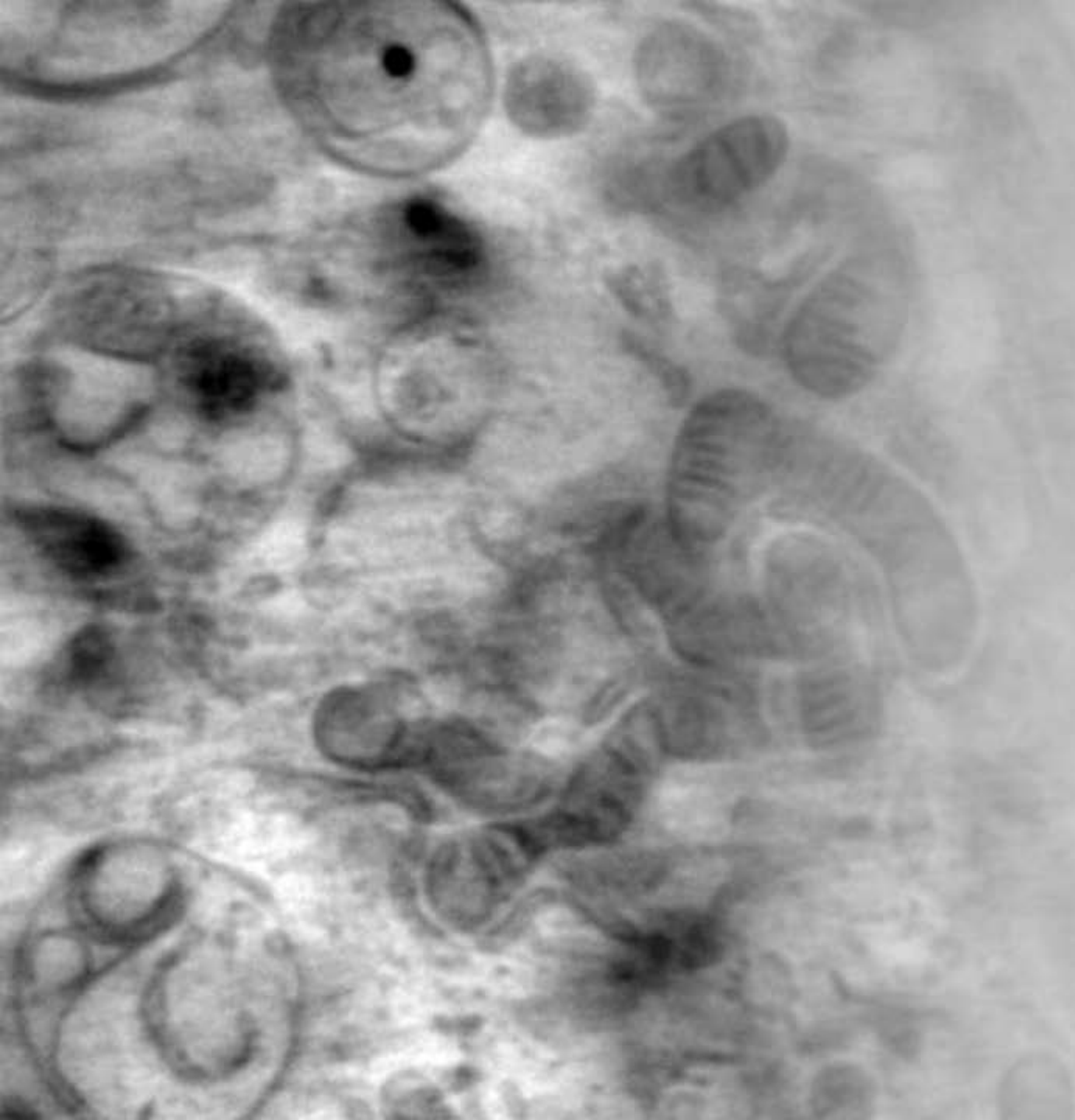


BioImaging UK meeting
MMC2023, Manchester 03/07/2023

Correlative **L**ight and **X**-ray **T**omography (CLXT)

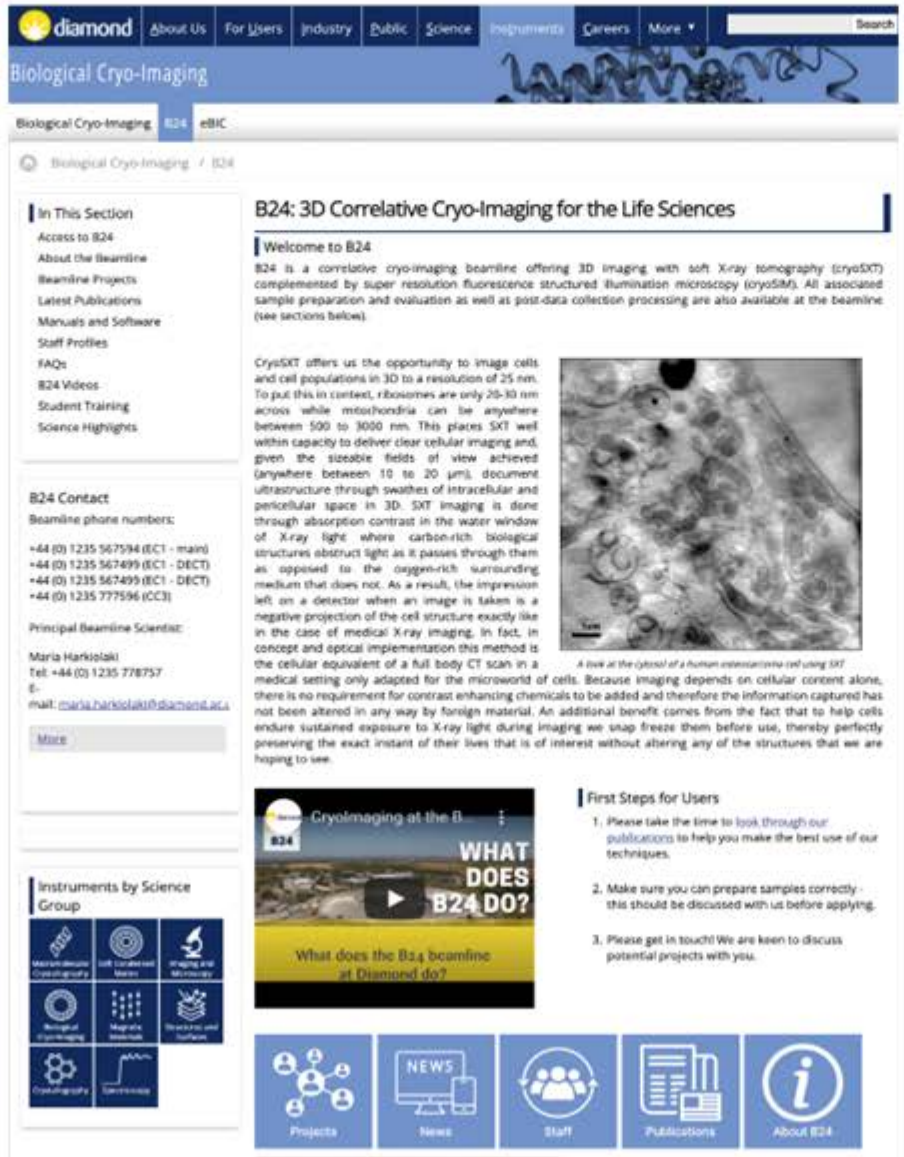
Imaging partnerships at a synchrotron setting

Maria Harkiolaki

Correlative Cryo-imaging Beamline B24
Diamond Light Source, UK

Beamline B24 @ Diamond Light Source

The Diamond Project & the correlative cryo-imaging beamline B24



SCAN ME

The B24 beamline areas today

Sample preparation areas @ B24

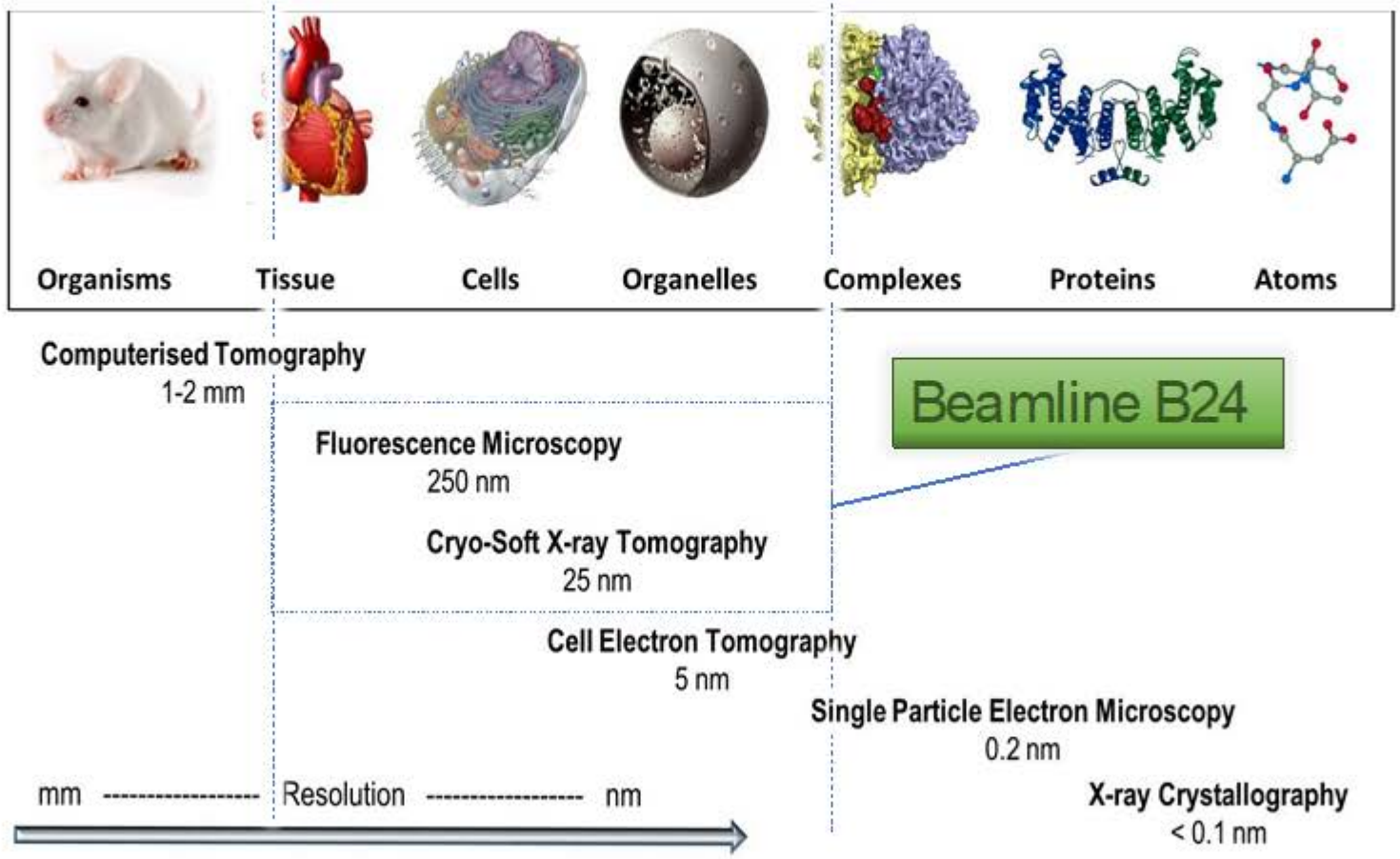
X-ray Imaging @ B24

Fluorescence Imaging @ B24

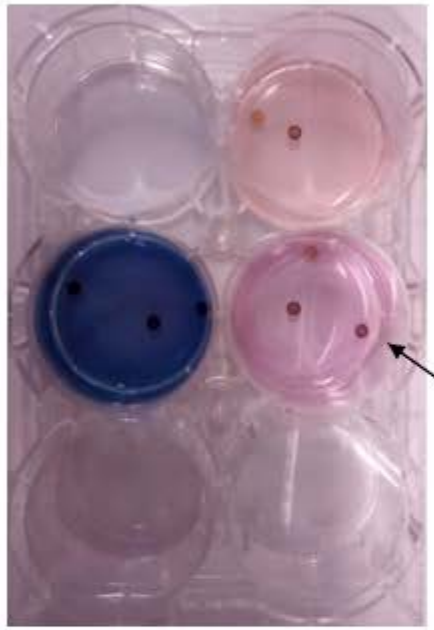
Why, Who & What

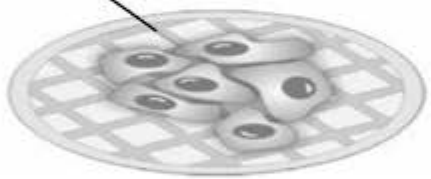
diamond

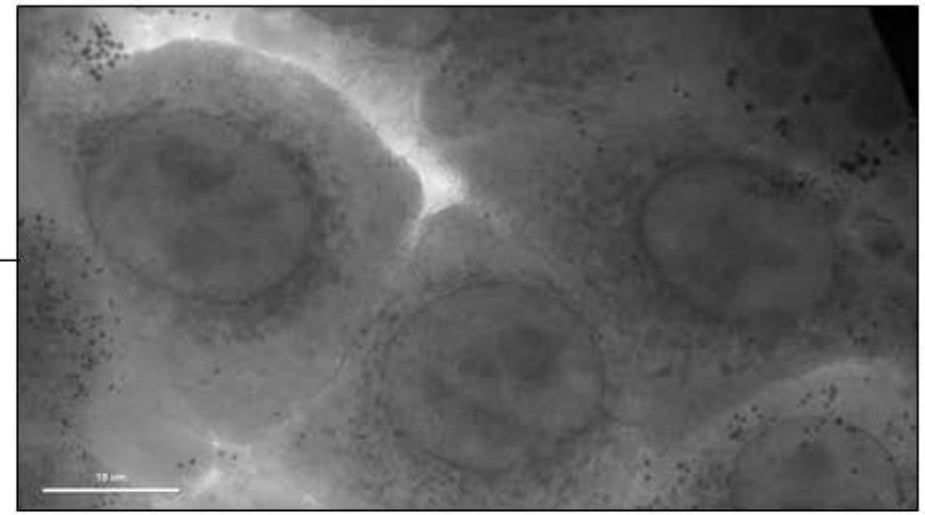
Imaging across scales for the biological sciences

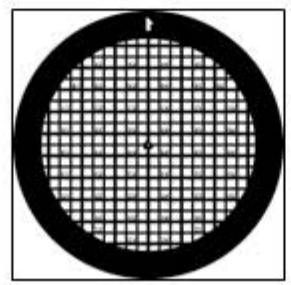


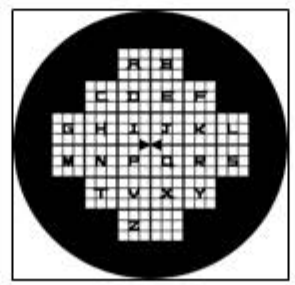
What is a sample for B24?

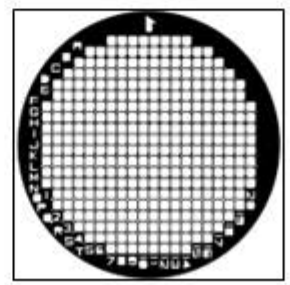


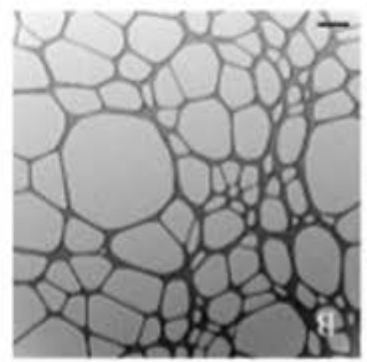


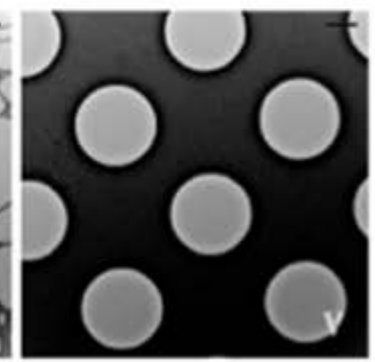






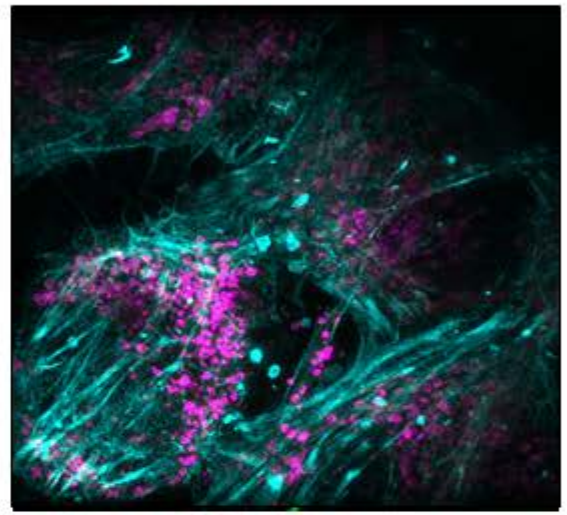


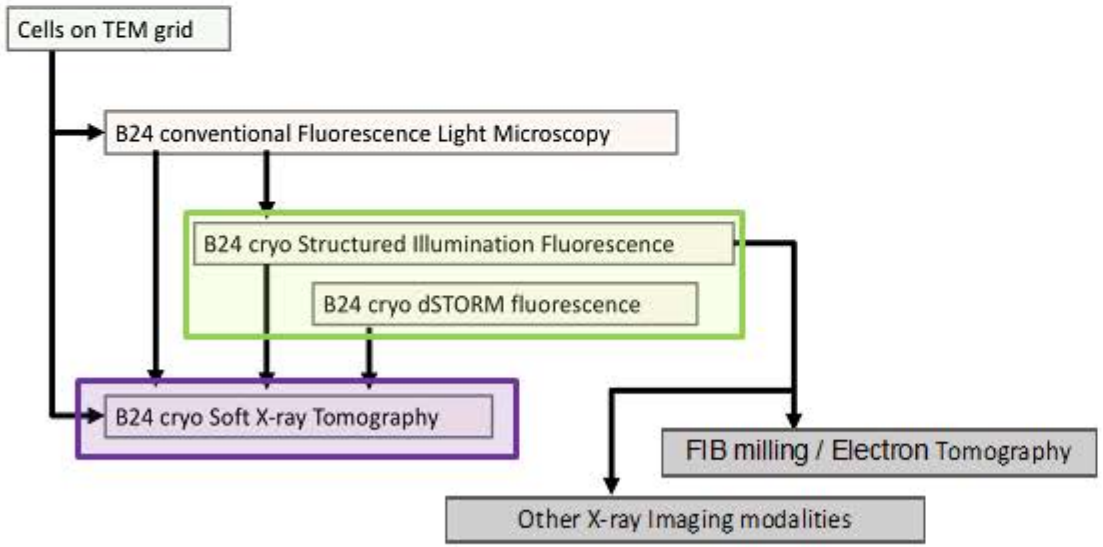


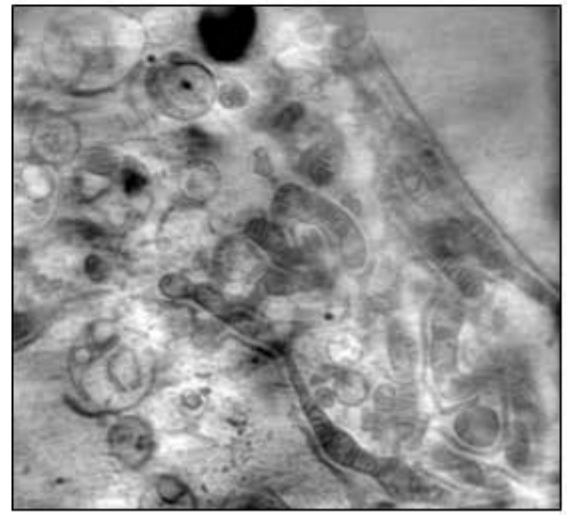


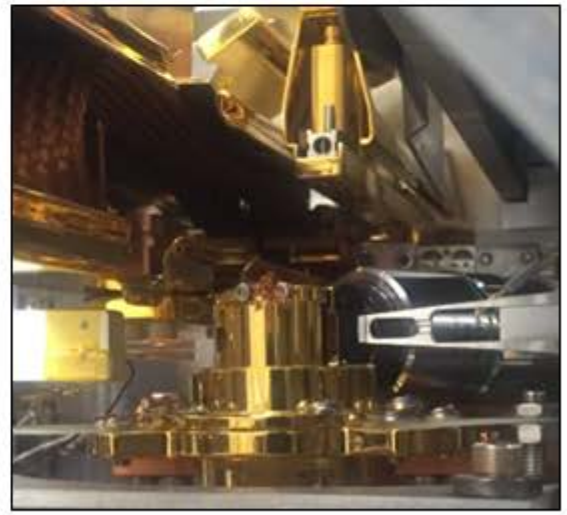
Correlative Light and X-ray cryo-Microscopy (CLXT) at beamline B24

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



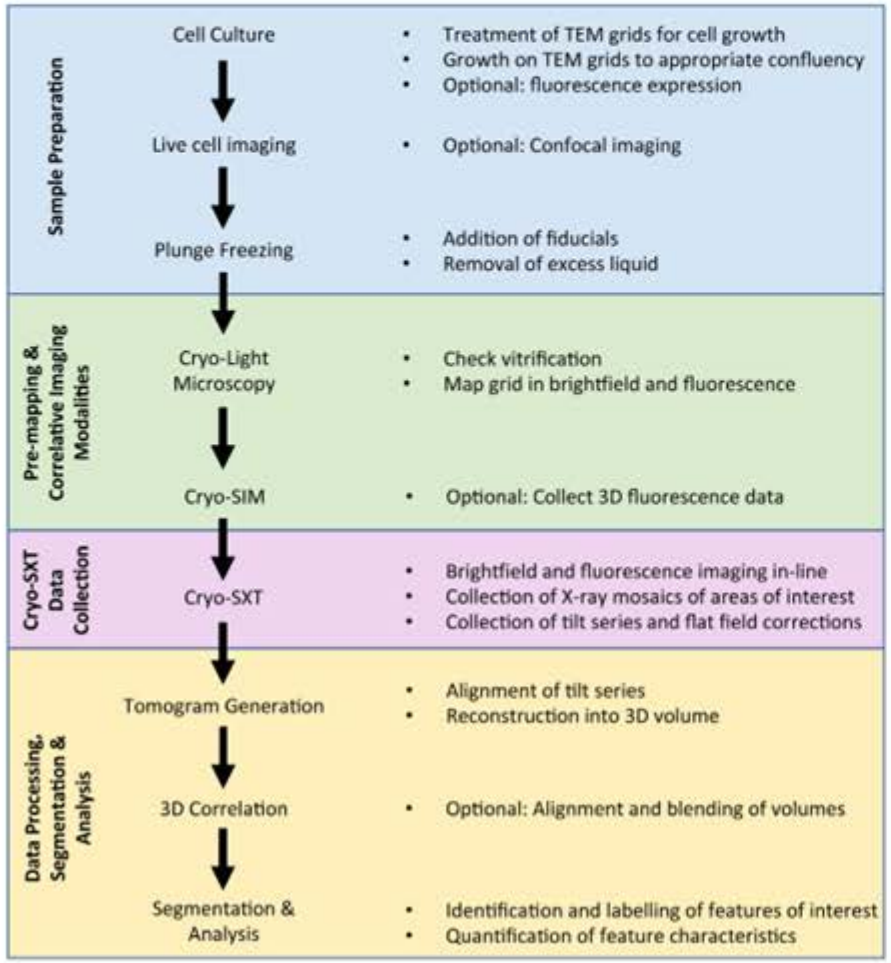






Correlative cryo-imaging (CLXT) workflow at B24 and access points

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

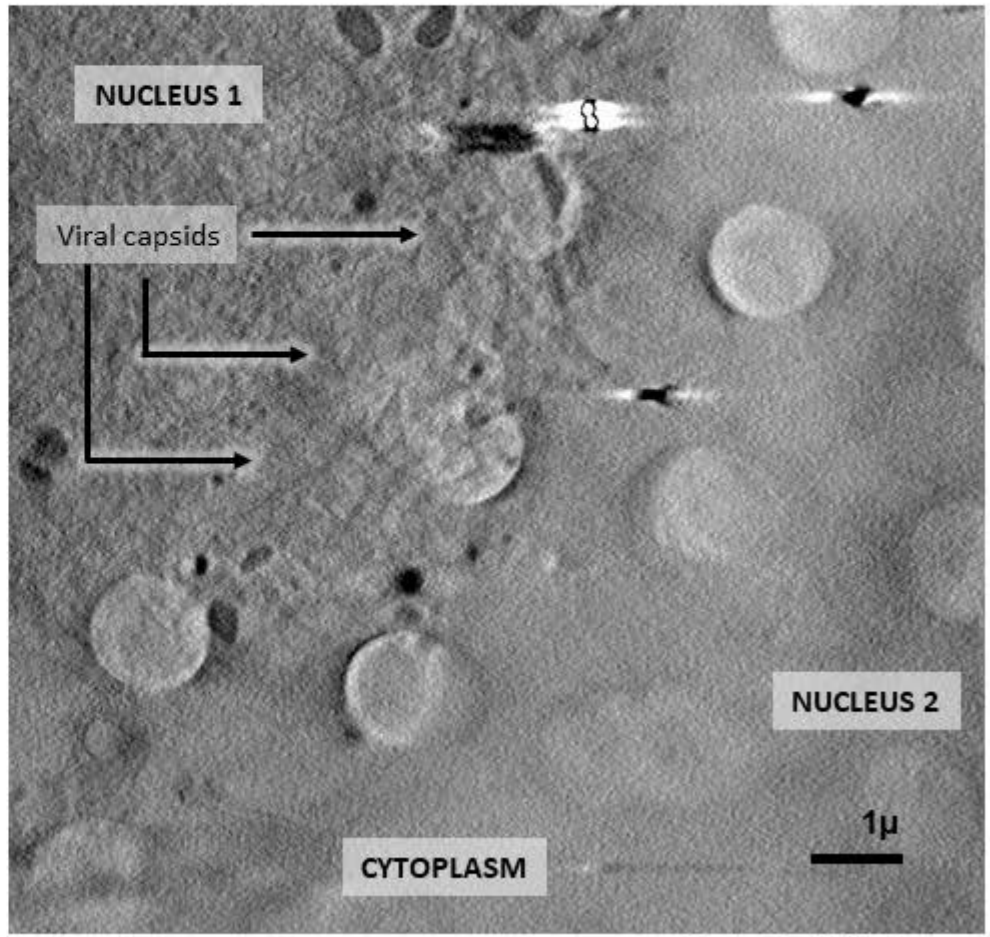
- Communication avenues
  - Websites: synchrotron and beamline
  - Public profile: beamline and staff
  - Availability of contacts

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 12um thick samples
- FOV: 10x10 or 16x16 µm
- Endstation: Zeiss Xradia 825 synchrotron TXM



Movie by K Nahas

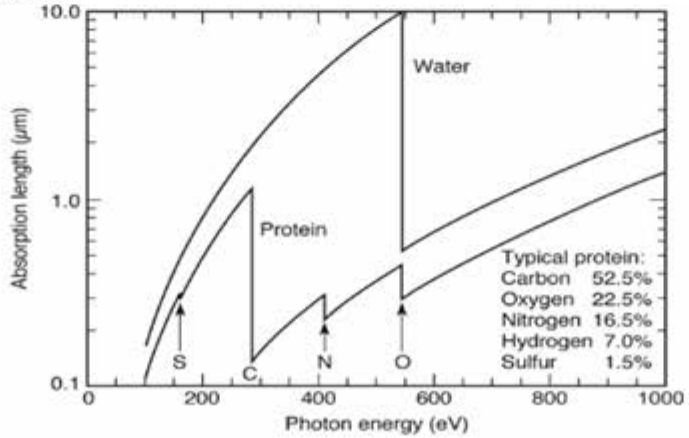
Scheduled beamline upgrades: XANES, phase contrast, Bio-Containement Level 3 operation…

Special thanks go to:

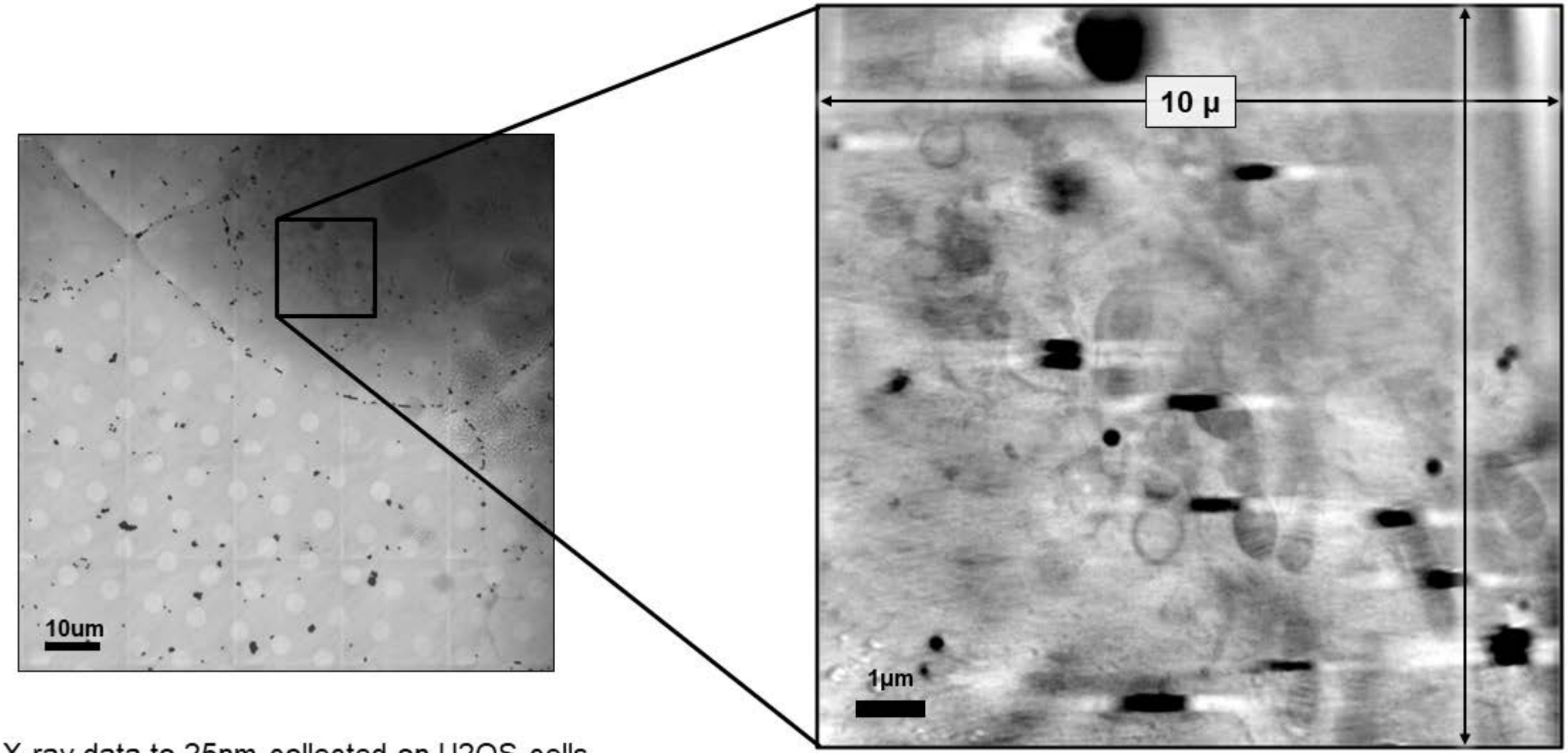

L Duke


J Irwin


E Pereiro



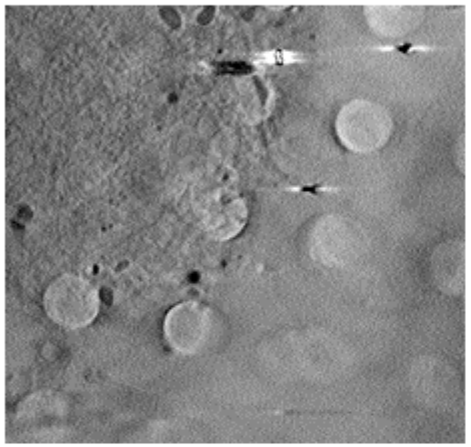
3D X-ray imaging at the water window



X-ray data to 25nm collected on U2OS cells

120º tilt series collected at 0.2º 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

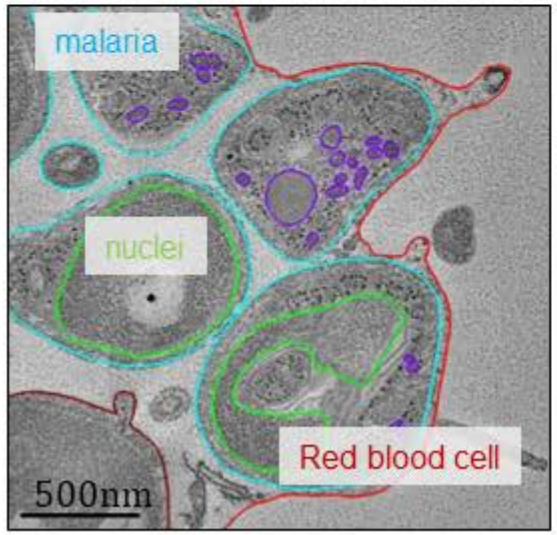
✓ **Post processing/analyses**

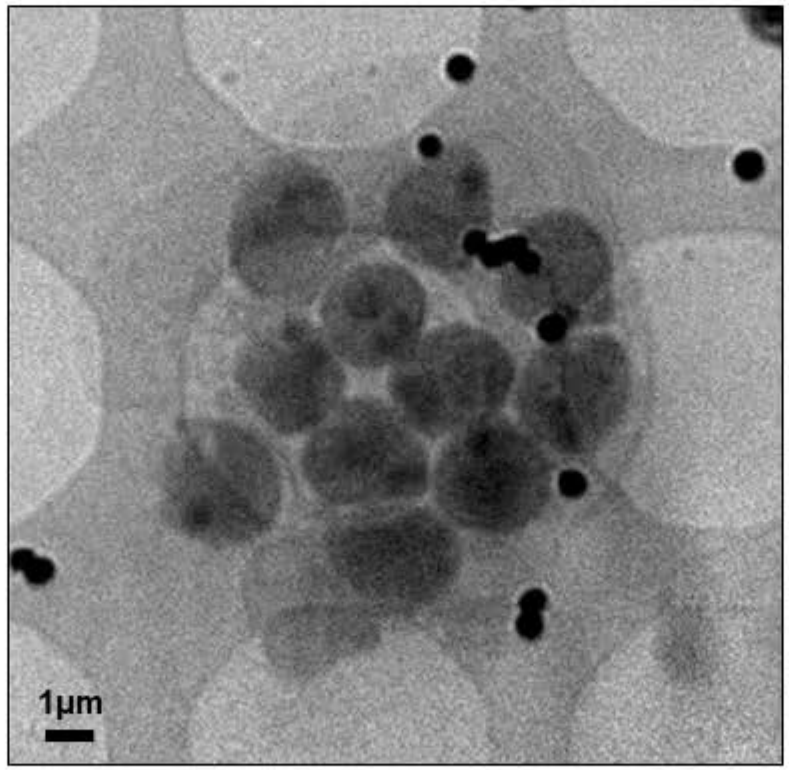
- Data mining — dependent on user requirements

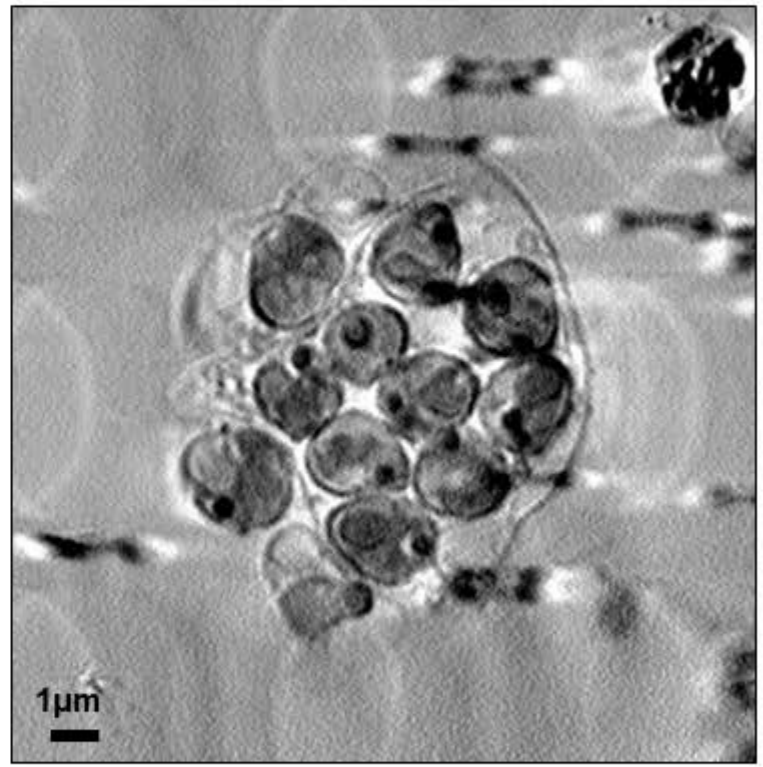
Science using SXT at B24

Plasmodium falciparum biology

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





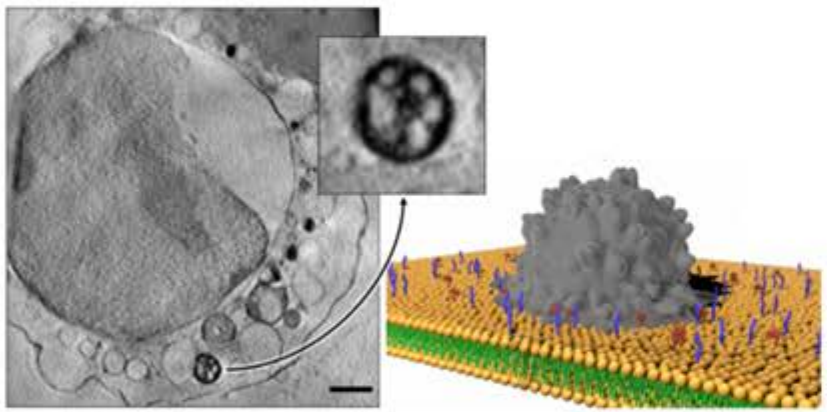


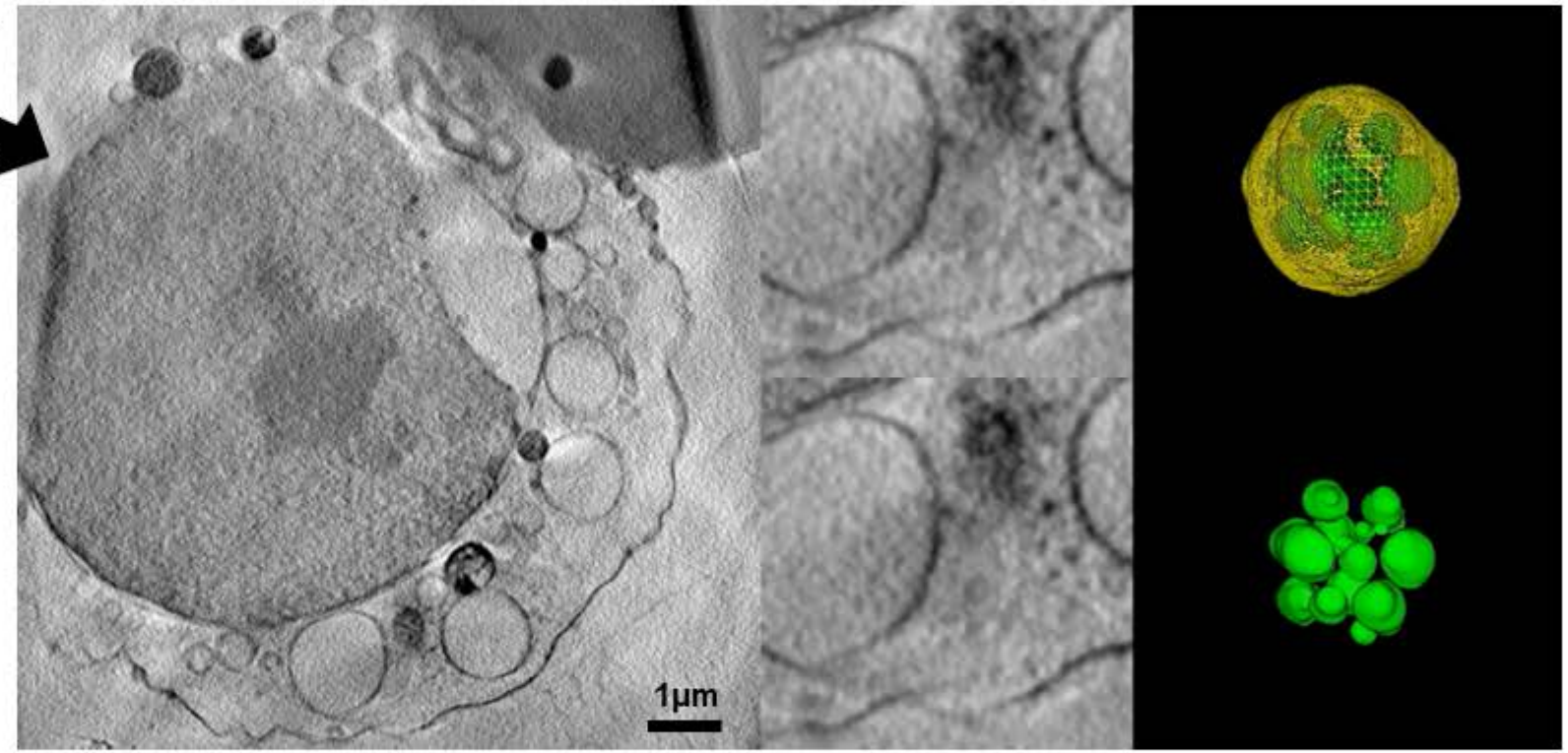
Hale V. L. et al.
Parasitophorous vacuole poration precedes its rupture and rapid host erythrocyte cytoskeleton collapse in *Plasmodium falciparum* egress
Proc Natl Acad Sci U S A. 2017 Mar 28;114(13):3439-3444.

Cell-cell killing and new cytotoxic organelles

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

Cytotoxic T cell





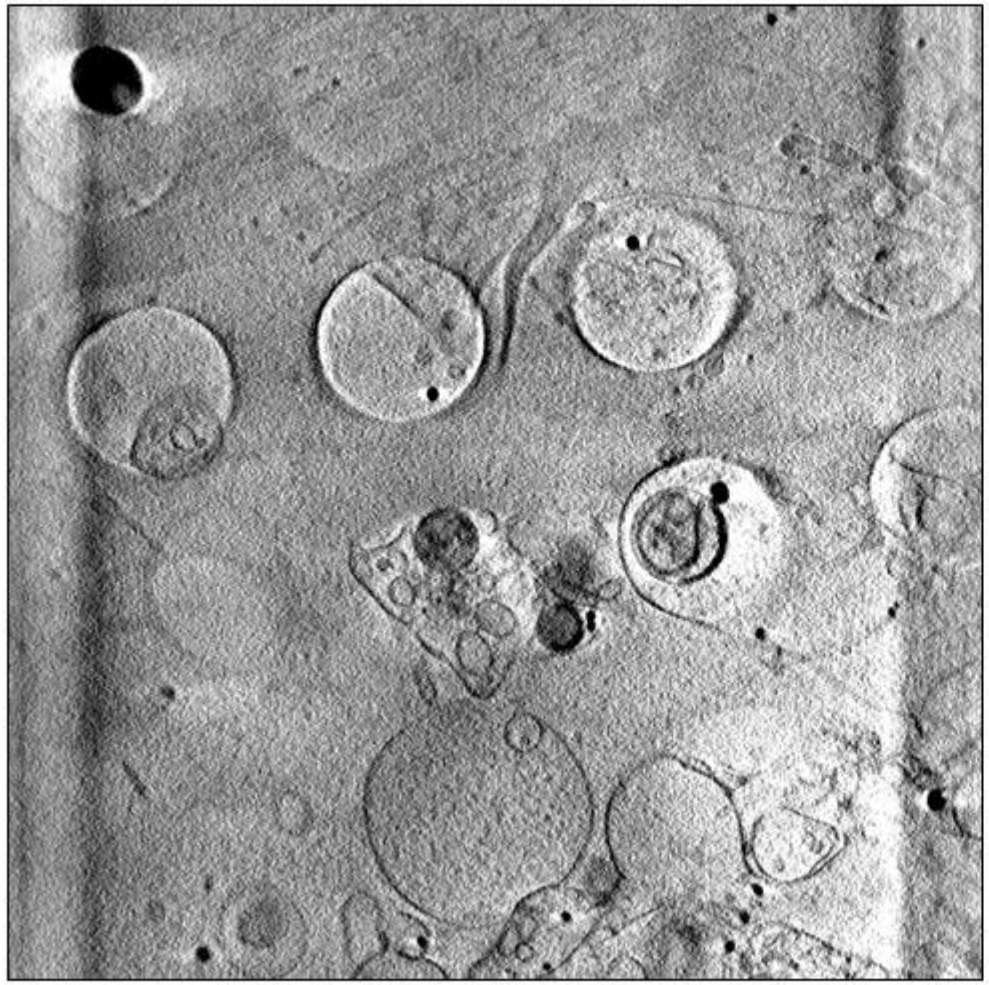
Balint S.et al.
Supramolecular attack particles are autonomous killing entities released from cytotoxic T cells
Science. 2020 May 22;368(6493):897-901.

Having SXT, do we really need further complementary information?

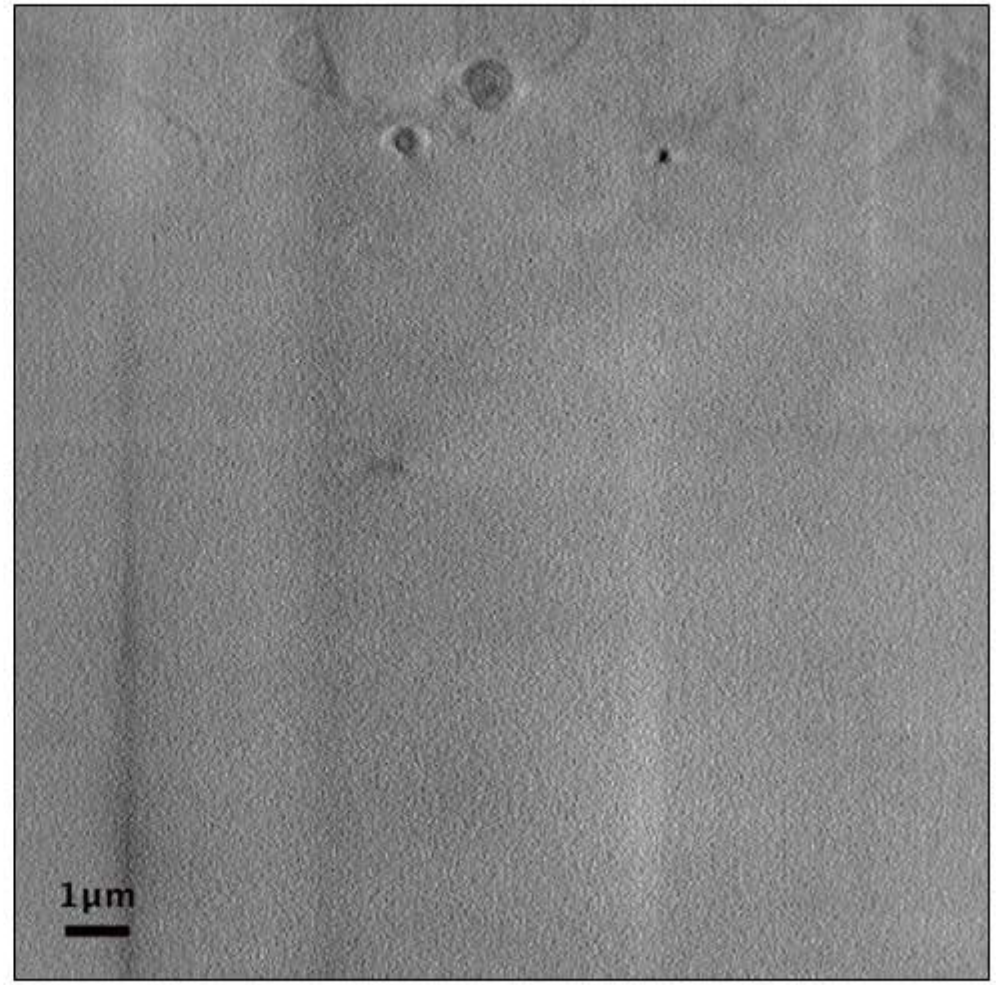
diamond

Reovirus infection in osteosarcoma cells

SXT



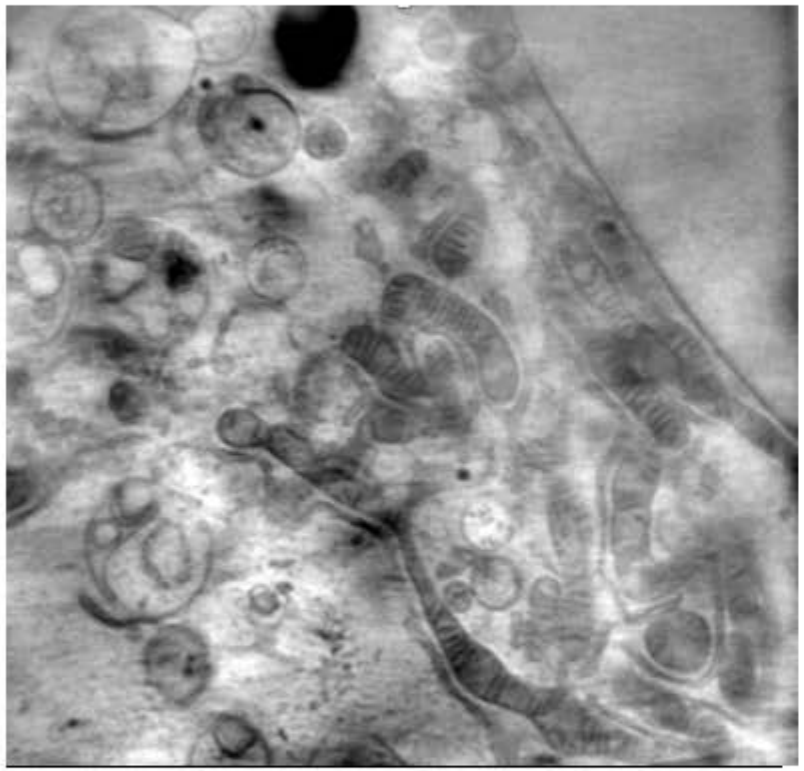
CLXT



2nd Microscope:

Cryo- Structure Illumination Microscope

Correlating microscopies for B24

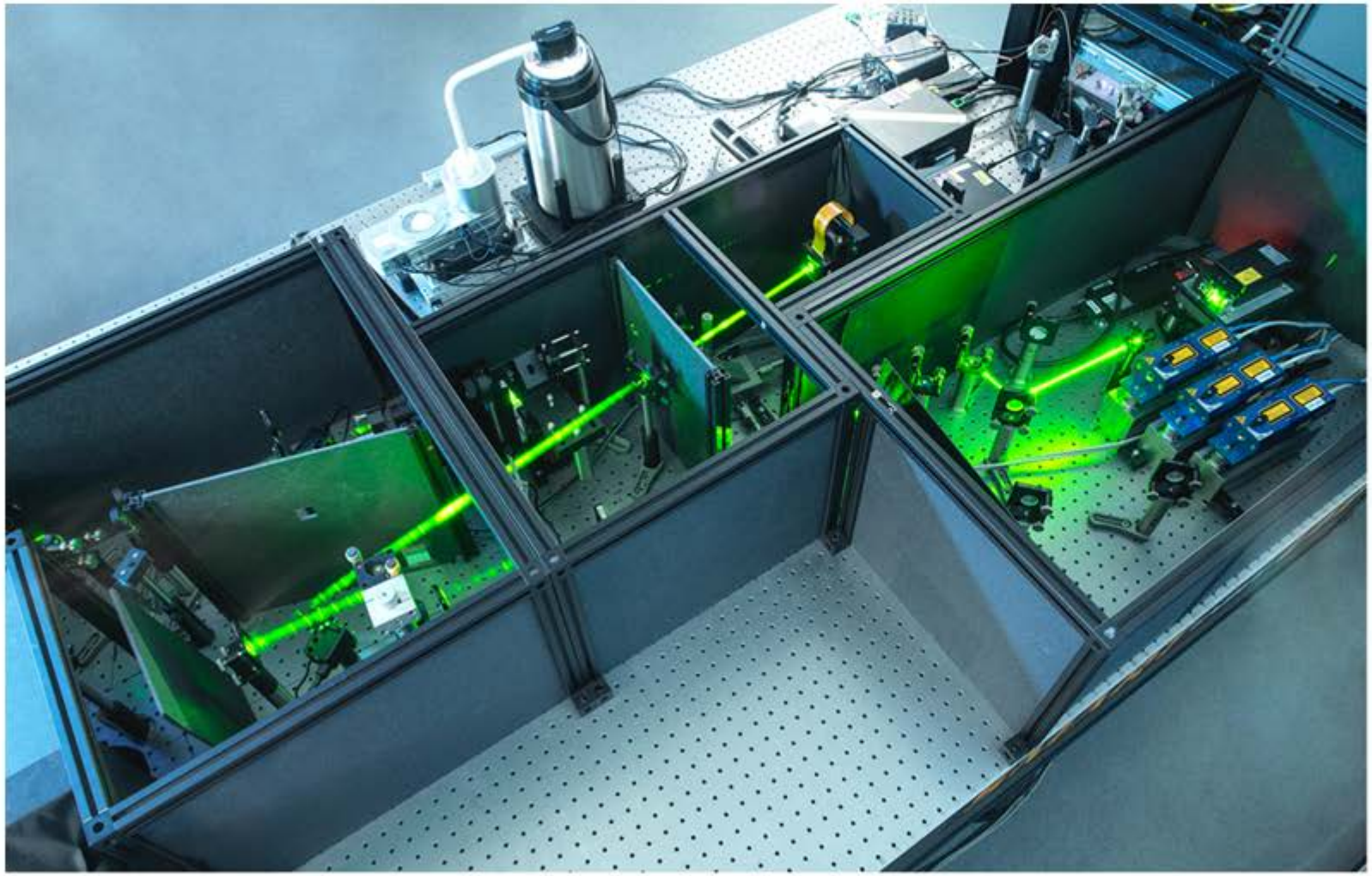


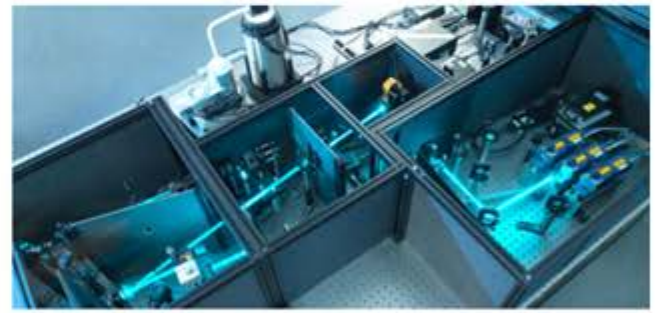
-Natural absorption
-25nm resolution
-sample cryo-fixed

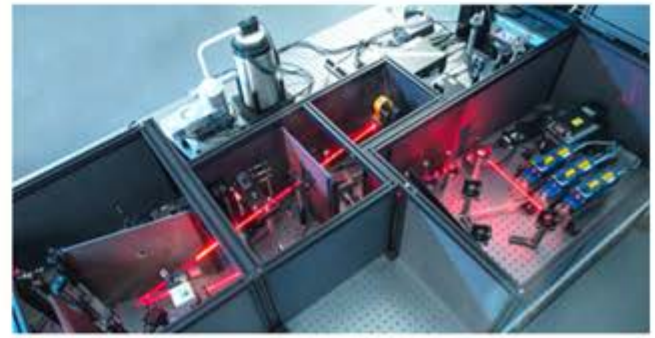
The requirements for a microscope to correlate:

- 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

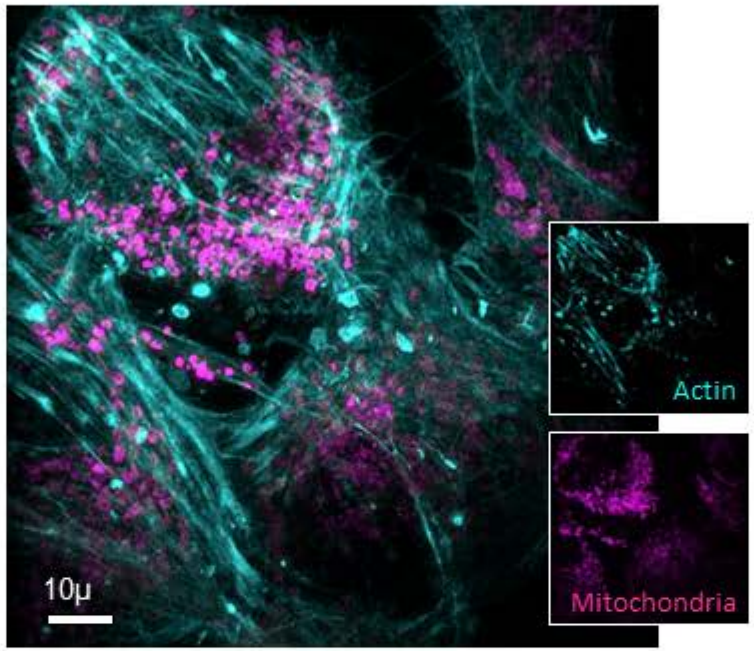
The B24 Structured Illumination Microscope

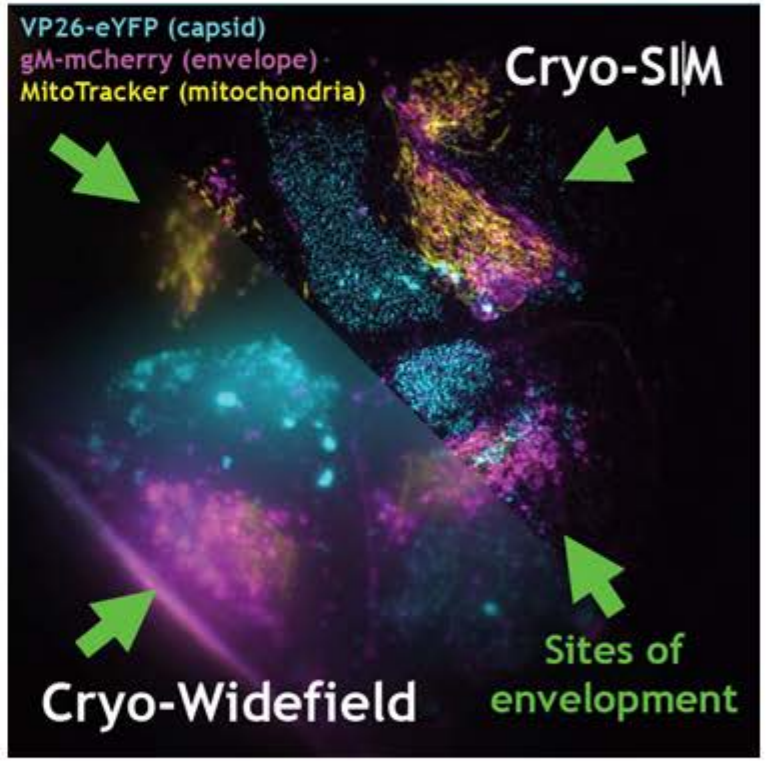






Super resolution 3D cryo-fluorescence imaging at B24

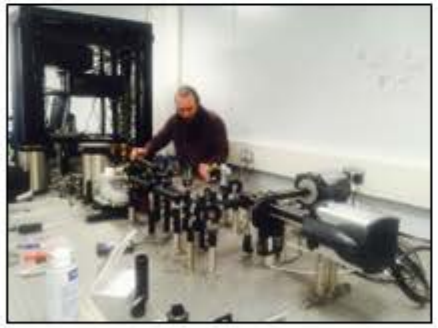


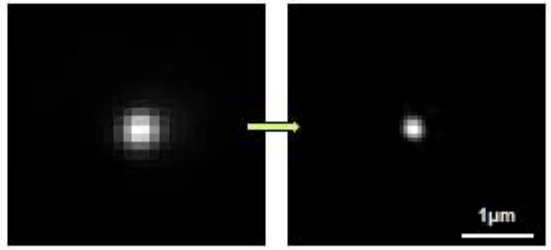


Special thanks go to:


Mick Phillips


Ian Dobbie

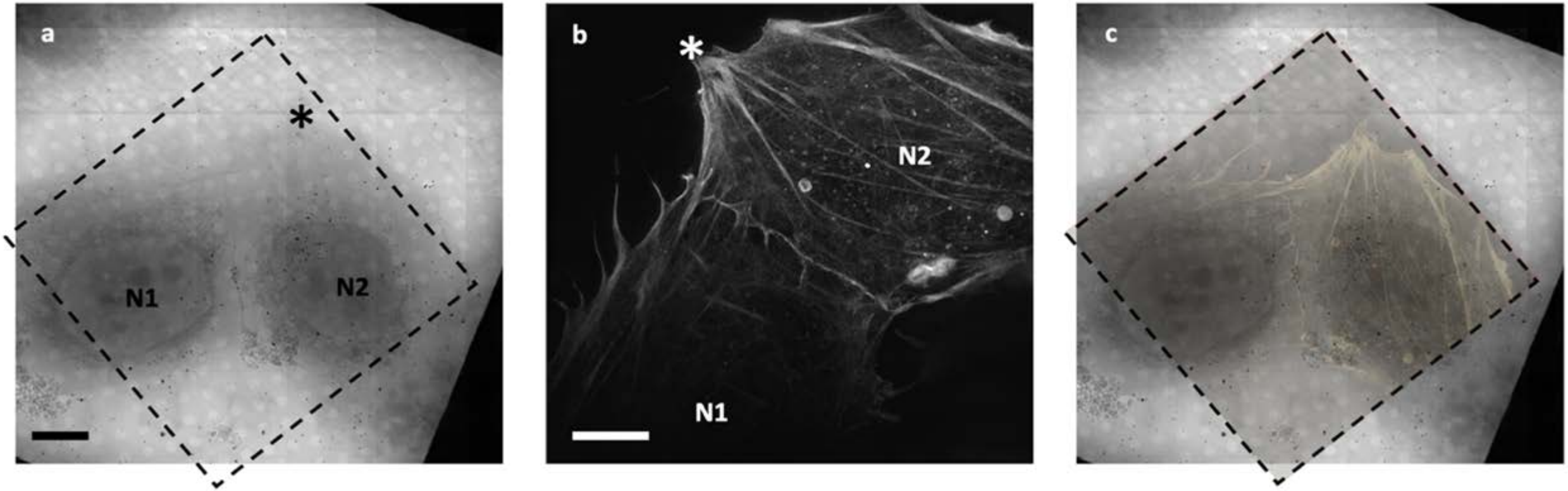




Cryo Structured Illumination

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

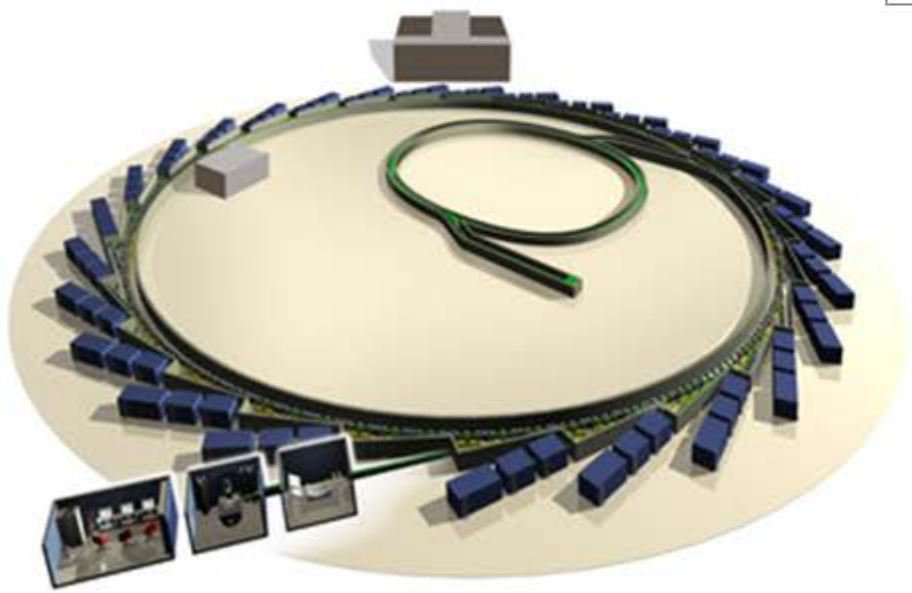
CLXT at B24: seeing the same thing with different eyes



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?

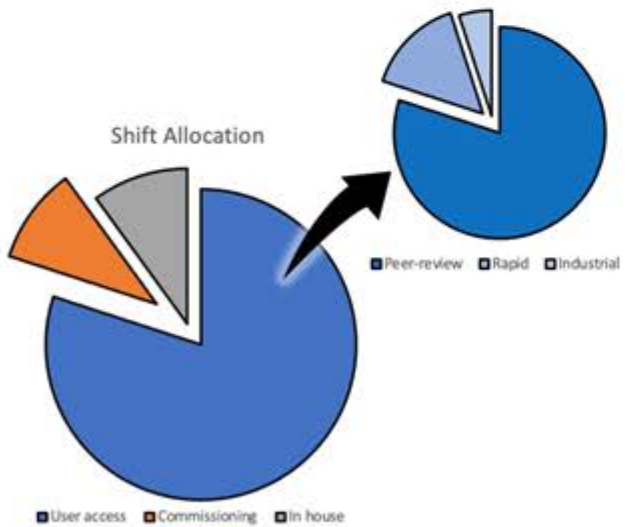
CLXT: Where can you find it?

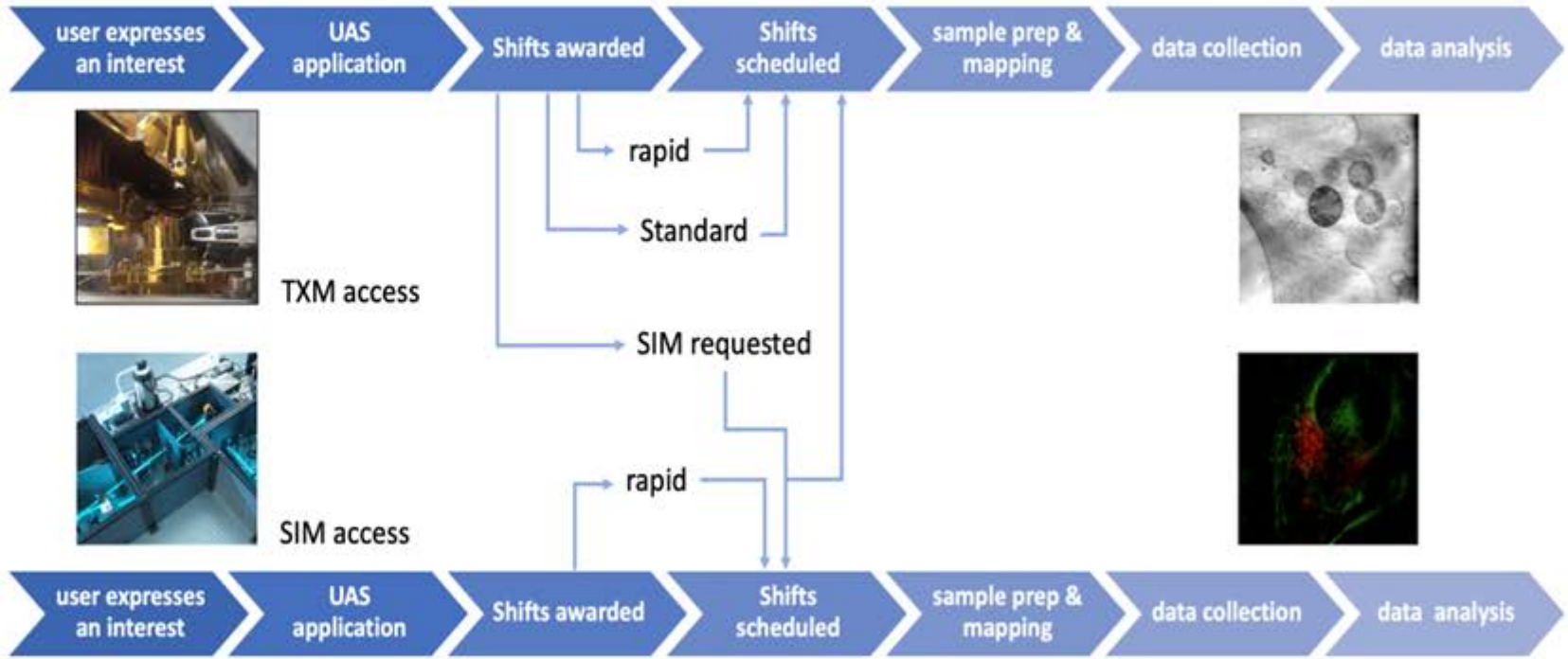


Beamline	Source	Energy range, eV	Spatial resolution (nm)	Additional techniques (to water window SXT)
Diamond, U.K. Beamline B24	Bending magnet	200–2600	25–40	CryoSIM Phase-contrast XANES
ALBA, Spain Beamline Mistral	Bending magnet	270–1200	25–40	XANES
NSRL, China SXM Beamline	Bending magnet	200–2500	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 @Birkberk, M Dustin @Oxford, A Wann @Oxford, P Zhang @eBIC, R Robinson @Okayama

Special thanks to our wonderful user community!!!!!

... and thank you for listening!