Correlative Light and X-ray Microscopy: CLXM

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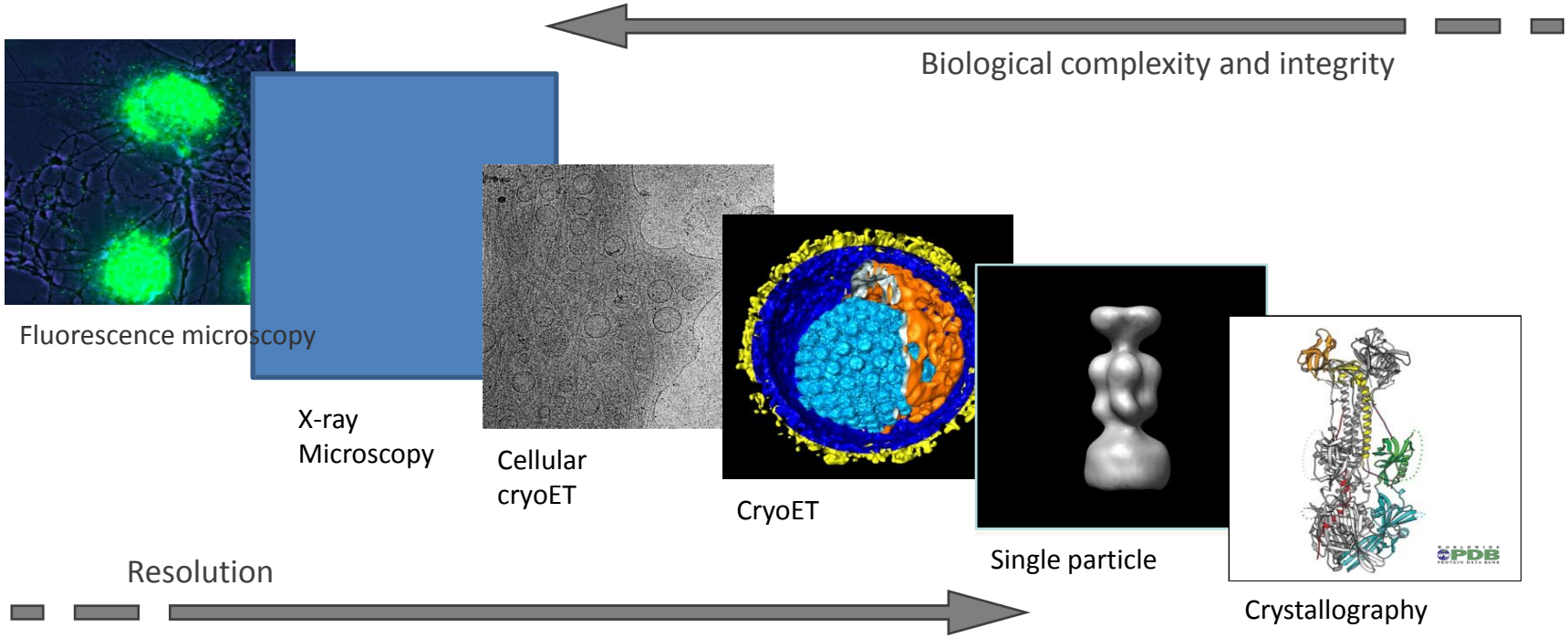
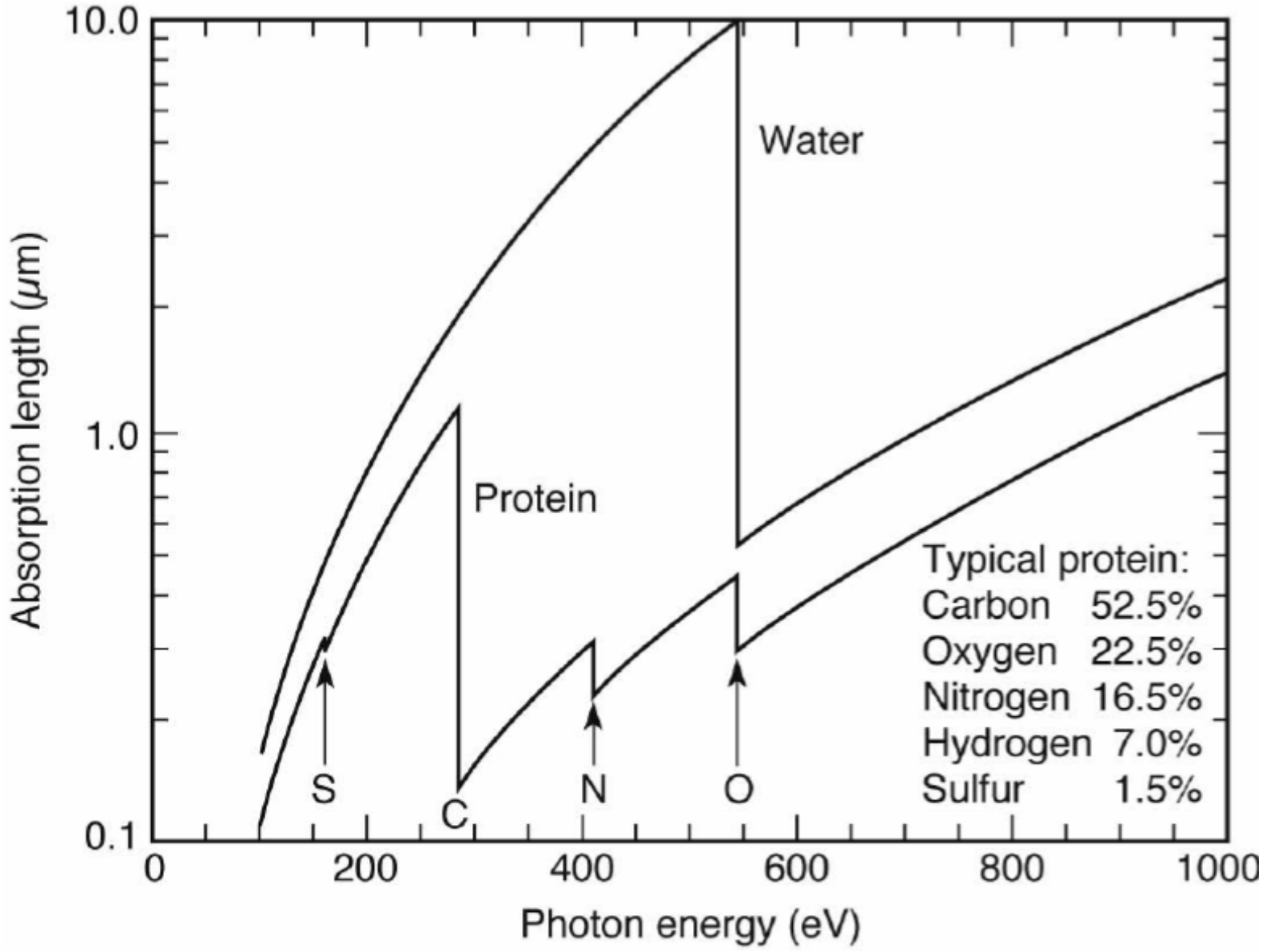
Talk Outline

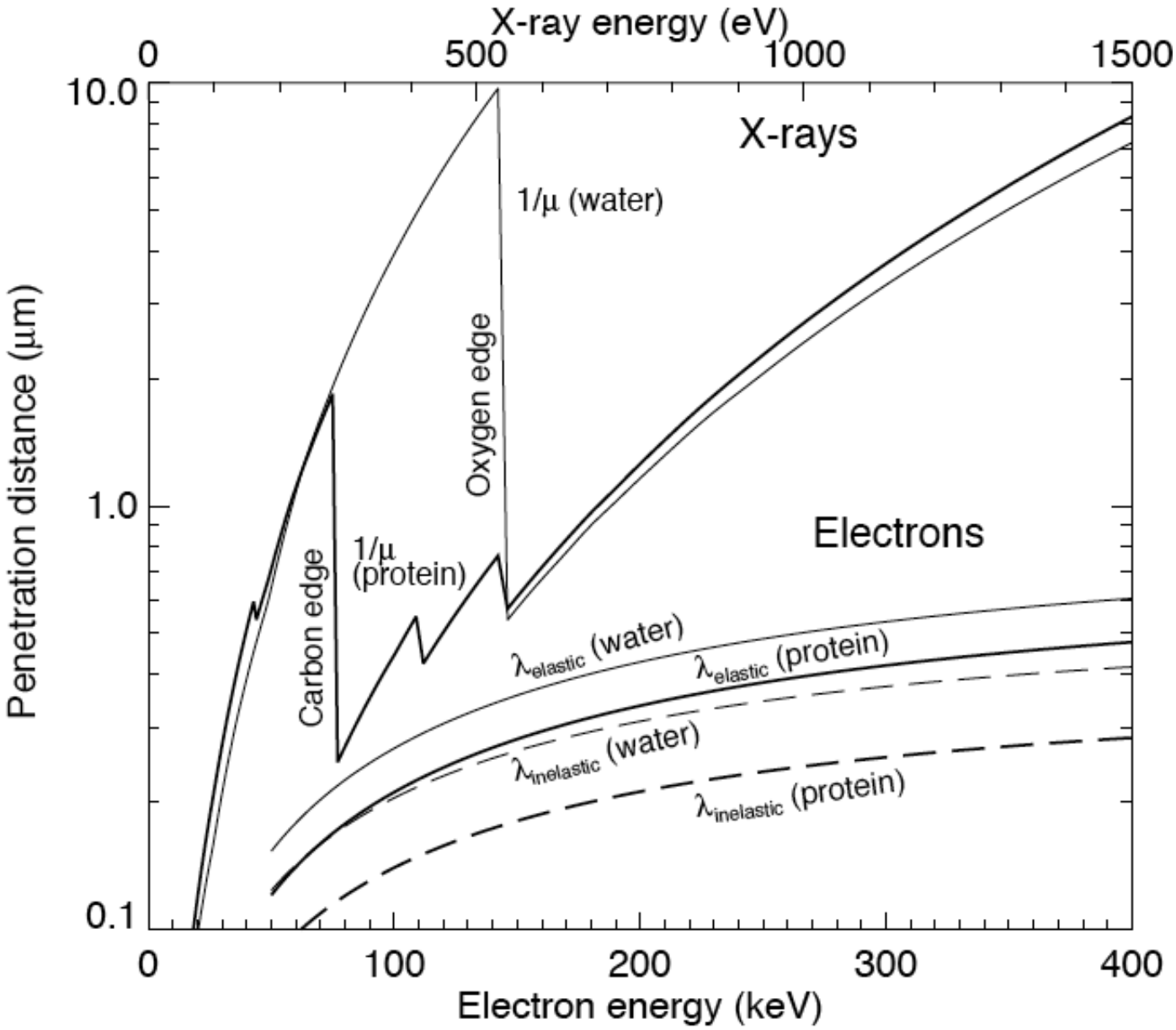
- Introduction to cryo soft X-ray tomography
- Sample Preparation and Workflow
- Some results
- Going forward

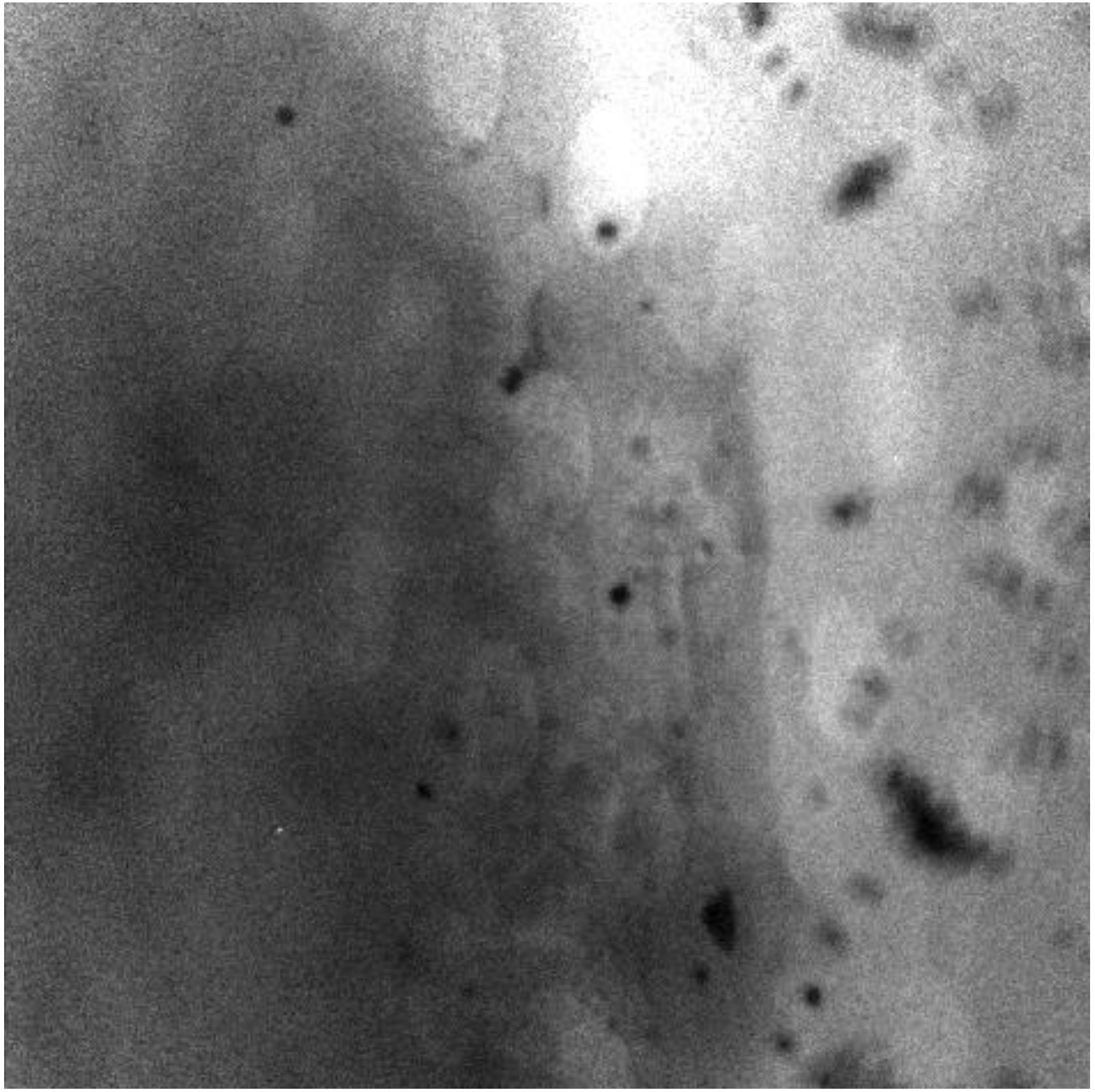
A bit of background

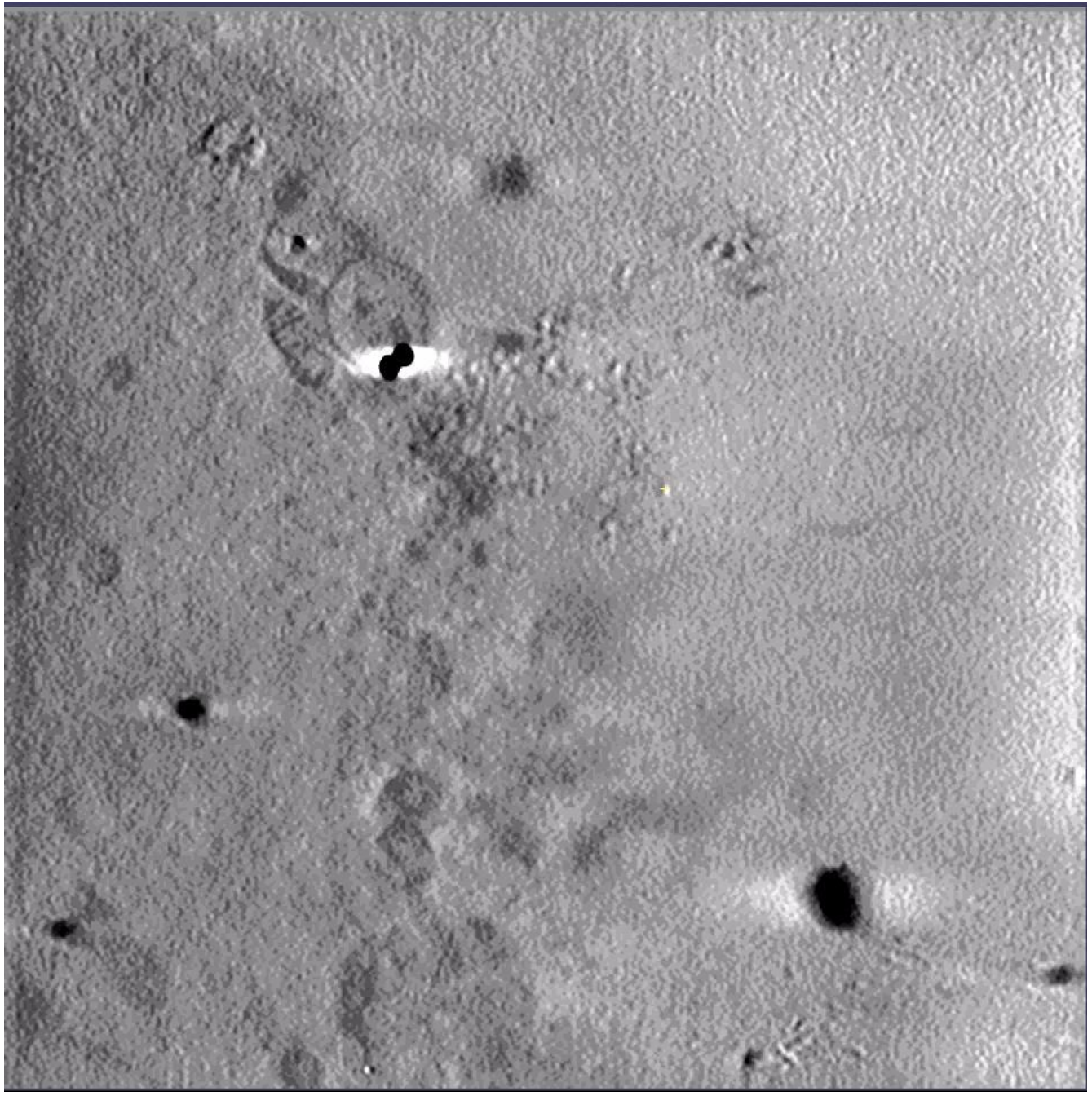
- New 3D technique in structural biology
- Biological imaging of cells with soft X-rays
- Complementary to EM and light microscopy



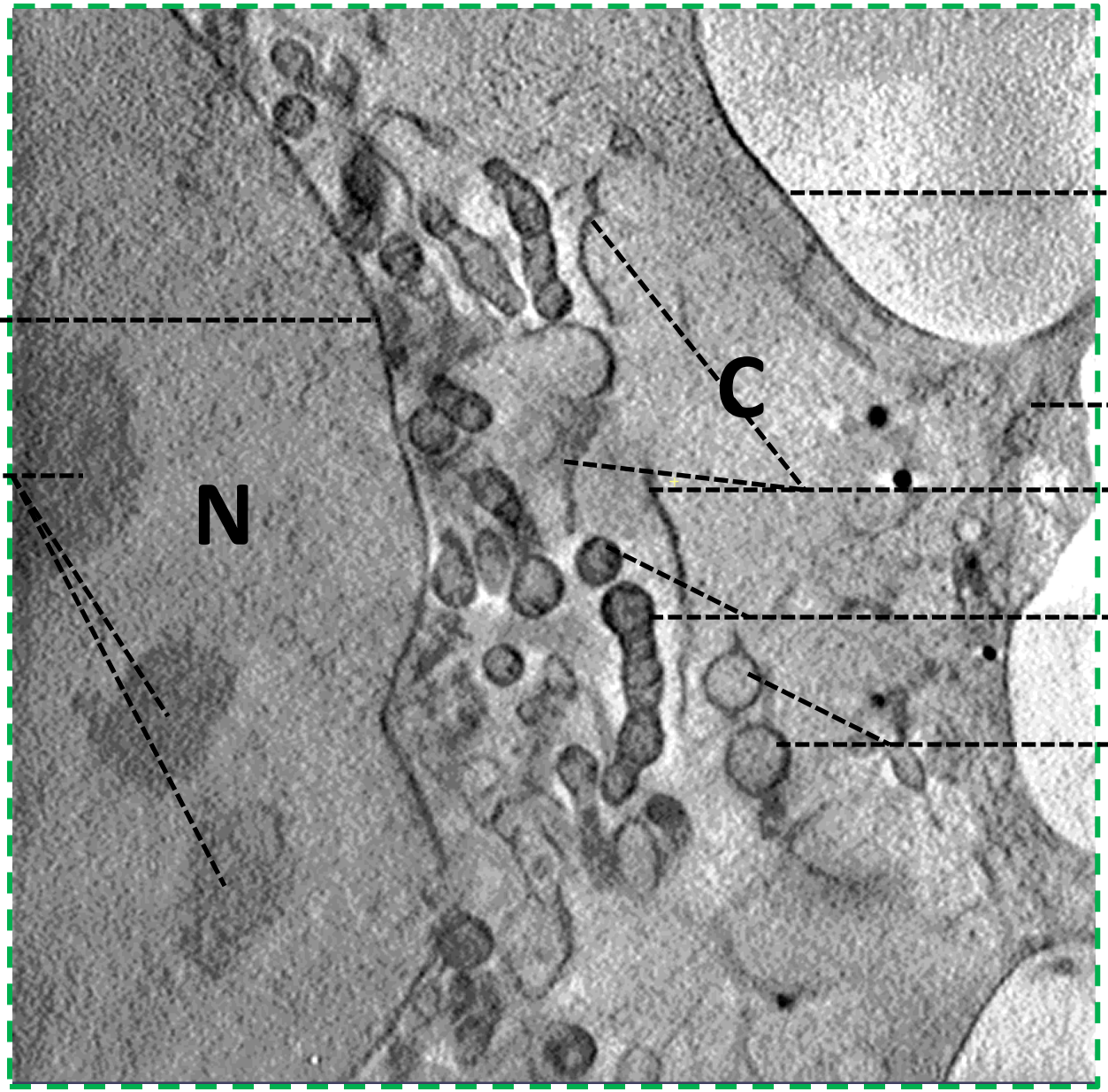

10.0
1.0
0.1
Absorption length (μm)
0
200
400
600
800
1000
Photon energy (eV)
Water
Protein
S
C
N
O
Typical protein:
Carbon 52.5%
Oxygen 22.5%
Nitrogen 16.5%
Hydrogen 7.0%
Sulfur 1.5%


X-ray energy (eV)
0
500
1000
1500
10.0
1.0
0.1
X-rays
1/μ (water)
Oxygen edge
Carbon edge
1/μ
(protein)
Electrons
λelastic (water)
λelastic (protein)
λinelastic (water)
λinelastic (protein)
Penetration distance (μm)
0
100
200
300
400
Electron energy (keV)





Analysing Cellular Features



Where can I collect data?


ENERGETIQ
xradia
xradia

Cryo-soft X-ray tomography

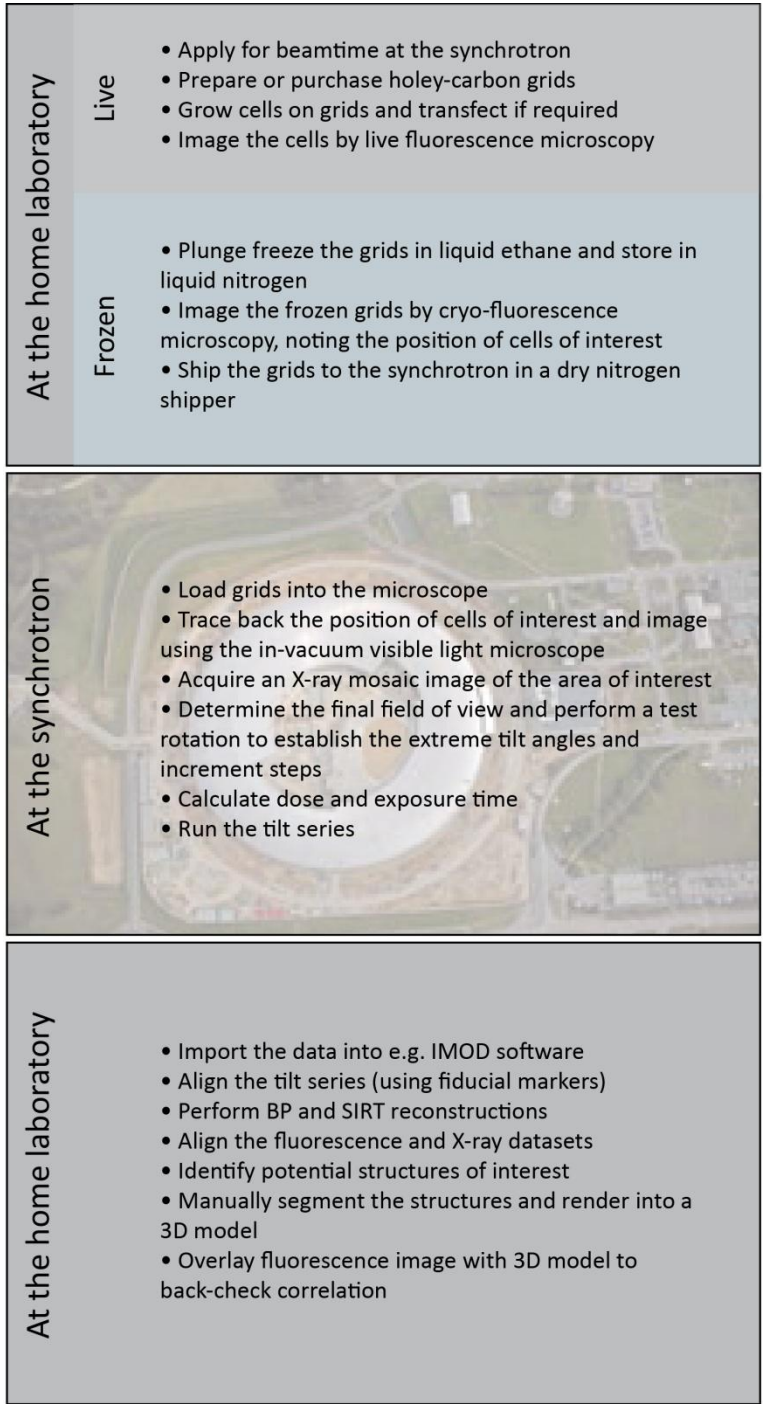
WHOLE CELLS

NO CHEMICAL FIXATIVES

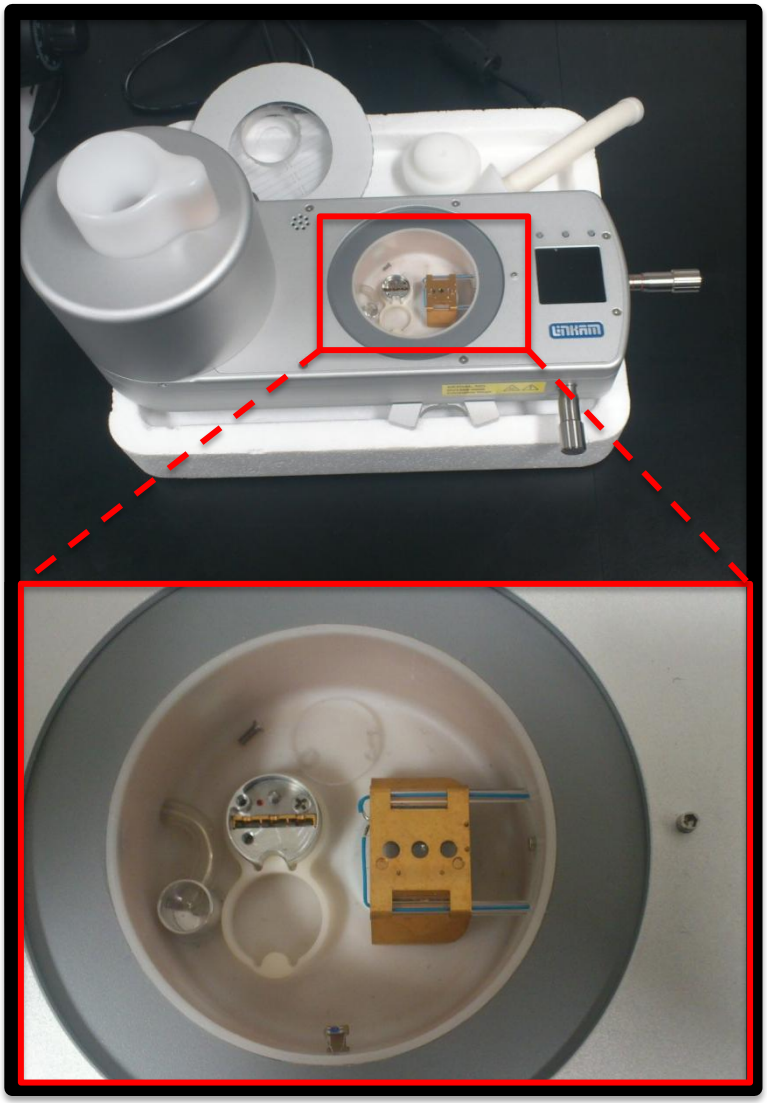
NO STAINS

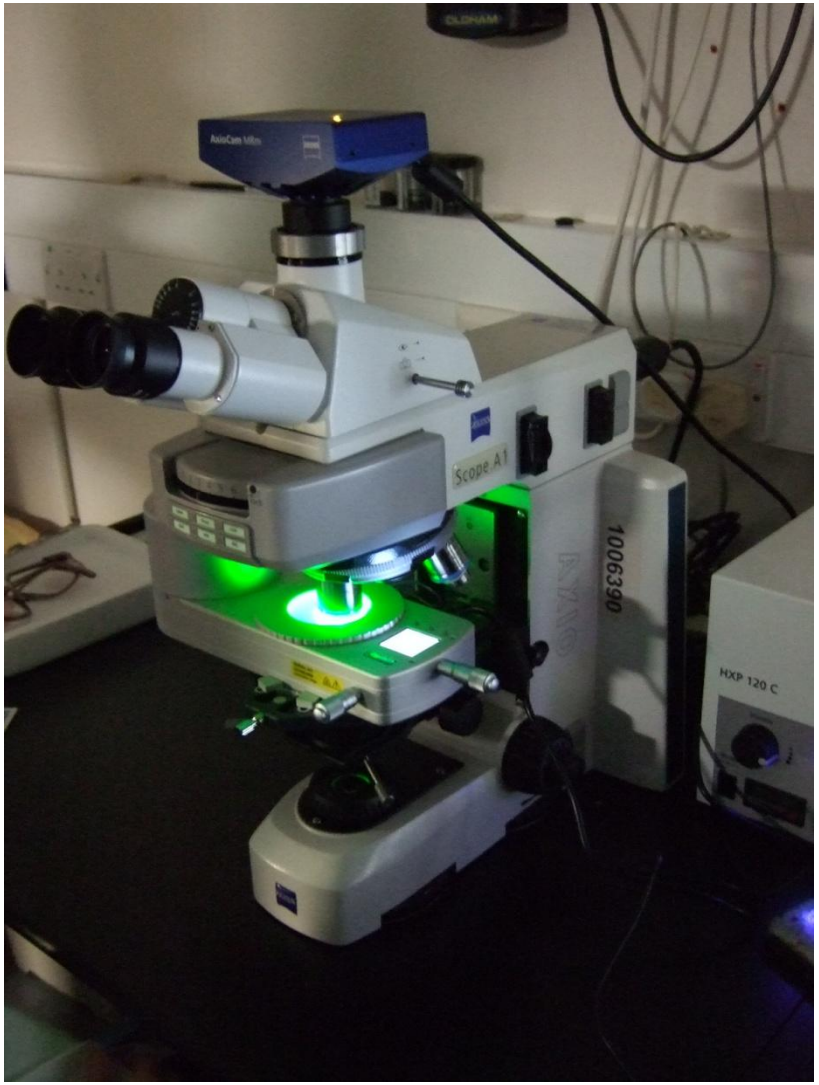
NO DEHYDRATION

‘Near Native-State Imaging’


At the home laboratory
Live
• Apply for beamtime at the synchrotron
• Prepare or purchase holey-carbon grids
• Grow cells on grids and transfect if required
• Image the cells by live fluorescence microscopy
Frozen
• Plunge freeze the grids in liquid ethane and store in liquid nitrogen
• Image the frozen grids by cryo-fluorescence microscopy, noting the position of cells of interest
• Ship the grids to the synchrotron in a dry nitrogen shipper
At the synchrotron
• Load grids into the microscope
• Trace back the position of cells of interest and image using the in-vacuum visible light microscope
• Acquire an X-ray mosaic image of the area of interest
• Determine the final field of view and perform a test rotation to establish the extreme tilt angles and increment steps
• Calculate dose and exposure time
• Run the tilt series
At the home laboratory
• Import the data into e.g. IMOD software
• Align the tilt series (using fiducial markers)
• Perform BP and SIRT reconstructions
• Align the fluorescence and X-ray datasets
• Identify potential structures of interest
• Manually segment the structures and render into a 3D model
• Overlay fluorescence image with 3D model to back-check correlation

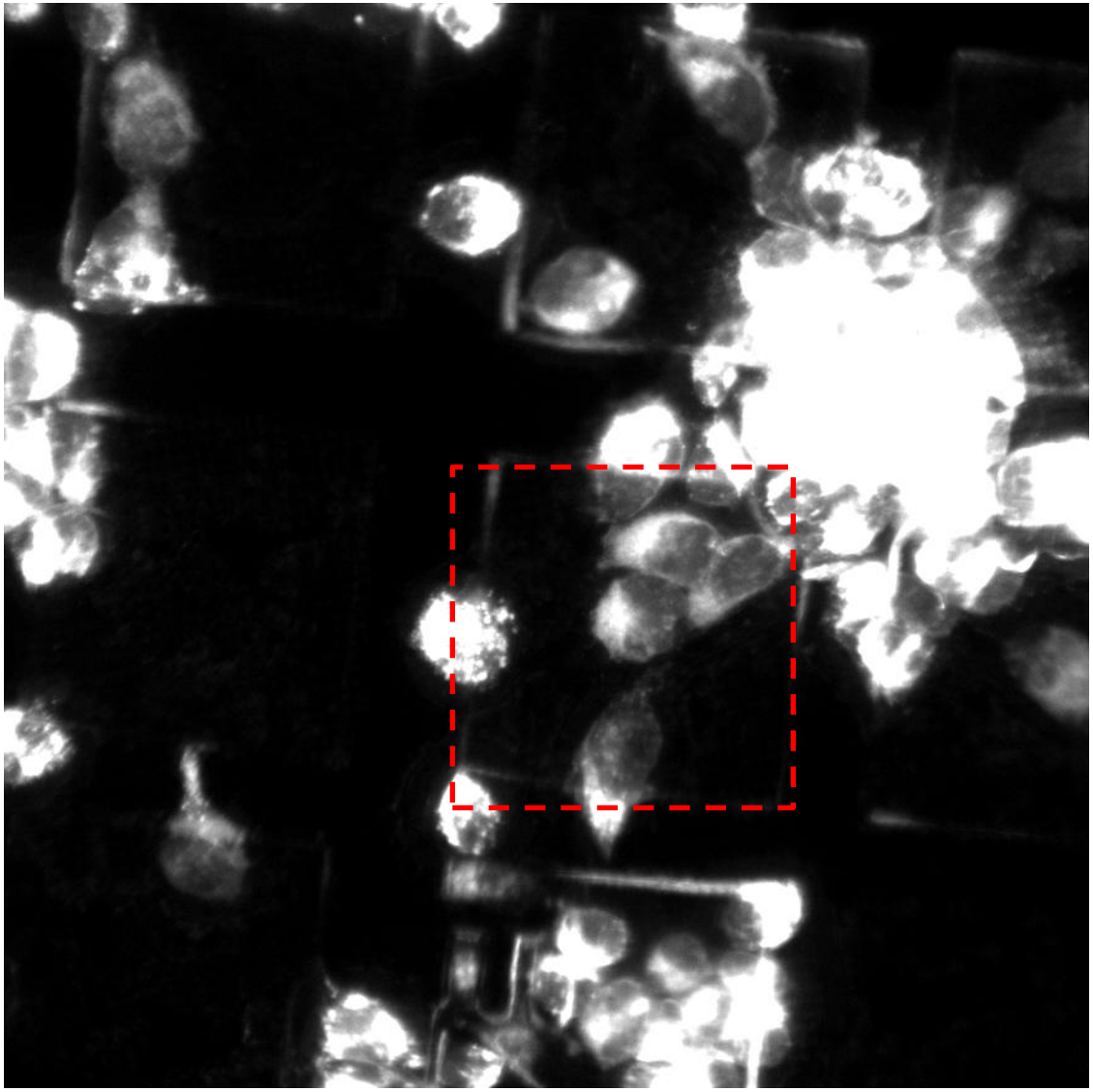
Using cryo-fluorescence imaging to screen grids prior to shipping

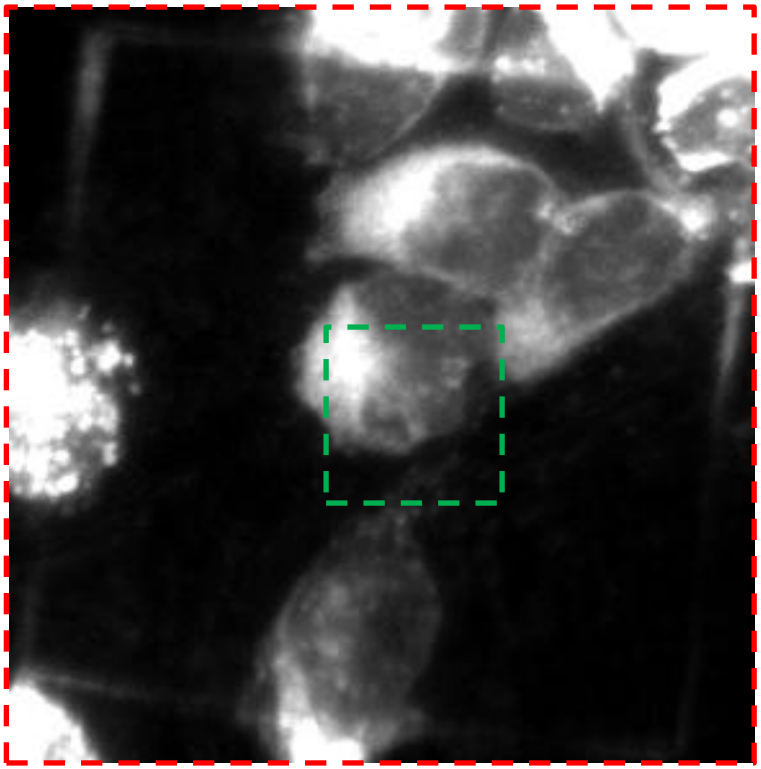




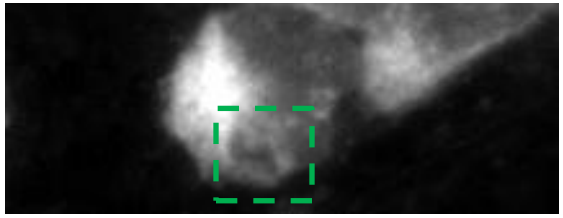
Van Driel, 2009

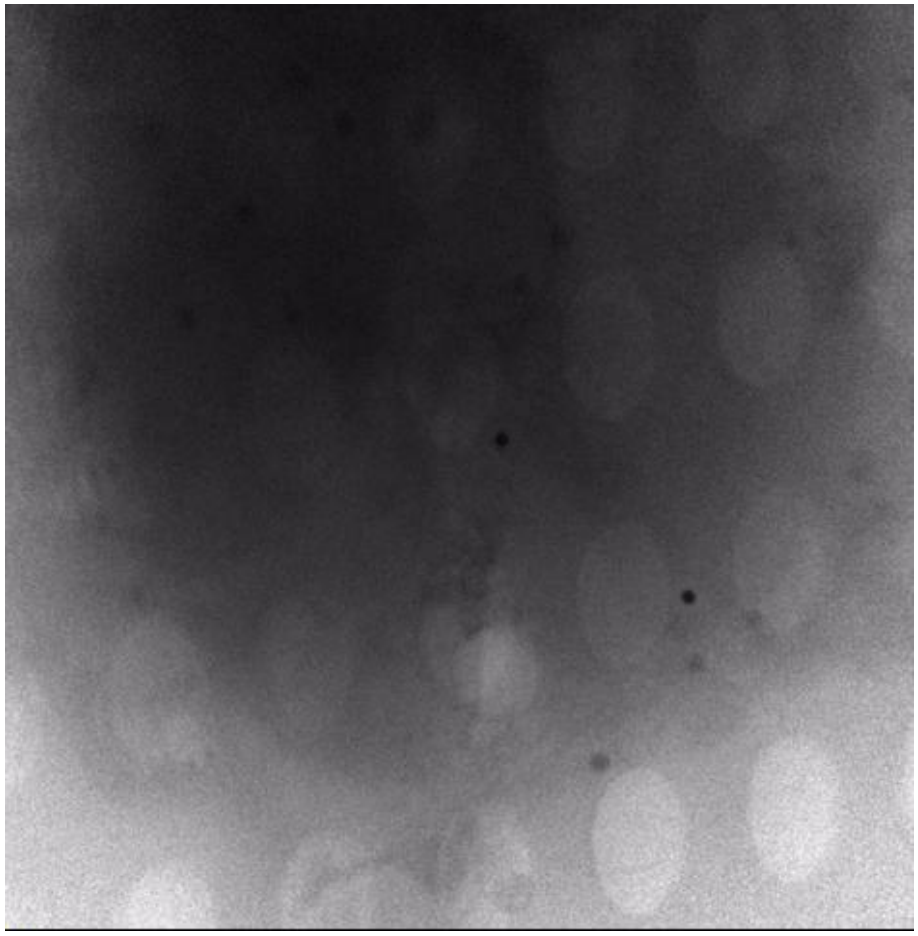
Correlative Example 1 – Fluorescence Search (20X Objective)

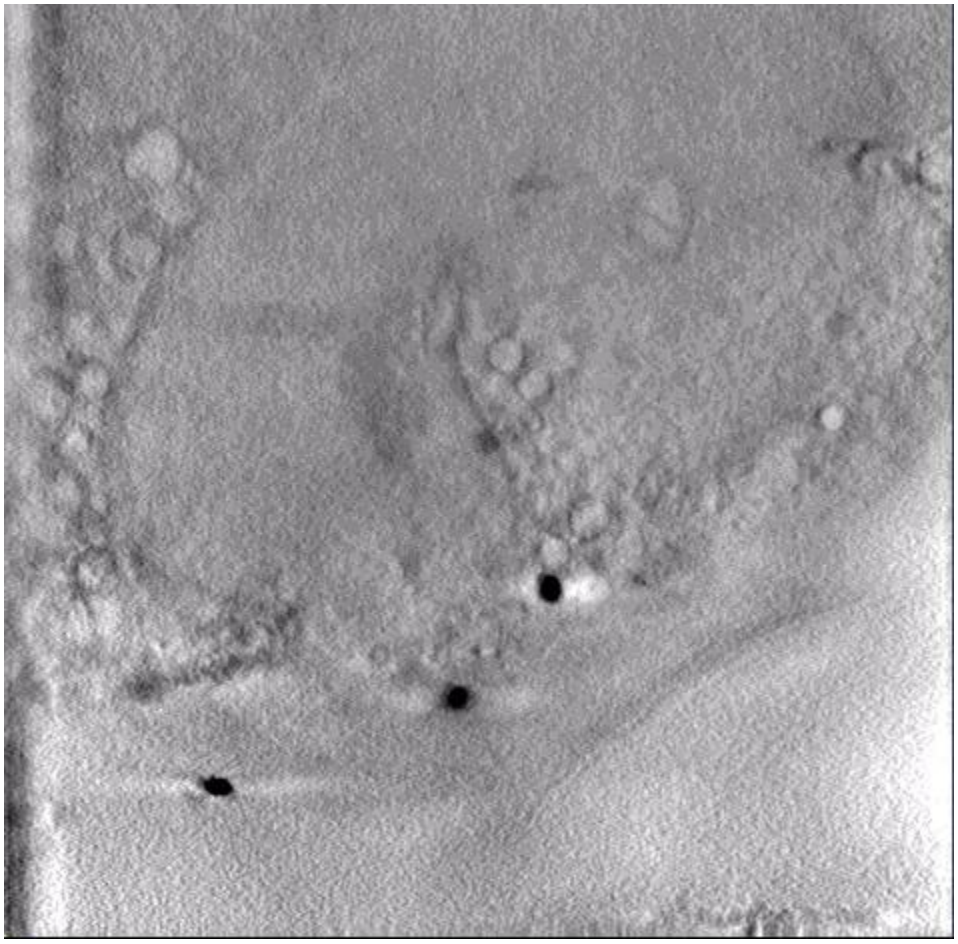




Correlative Example 1 – Soft X-ray Imaging

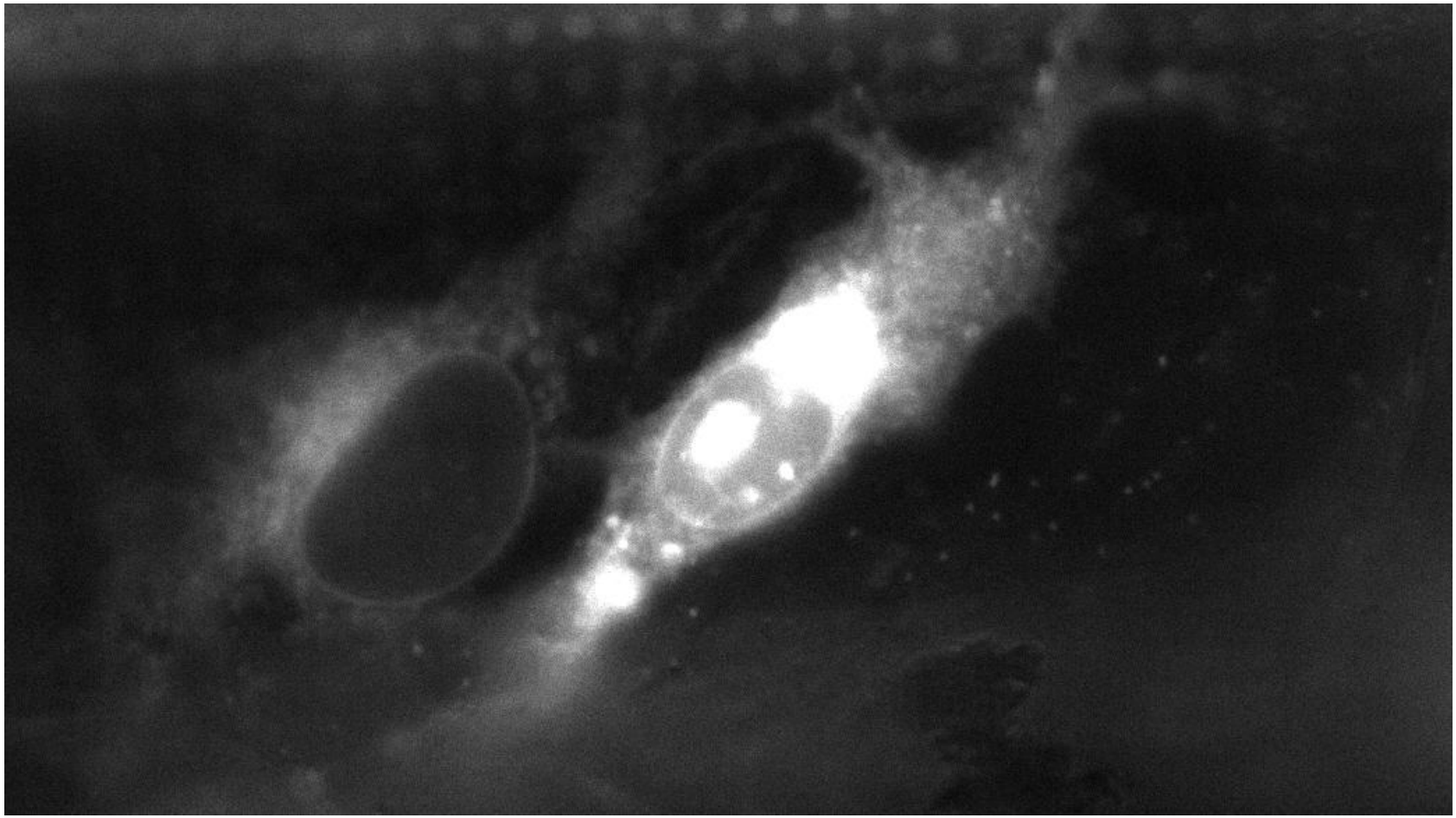




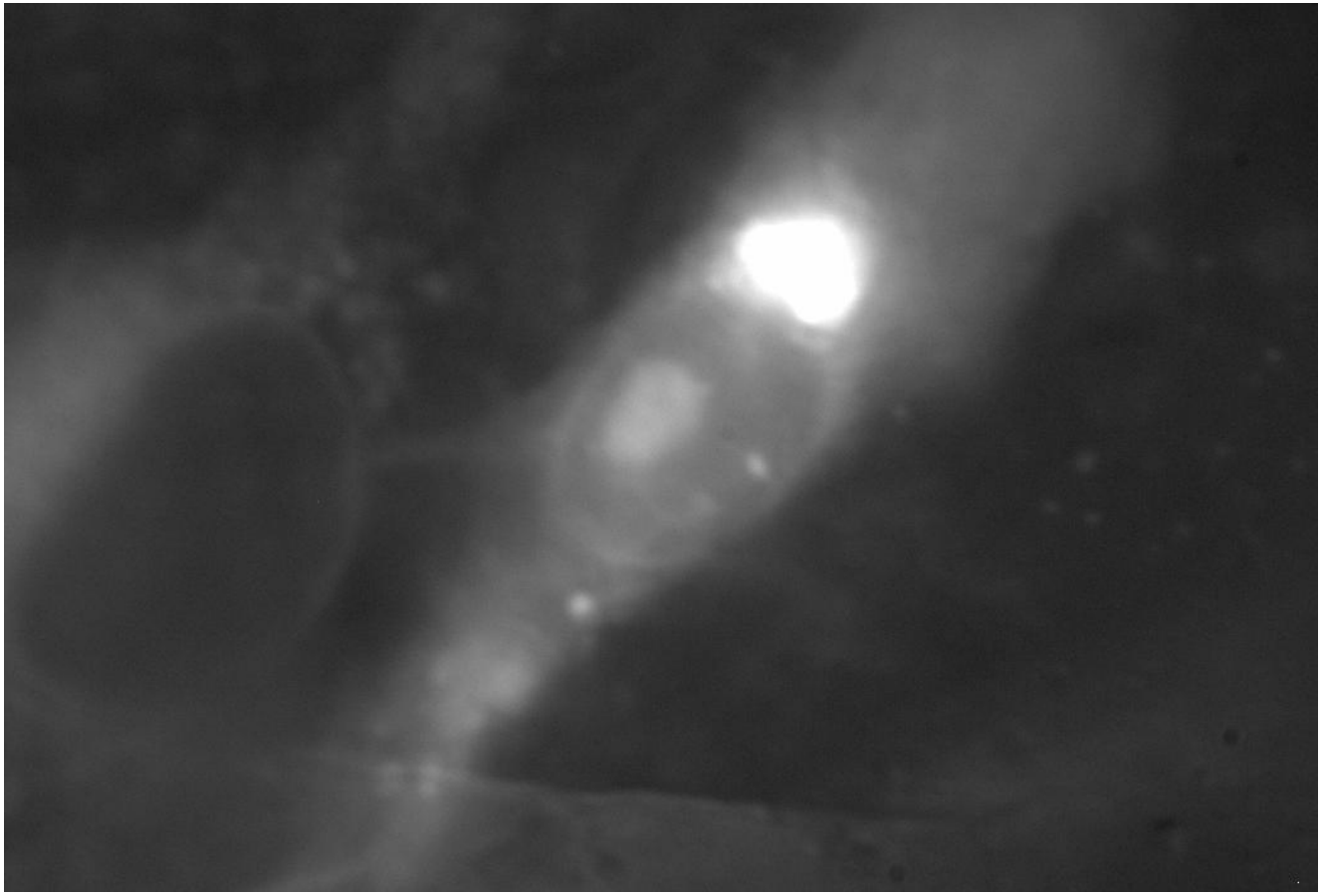


- ~4 µm thick peri-nuclear region with biological event of interest

Cryo fl using Cryostage @ LRI



In microscope VLM @ BESSY



Going Forward

- New technique:
  - Instrumentation
  - Sample Preparation
- 2D into 3D
- Super resolution