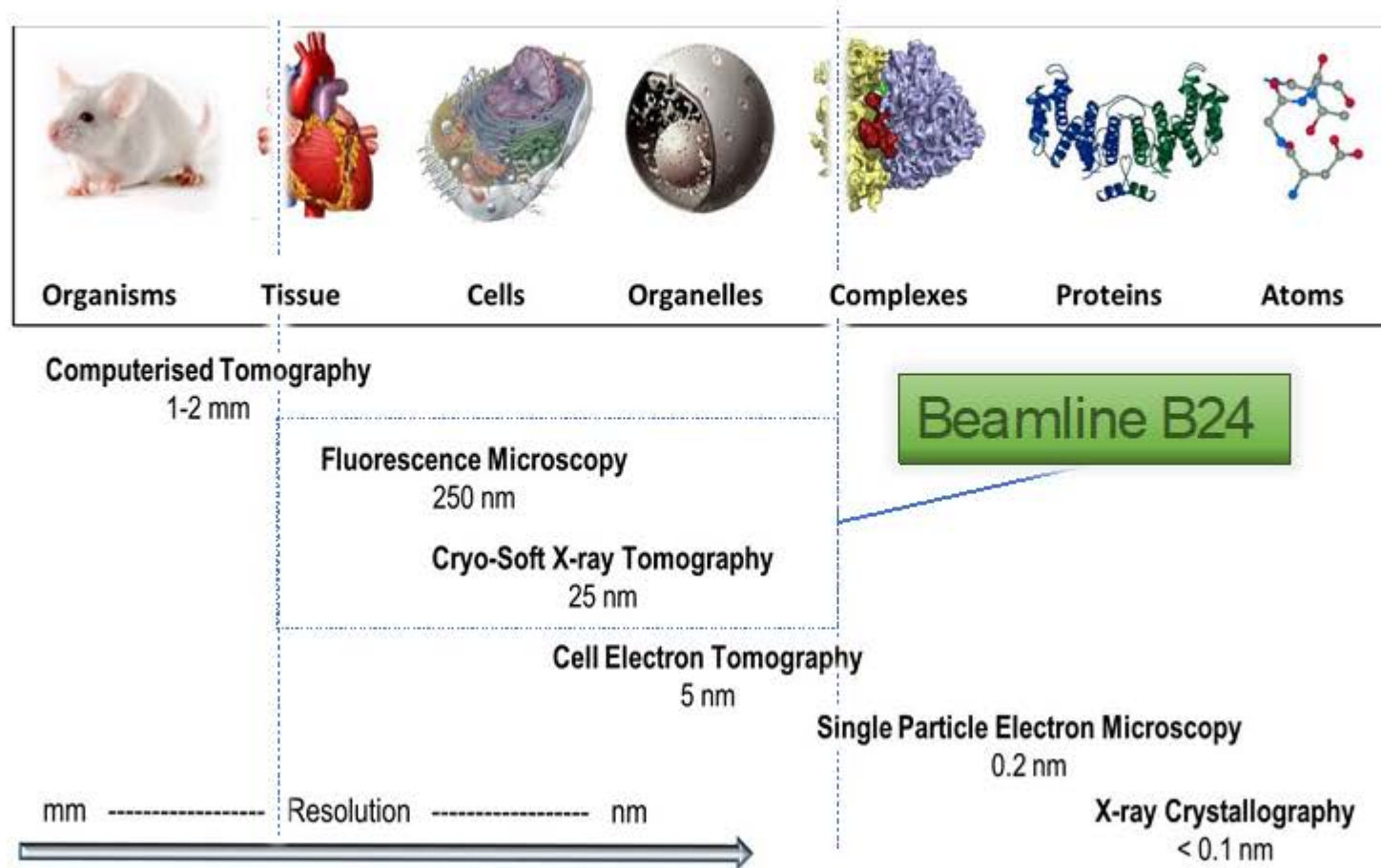


Fluorescence Imaging @ B24

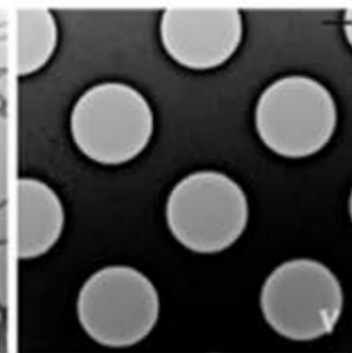
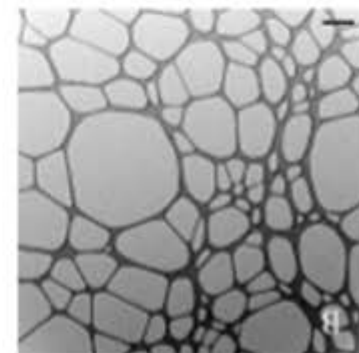
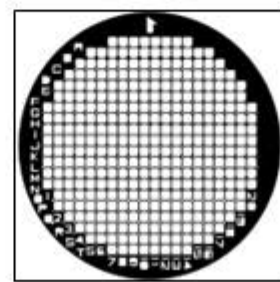
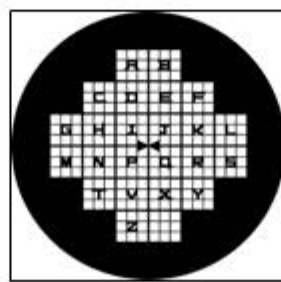
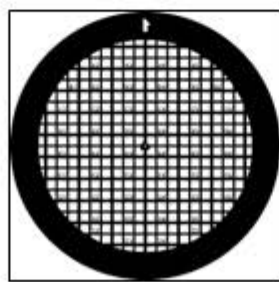
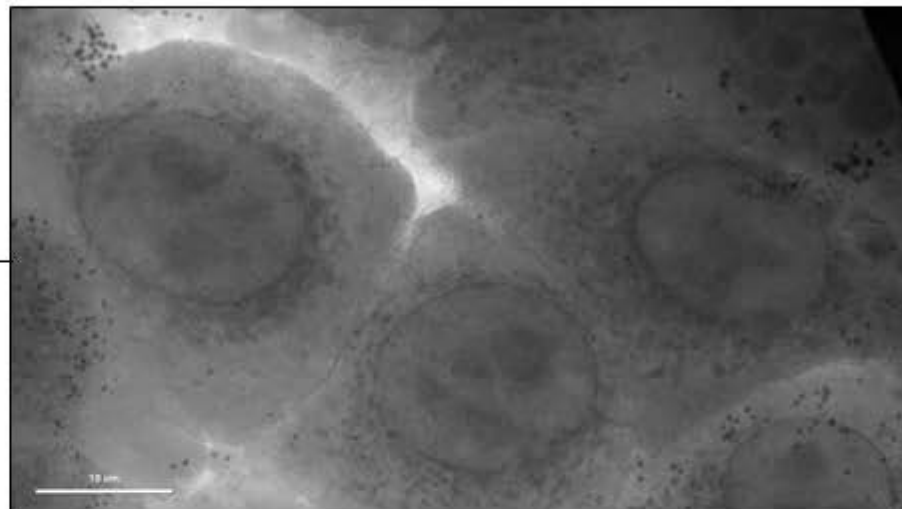
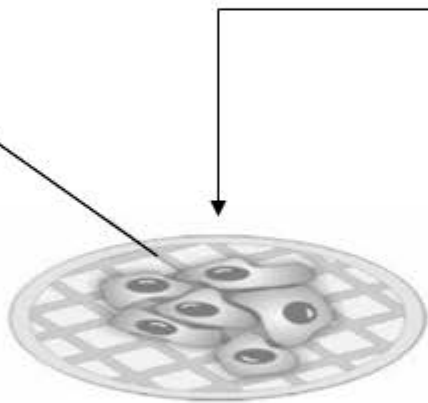
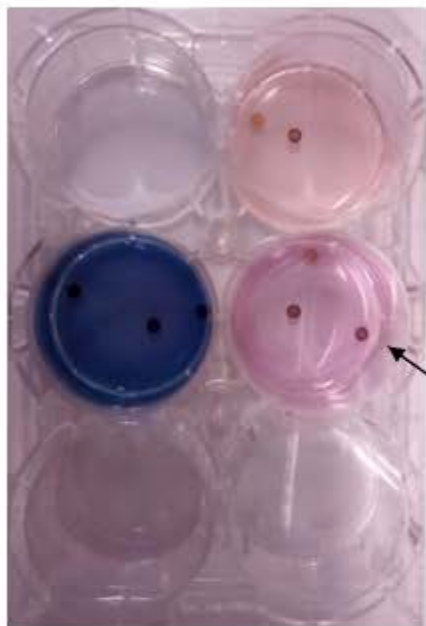


Why, Who & What

Imaging across scales for the biological sciences

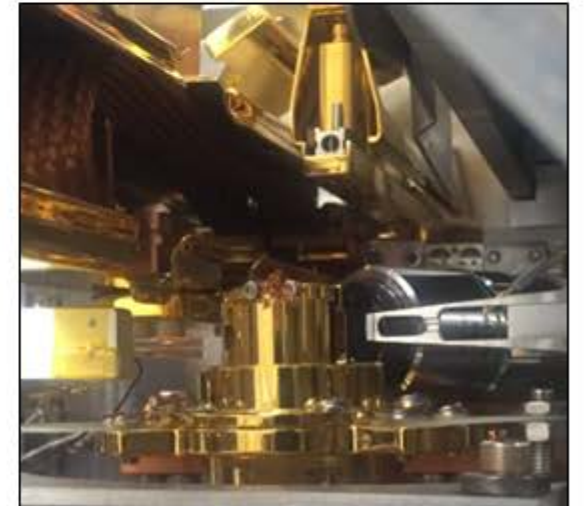
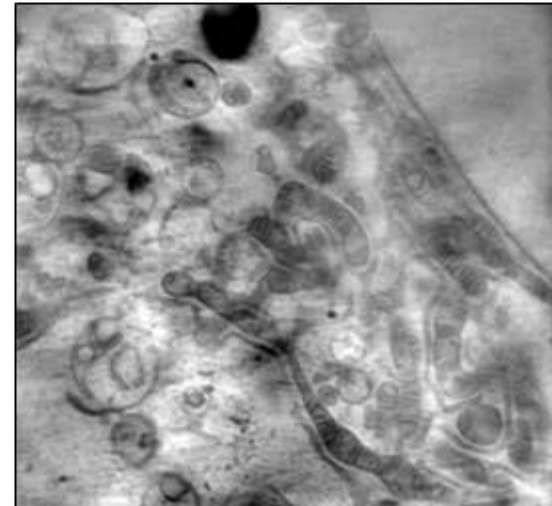
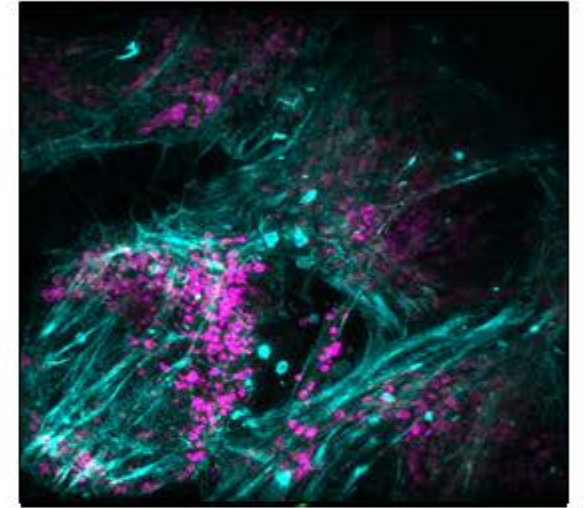
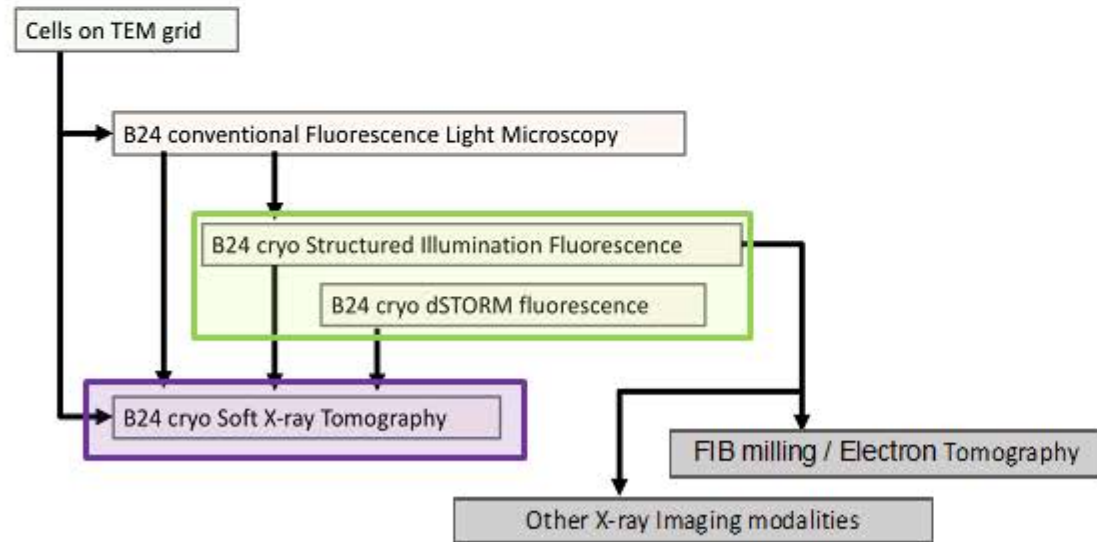


What is a sample for B24?



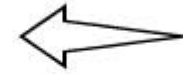
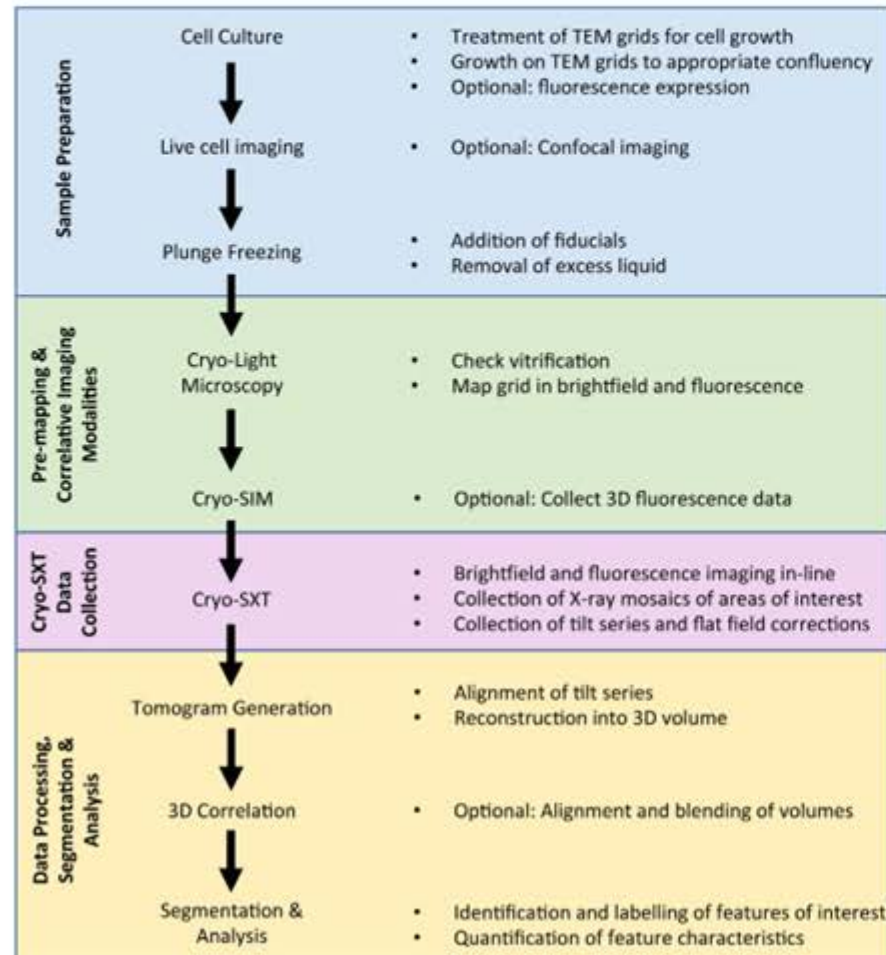
Correlative Light and X-ray cryo-Microscopy (CLXT) at beamline B24

- cryo Soft X-ray Tomography (SXT)
- cryo Structured Illumination Microscopy (SIM)



Correlative cryo-imaging (CLXT) workflow at B24 and access points

The difference between building a method that works and a method that works for all



- Work out the pipeline and use it (!)
- Accessible/published peer-reviewed protocols
 - No tricky reagents
 - No hidden steps
 - No user-dependent variability
- Published peer-reviewed case studies
 - High biomedical relevance
 - High impact
- Communication avenues
 - Websites: synchrotron and beamline
 - Public profile: beamline and staff
 - Availability of contacts

1st Microscope:

Full-field transmission soft X-ray microscope



Soft X-ray Tomography at B24

- 3D Imaging technique → ultrastructure of vitrified whole cells at near-native states (no fixation or staining required)
- Absorption contrast imaging at ~500eV (water window)
- Limited angle tomography (140 degrees total tilt)
- Resolution: 25 & 40nm in up to 12μm thick samples
- FOV: 10x10 or 16x16 μm
- Endstation: Zeiss Xradia 825 synchrotron TXM

Scheduled beamline upgrades: XANES, phase contrast, Bio-Containment Level 3 operation...

Special thanks go to:



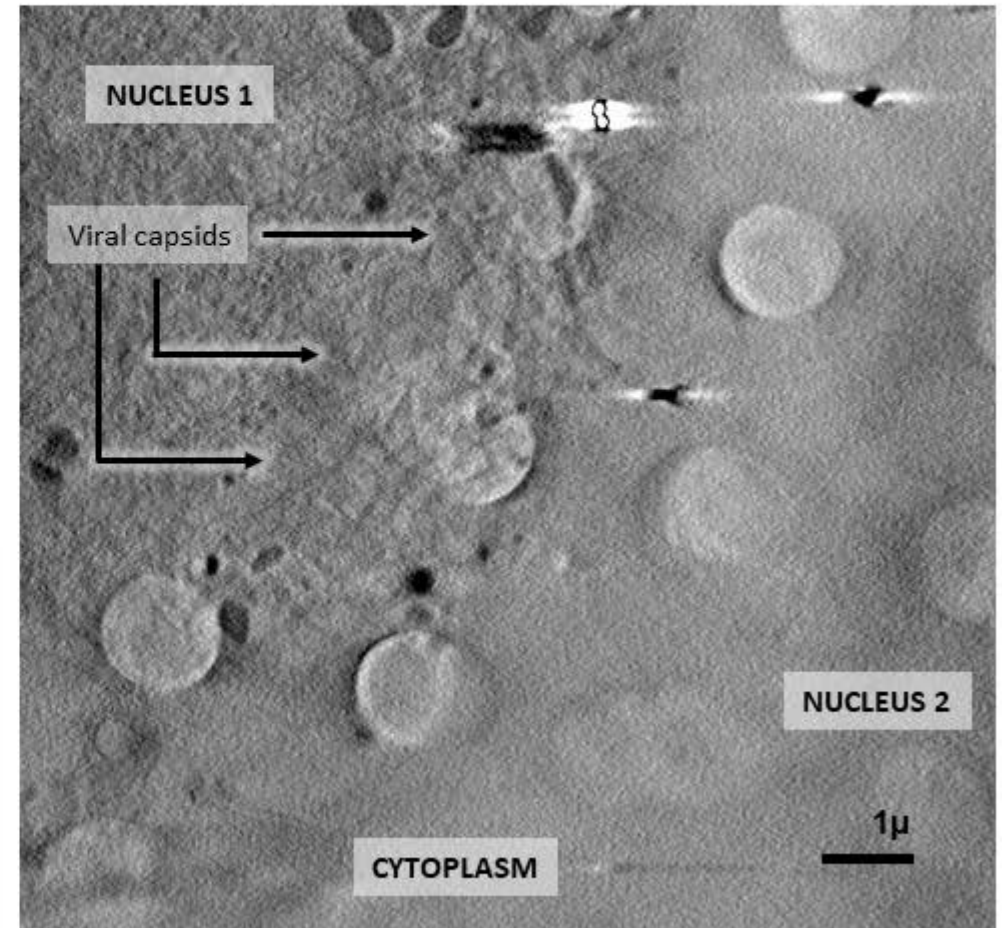
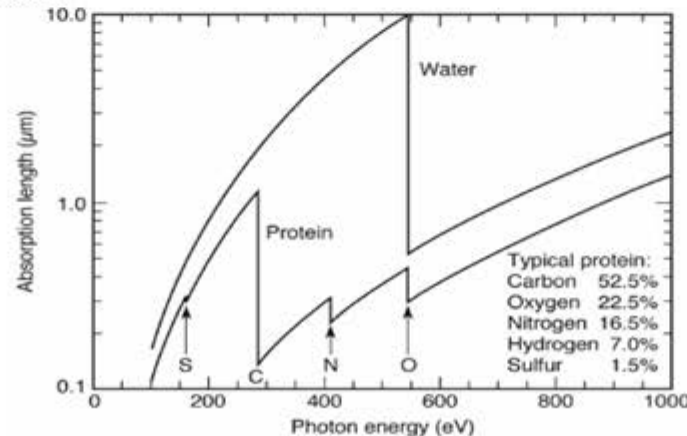
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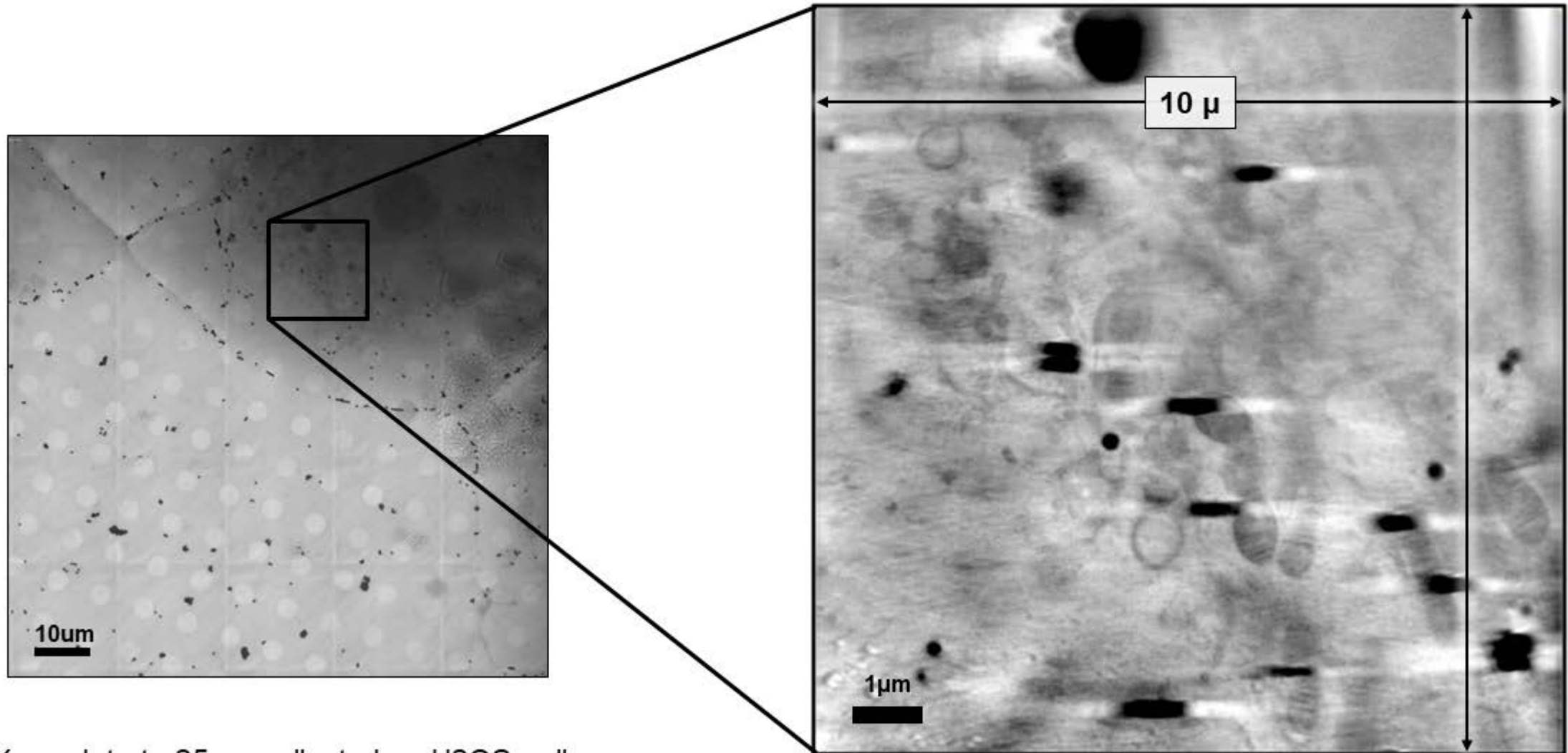


E Pereiro



Movie by K Nahas

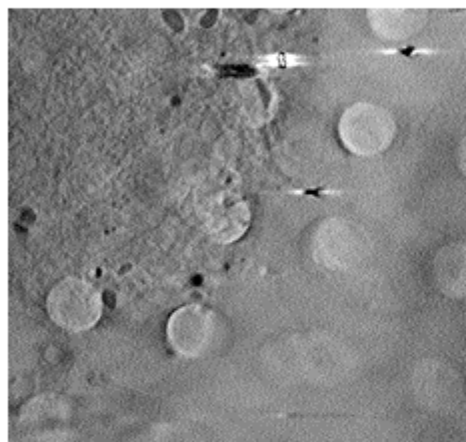
3D X-ray imaging at the water window



X-ray data to 25nm collected on U2OS cells

120° tilt series collected at 0.2° steps; Total data collection time 20min; Tomogram generated with IMOD.

SXT how long does it take?



Reconstructed SXT data

HSV1-infected HFFF cells
FOV 16x16 μm

- | | |
|---|--------------------------------|
| ✓ Experiment design | dependent on user requirements |
| ✓ Sample Preparation | |
| - Cell culture/maintenance | 0-6 days |
| - Cell seeding on TEM grids (when needed) | 6-24 h |
| - Cell treatment (dependent on user requirements) & labelling | 0.5-1 h |
| - Cryopreservation (snap freezing) | 0.5-1 h |
| ✓ Data collection | |
| - Samples loaded to TXM (air to vacuum; sample remains vitrified) | 1-2 h |
| - Sample (TEM grid with multiple imaging areas) loaded and mapped | 15-30 min |
| - Single tilt series acquisition | 10-30 min |
| - Complete grid data collection (several tilt series; 20-50 Gbytes) | 3-6 h |
| - Data processing and reconstruction (concurrent to data acquisition) | 3-6 h |
| ✓ Post processing/analyses | |
| - Data mining | dependent on user requirements |

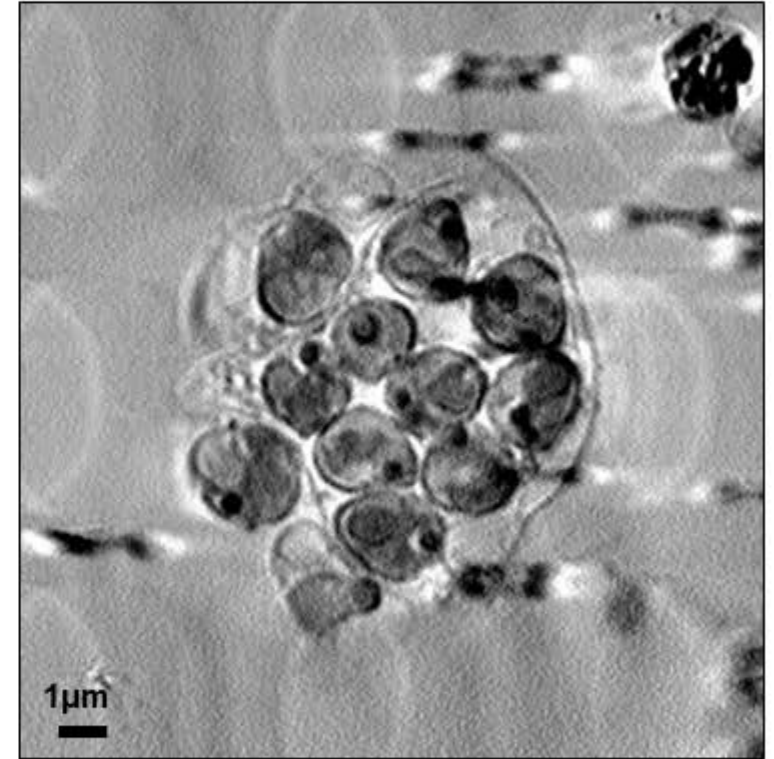
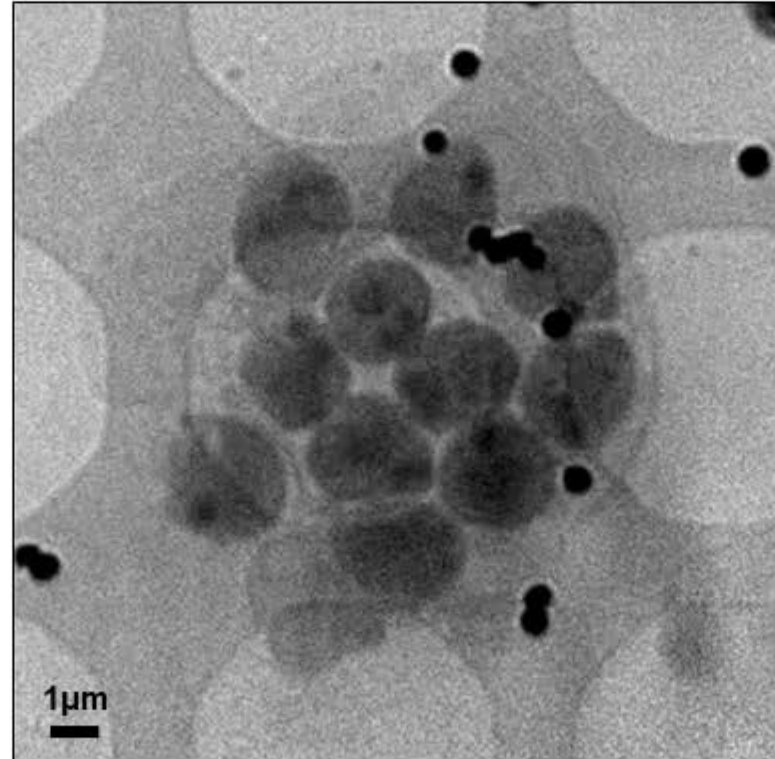
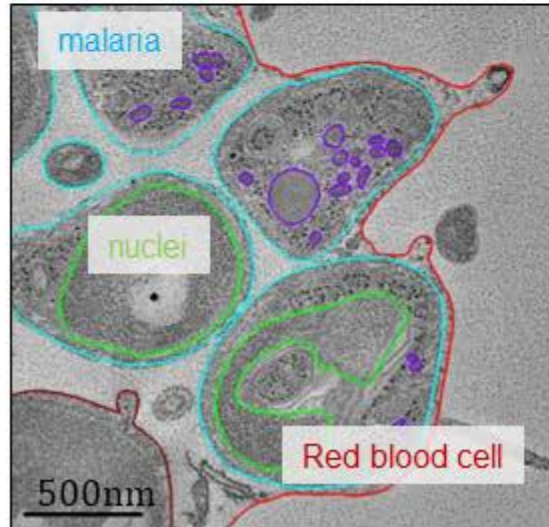
Science using SXT at B24



Plasmodium falciparum biology



Prof Helen Saibil
Institute of Structural and
Molecular Biology
Birkbeck College. UK



Hale V. L. et al.

Parasitophorous vacuole poration precedes its rupture and rapid host erythrocyte cytoskeleton collapse in *Plasmodium falciparum* egress

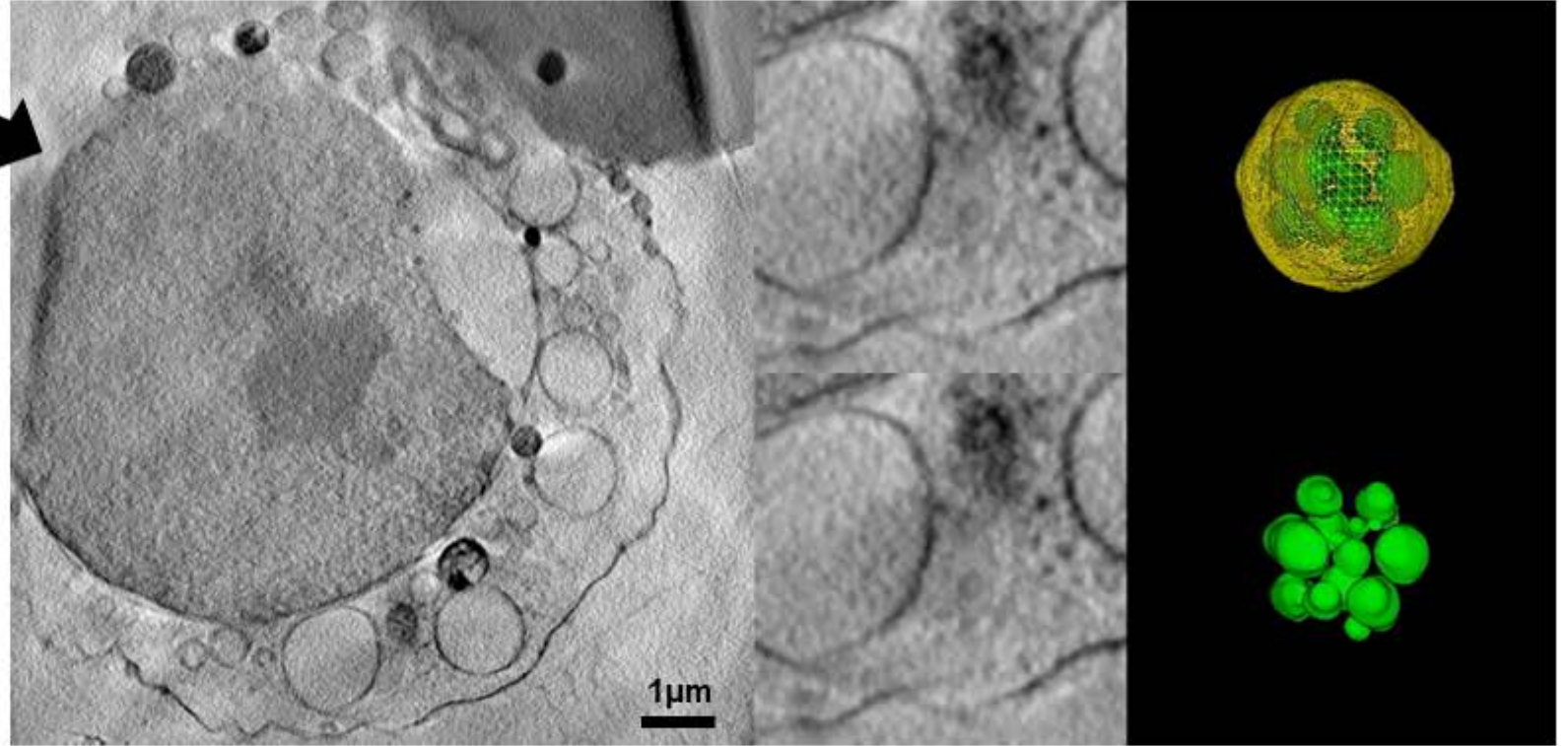
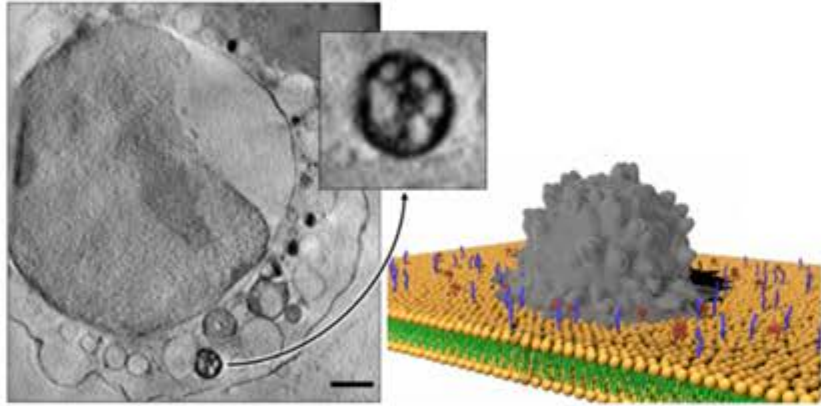
Proc Natl Acad Sci U S A. 2017 Mar 28;114(13):3439-3444.

Cell-cell killing and new cytotoxic organelles



Prof Mike Dustin
Kennedy Institute of Rheumatology
University of Oxford, UK

Cytotoxic T cell



Balint S. et al.

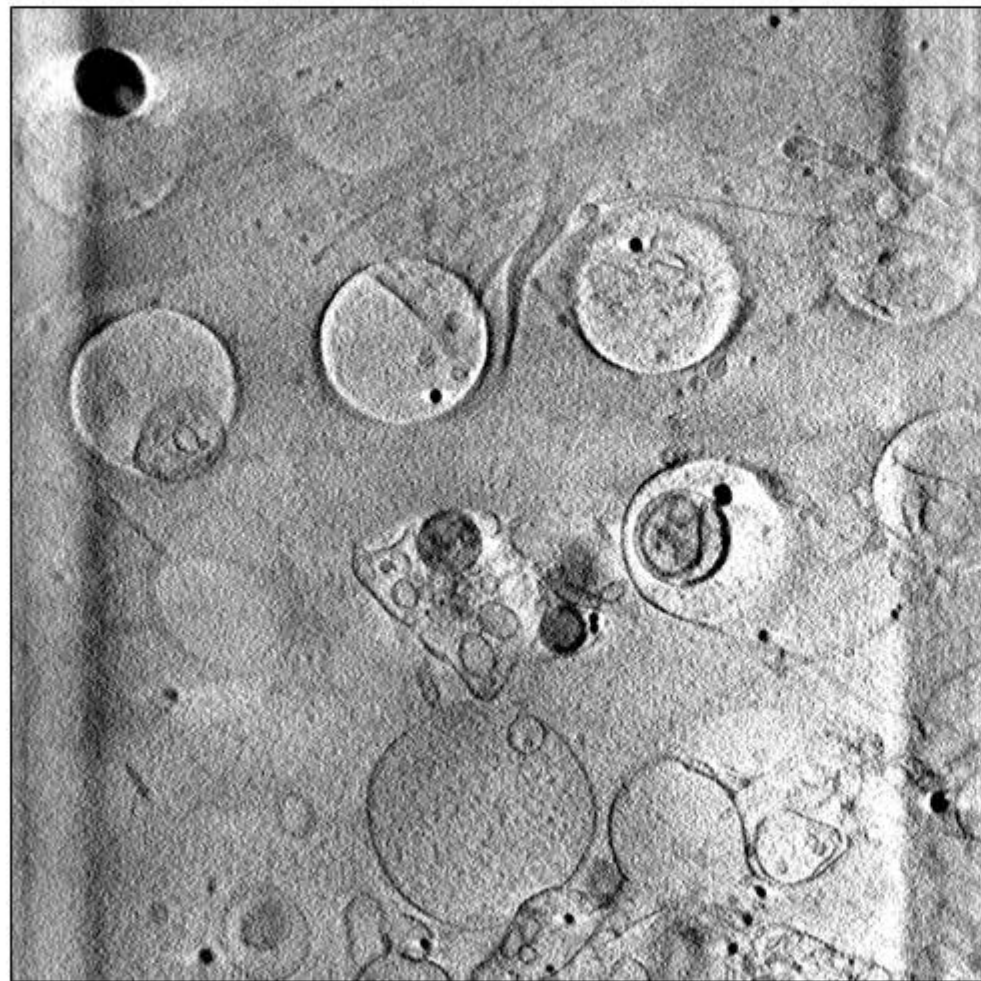
Supramolecular attack particles are autonomous killing entities released from cytotoxic T cells

Science. 2020 May 22;368(6493):897-901.

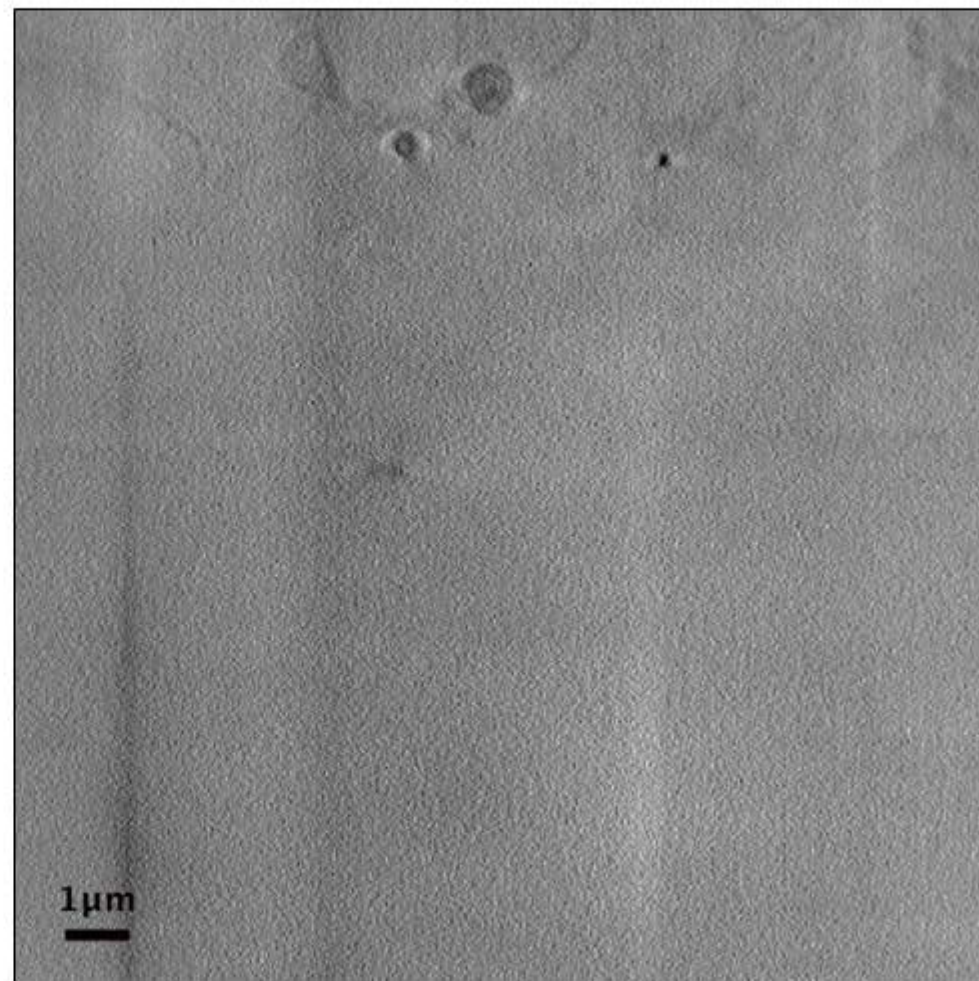
Having SXT, do we really need further complementary information?

Reovirus infection in osteosarcoma cells

SXT



CLXT

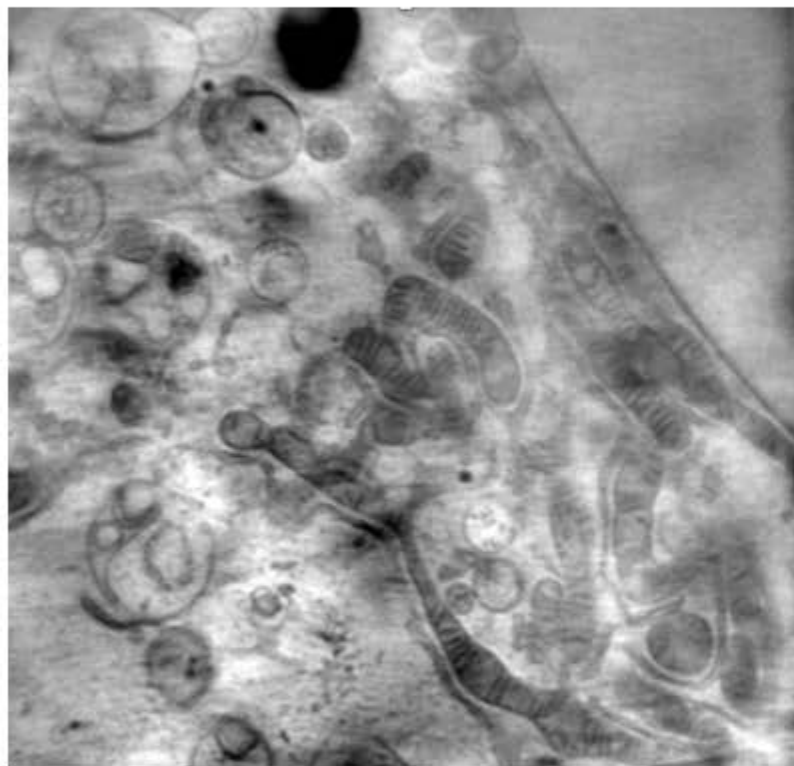


2nd Microscope:

Cryo- Structure Illumination Microscope



Correlating microscopies for B24



- Natural absorption
- 25nm resolution
- sample cryo-fixed

The requirements for a microscope to correlate:

- Provide chemical localization in SXT data
- Same sample imaging at cryogenic temperatures
- Non-destructive
- No need for further sample processing
- Beyond the diffraction limit
- Ensure ongoing support
- Pre-shift assessment of experiment & samples
- 2D mapping and 3D imaging
- Accessible & user-friendly

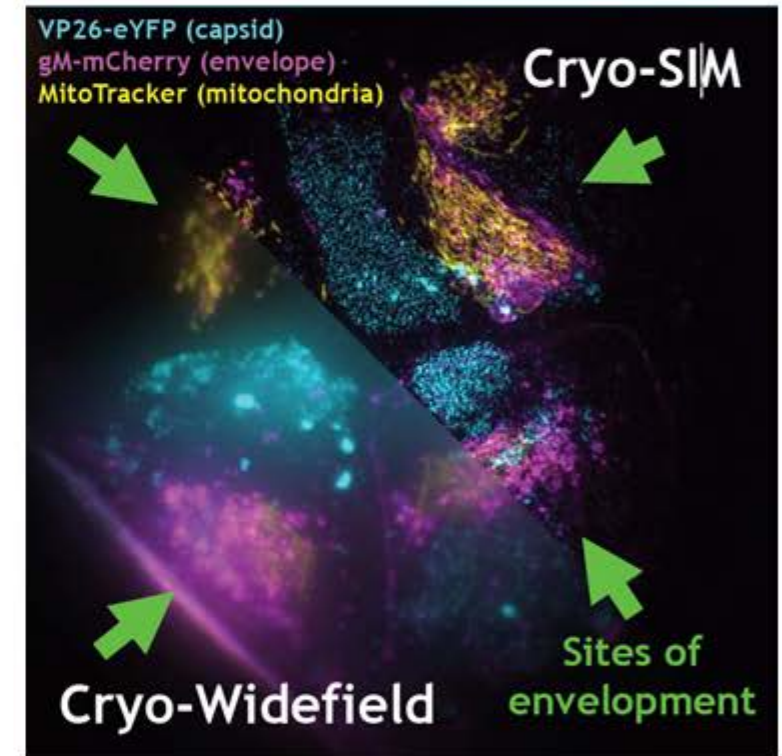
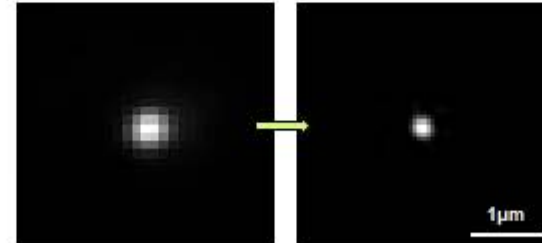
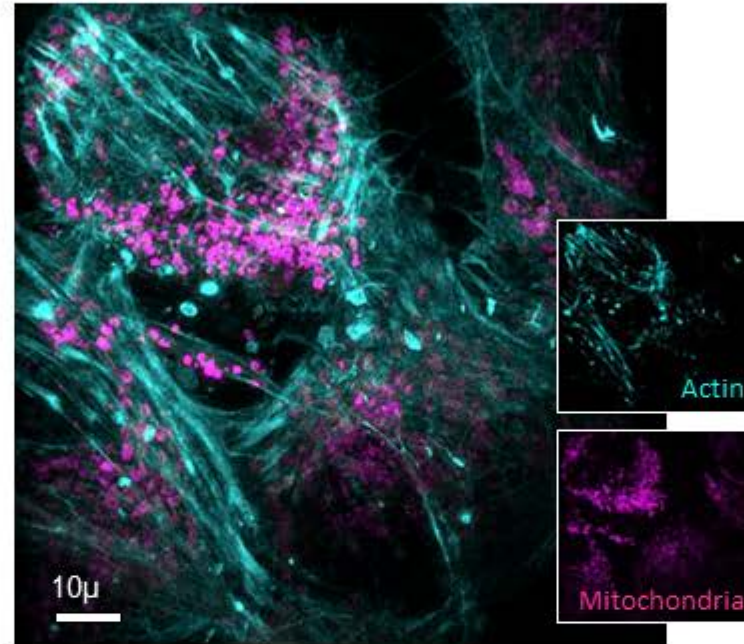
The B24 Structured Illumination Microscope



Super resolution 3D cryo-fluorescence imaging at B24

Micron
OXFORD

LINKAM



Special thanks go to:



Mick
Phillips



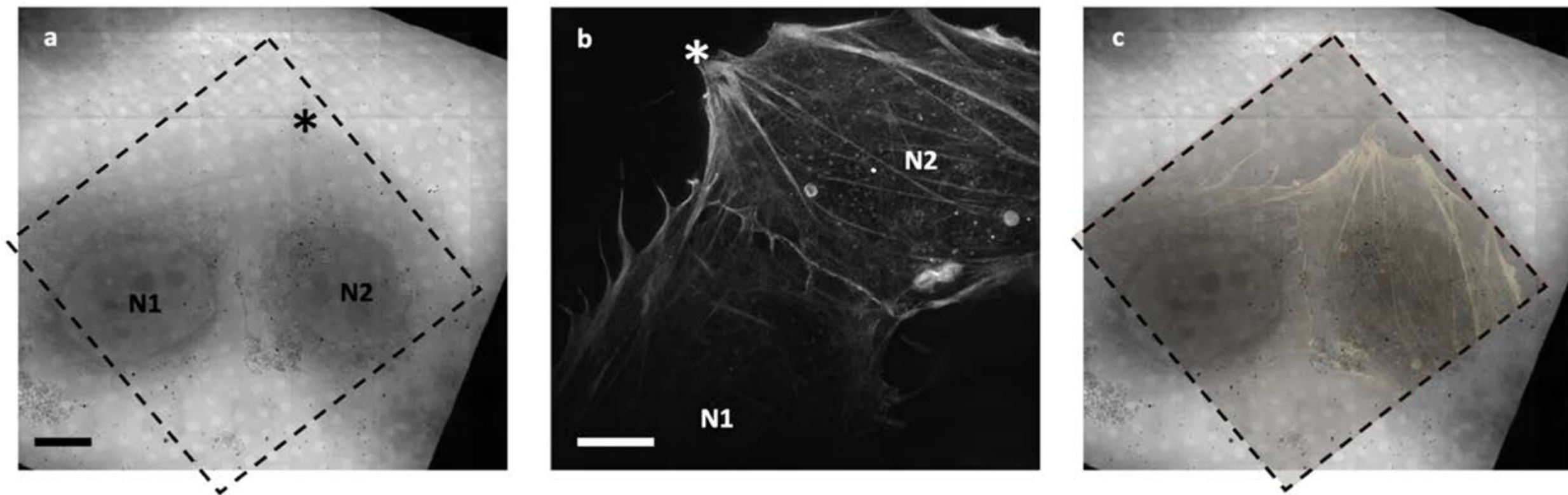
Ian
Dobbie



Cryo Structured Illumination

- Vitrified samples on grids
- Excitation energies = visible spectrum
- Dual fluorescence register
- Z stack height of over 12microns
- Resolution to less than 200nm

CLXT at B24: seeing the same thing with different eyes

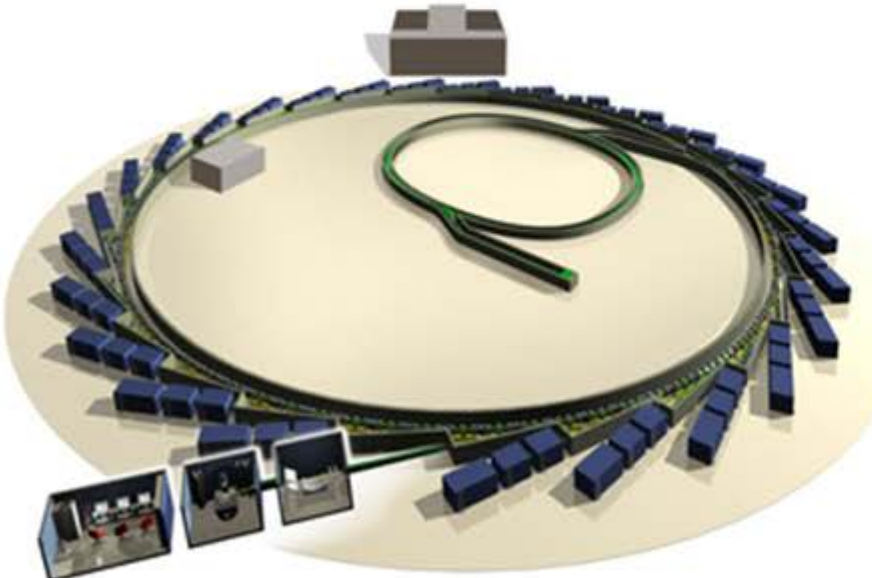


U2OS cells with F-actin fluorescent labelling views using the B24 TXM & SIM (Panels a & b respectively) and the added value of their combination

Where are all the microscopes?



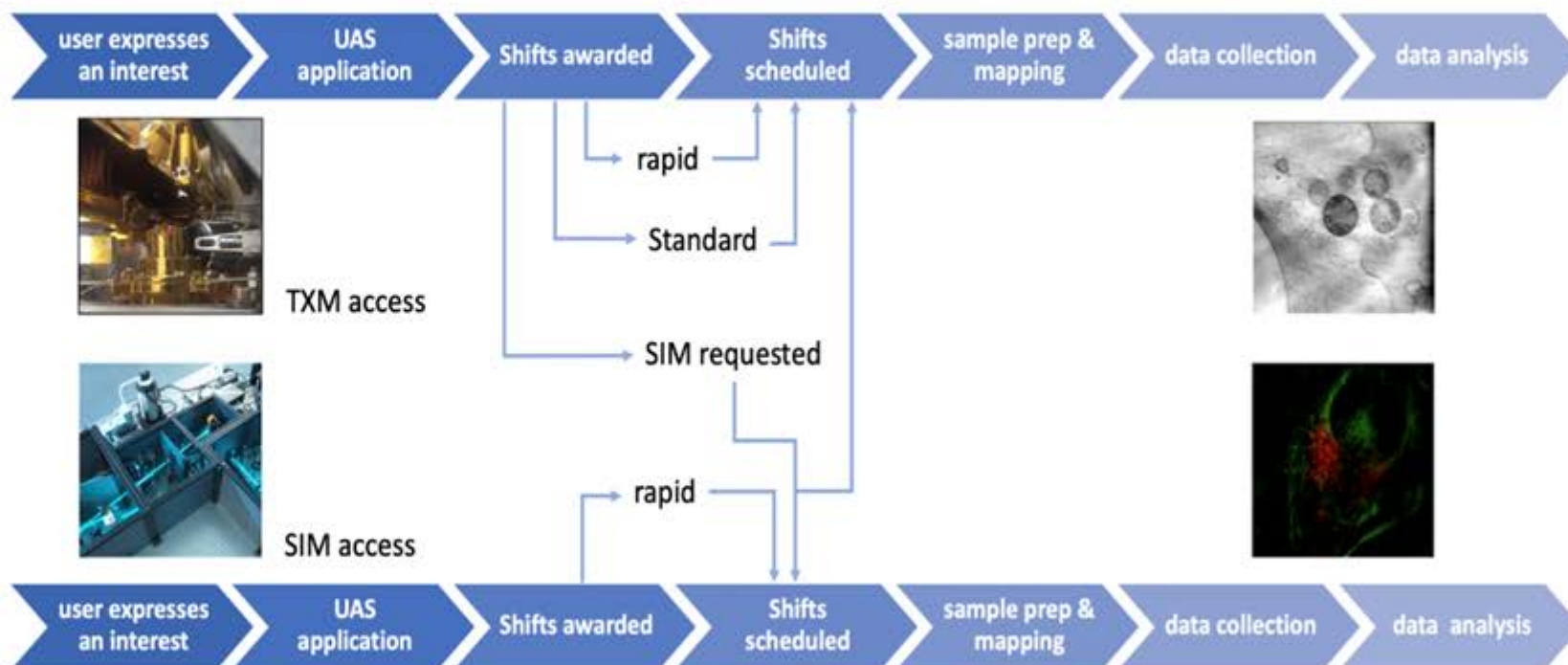
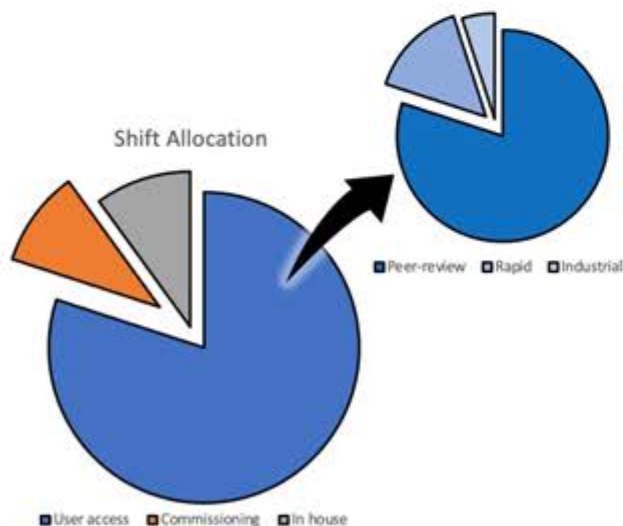
CLXT: Where can you find it?

	Beamline	Source	Energy range, eV	Spatial resolution (nm)	Additional techniques (to water window SXT)
	 Diamond, U.K. Beamline B24	Bending magnet	200–2600	25–40	CryoSIM Phase-contrast XANES
	 ALBA, Spain Beamline Mistral	Bending magnet	270–1200	25–40	XANES
	NSRL, China SXM Beamline	Bending magnet	200–2500	30	Phase-contrast
	HBZ, Germany Beamline U41	Undulator	180–2800	25–40	NEXAFS* Phase-contrast
	ALS, U.S.A. Beamline 2.1	Bending magnet	400–1300	35–80	Cryogenic fluorescence microscope
	TBS, Taiwan Beamline 24A	Bending magnet	260–2600	50	

Who can use all these microscopes?



CLXT: How is B24 expertise & instruments accessed?



Acknowledgements

Diamond Light Source

past and present team members, technical support and scientific advisors and especially C Pizzey, M Dumoux, A Ashtun, *and many many *many!** more...

International synchrotron community

Especially the team @ Mistral, ALBA

Imaging instrumentation and software development experts

DLS scientific software team, Micron @University of Oxford, Linkam Scientific Instruments, Zeiss X-ray Imaging team, ecCLEM developers

B24 Users and collaborators and their respective teams

J Grimes group @ Oxford, S Boulant @Heidelberg, S Graham & C Crump @Cambridge, H Saibil @Birkbeck, M Dustin @Oxford, A Wann @Oxford, P Zhang @eBIC, R Robinson @Okayama

Special thanks to our wonderful user community!!!!

... and thank you for listening!

