

# A laboratory-based soft X-ray microscope for whole cell imaging

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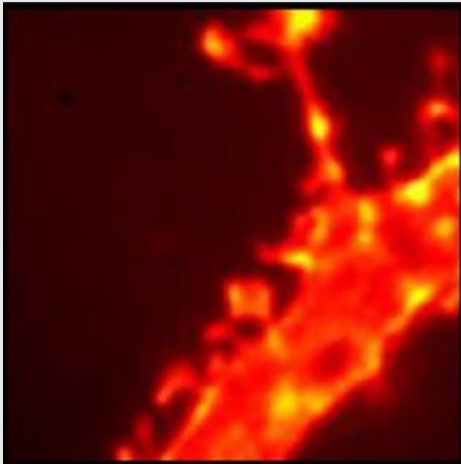


# COMMONLY USED IMAGING METHODS

Labelled content only

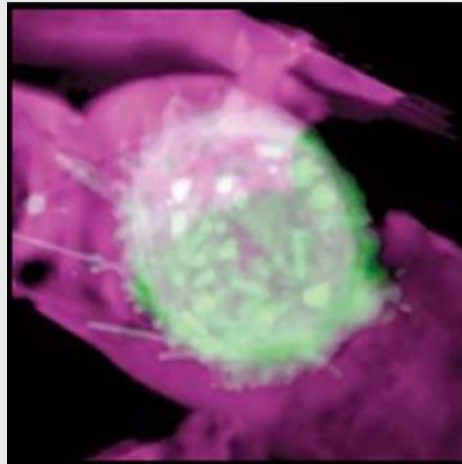
Whole structure

Wide-field  
fluorescence



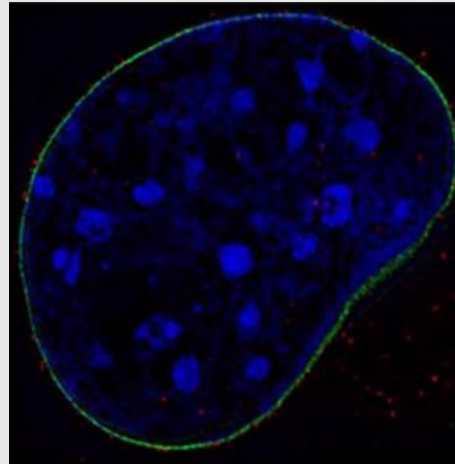
Hafi et al. (2014)

Light Sheet



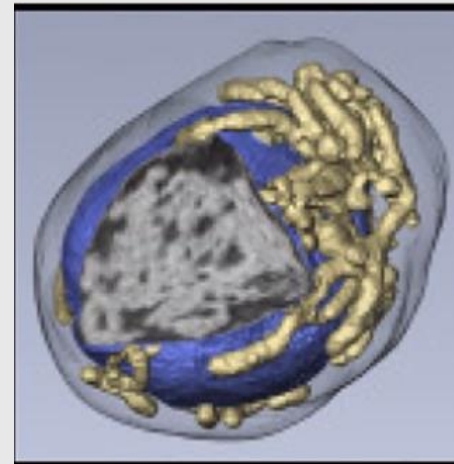
Liu et al. (2018)

Super-resolution  
fluorescence



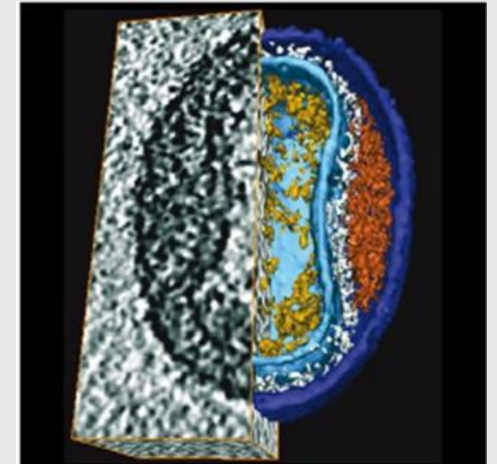
Schermelleh et al. (2009)

Cryo-soft x-ray  
tomography



Le Gros et al. (2016)

Cryo-electron  
tomography



Cyrklaff et al. (2007)

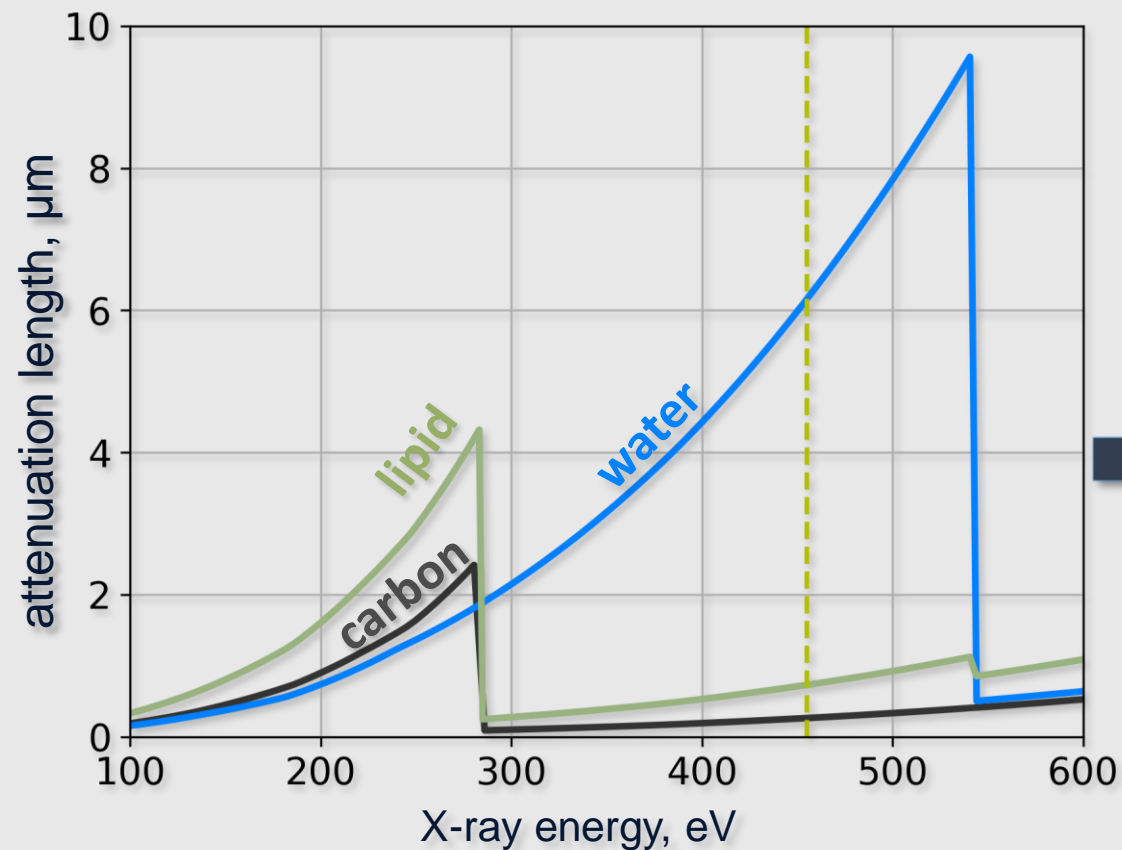
Resolution

70-250nm

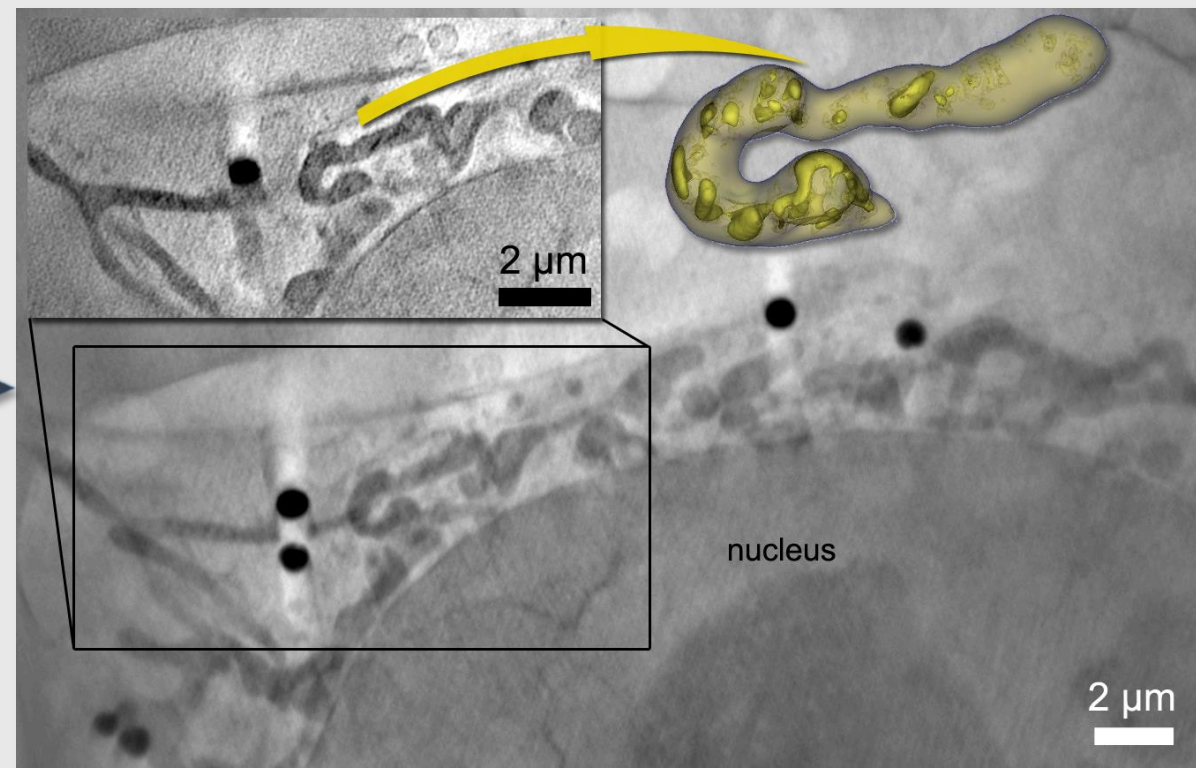
50nm

2-5nm

# SOFT X-RAY TOMOGRAPHY CONTRAST MECHANISM



Lab-based SXT of a HeLa cell



**Cellular structure in 3D + Element-specific contrast from cells + Native environment**

Fully hydrated

Whole cell

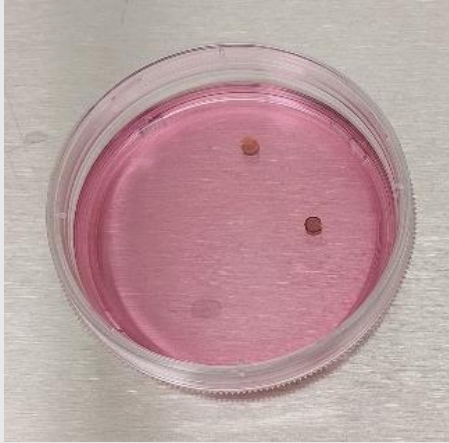
No staining

No chemical fixation

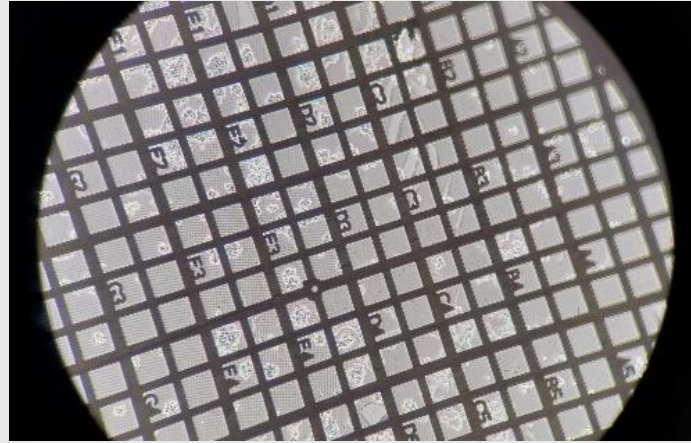
Cryo

# SAMPLE PREPARATION: JUST FREEZE

## EM Grids

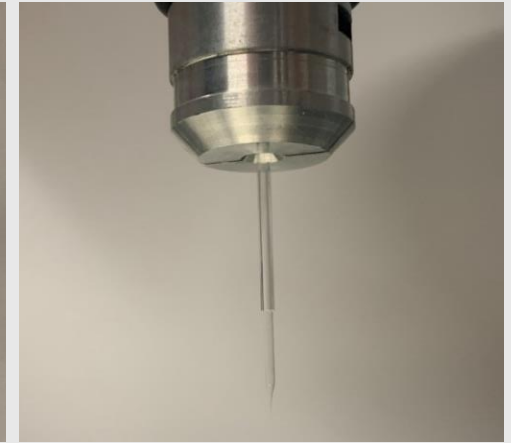


Grids in culture



Cells on grid

## Plunge freezing grids and capillaries



## Glass capillaries



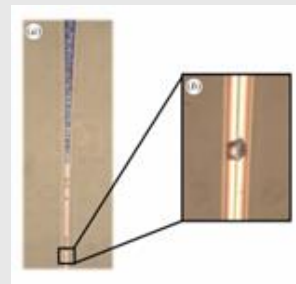
Harvesting cells



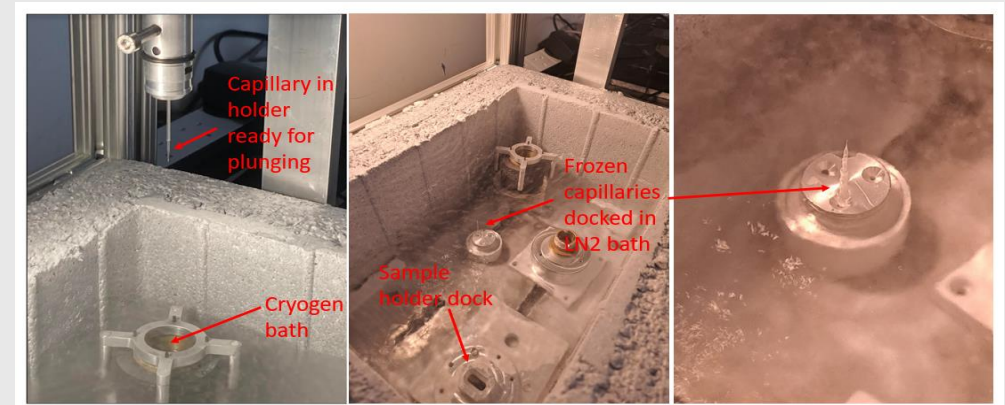
Harvesting cells



Loading

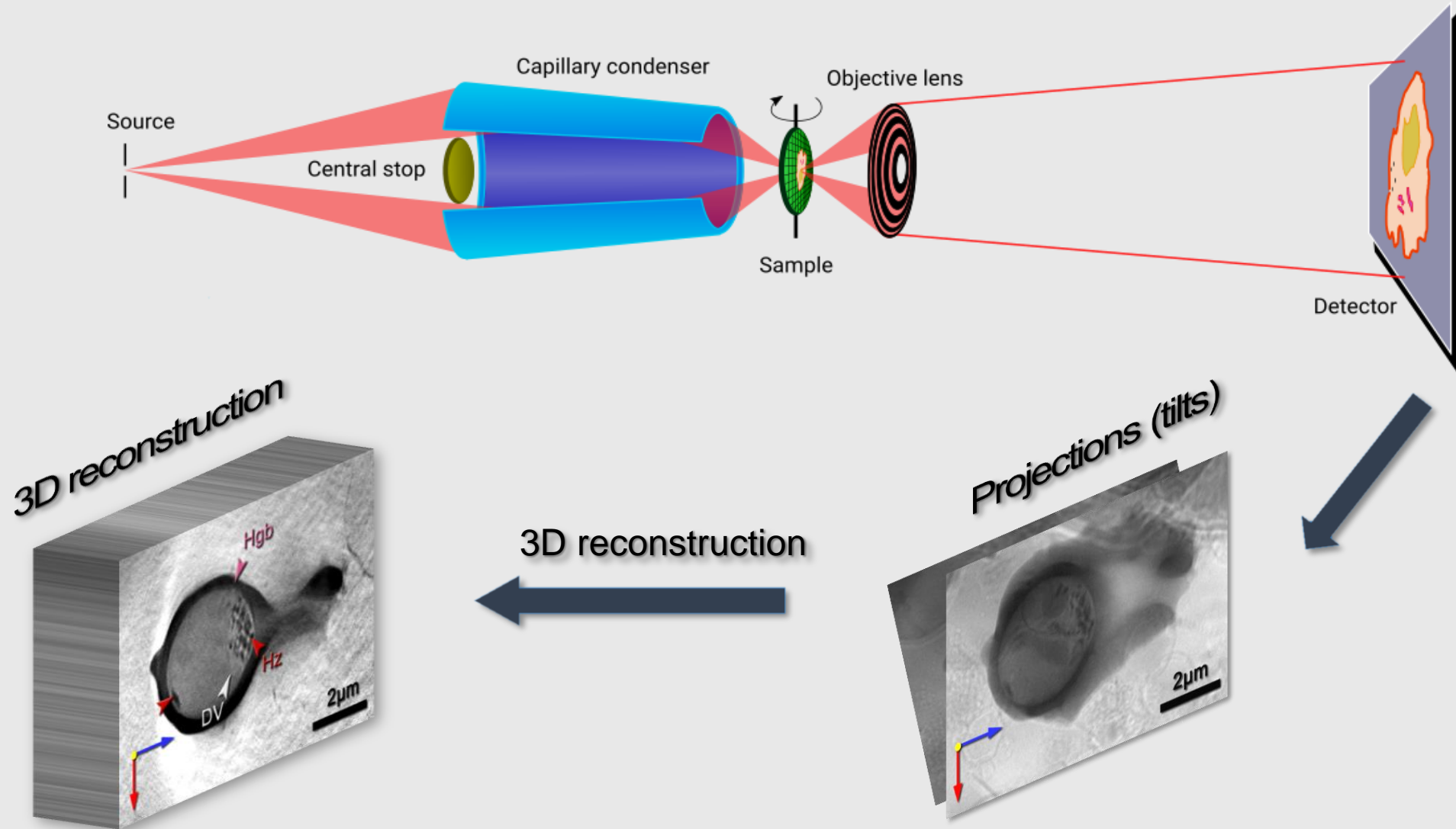


Cells in capillary tip



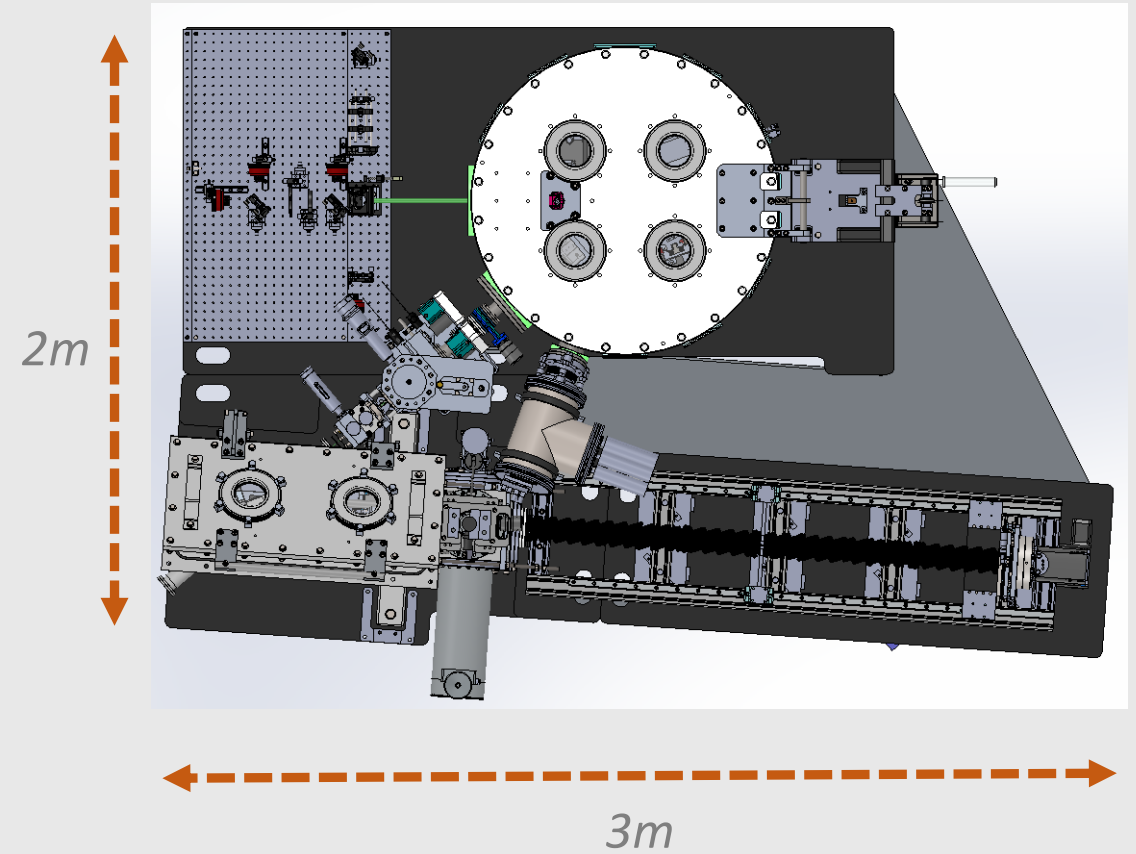
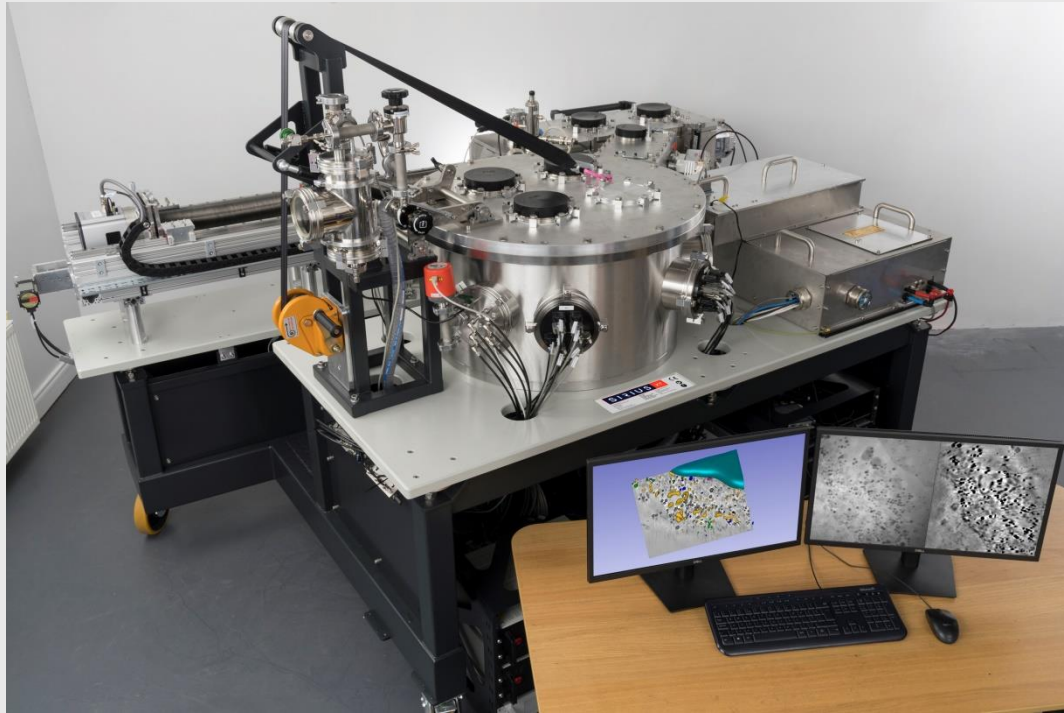
# SOFT X-RAY TOMOGRAPHY OVERVIEW

SIRIUS XT



# — THE SXT-100 LABORATORY MICROSCOPE

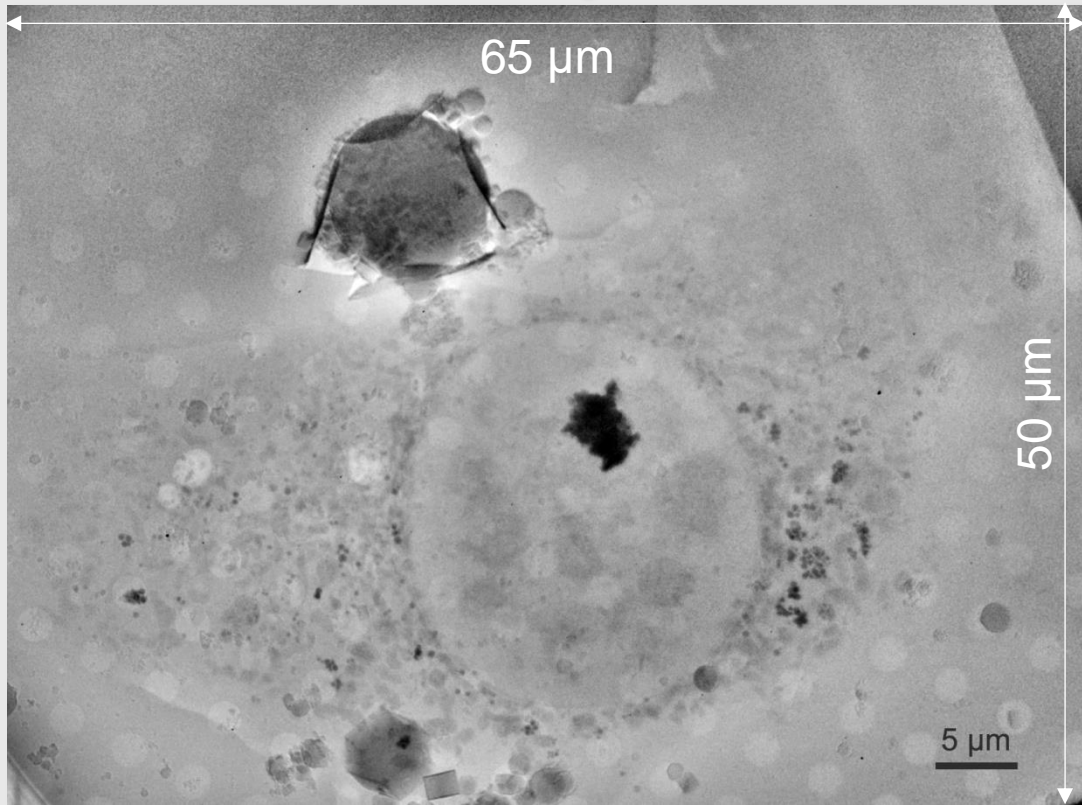
SIRIUS XT



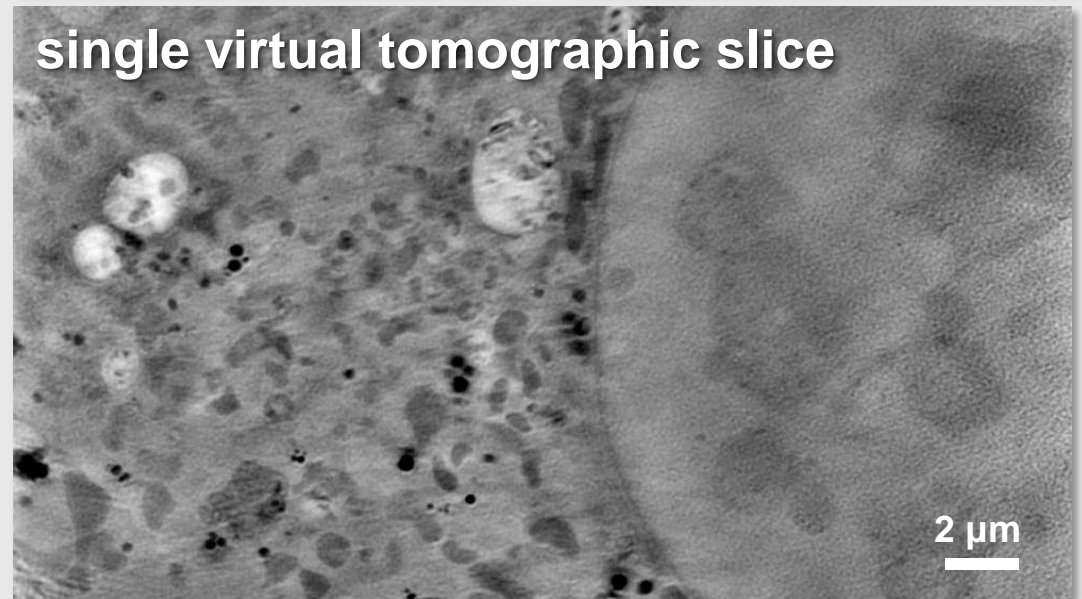
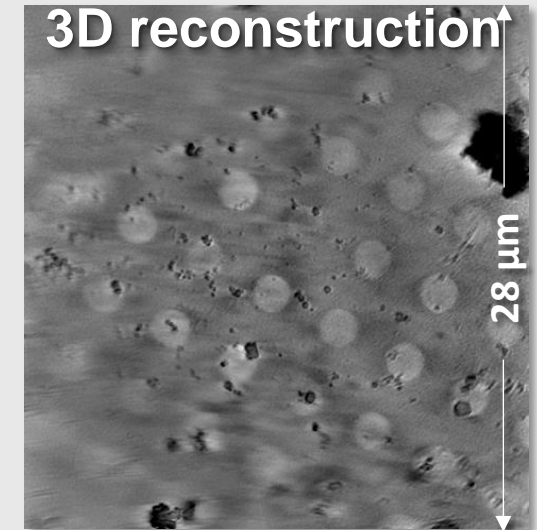
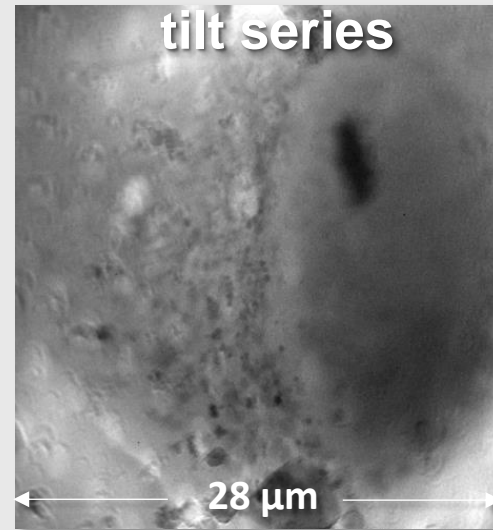
# CRYO-SXT OF MAMMALIAN CELLS

SIRIUS XT

2D soft X-ray overview

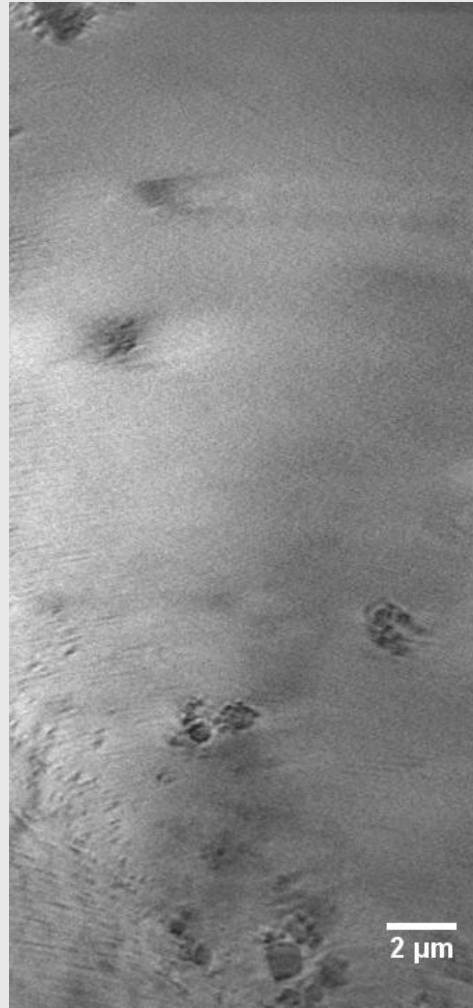
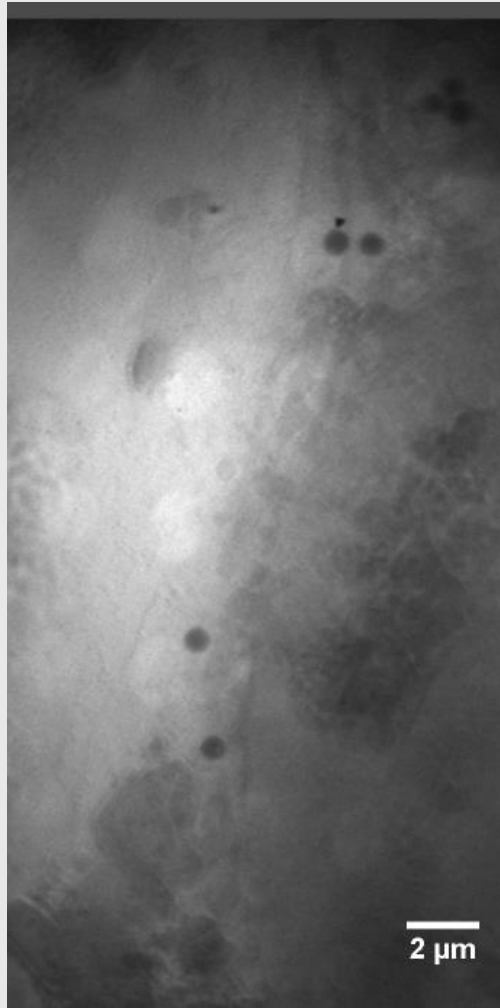


NIH-3T3 cell

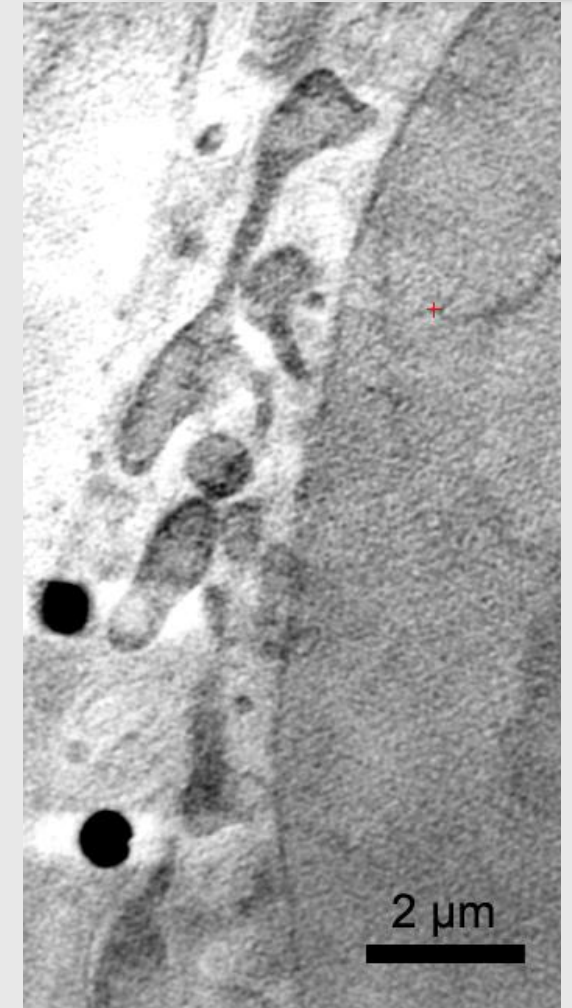
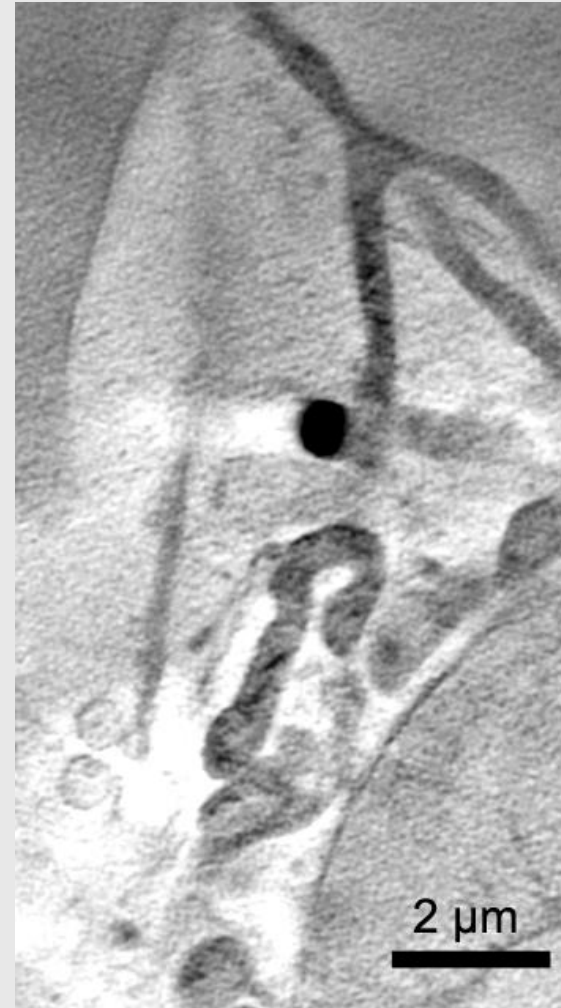


# CRYO-SXT OF HELA CELLS

SIRIUS XT



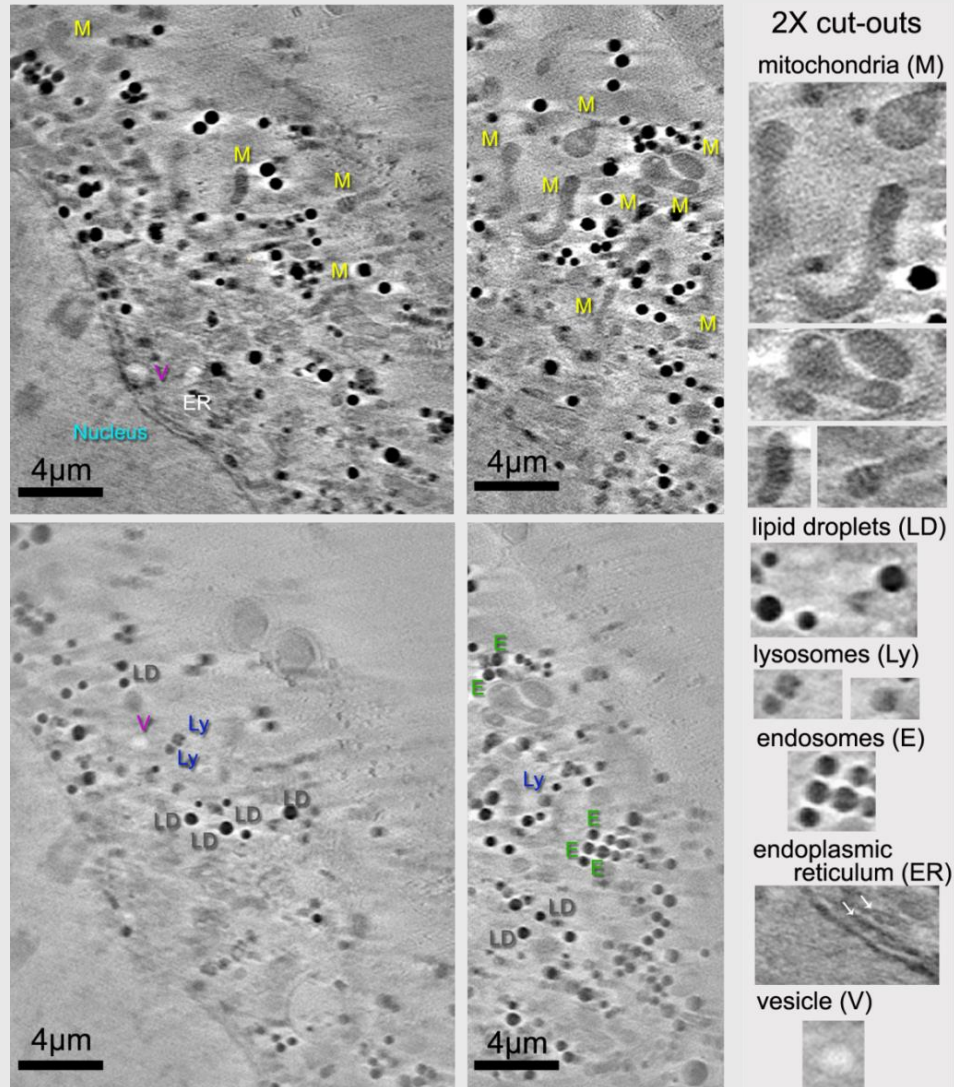
reconstruction 20x20x20 nm voxel size



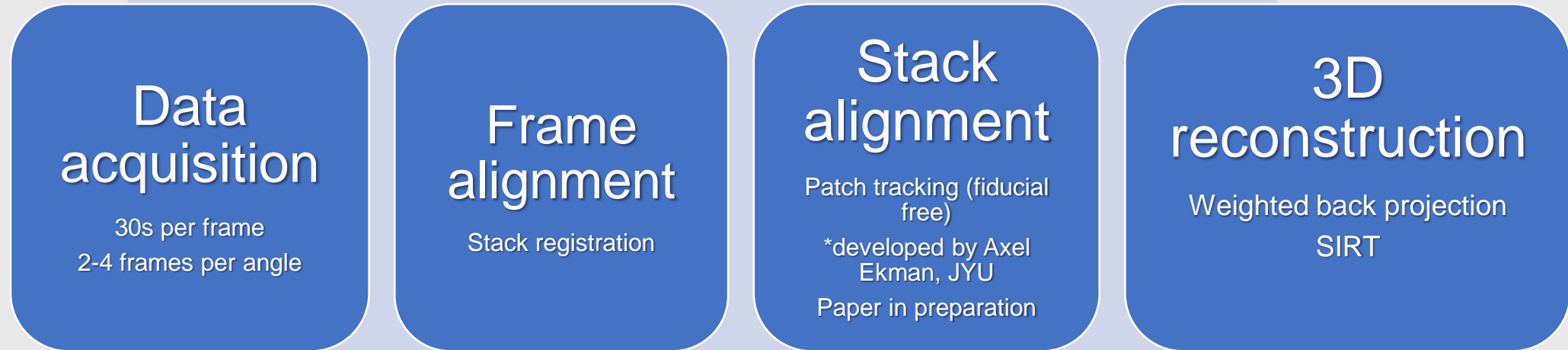
two slices through the reconstruction

Sample prepared by Dr. Maria Harkiolaki, Diamond Light Source





Samples from Dr. Chris Evans UCD (CoCID)

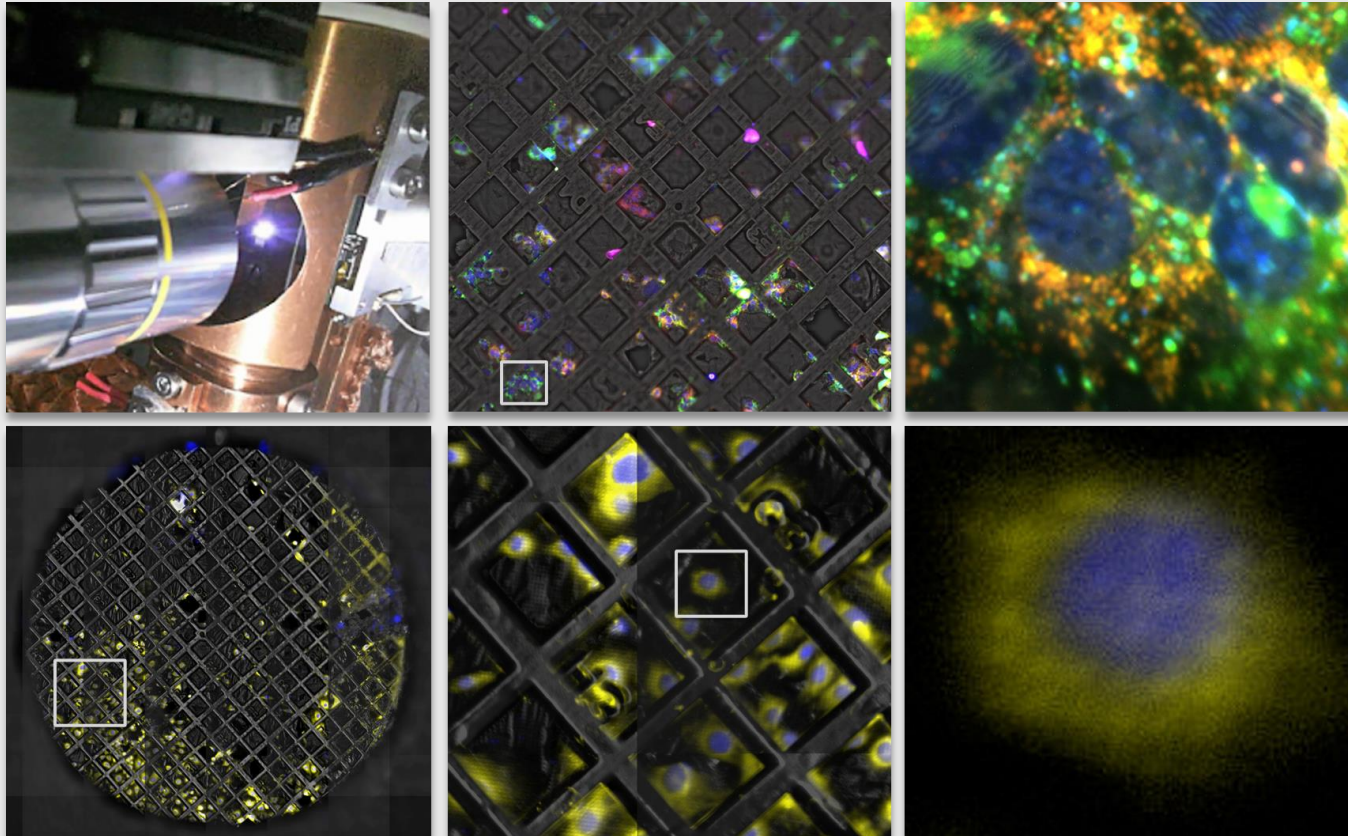


*In collaboration with Axel Ekman, JYU Finland*

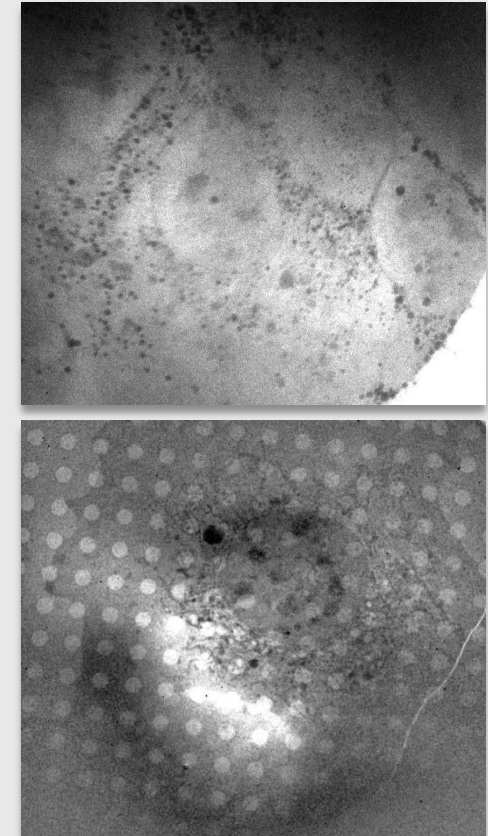
# — INTEGRATED FLUORESCENCE MICROSCOPE

SIRIUS XT

Dual magnification epi-fluorescence microscope



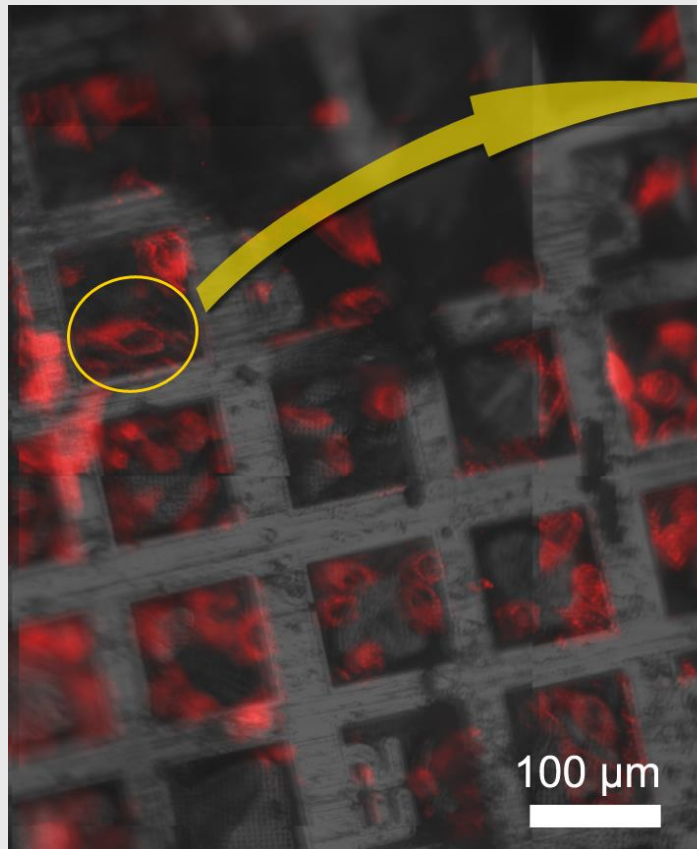
Soft X-ray low mag overview



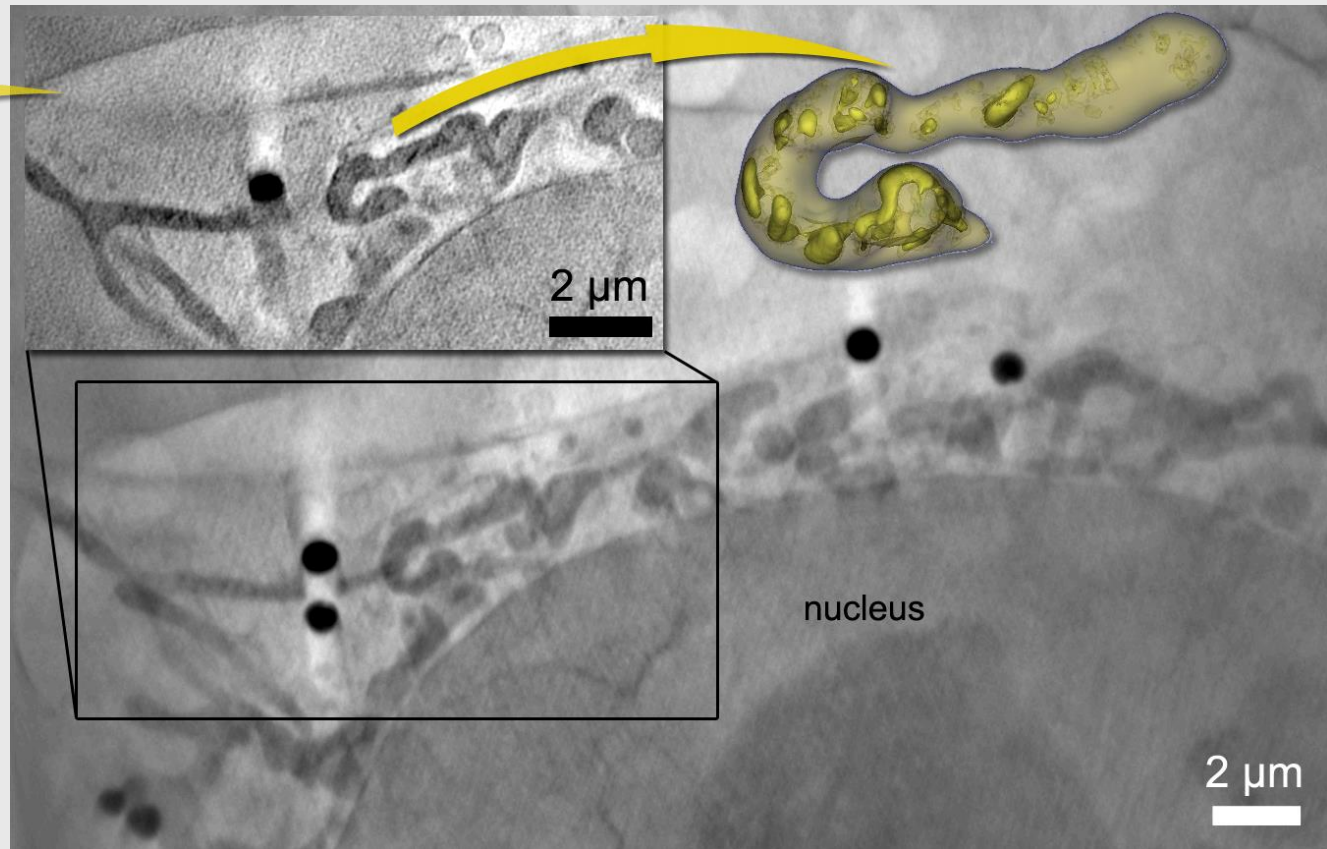
- Increased SXT throughput
- Correlating cell structure and function

Collaboration with Dr. Chris Evans, UCD Dublin and Dr. Simon Leclerc, JYU Finland

High throughput location of cells followed by SXT imaging

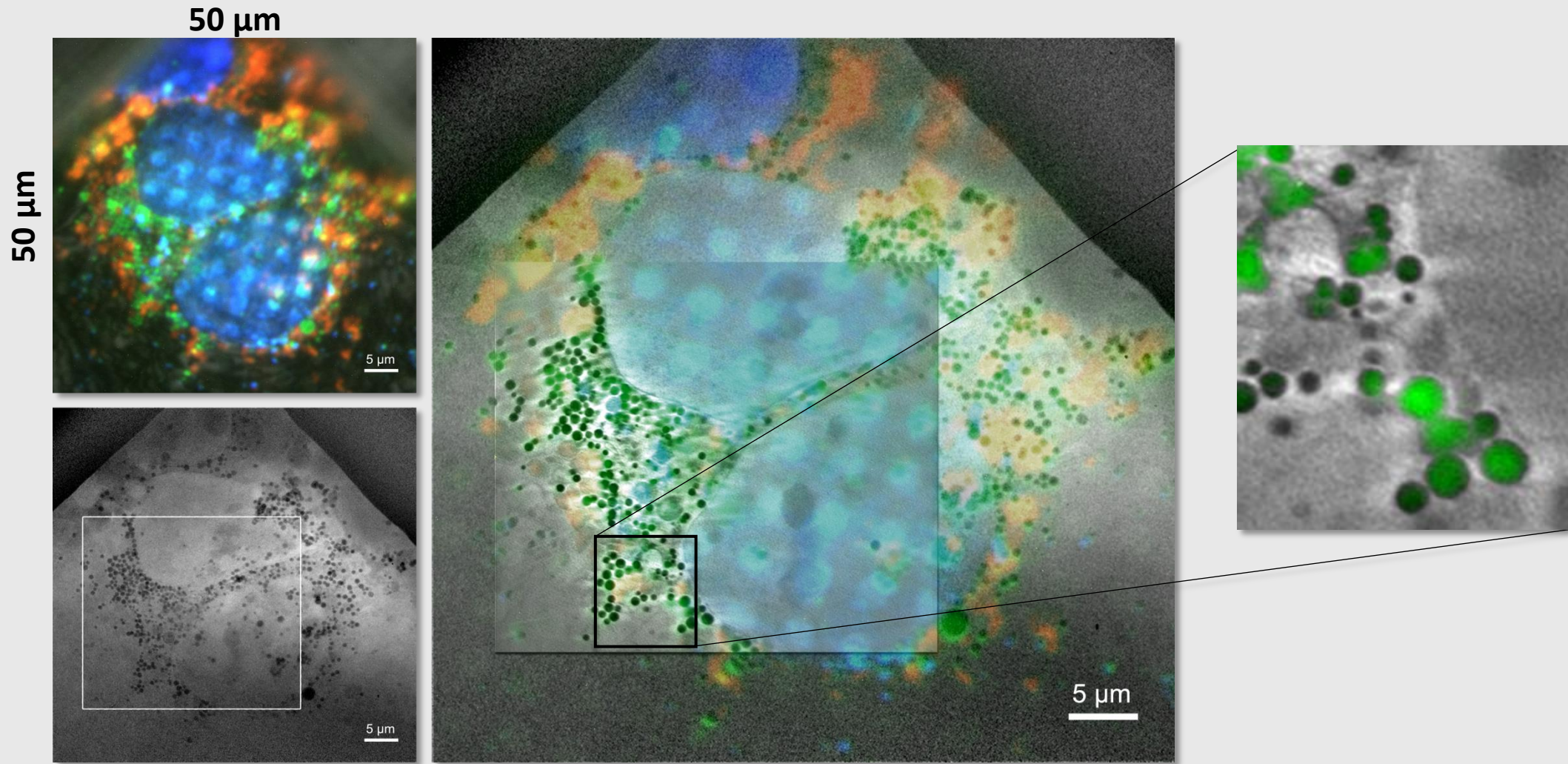


10X fluorescence image

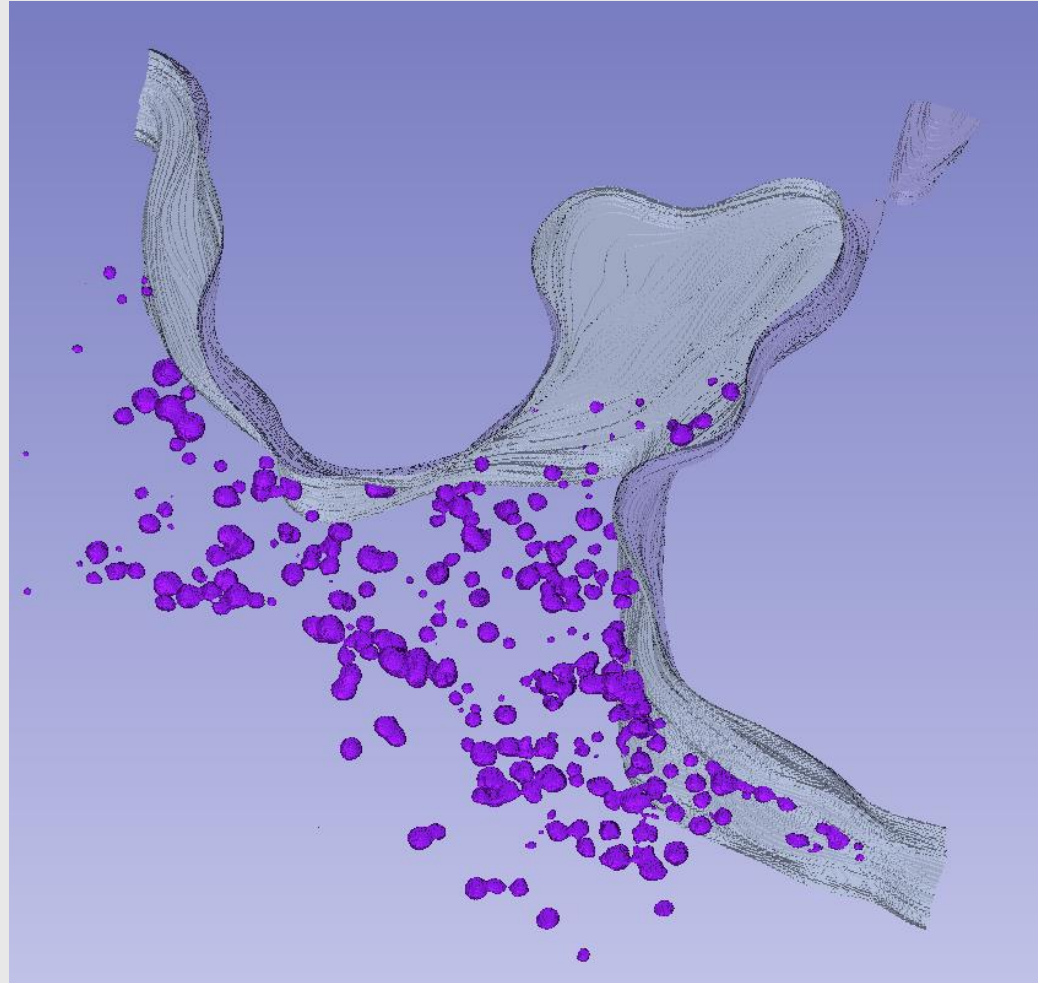


HeLa cell: 3D reconstruction slab

# CORRELATING SPECIFIC ORGANELLES

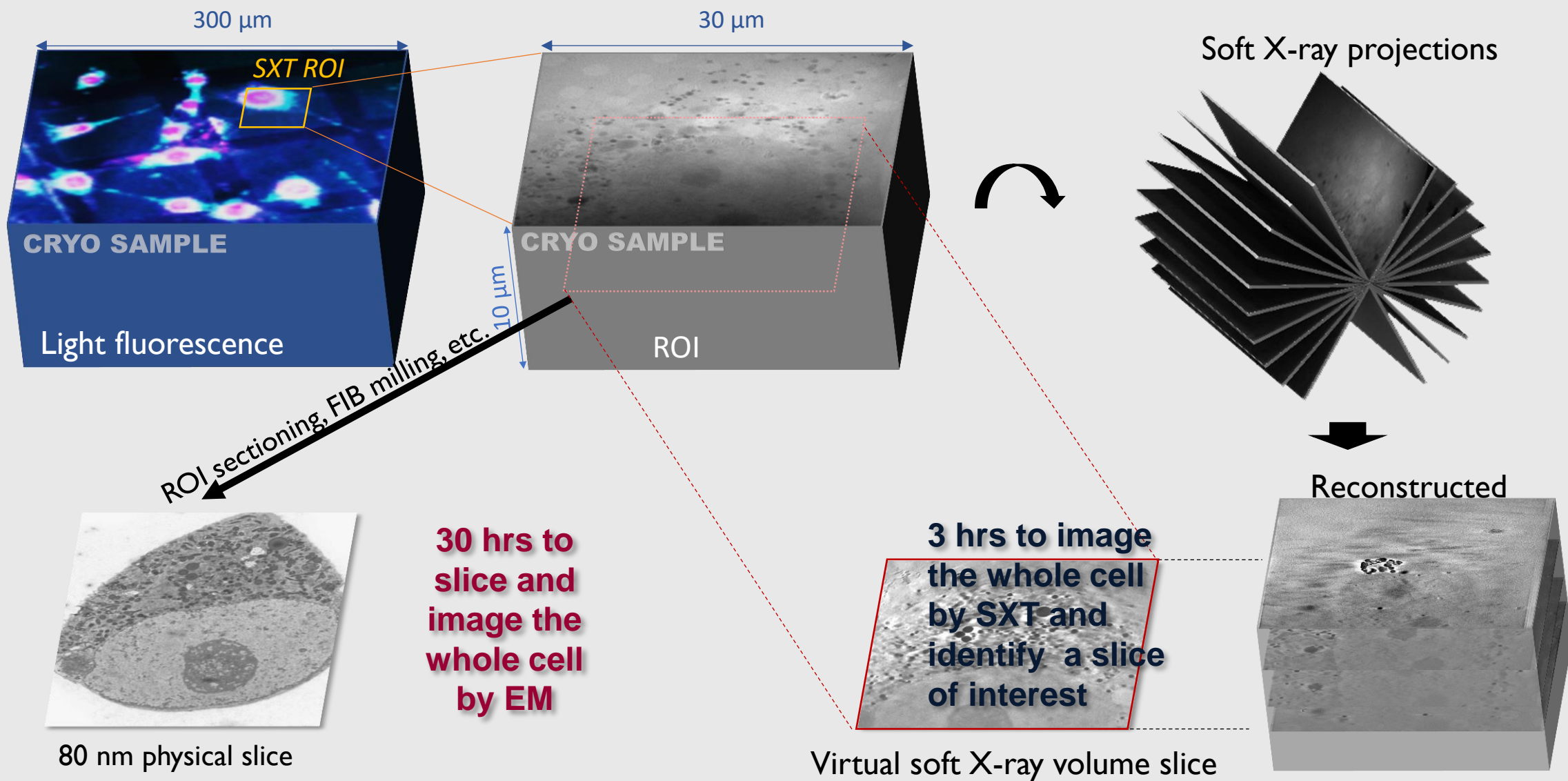


Collaboration with Dr. Chris Evans, UCD Dublin

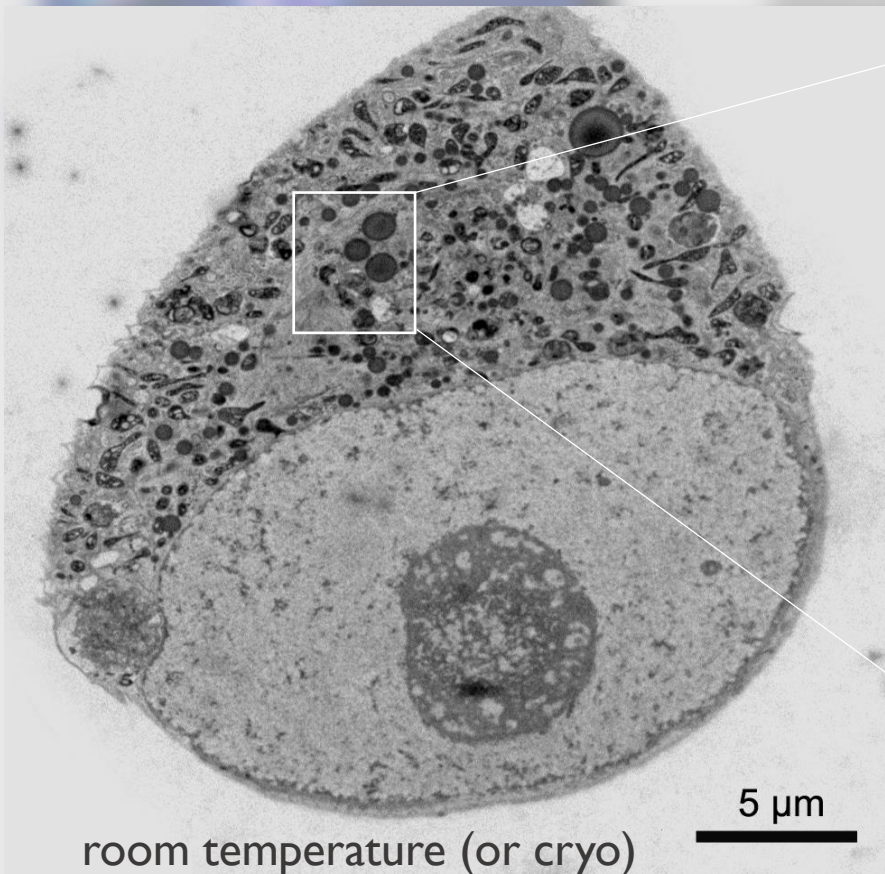


Segmented using Ilastik

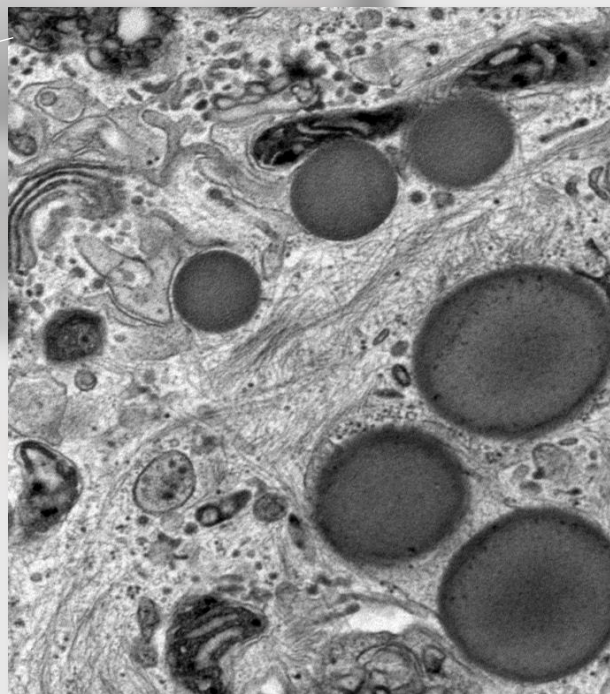
# CORRELATIVE LIGHT ELECTRON AND X-RAY MICROSCOPY



# EM

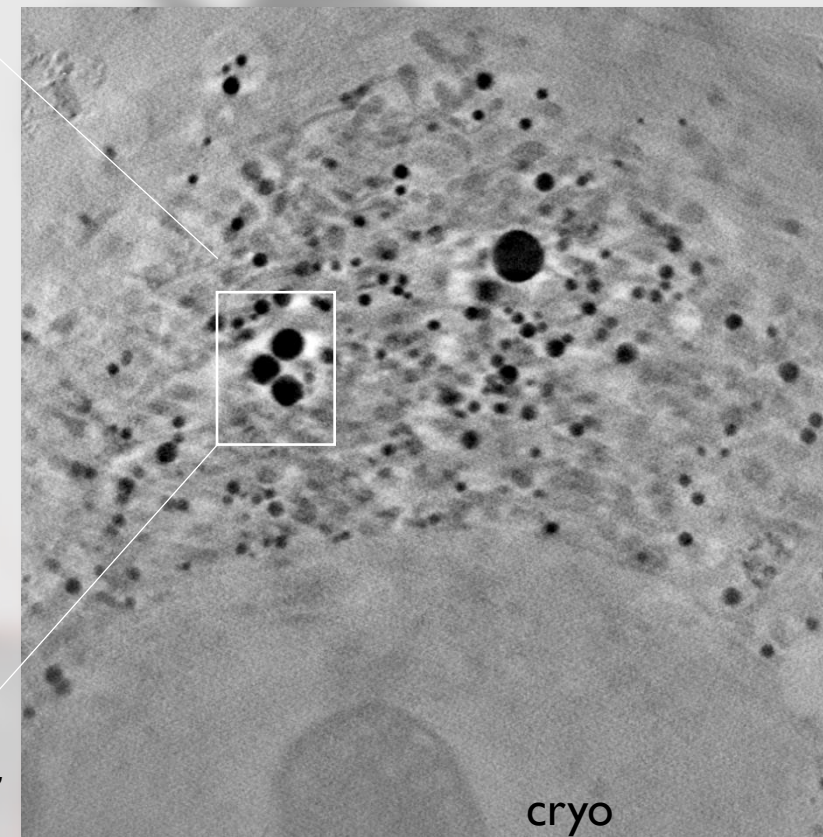


# EM zoom-in



*Collaboration with Dimitri Scholz and Tiina O'Neill, UCD Dublin*

# SXT



**Cryo-SXT can identify structures for EM sectioning or cryo lamellae**



— CORRELATIVE LIGHT ELECTRON AND X-RAY MICROSCOPY —

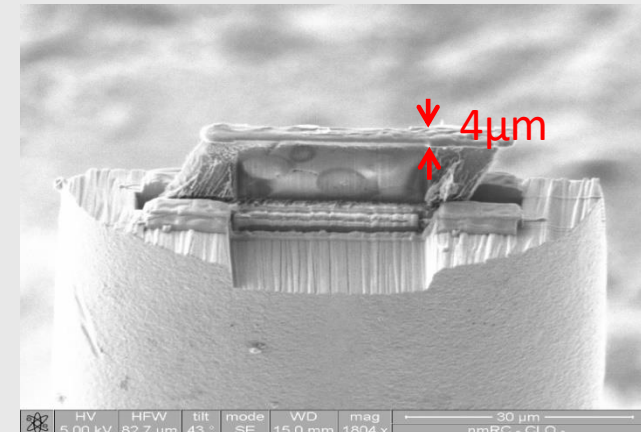
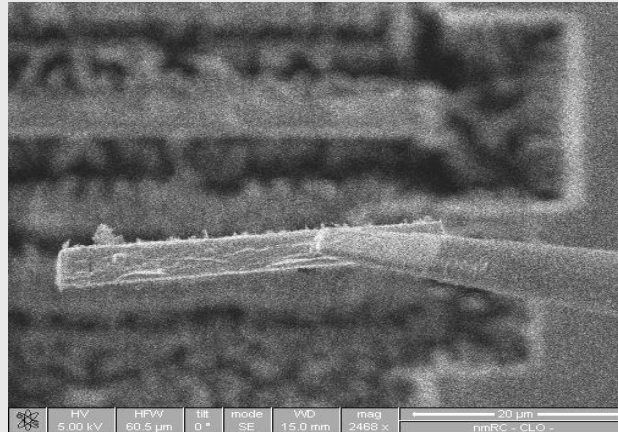
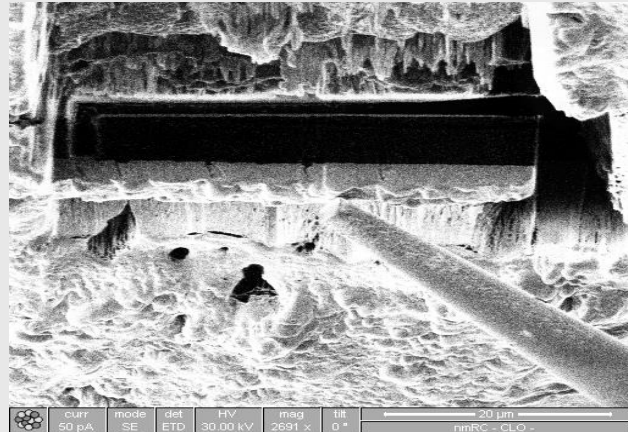
SIRIUS XT



[clexm.eu](http://clexm.eu)



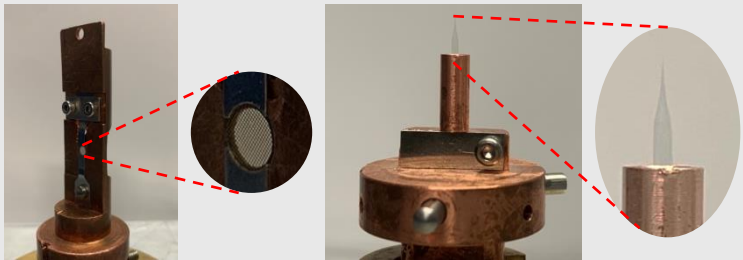
## Workflow for imaging bulk fully hydrated tissue samples



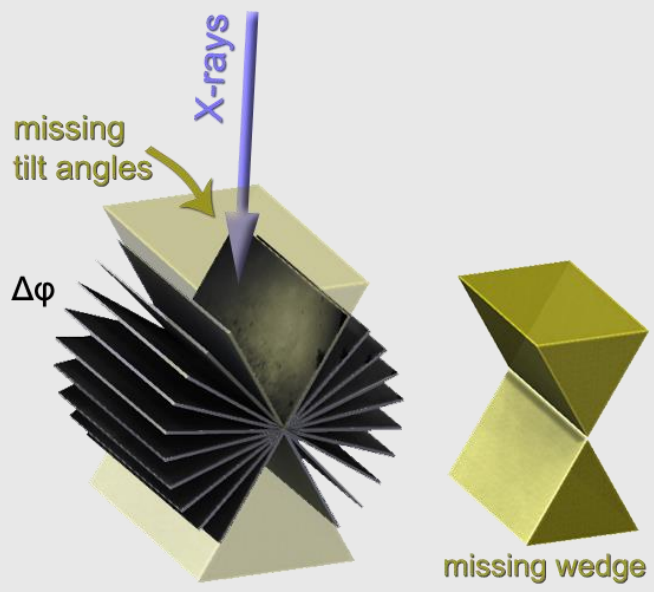
Collaboration with Chris Parmenter, University of Nottingham

# — FULL TILT TOMOGRAPHY WITH GLASS CAPILLARIES —

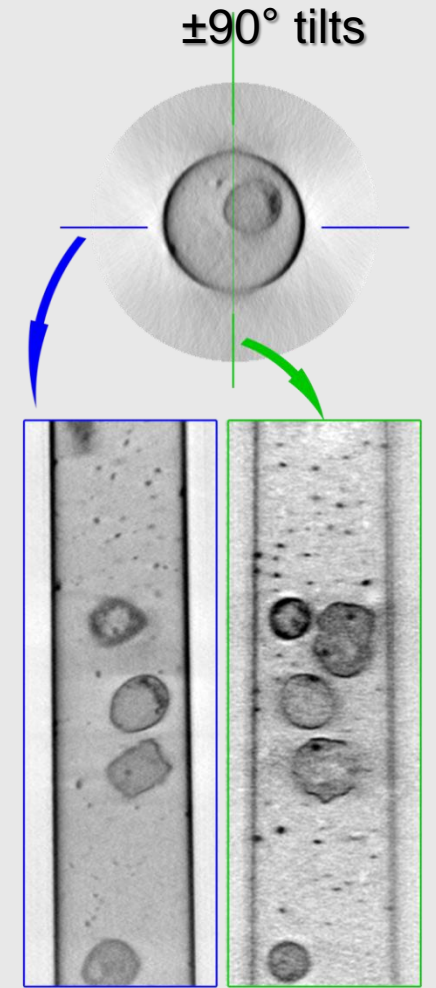
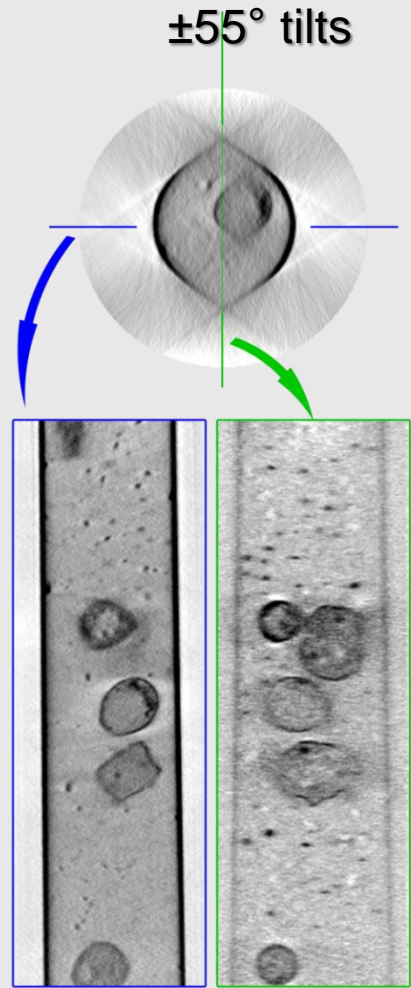
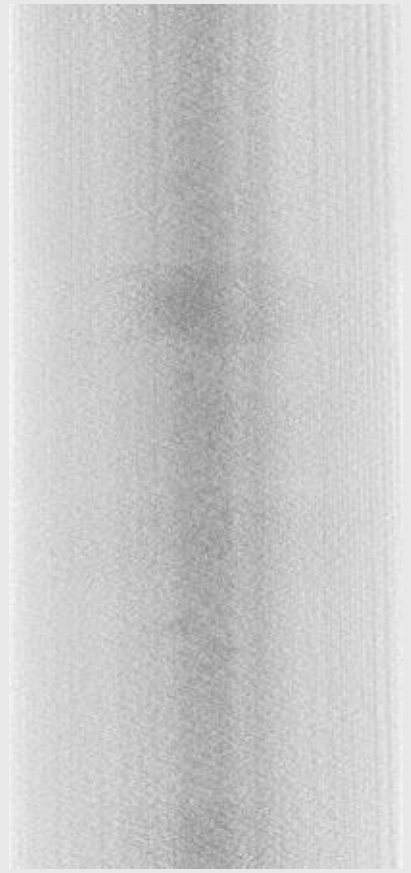
SIRIUS XT



partial-tilt tomography



sample tilt projections



Collaboration with Venera Weinhardt, Heidelberg University

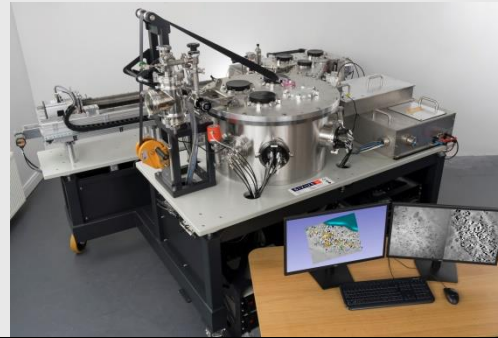


# — FIRST COMMERCIAL DEPLOYMENT, AUGUST 2023 —

**SIRIUS XT**



Conway Core Imaging Facility  
Dr. Dimitri Scholz



- 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

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  - Core Imaging lab at Conway Institute of Biomedical and Biomolecular Sciences



- Dr. Eva Pereiro
- Dr. Ana Perez-Berna



- Prof. Nicola Fletcher
- Dr Christopher Evans
- Dr. Dimitri Scholz
- Dr. Tiina O'Neill

## Thank you for listening!



**B08 – Biological Soft X-ray Tomography**  
**26<sup>th</sup> July**  
**Booth #532**

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- Dr. Simon Leclerc
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- Dr. Visa Ruokolainen