Cryo-EM Course at LBMS

Date/Time: June 20-23, 2023, 10:00 am - 5:00 pm ET

Cryo-Electron Tomography

— *Imaging Cells and Molecules at High Resolution*

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Outline

★Brief introduction of electron tomography

★Practical aspects in cryo-electron tomography

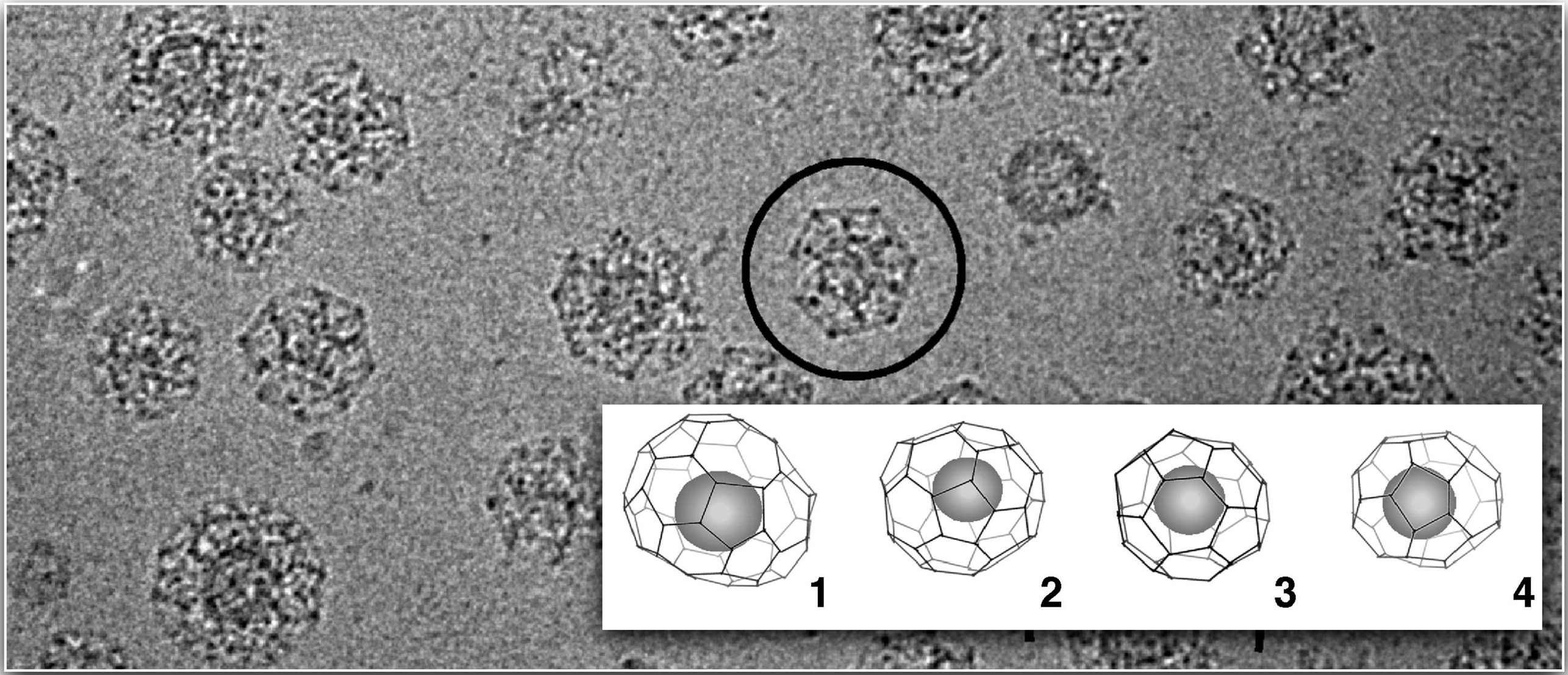
- Sample preparation
- Data collection
- Image analysis

★Frontiers in cryo-electron tomography

The power of cryo-ET

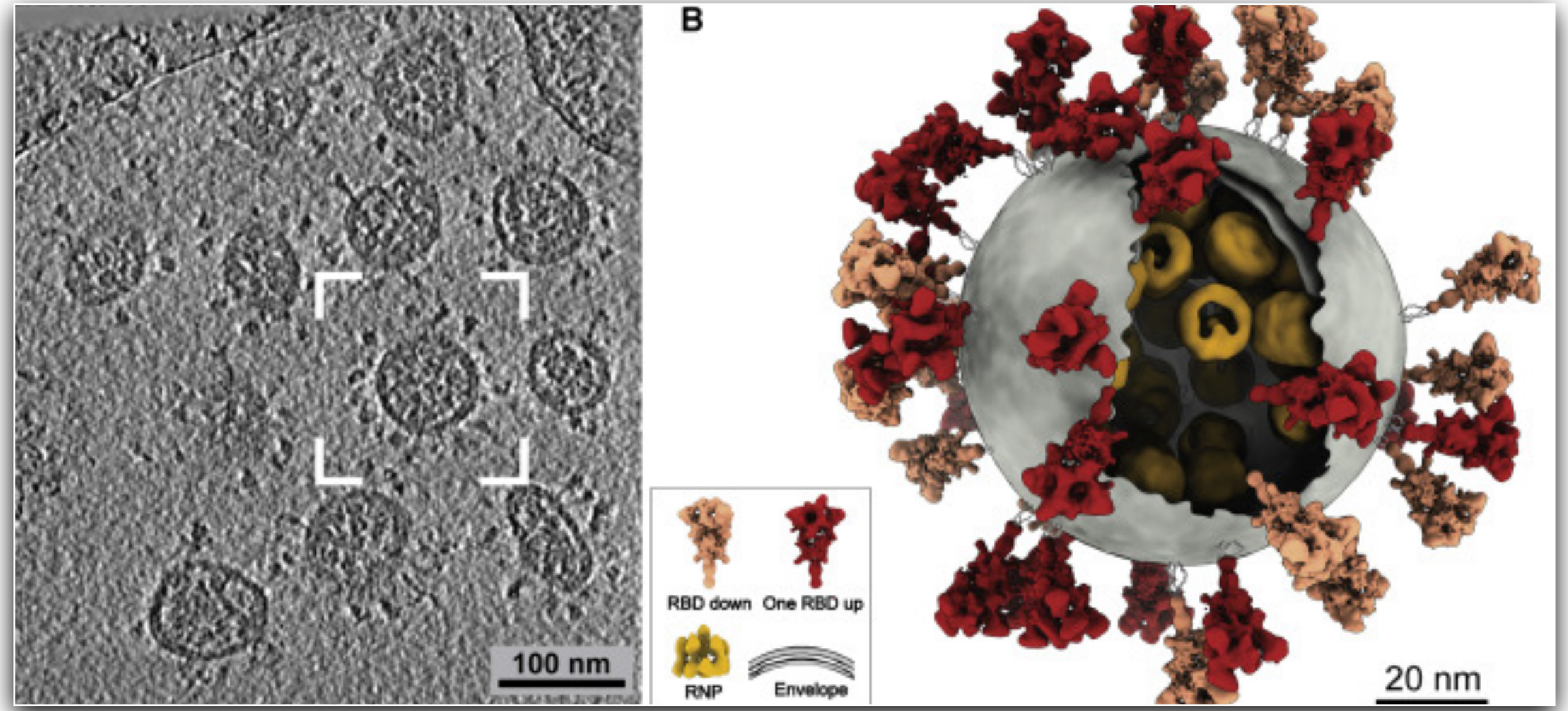
- Providing 3D snapshots of unique biomedical complexes in their functional environments.
- Bridging the information gap between light microscopy and near-atomic resolution techniques (such as cryo-EM or X-ray crystallography).

Large protein complexes



Cheng et al., JMB 2007

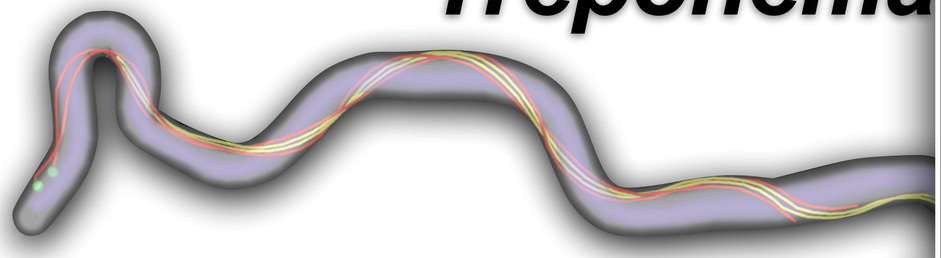
Viruses



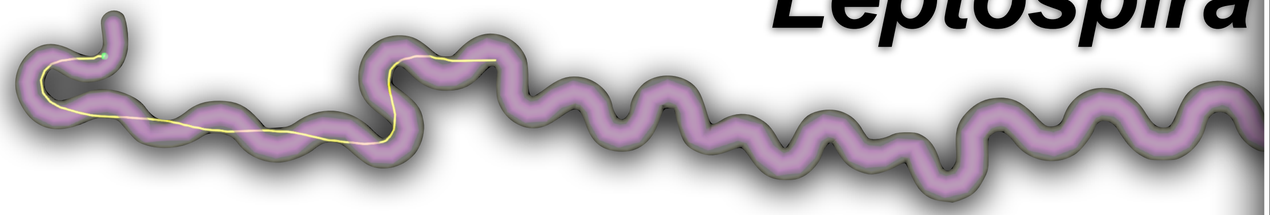
Yao et al., Cell 2020

Spirochetes

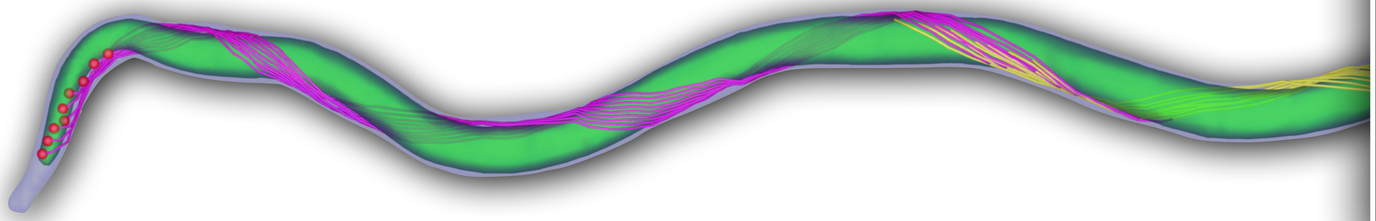
Treponema

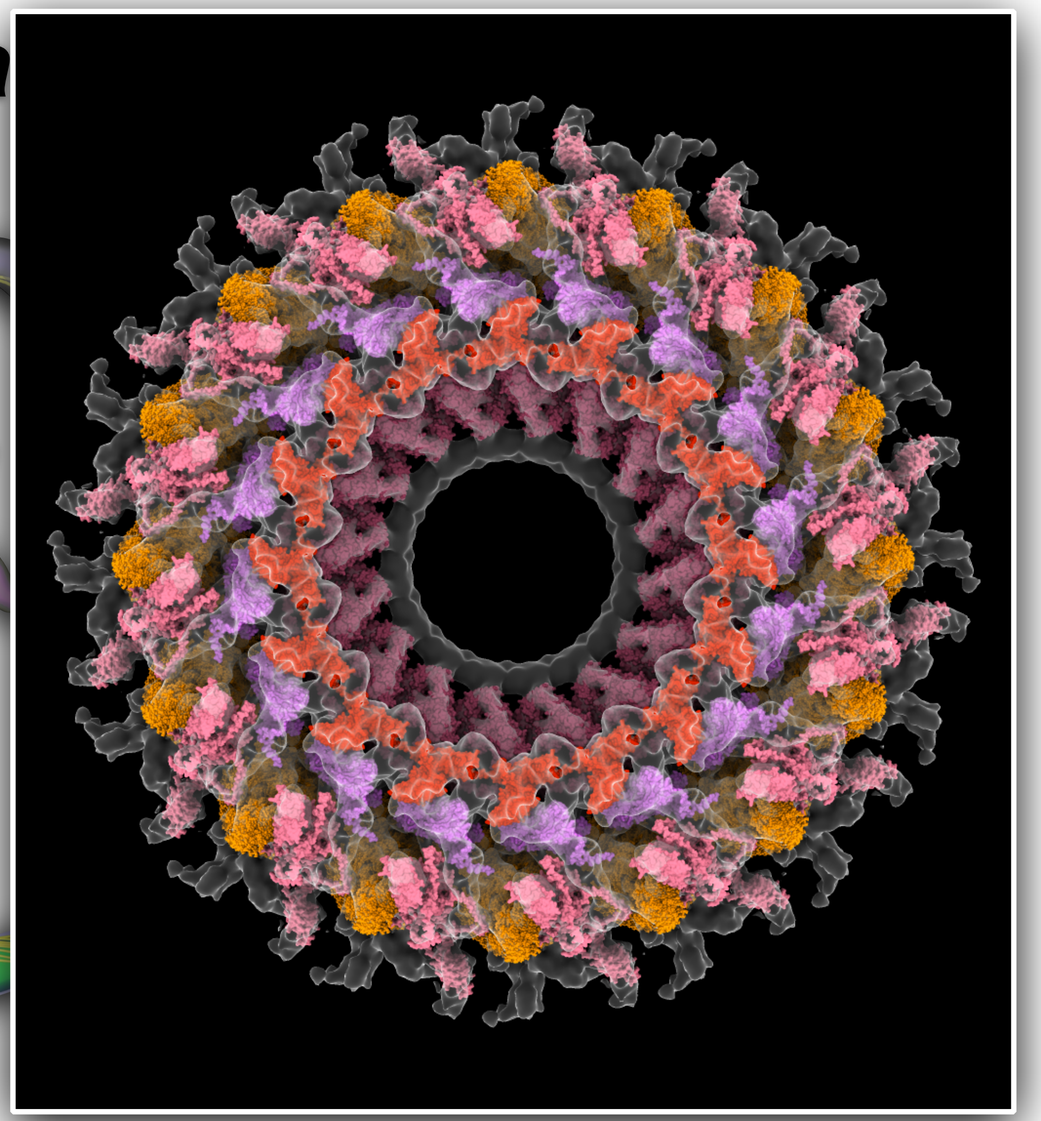


Leptospira

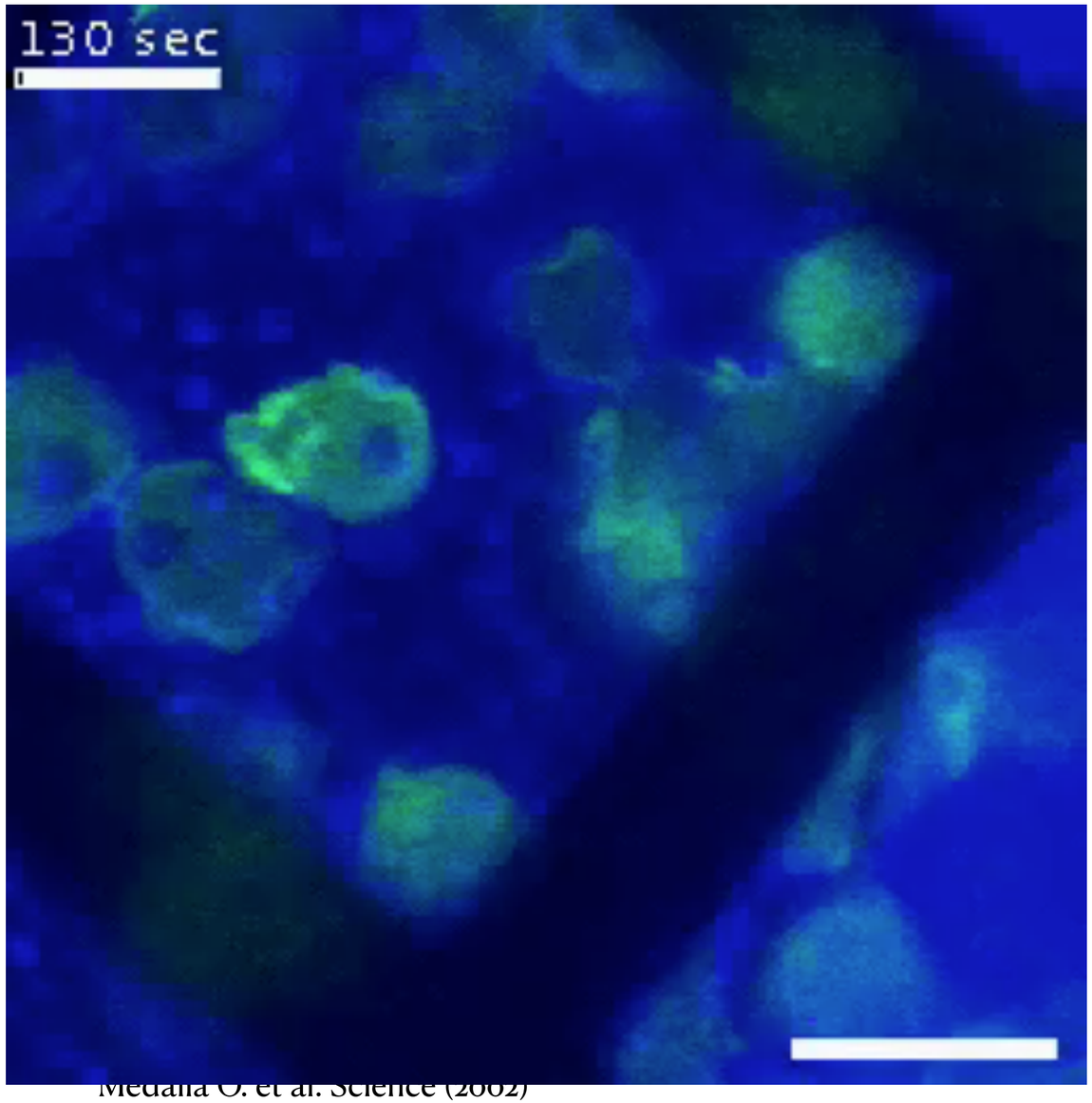


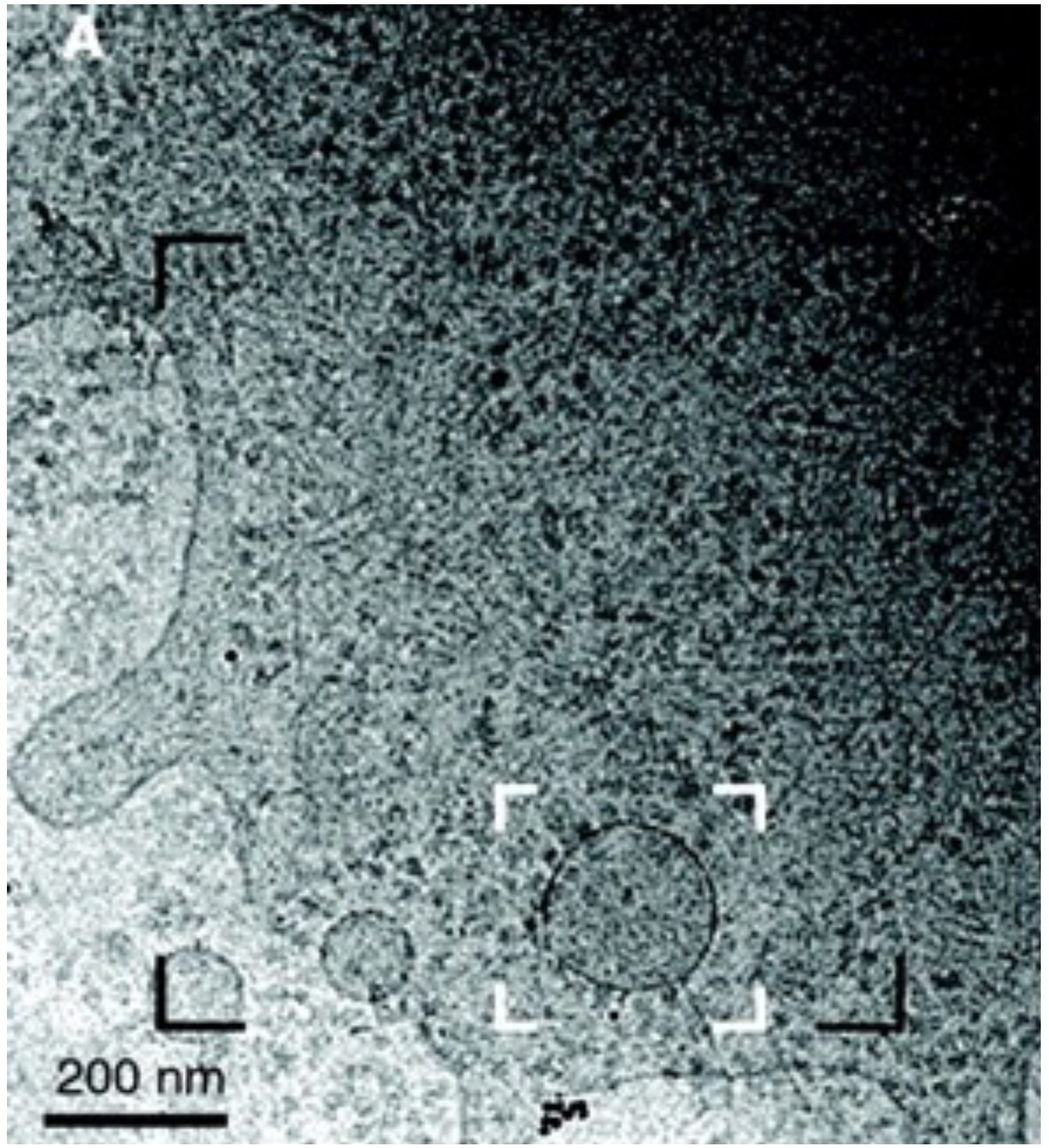
Borrelia





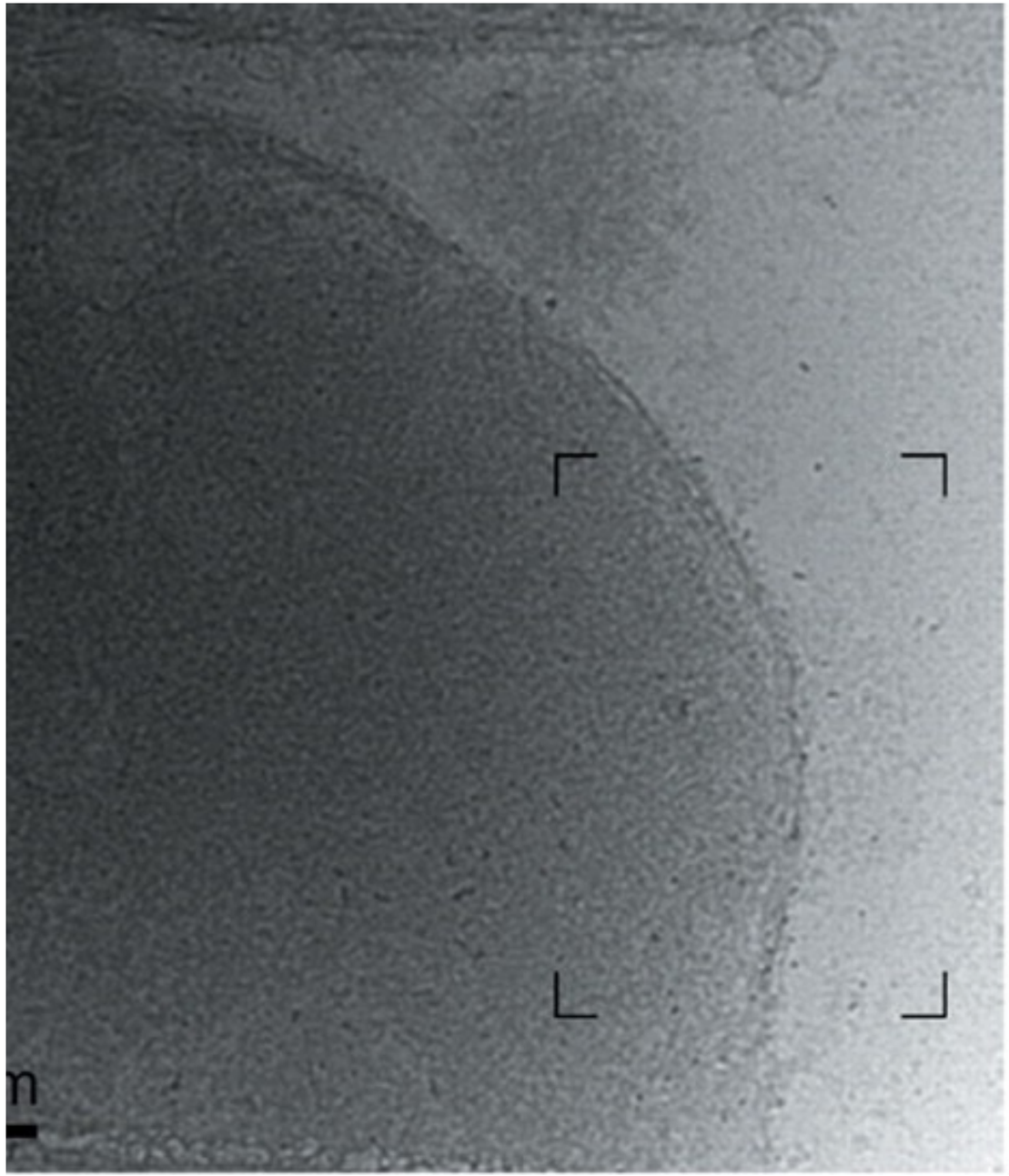
Cryo-ET imaging of eukaryotic cells

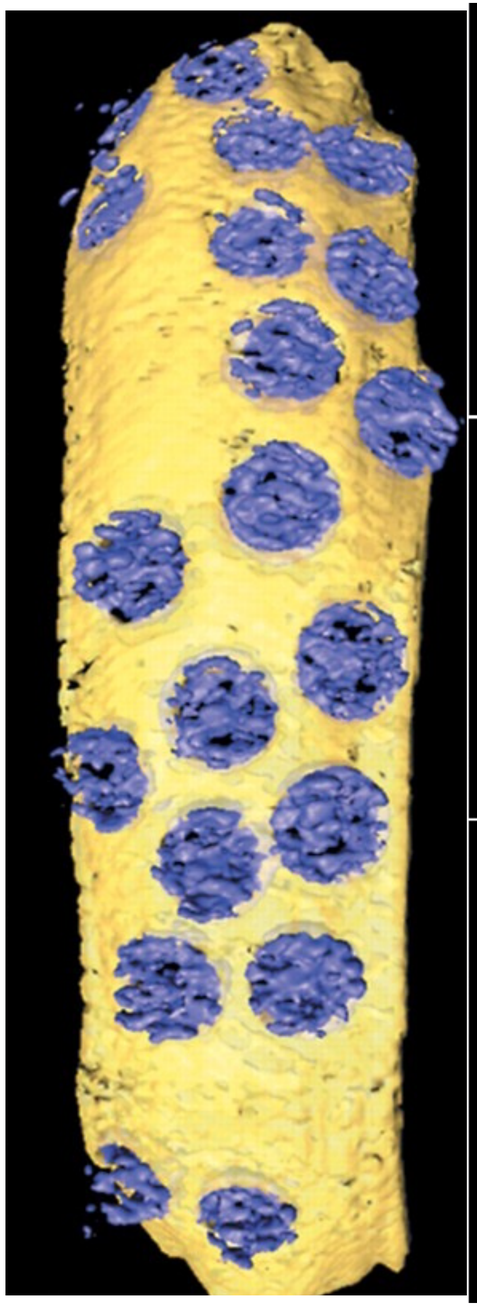


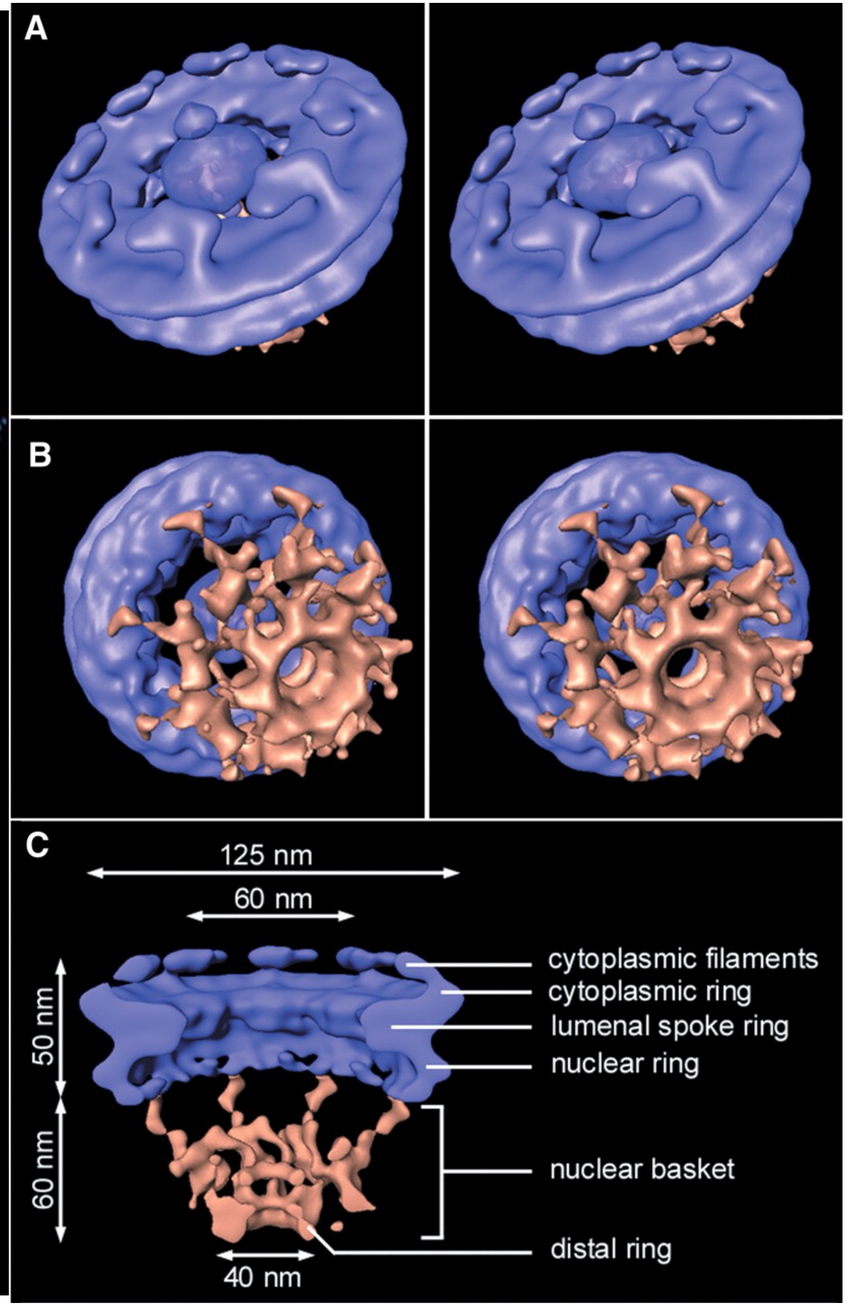


Medalia O. et al. Science (2002)

NPC structure in situ







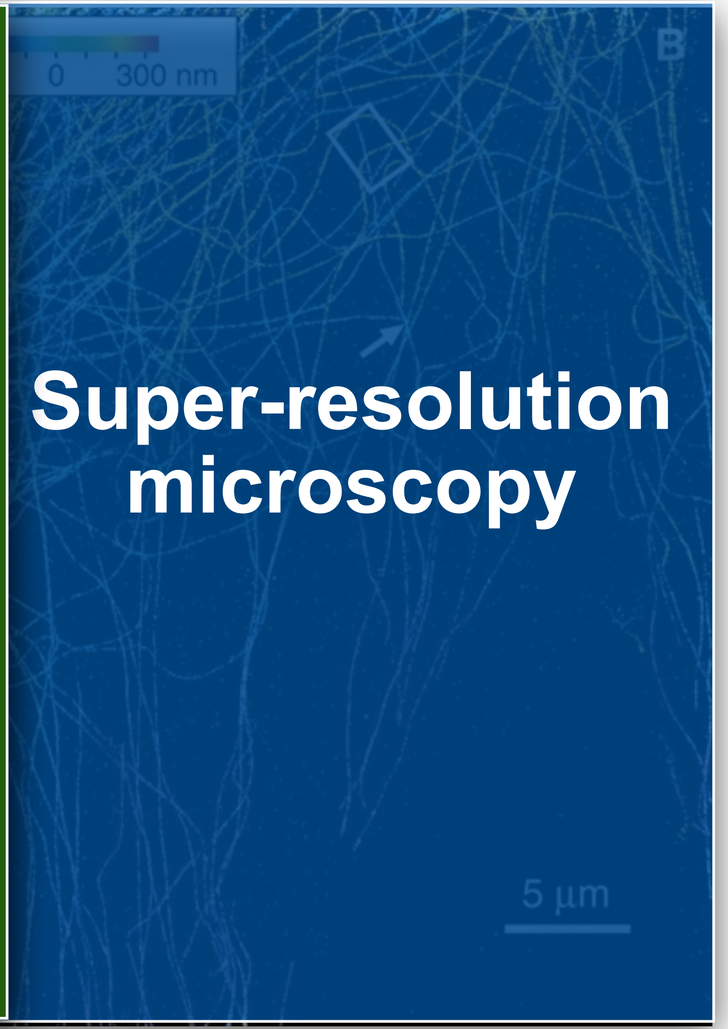
Cryo-ET — bridging the information gap

Cryo-EM
NMR
Crystallography

Alphafold2

Cryo-ET

(Cryo-FIB, Cryo-CLEM, Sub-tomogram averaging)



Super-resolution microscopy

Kellogg et al., Science 2018

Watanabe et al., Cell 2020

Huang et al., Science 2008

Single-Particle Cryo-EM

THE REVOLUTION WILL NOT BE CRYSTALLIZED

MOVE OVER X-RAY CRYSTALLOGRAPHY. CRYO-ELECTRON MICROSCOPY IS KICKING UP A STORM IN STRUCTURAL BIOLOGY BY REVEALING THE HIDDEN MACHINERY OF THE CELL.

BY EWEN CALLAWAY

In a basement room, deep in the bowels of a steel-clad building in Cambridge, a major insurgency is under way.

A hulking metal box, some three metres tall, is quietly beaming terabytes' worth of data through thick orange cables that disappear off through the ceiling. It is one of the world's most advanced cryo-electron microscopes: a device that uses electron beams to photograph frozen biological molecules and lay bare their molecular shapes. The microscope is so sensitive that a shout can ruin an experiment, says Sjors Scheres, a structural biologist at the UK Medical Research Council Laboratory of Molecular Biology (LMB), as he stands dwarfed beside the £5-million (US$7.7-million) piece of equipment. "The UK needs many more of these, because there's going to be a boom," he predicts.

In labs around the world, cryo-electron microscopes such as this one are sending tremors through the field of structural biology. In the past three years, they have revealed exquisite details of protein-making ribosomes, quivering membrane proteins and other key cell molecules,

172 | NATURE | VOL 525 | 10 SEPTEMBER 2015

Nature 2015

The Nobel Prize in Chemistry 2017

© Nobel Media. Ill. N. Elmehed

Jacques Dubochet

Prize share: 1/3

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Joachim Frank

Prize share: 1/3

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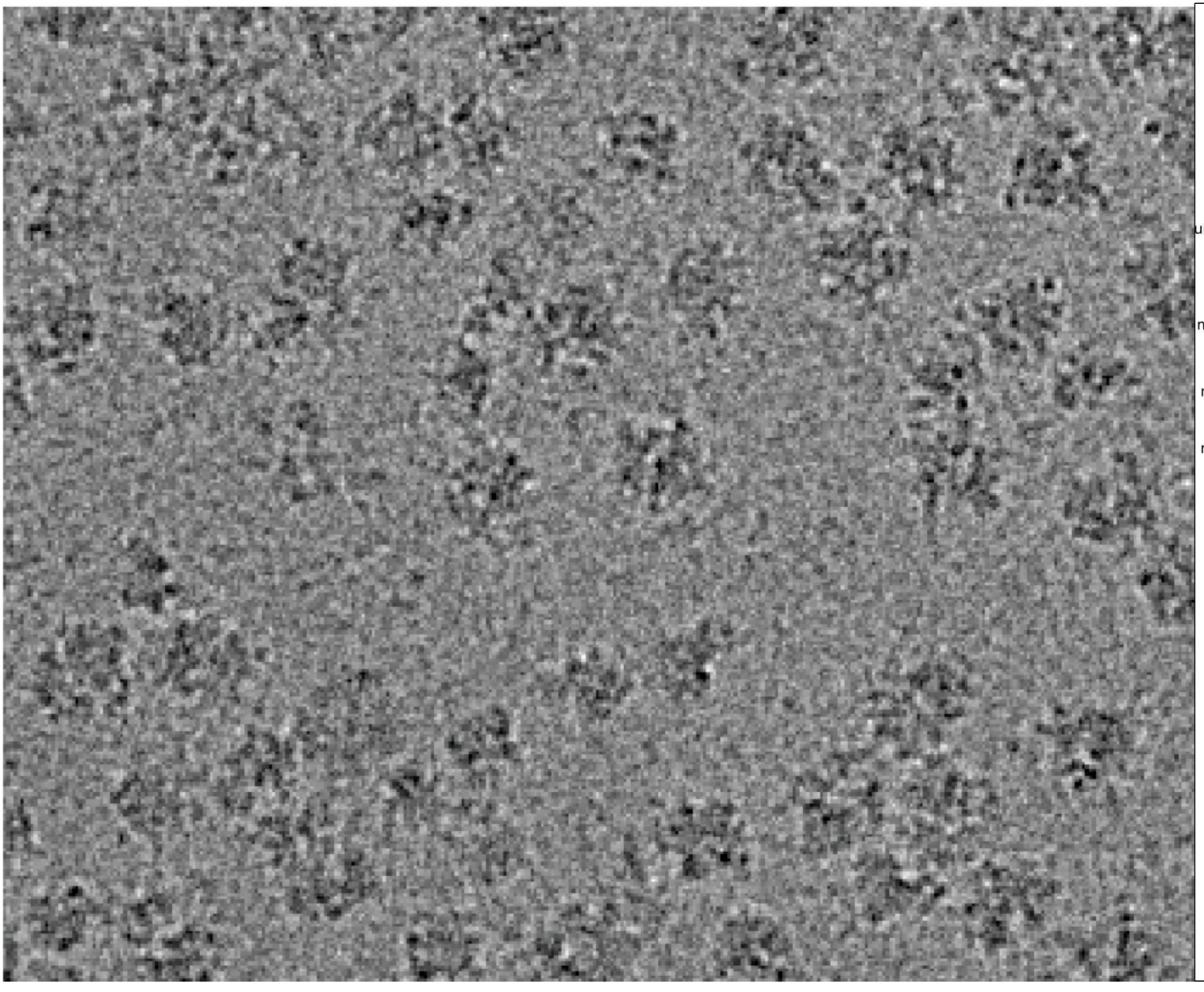
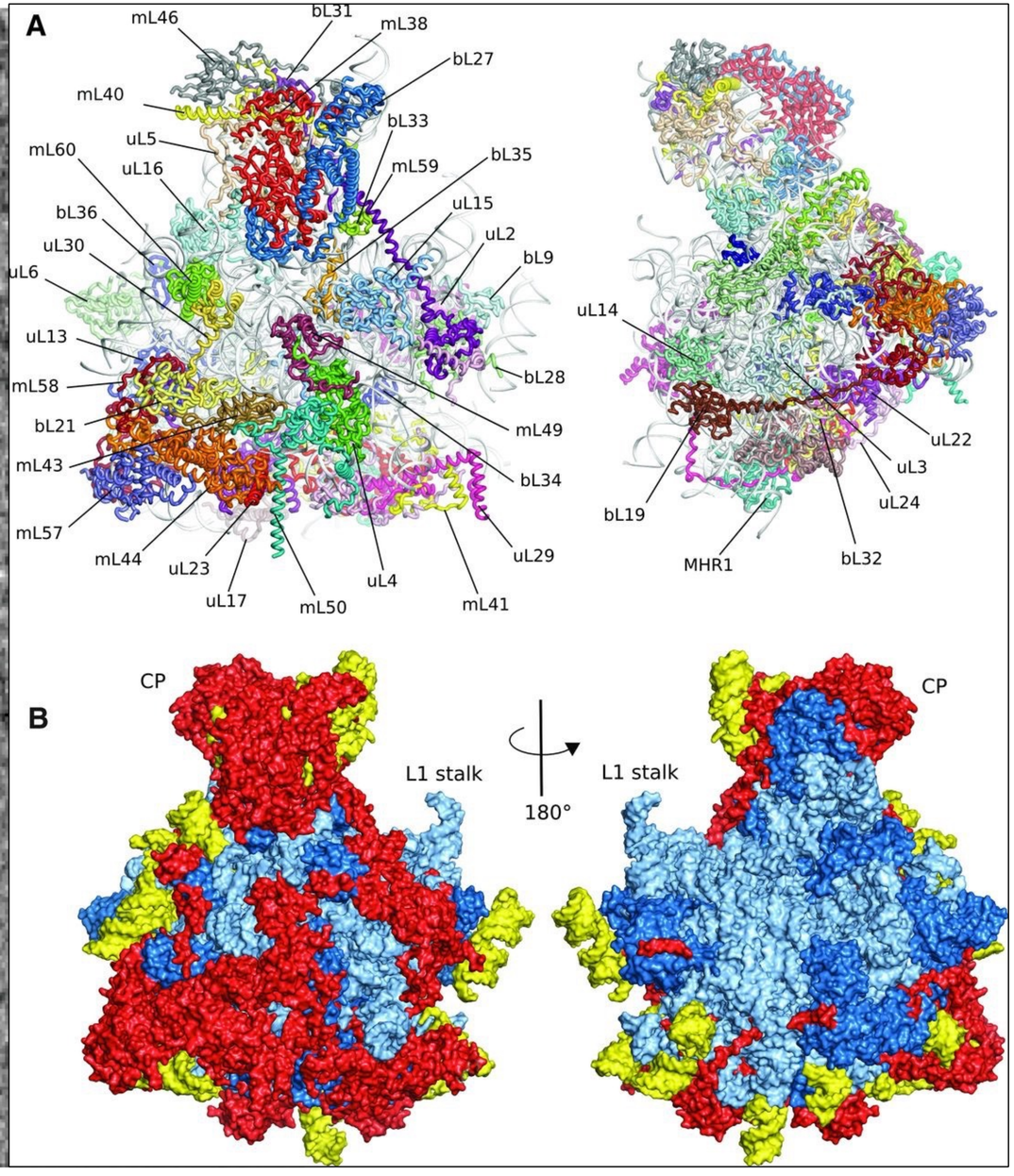
Richard Henderson

Prize share: 1/3

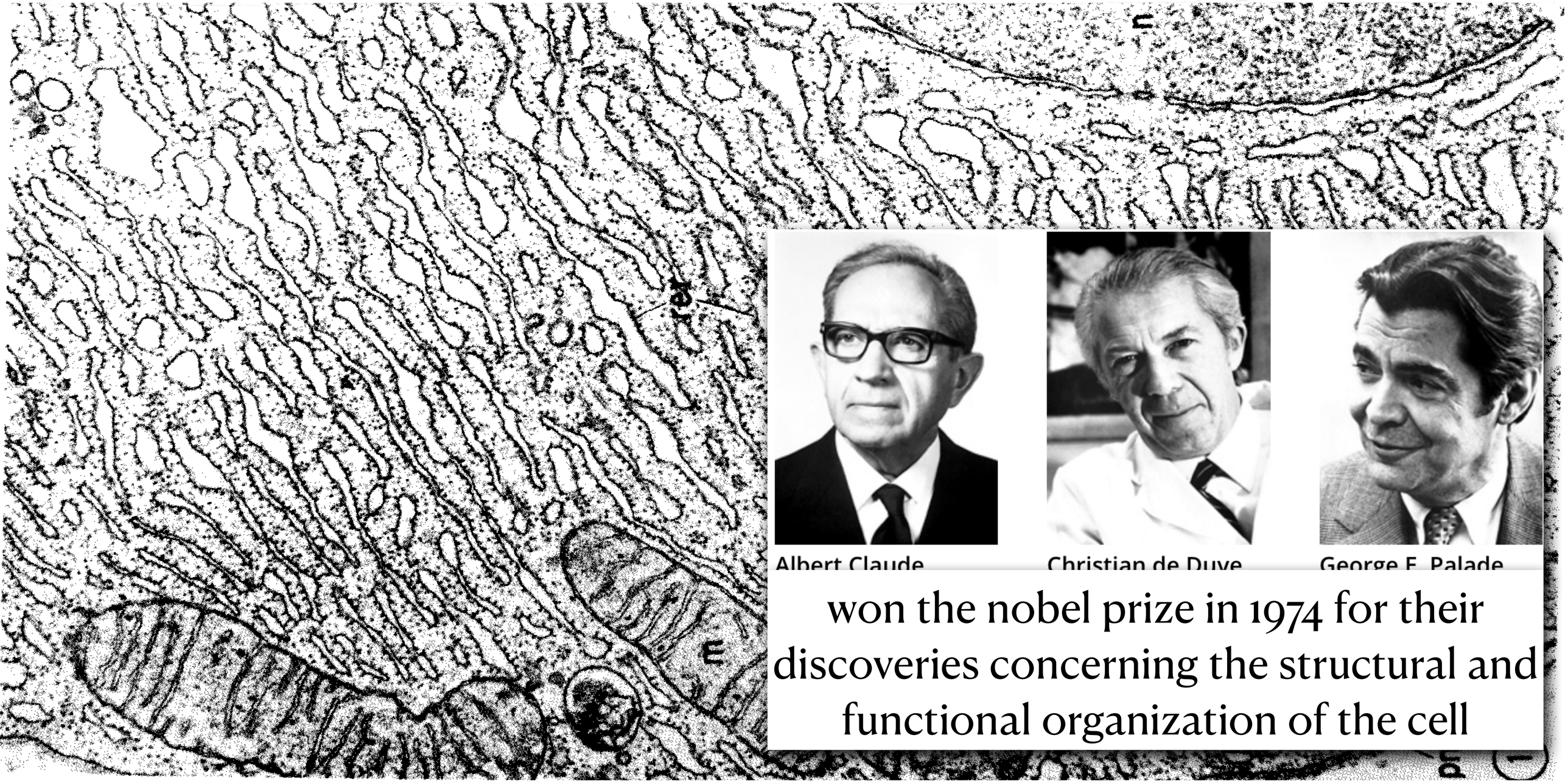
The Nobel Prize in Chemistry 2017 was awarded to Jacques Dubochet, Joachim Frank and Richard Henderson *"for developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"*.

Single-Particle Cryo-EM





Traditional thin section EM



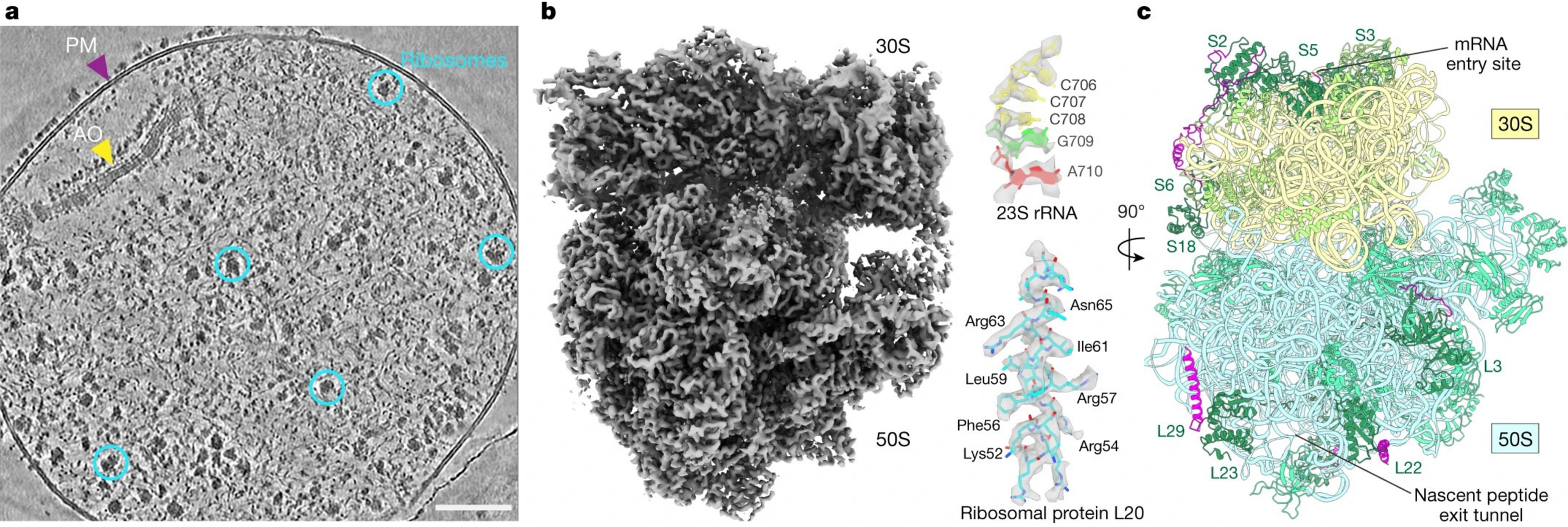
Albert Claude

Christian de Duve

George E. Palade

won the nobel prize in 1974 for their discoveries concerning the structural and functional organization of the cell

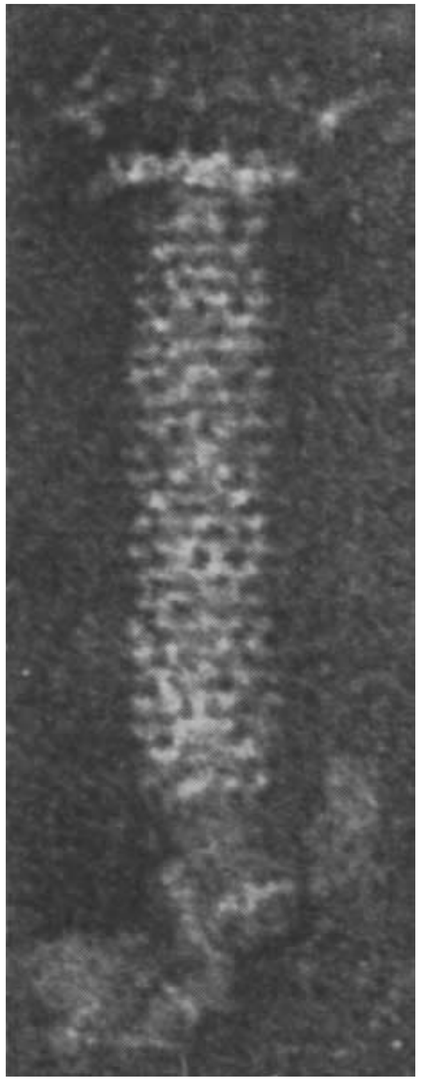
In situ structure revealed by cryo-ET

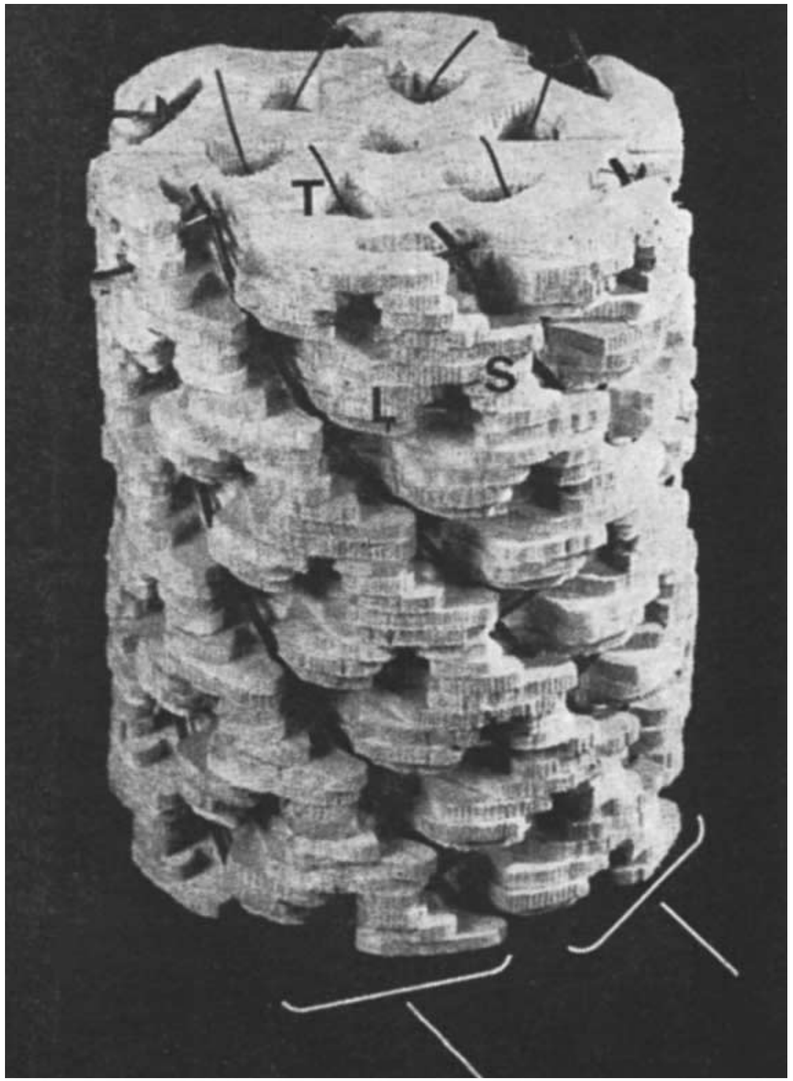


Visualizing translation dynamics at atomic detail inside a bacterial cell

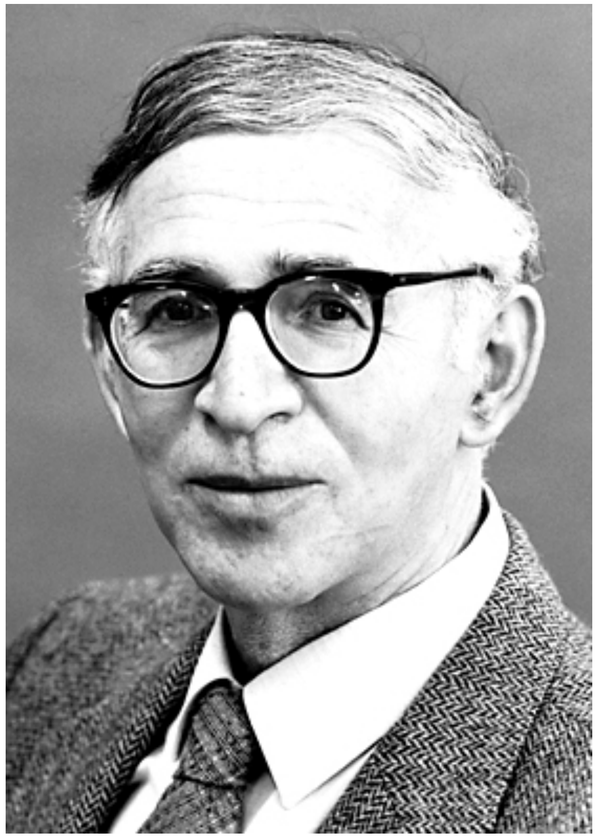
Xue et al. Nature 2022

Tomography—3D reconstruction from 2D images





De Rosier & Klug, 1968



Aaron Klug
The Nobel Prize in Chemistry 1982 was awarded to Aaron Klug *"for his development of crystallographic electron microscopy …"*

David DeRosier
Professor Emeritus
Brandeis University

Tomography—3D reconstruction from 2D images

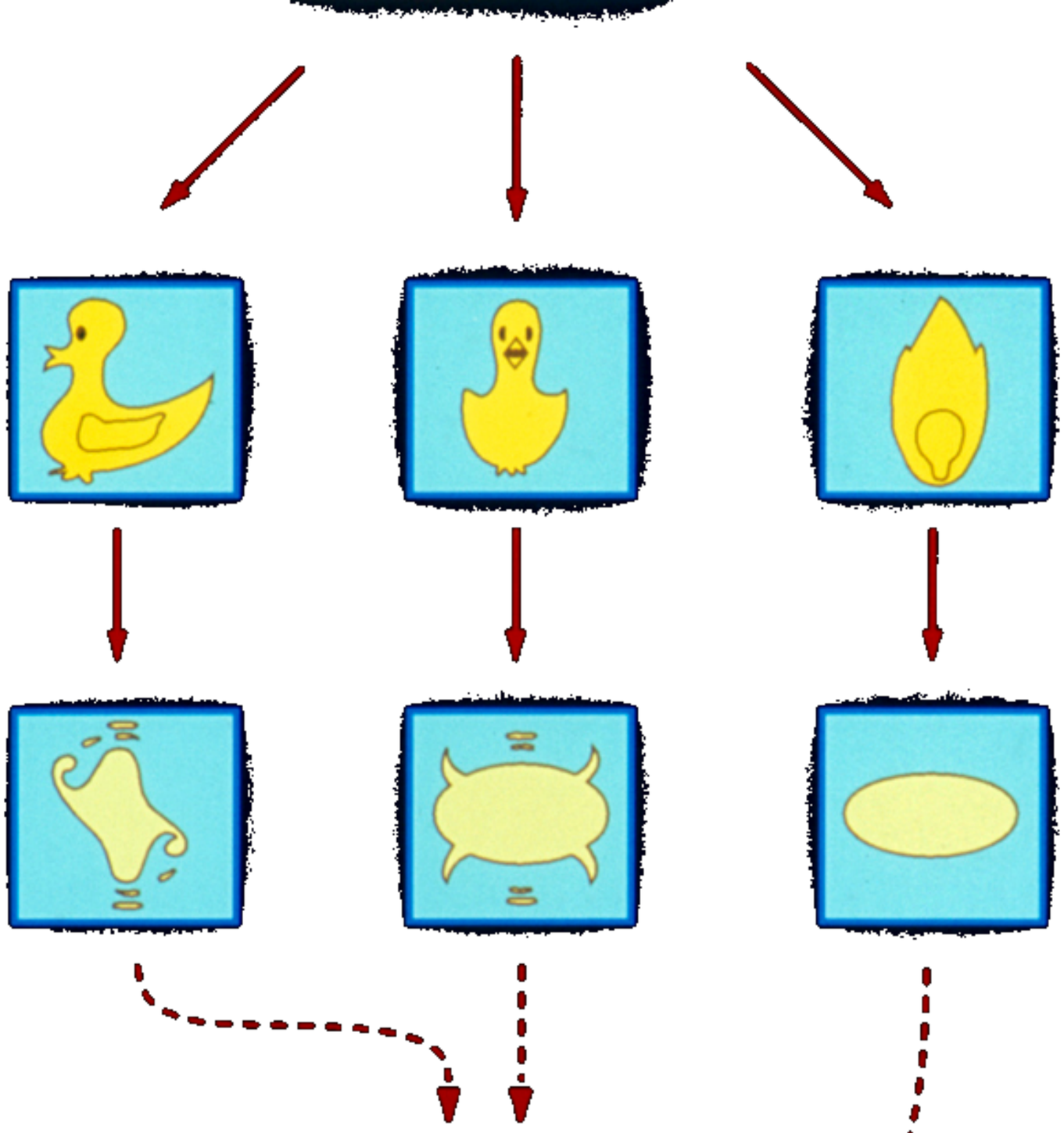
3D Specimen

Different 2D Projected Images

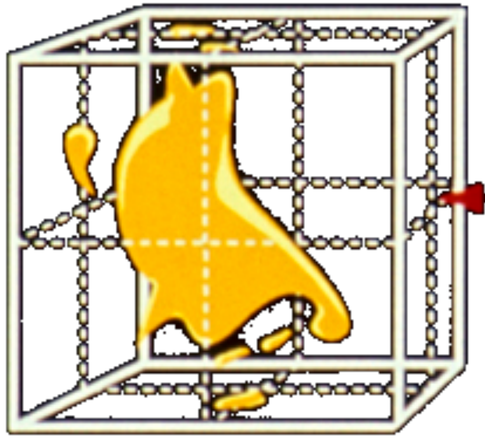
Different 2D Projected Images

2D Fourier transforms

are



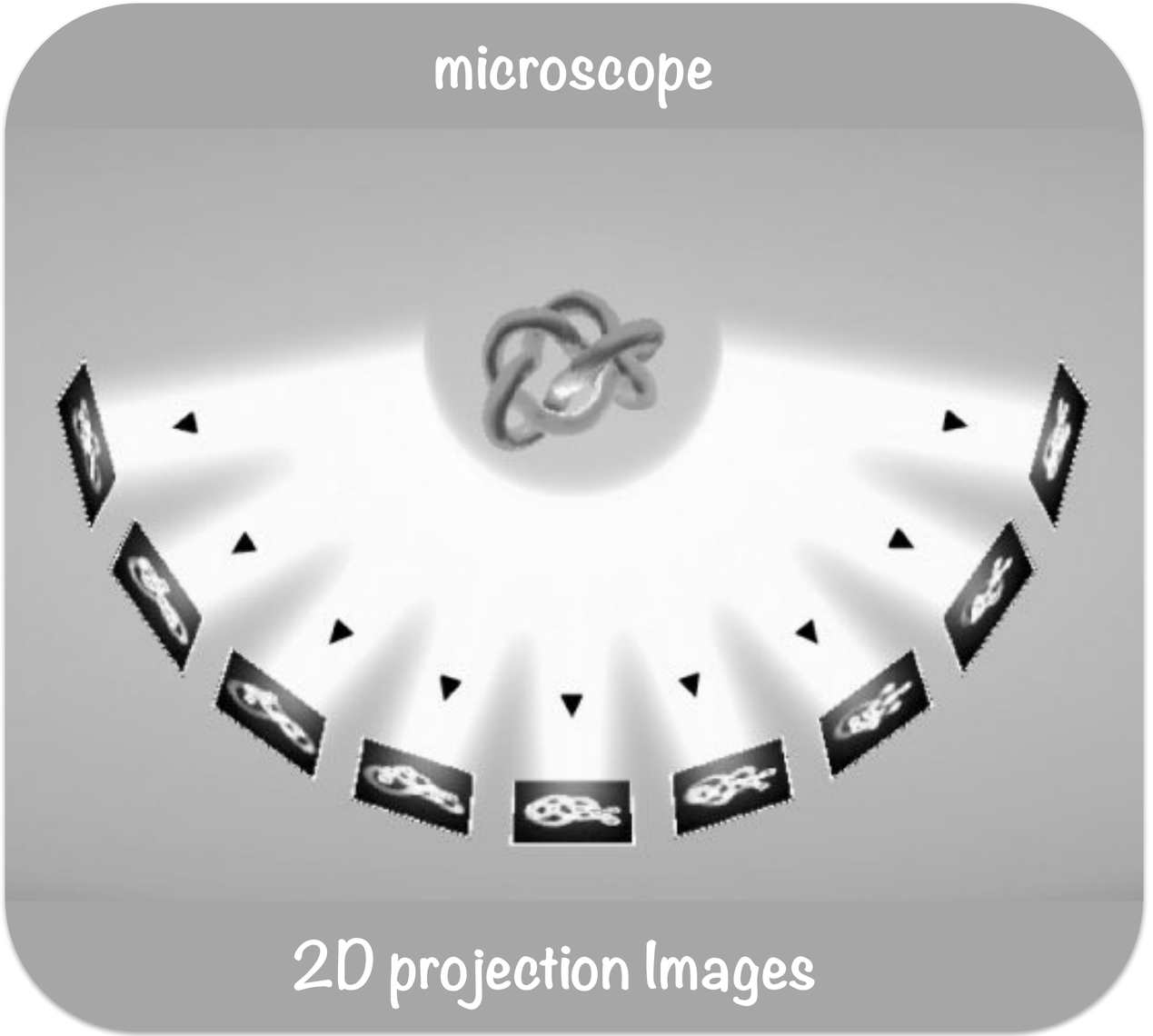
Sections of 3D Fourier Transform

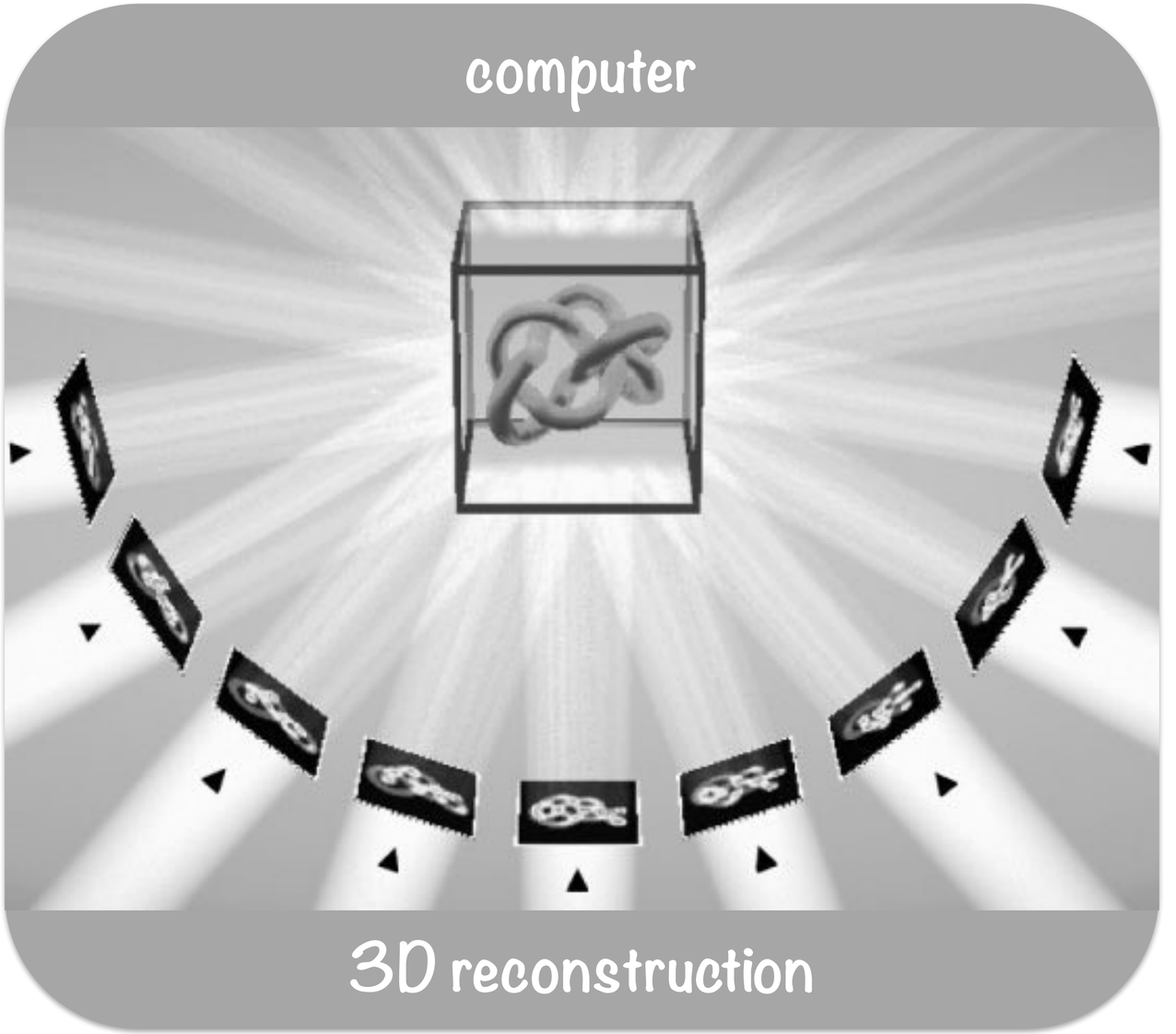


FOURIER | INVERSION

3D Map

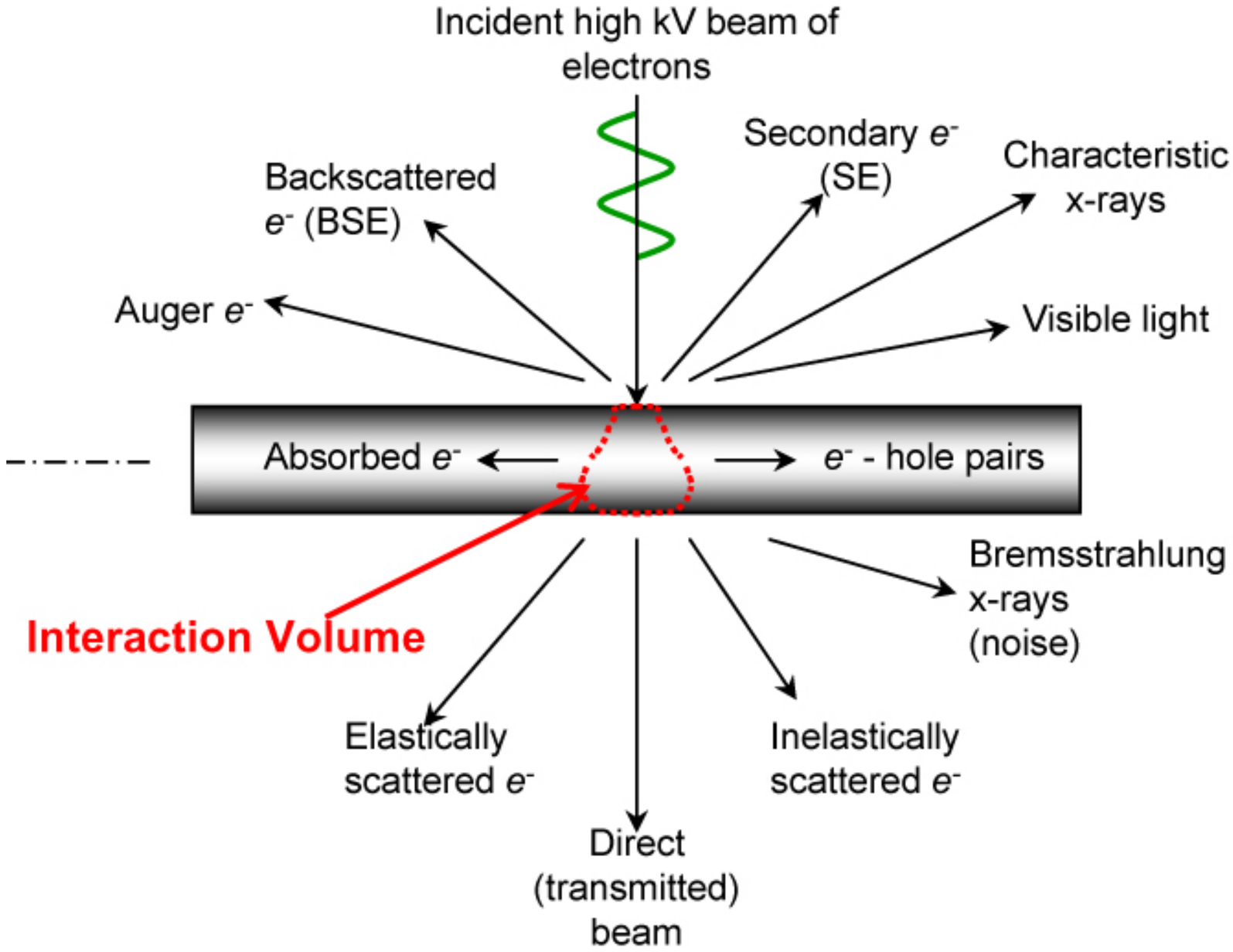
Two major steps in Tomography





Sali A. et al. Nature (2003)

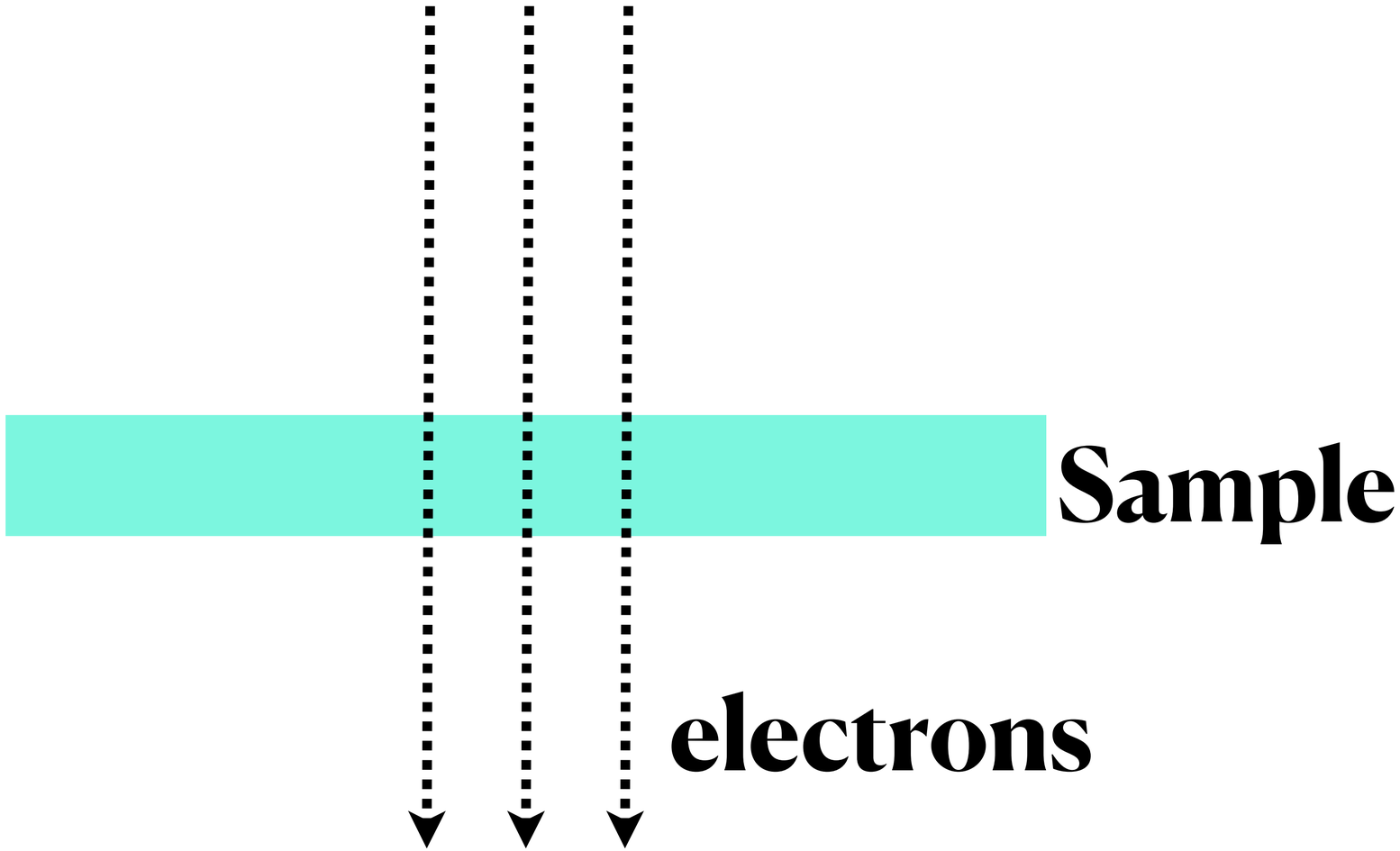
Strong electron-specimen interactions



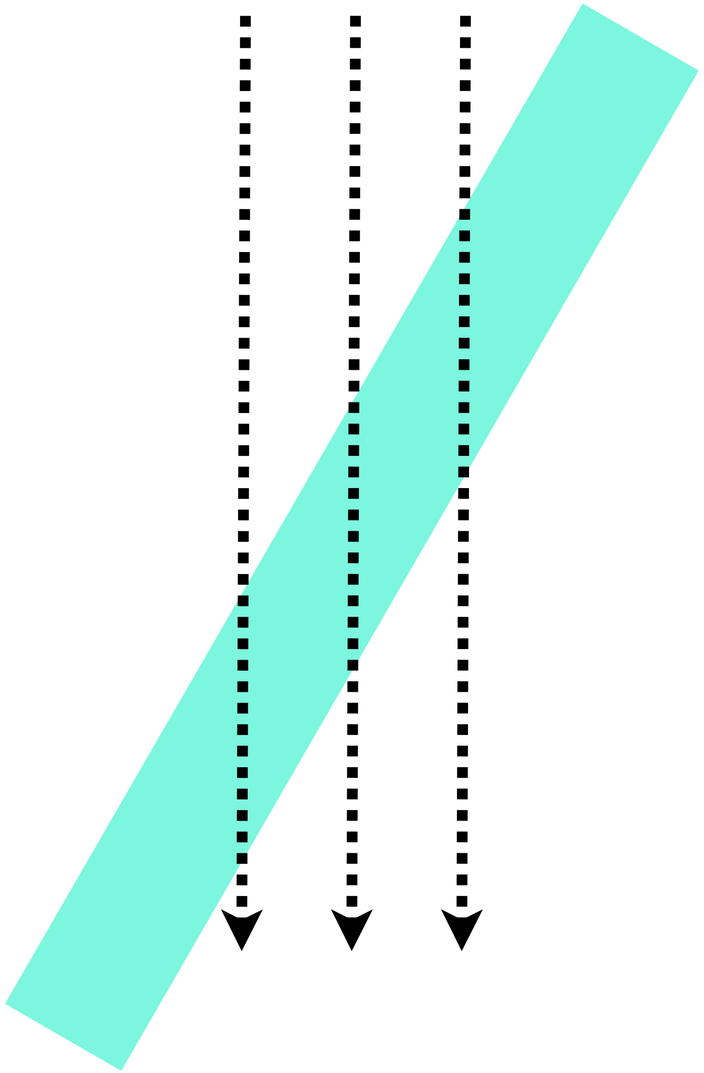
For any frozen-hydrated specimen, the mean free path for 200–300 keV electrons is estimated to be about 200–300 nm.

Cryo-ET samples should be less than 300nm!!

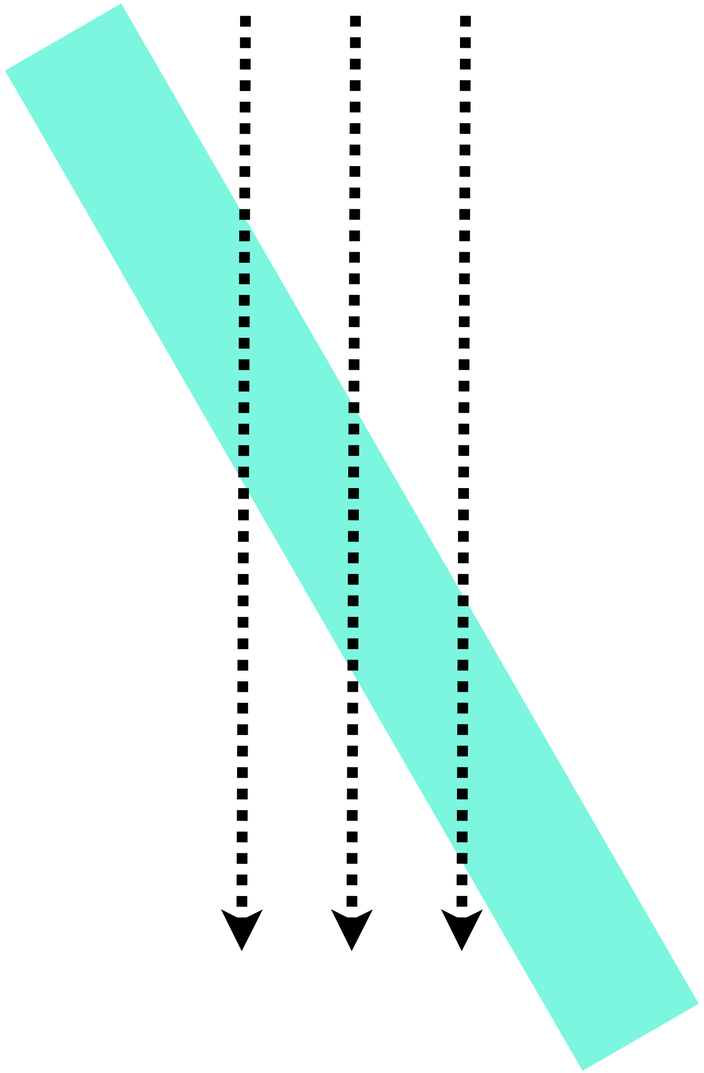
Limited sample thickness and tilt range



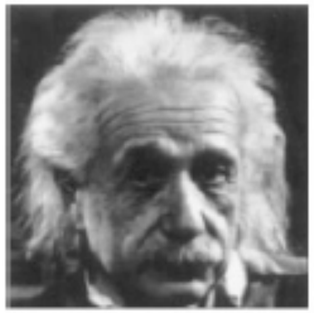
Limited sample thickness and tilt range



Limited sample thickness and tilt range

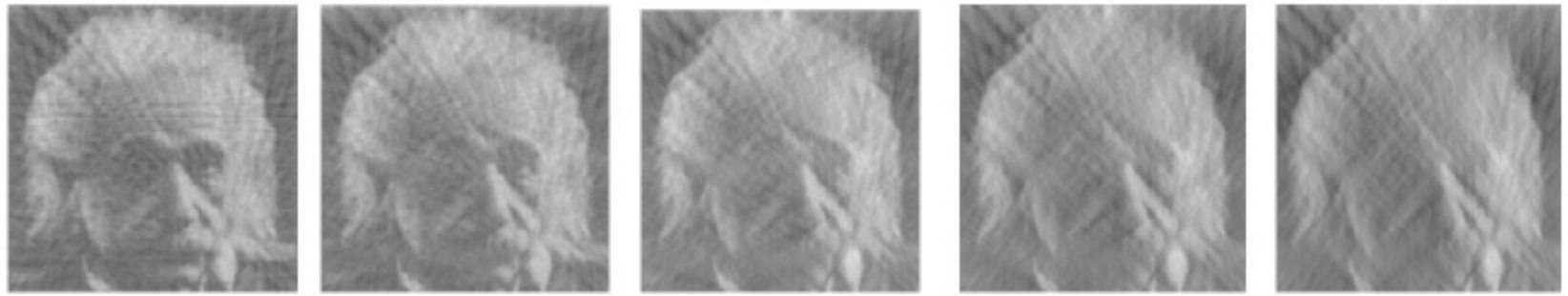


Missing wedge artifact



original image

5 deg increment



−90 − 90 deg −80 − 80 deg −70 − 70 deg −60 − 60 deg −50 − 50 deg

2 deg increment

Outline

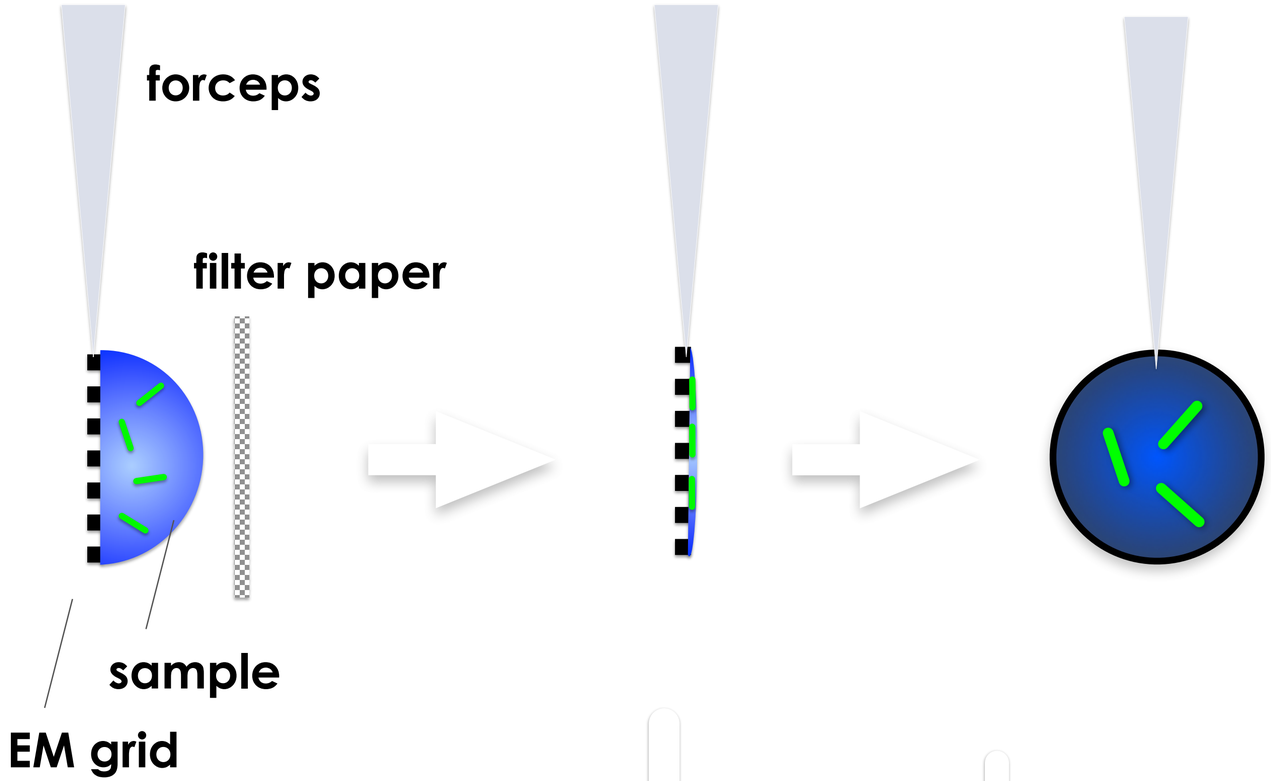
★Brief introduction of electron tomography

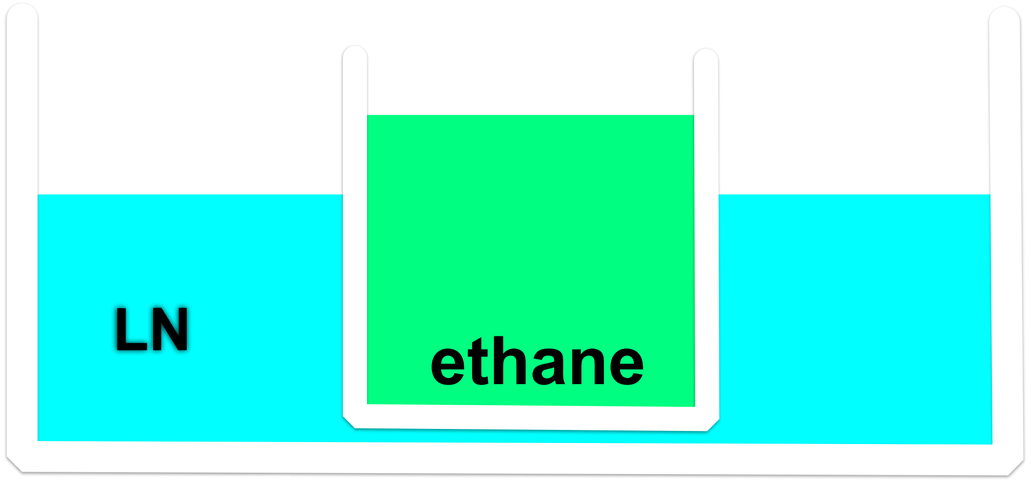
★Practical aspects in cryo-electron tomography

- **Sample preparation**
- Data collection
- Image analysis

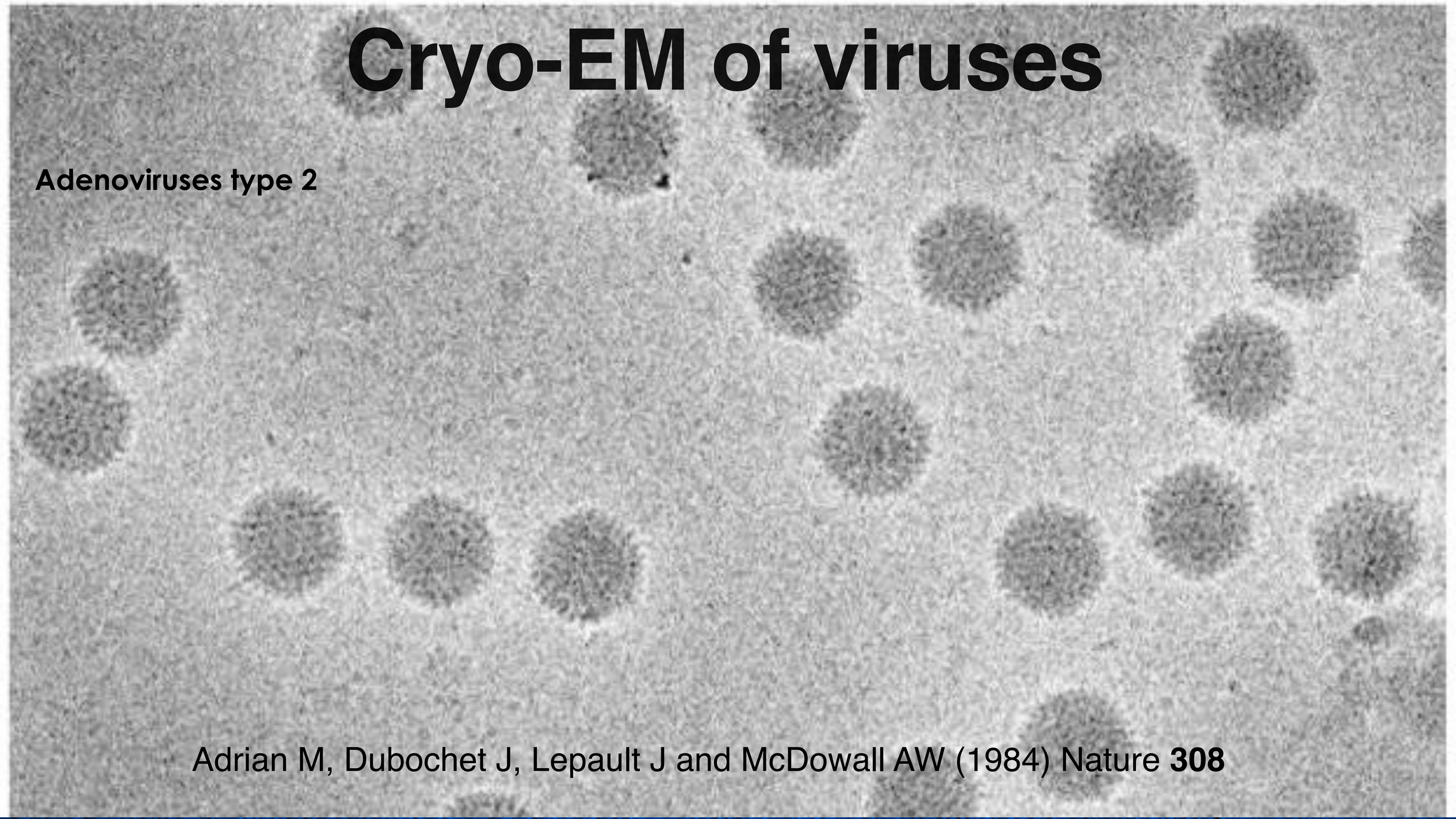
★Frontiers in cryo-electron tomography

Cryo-ET specimen preparation



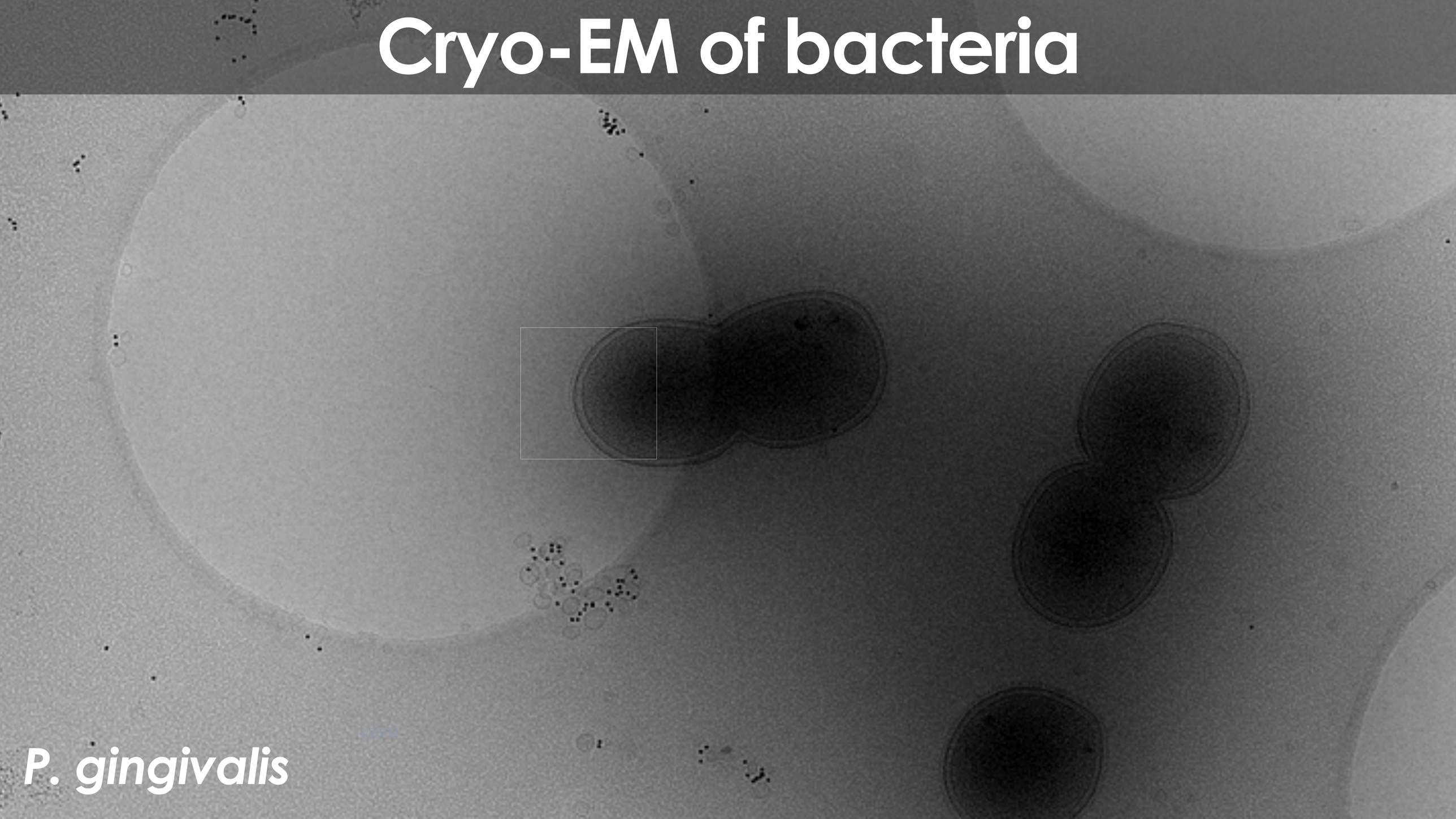


Cryo-EM of viruses



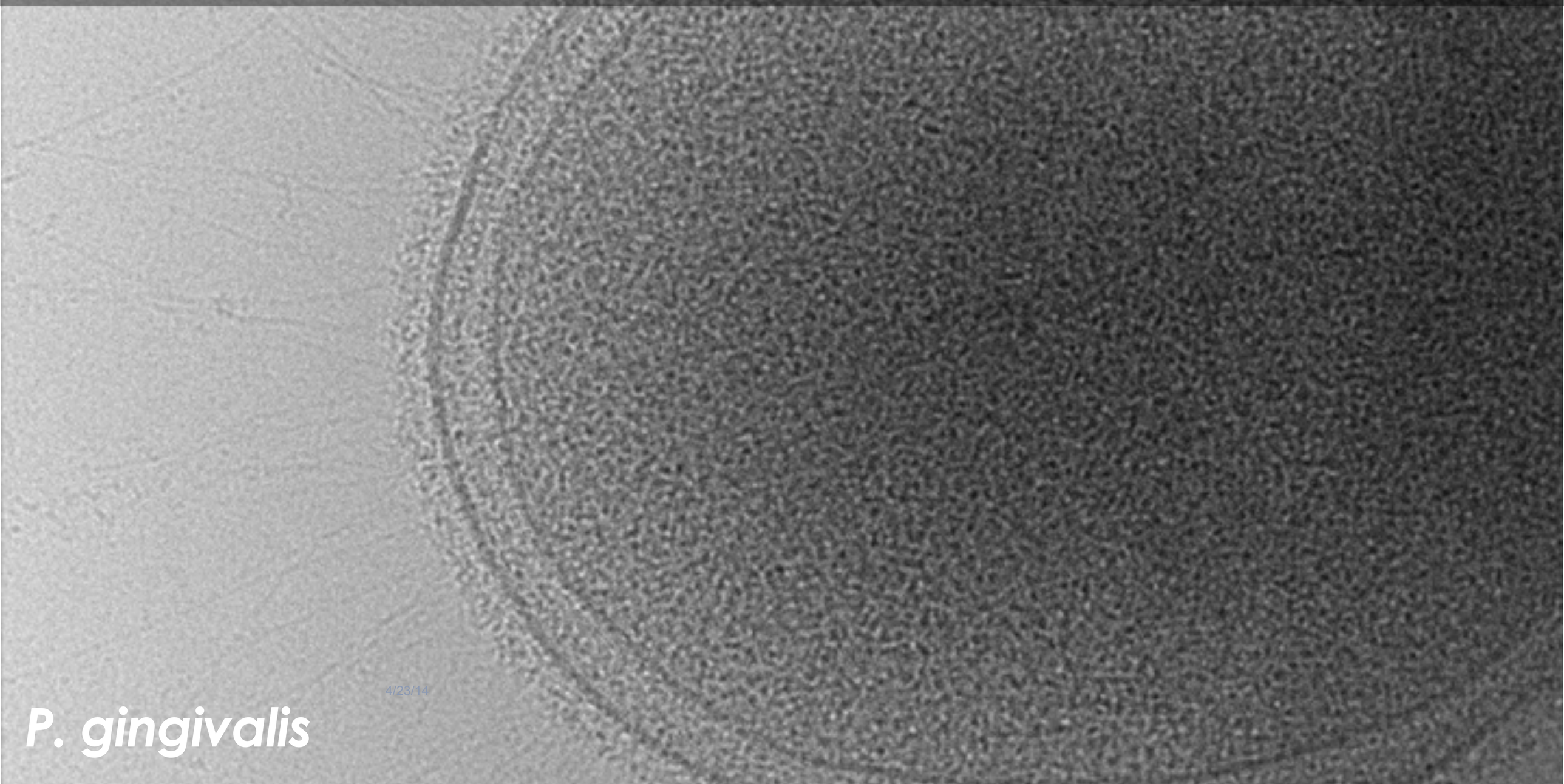
Adrian M, Dubochet J, Lepault J and McDowall AW (1984) Nature **308**

Cryo-EM of bacteria



P. gingivalis

Cryo-EM of bacteria



4/23/14

P. gingivalis

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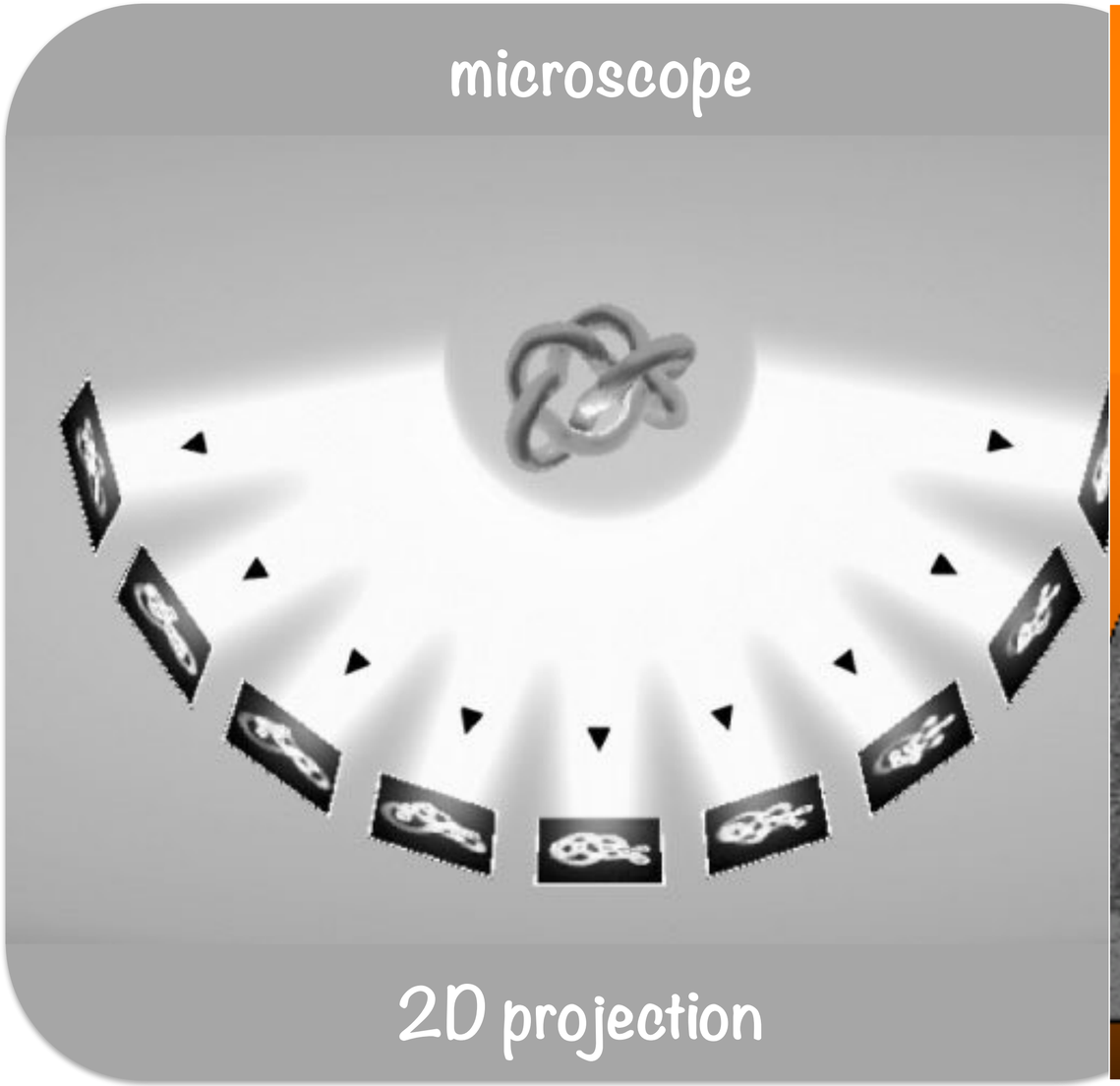
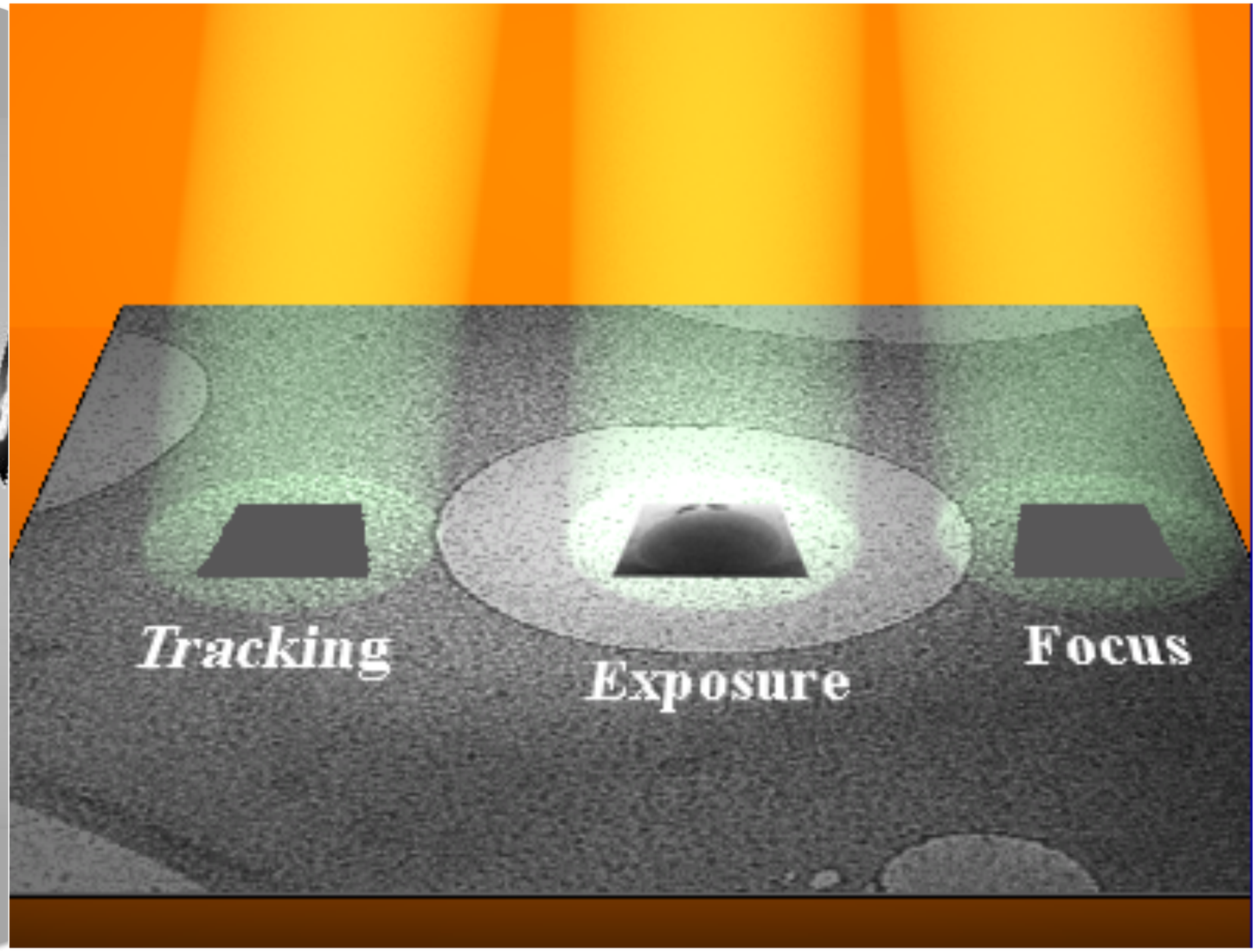
- Image analysis

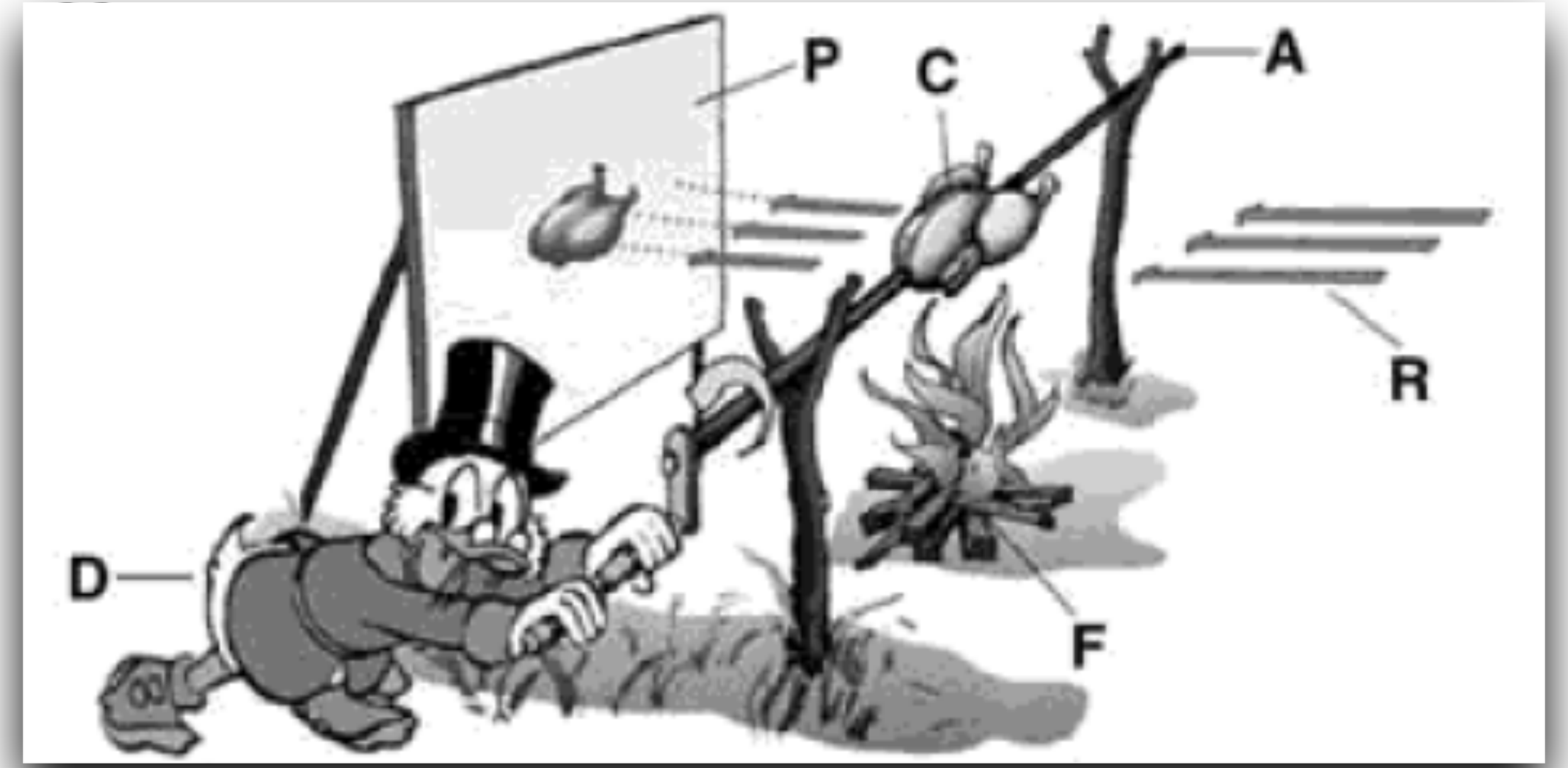
★Frontiers in cryo-electron tomography

How to collect cryo-ET data?



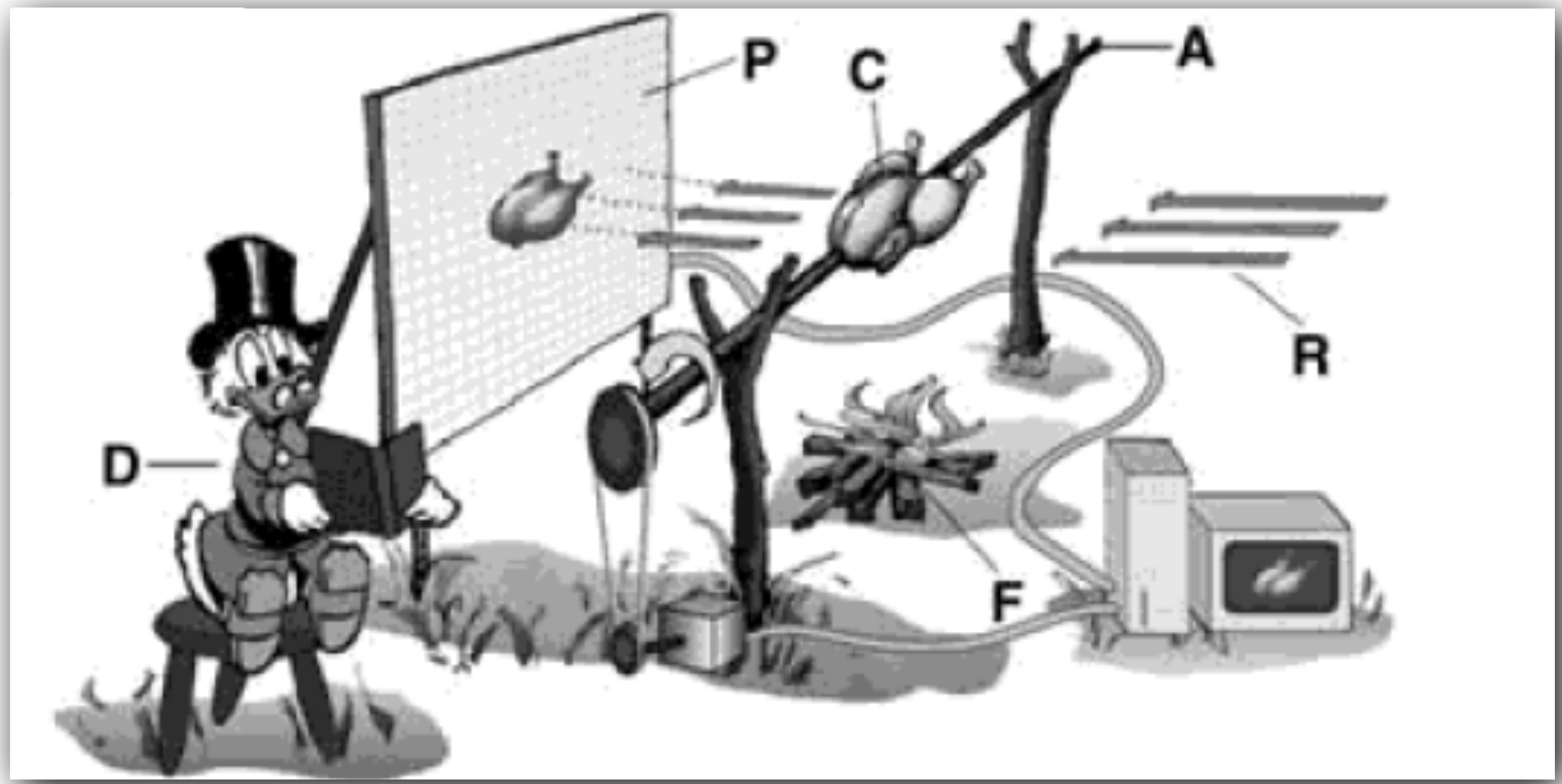


Data collection could be tedious



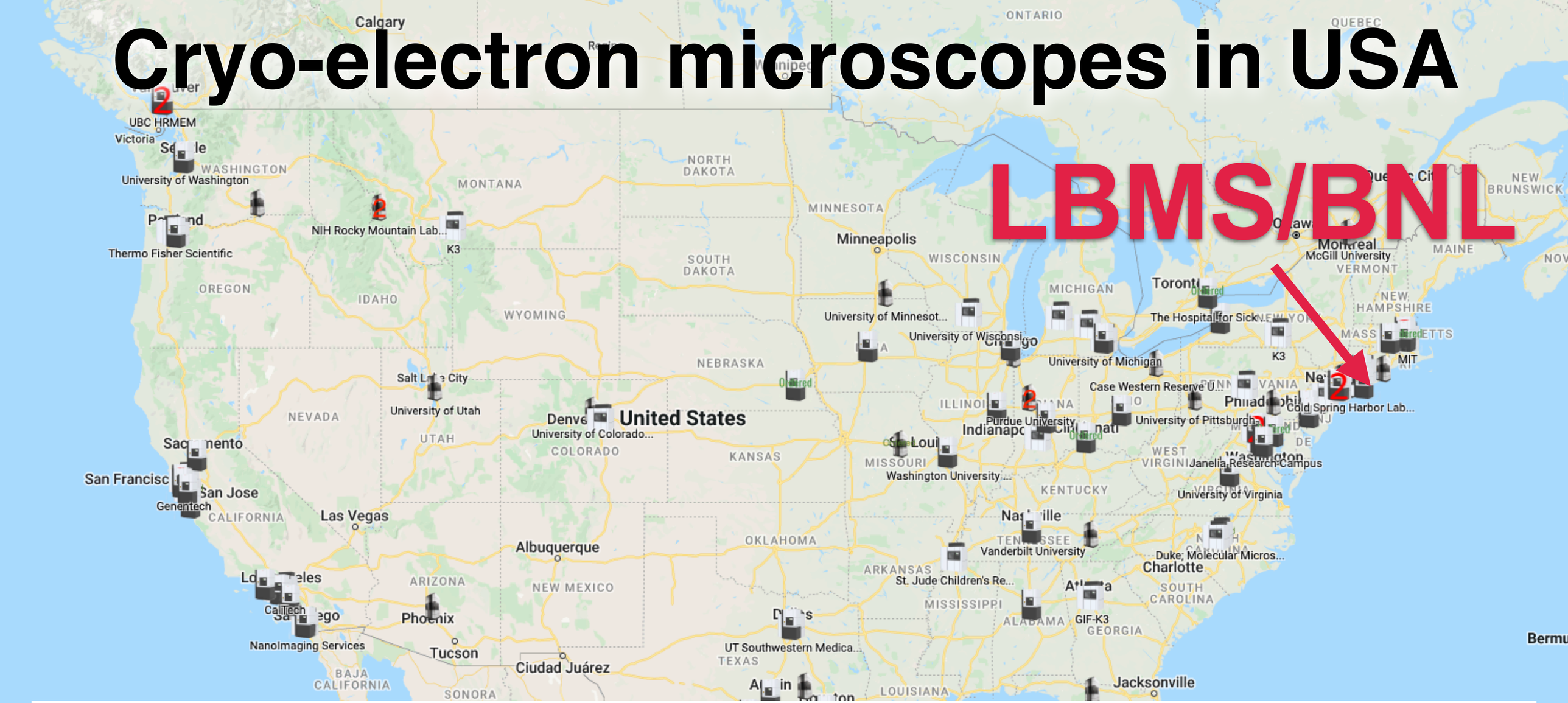
Frank: Electron Tomography

Automation is essential



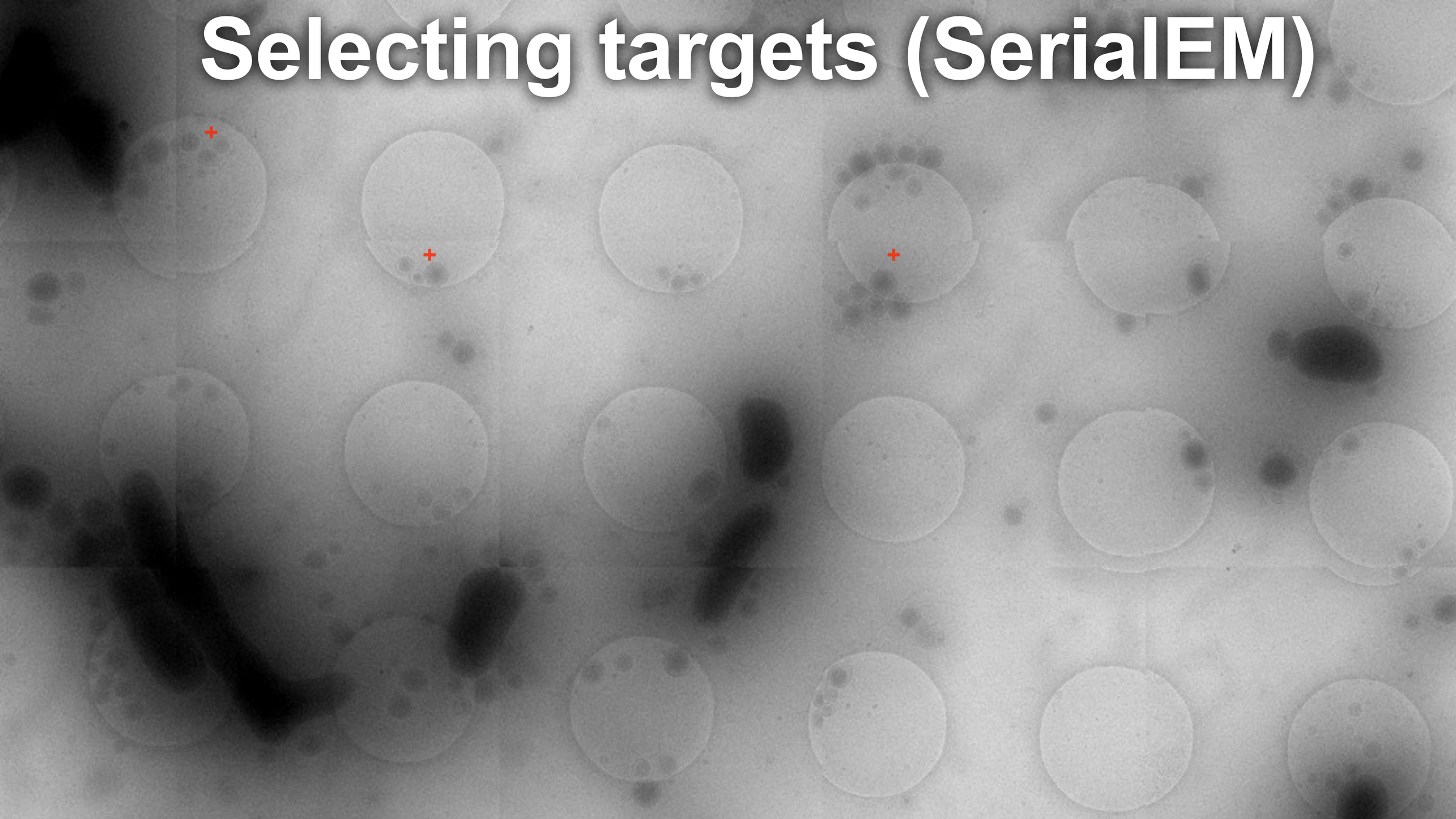
Frank: Electron Tomography

Cryo-electron microscopes in USA

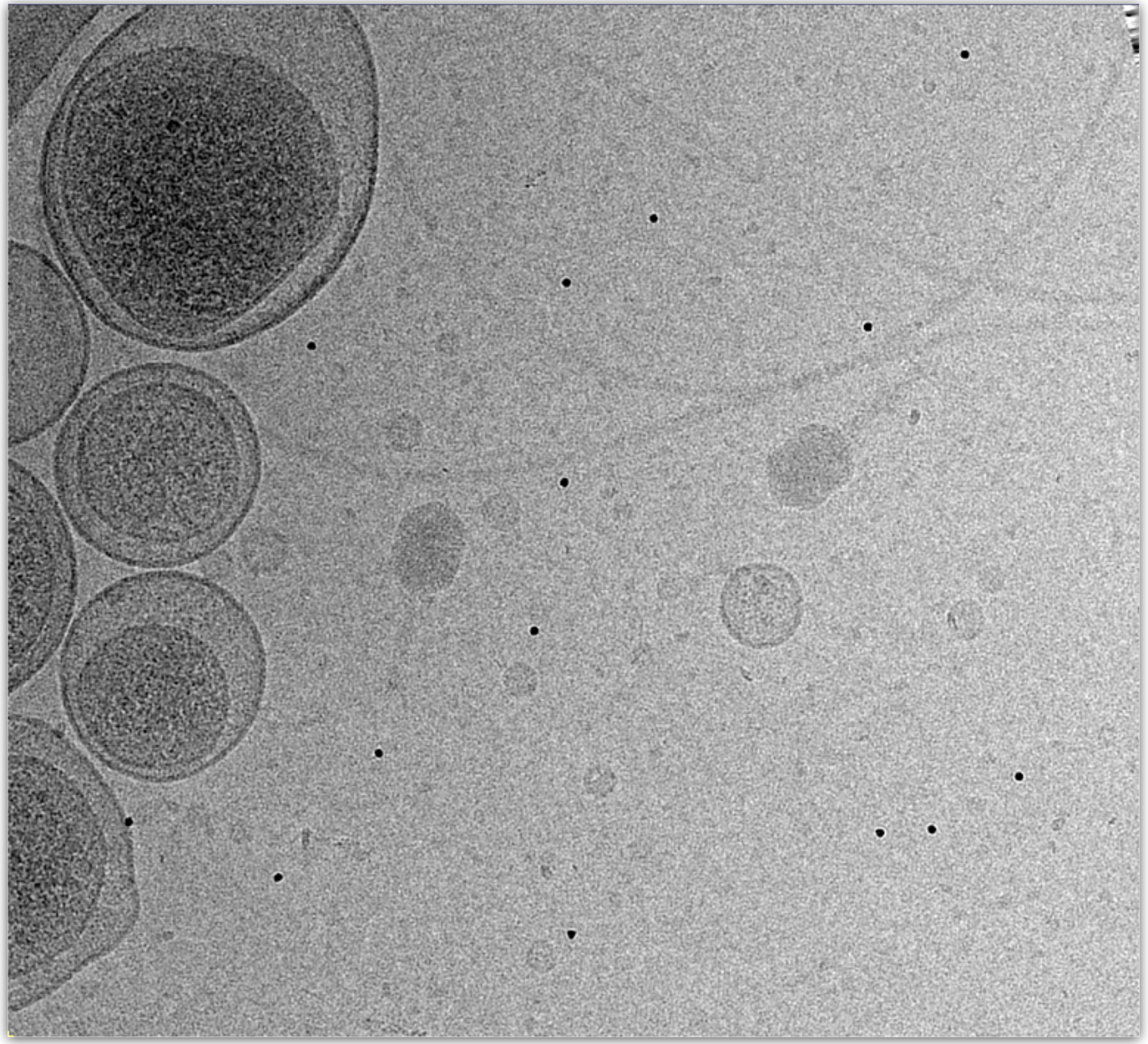


Multiple National Centers for Cryo-EM have been established.
Most microscopes can be controlled remotely.
Basic microscope operations are similar.

Selecting targets (SerialEM)



Collecting tilt series



SerialEM

David Mastronarde

Univ. of Colorado

Boulder

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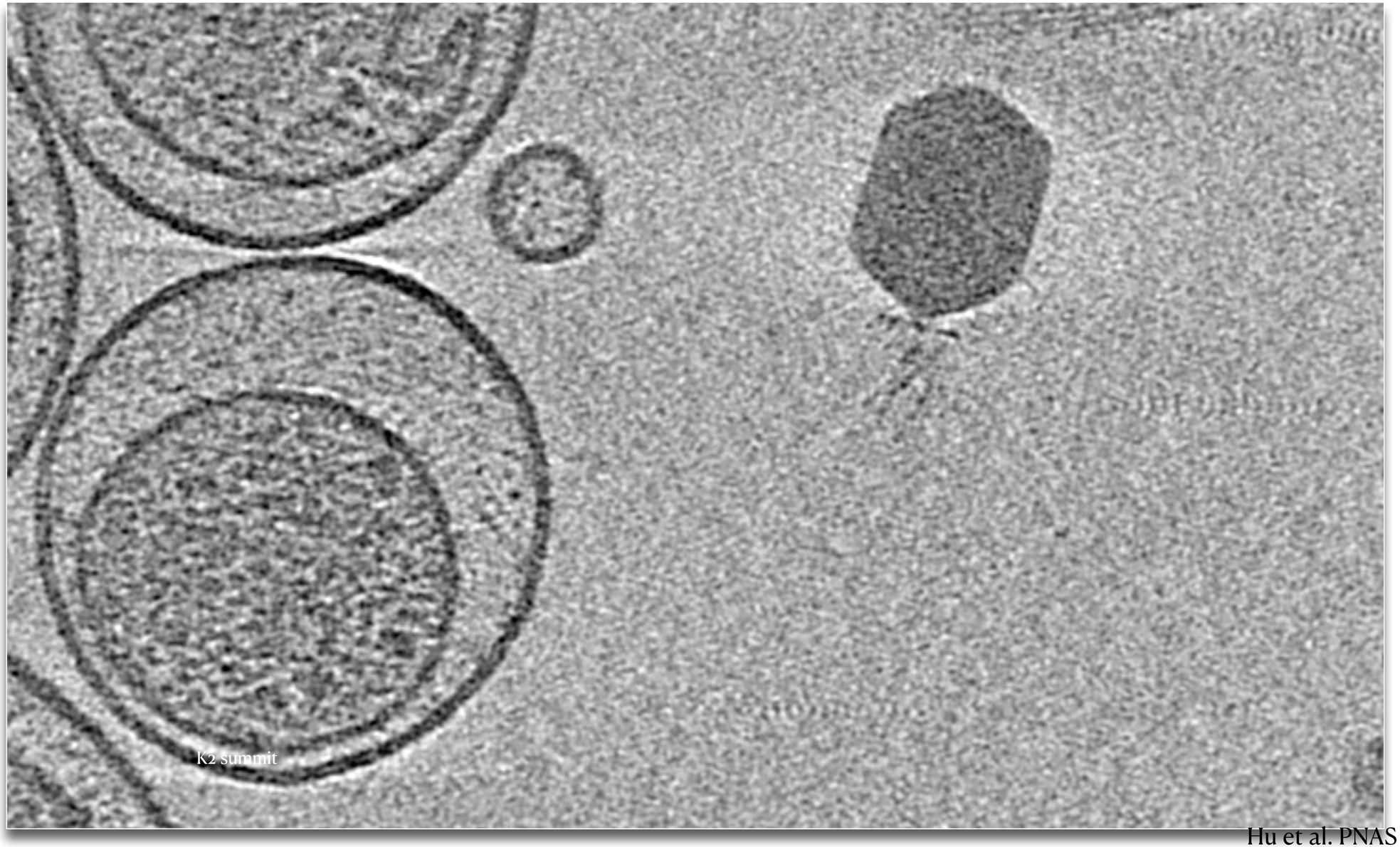
- Data collection
- **Image analysis**

★Frontiers in cryo-electron tomography

Alignment and reconstruction (IMOD)



Hu et al. PNAS (2015)

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Automated tilt series alignment and tomographic reconstruction in IMOD

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ARTICLE INFO

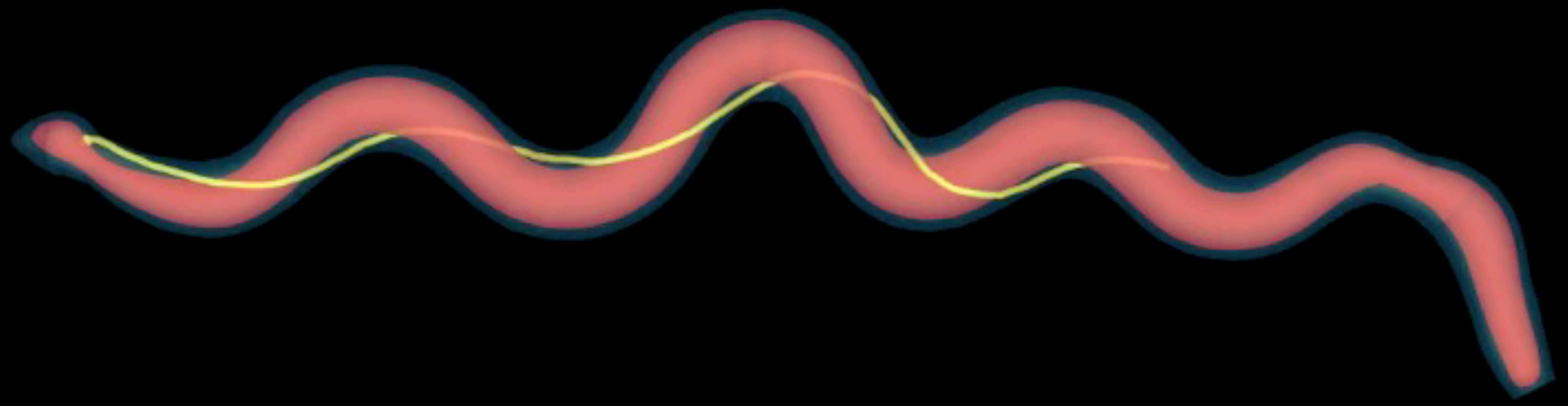
Article history:
Received 12 May 2016
Received in revised form 15 July 2016
Accepted 18 July 2016
Available online 19 July 2016

Keywords:
Electron tomography
Tilt series alignment

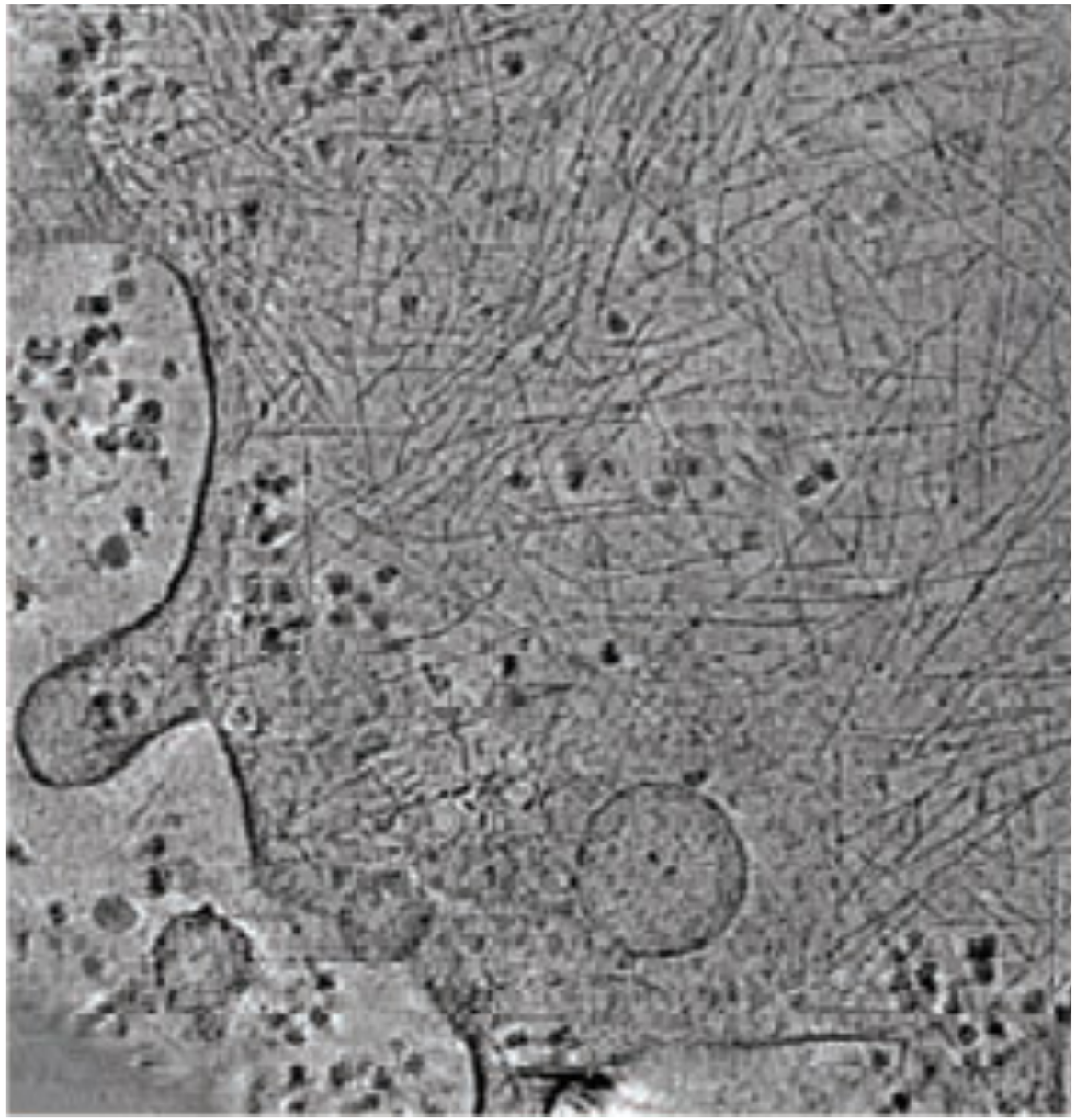
ABSTRACT

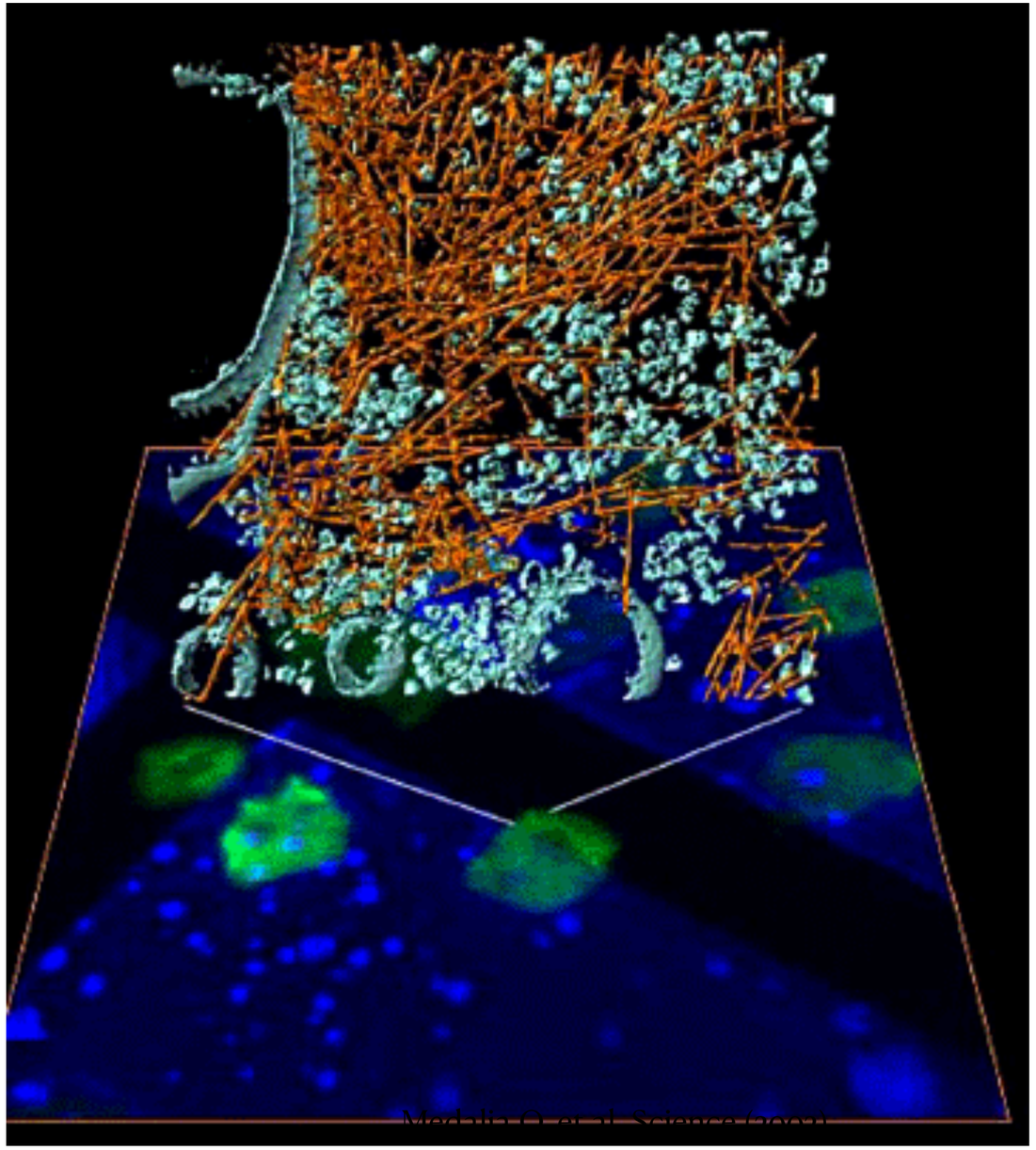
Automated tomographic reconstruction is now possible in the IMOD software package, including the merging of tomograms taken around two orthogonal axes. Several developments enable the production of high-quality tomograms. When using fiducial markers for alignment, the markers to be tracked through the series are chosen automatically; if there is an excess of markers available, a well-distributed subset is selected that is most likely to track well. Marker positions are refined by applying an edge-enhancing Sobel filter, which results in a 20% improvement in alignment error for plastic-embedded samples and 10% for frozen-hydrated samples. Robust fitting, in which outlying points are given less or no weight in computing the fitting error, is used to obtain an alignment solution, so that

3D visualization of a spirochete

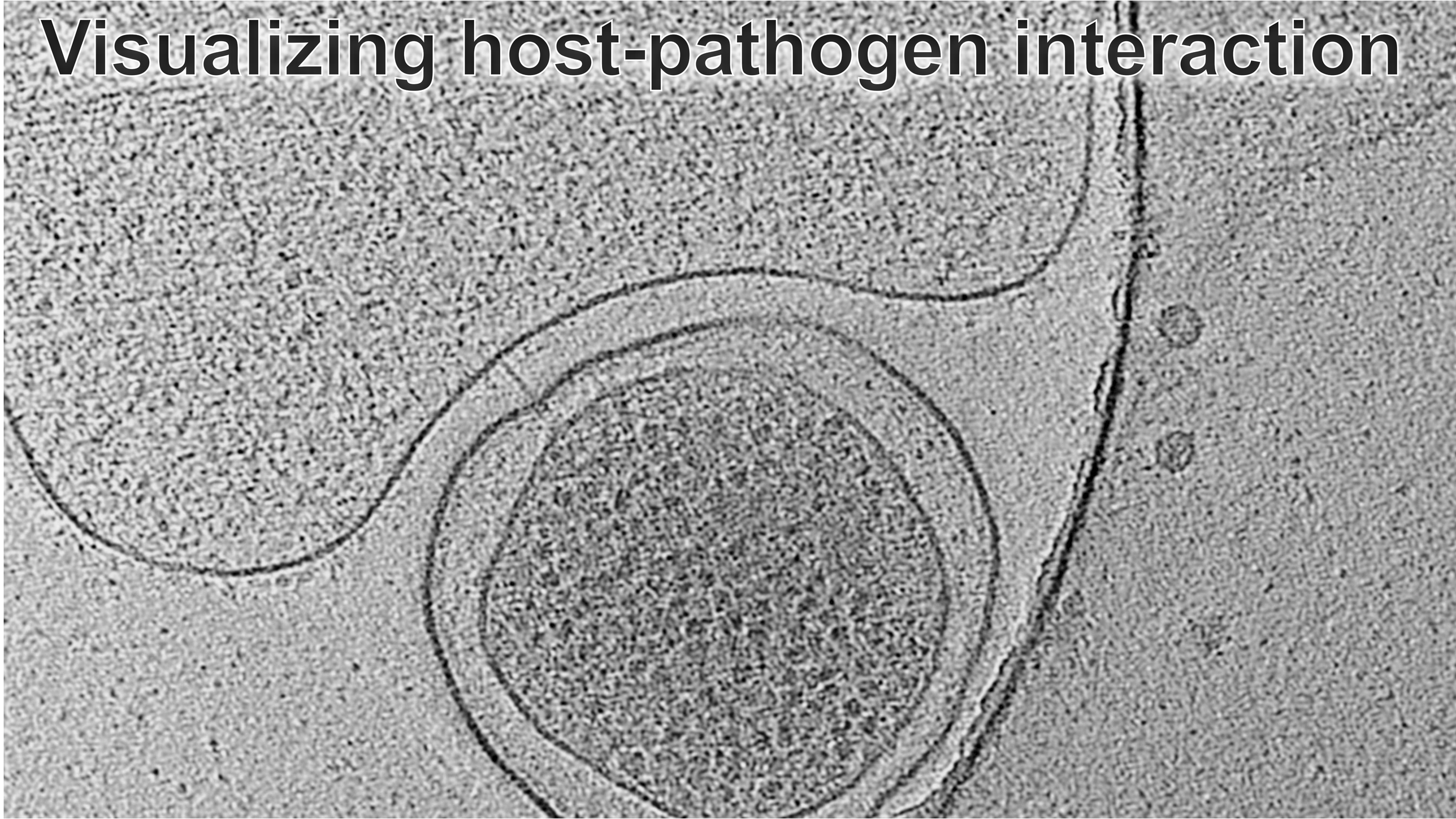


Actin cytoskeleton in eukaryotic cells

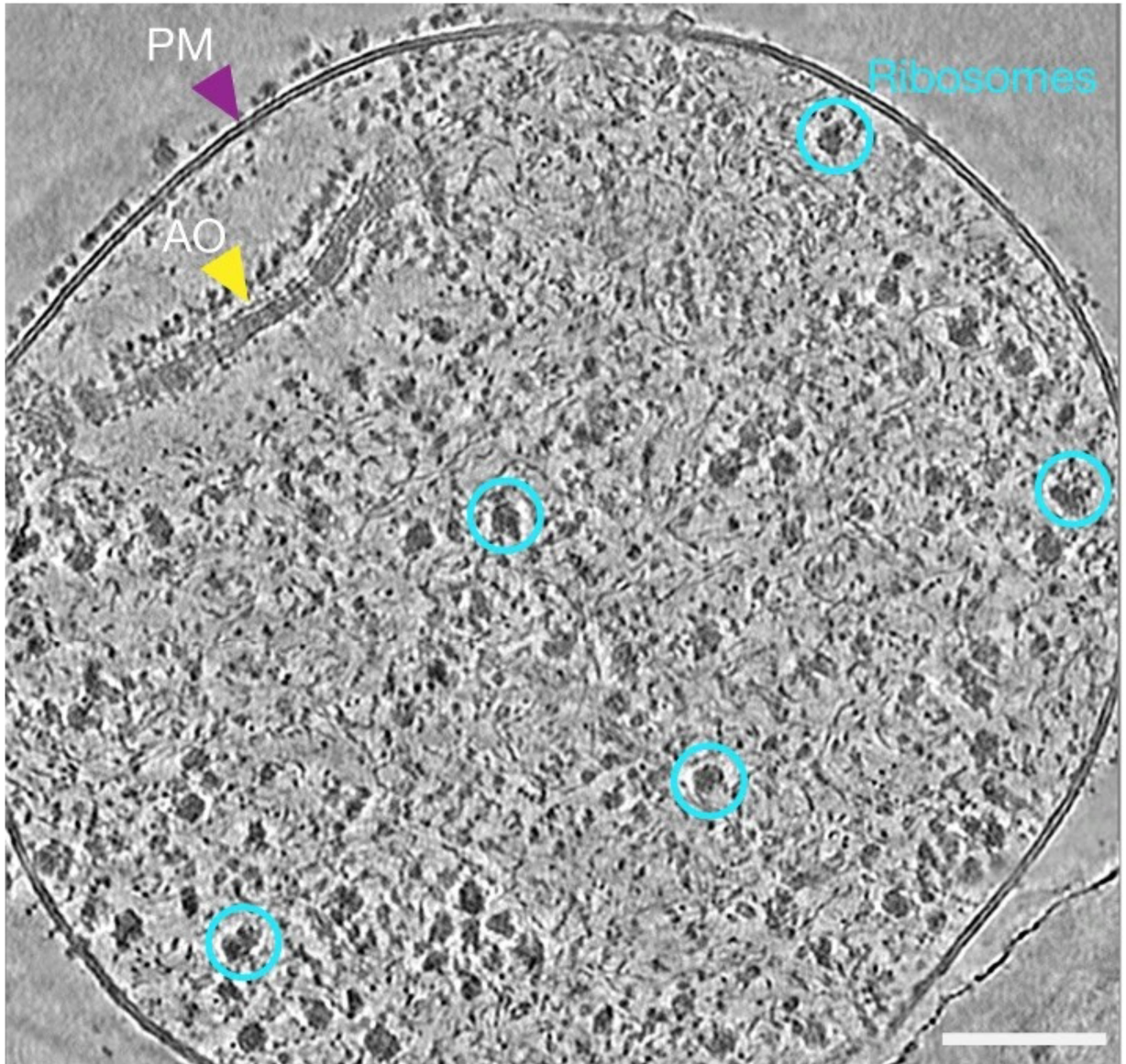


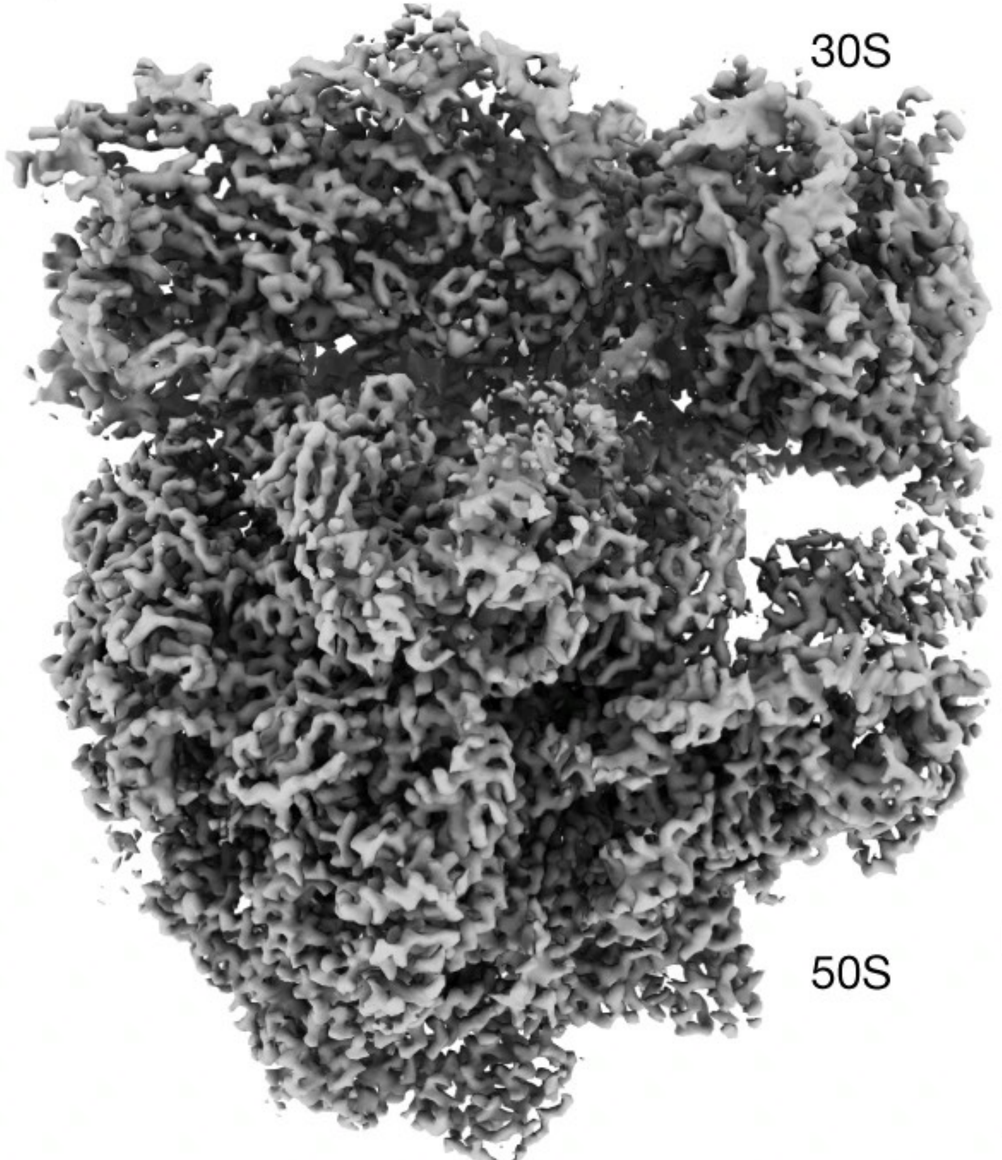


Visualizing host-pathogen interaction

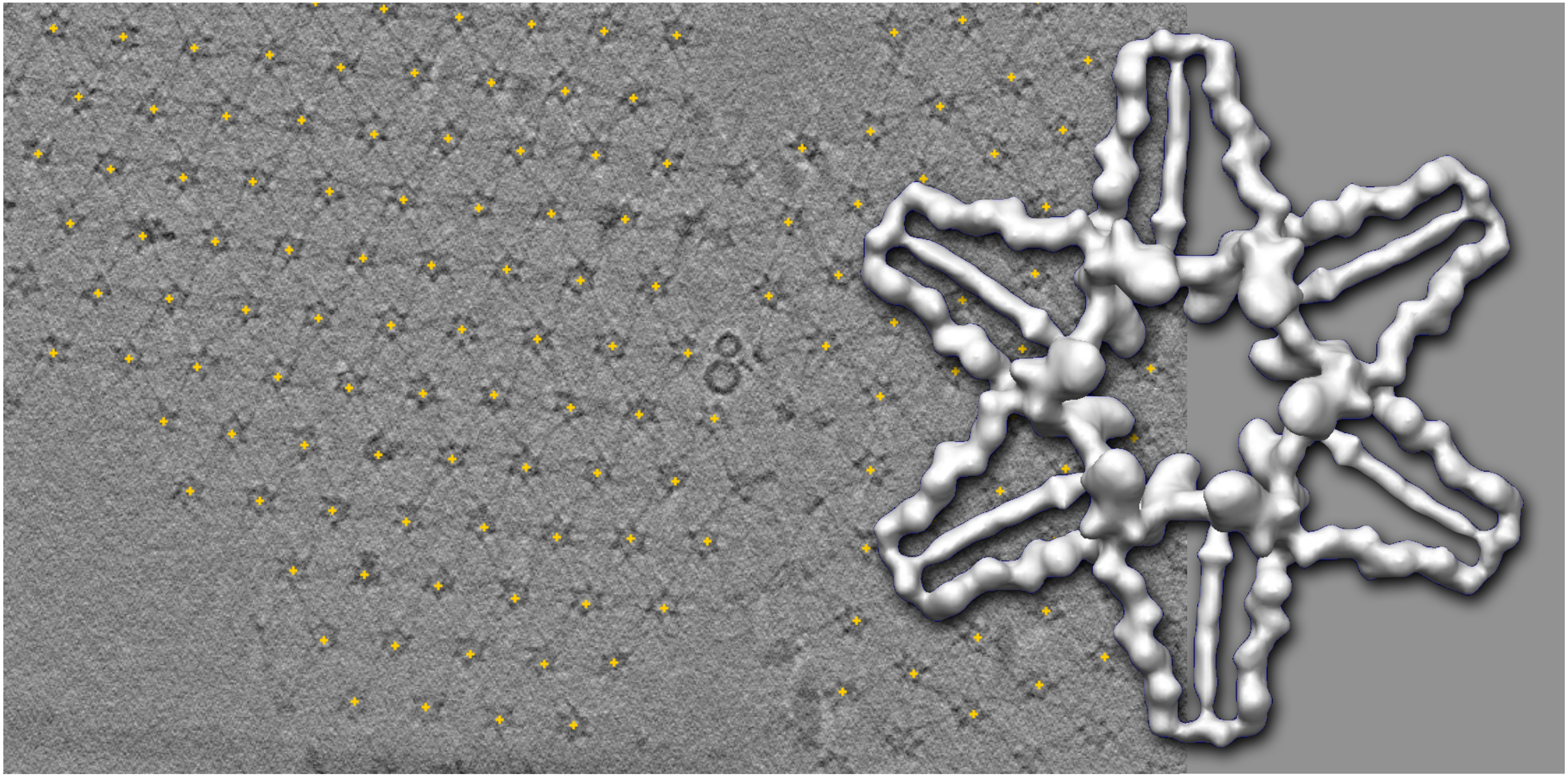


Sub-tomogram averaging
- towards high resolution in-situ structure dermination

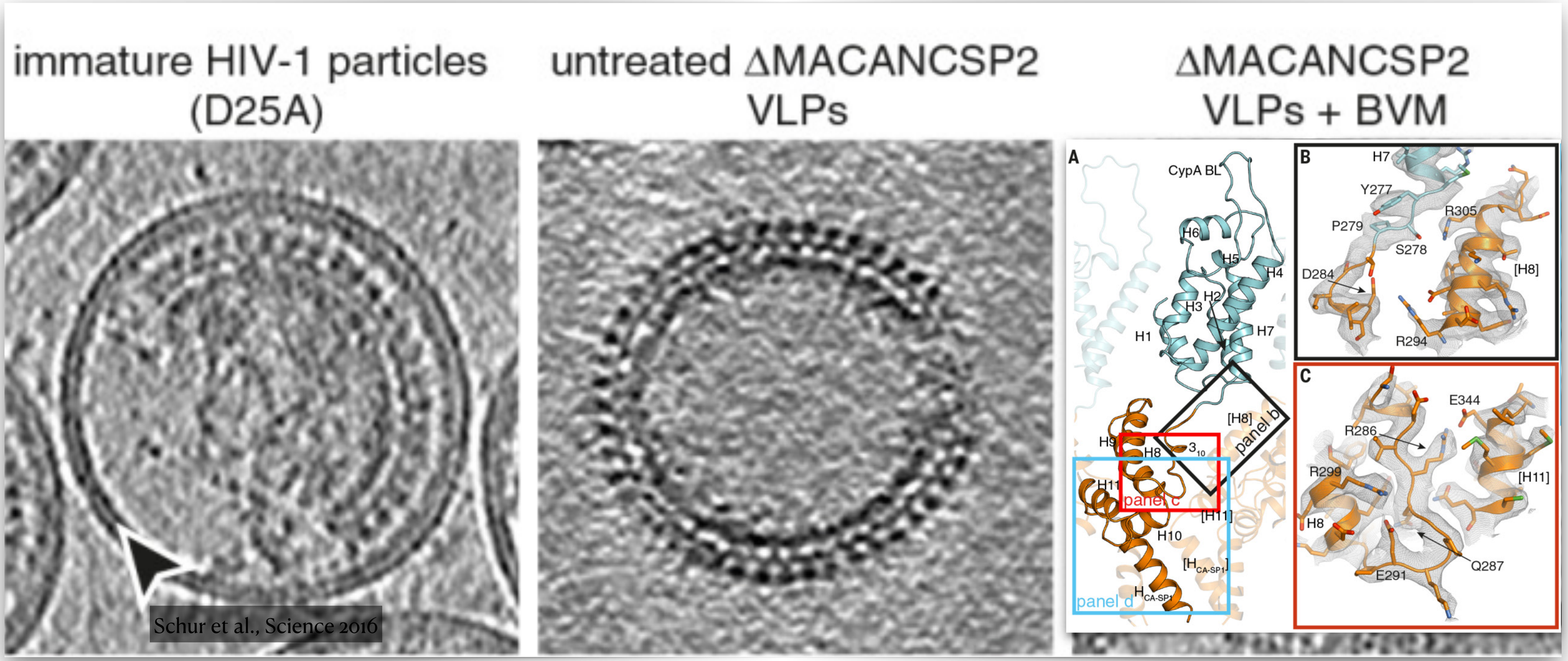




Picking 3-D sub-tomograms

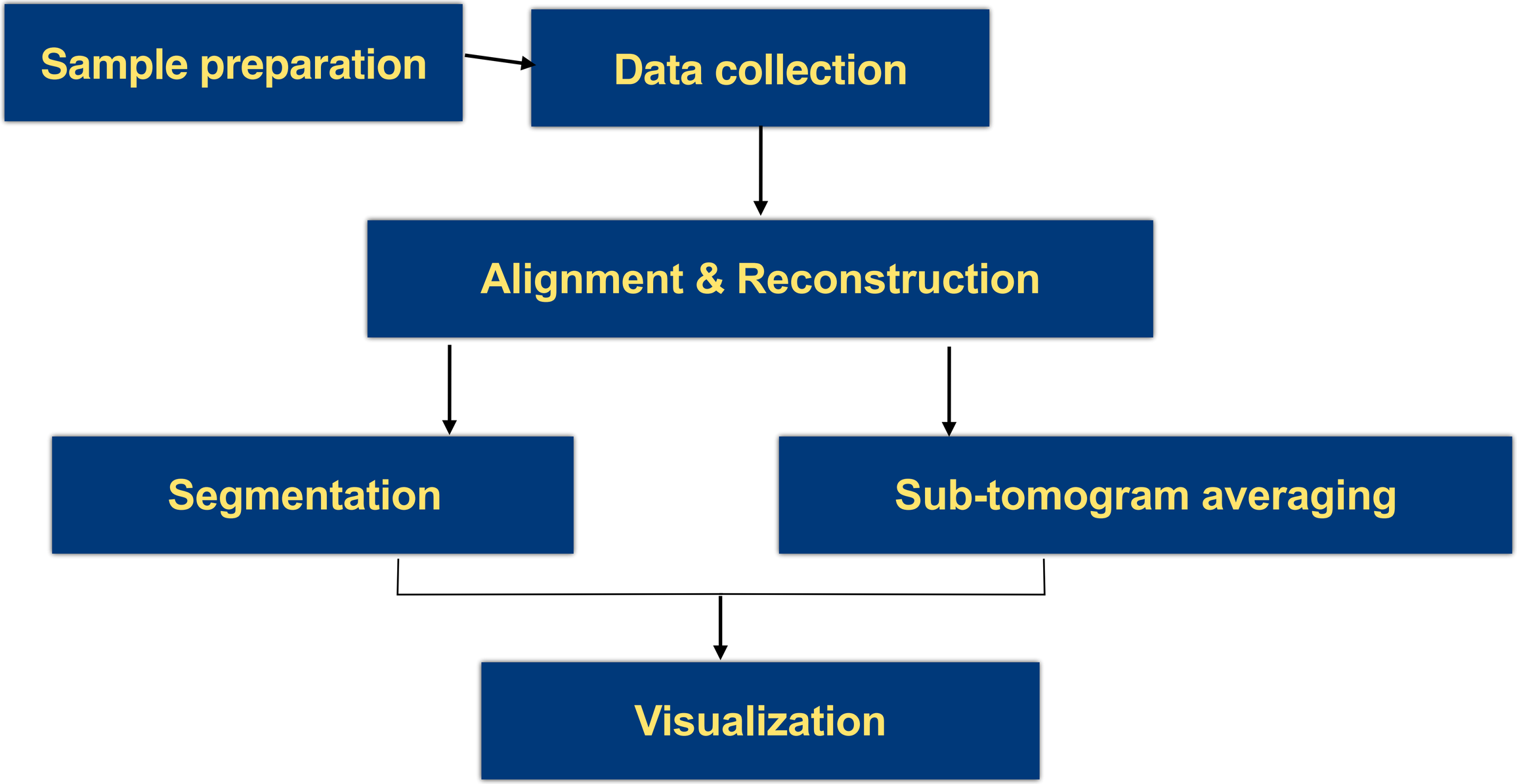


In situ structure at near atomic resolution



~300,000 asymmetric units were used to determine the 3.9Å resolution structure.

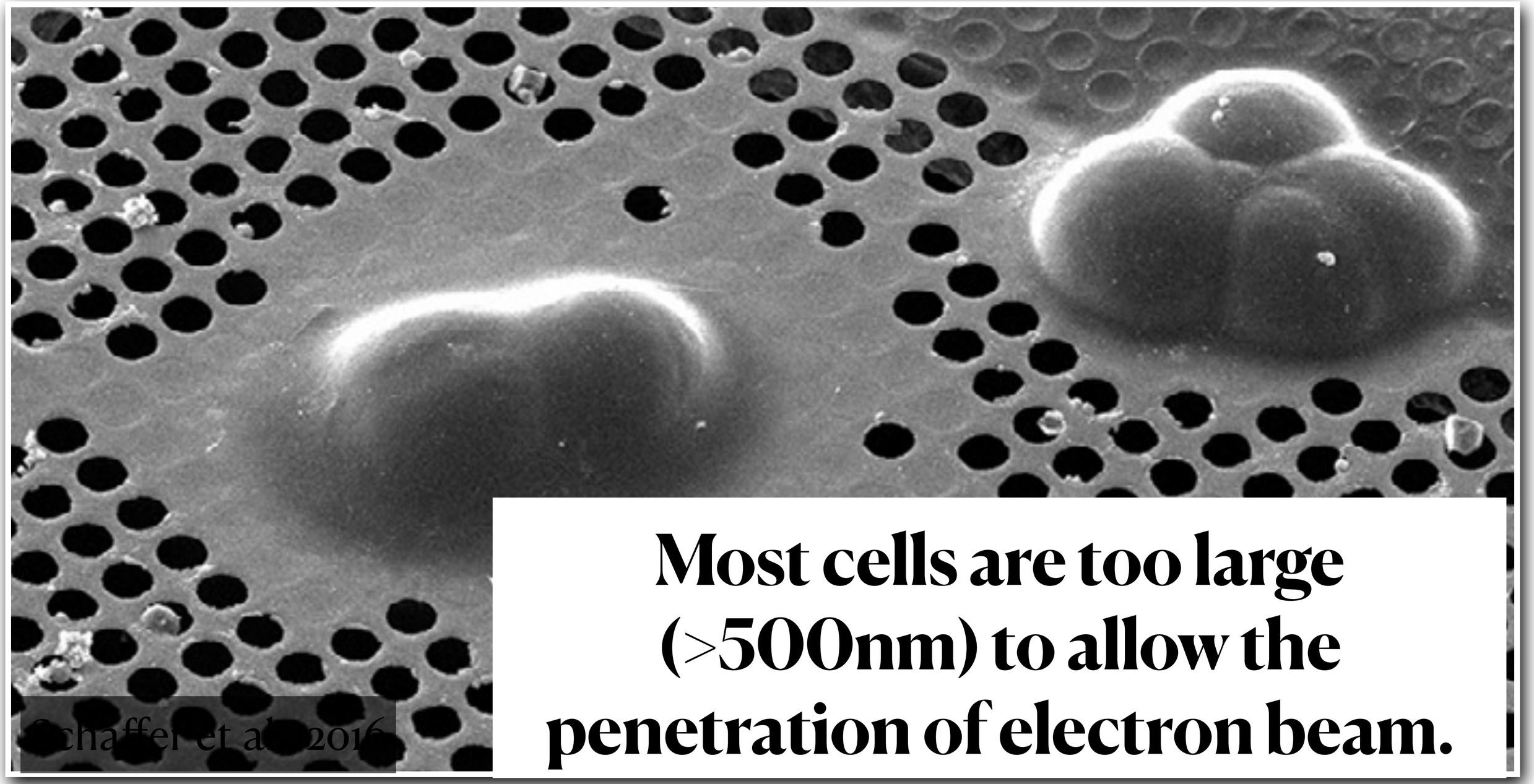
Cryo-ET workflow



Frontiers in cryo-ET

- Cryo-FIB milling
- New methods for data collection and image analysis
- Towards higher resolution and throughput

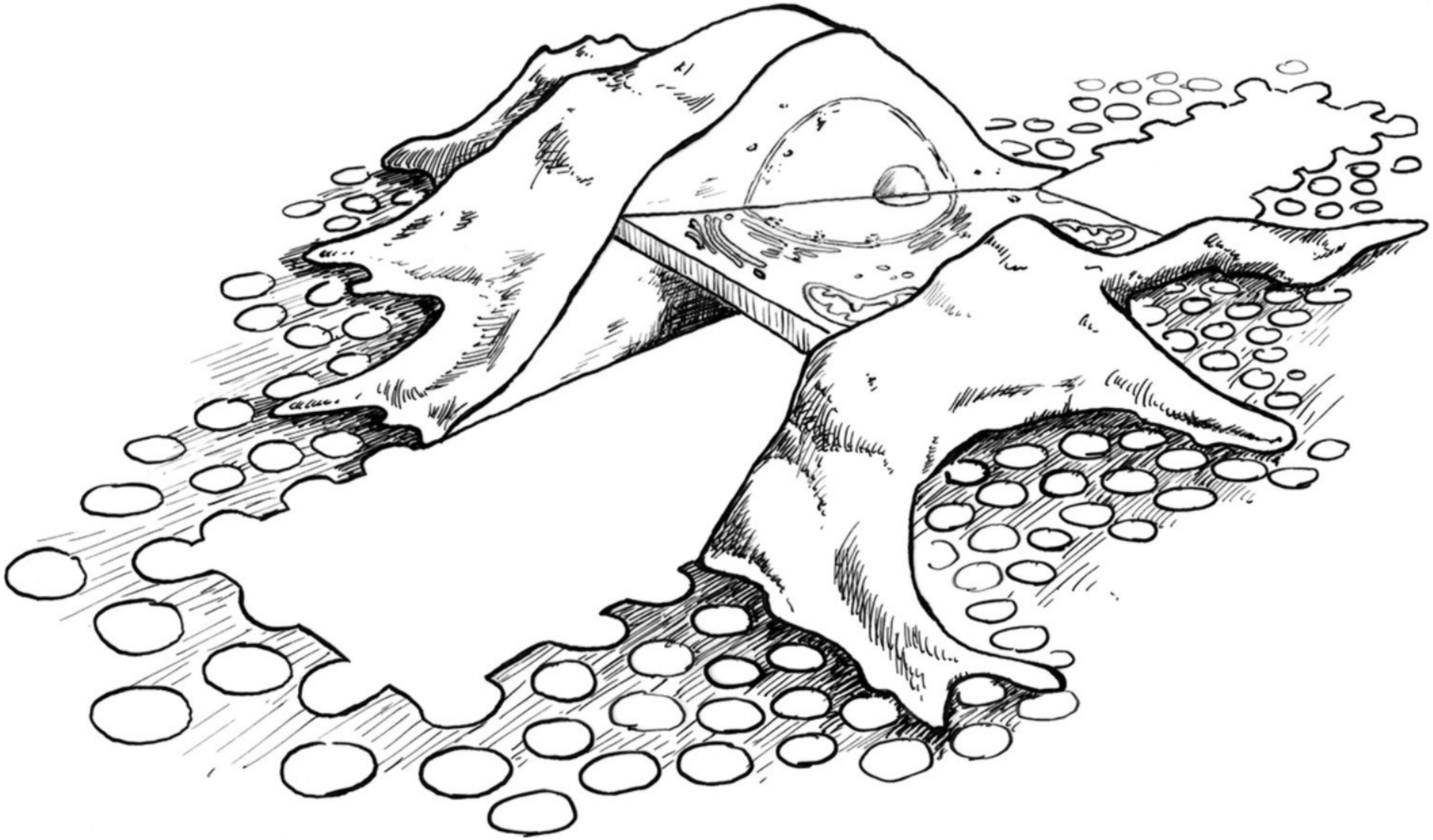
From small bacteria to large cells



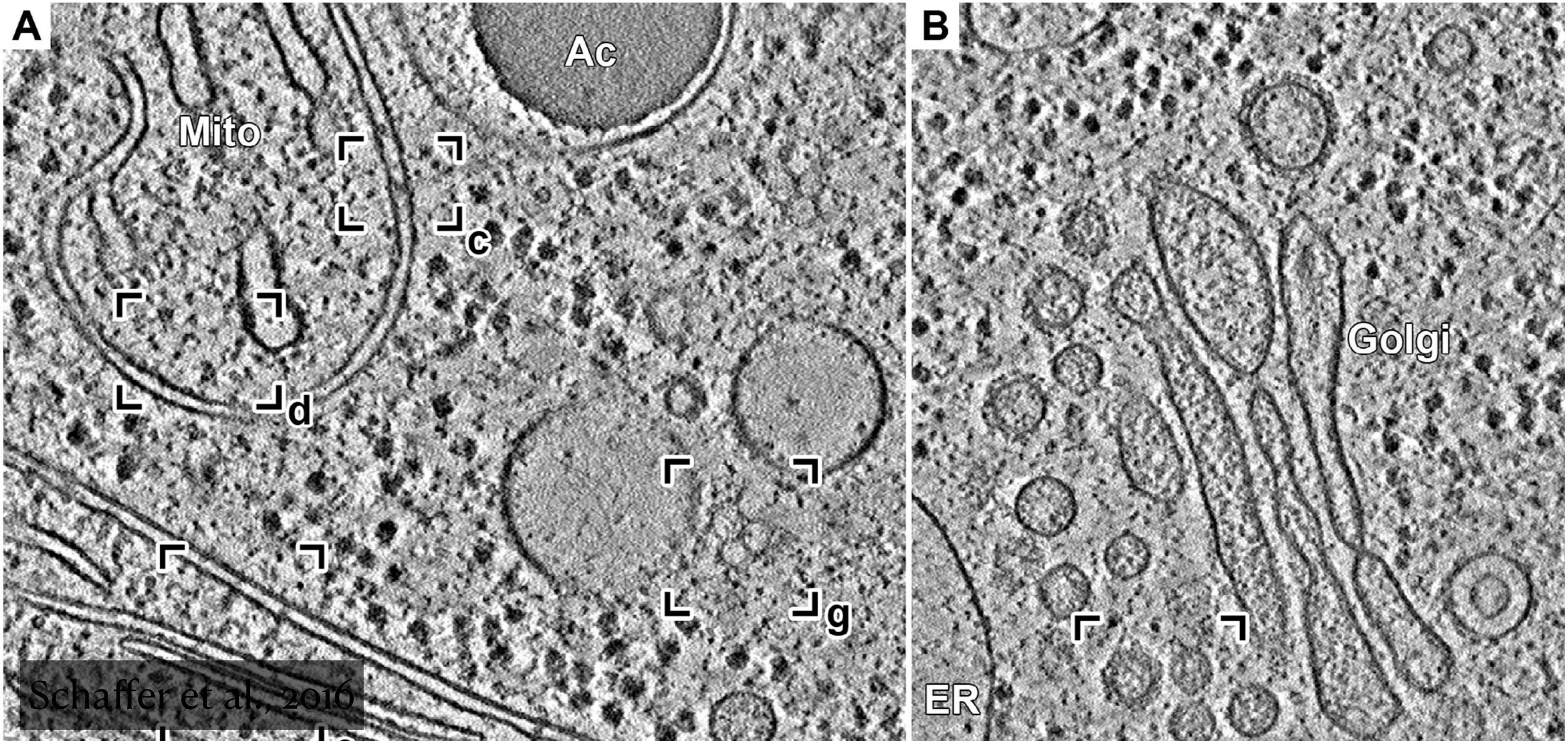
Most cells are too large (>500nm) to allow the penetration of electron beam.

Schaffer et al., 2016

Opening windows into the cell by focused-ion-beam milling

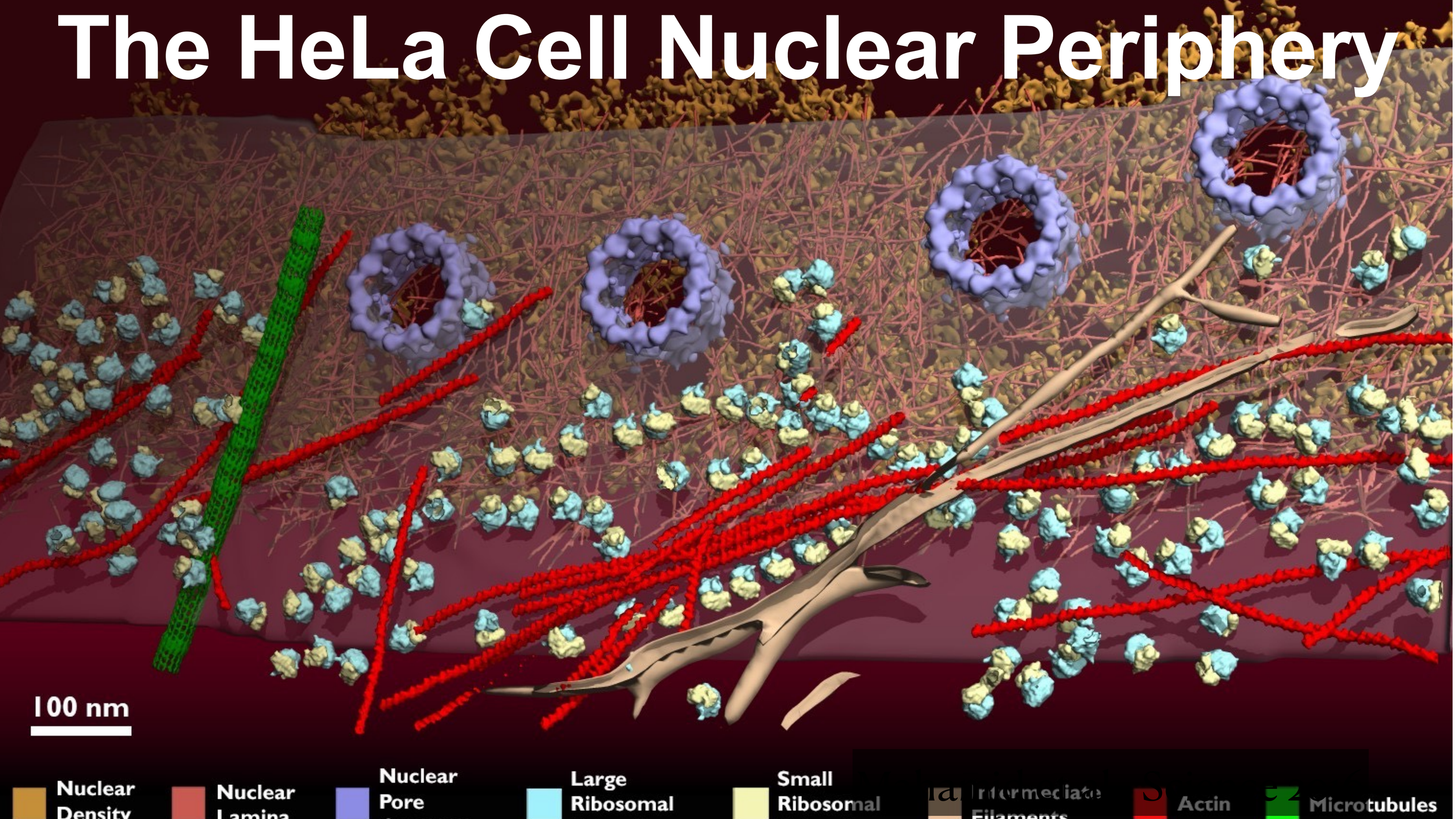


Imaging cellular features in situ

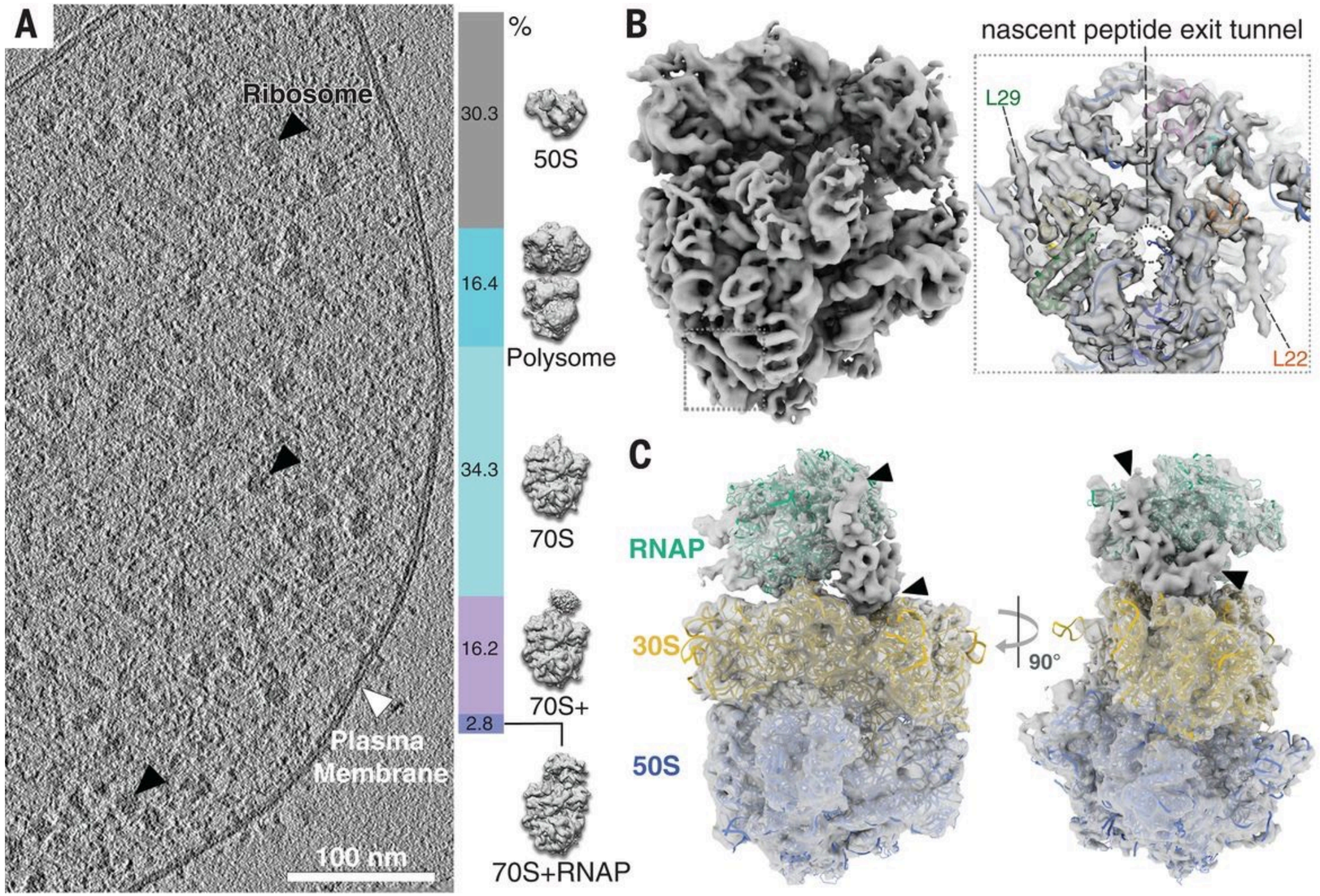


Schaffer et al., 2016

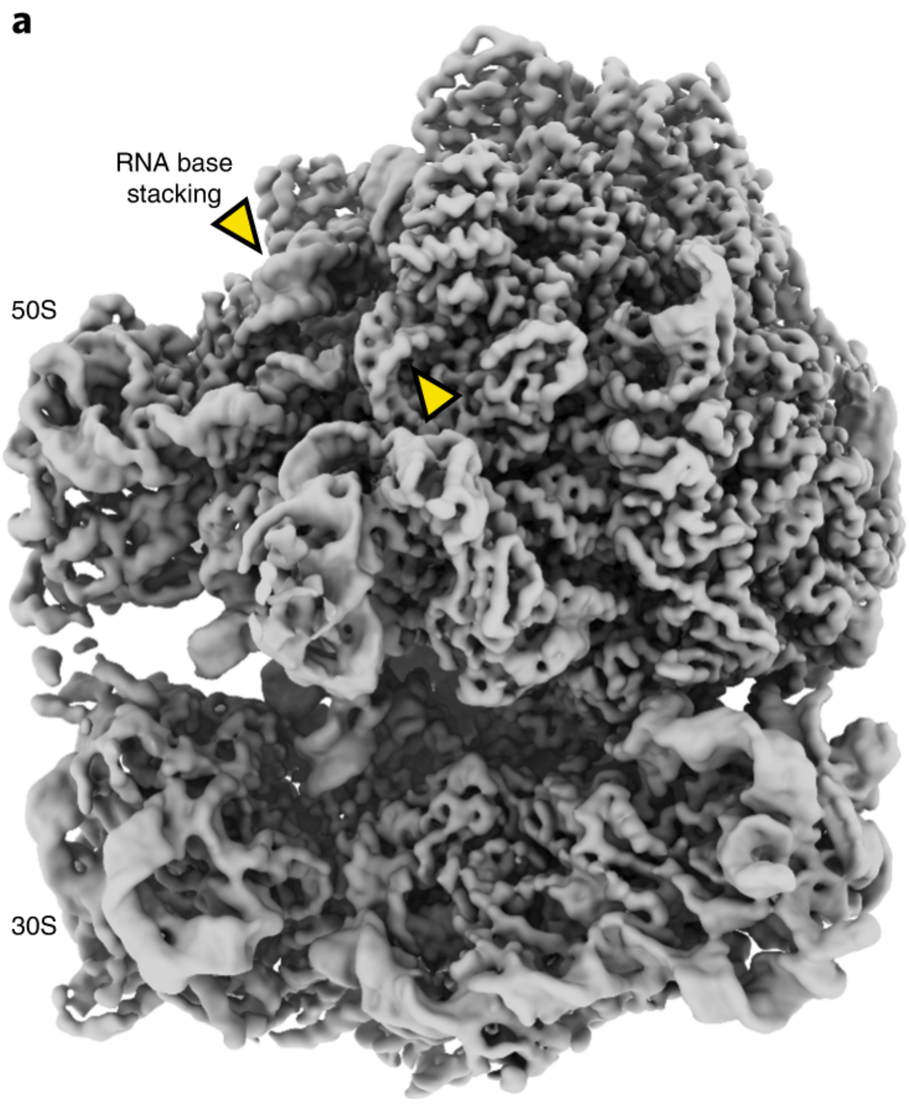
The HeLa Cell Nuclear Periphery



In-cell atomic architecture of large complexes

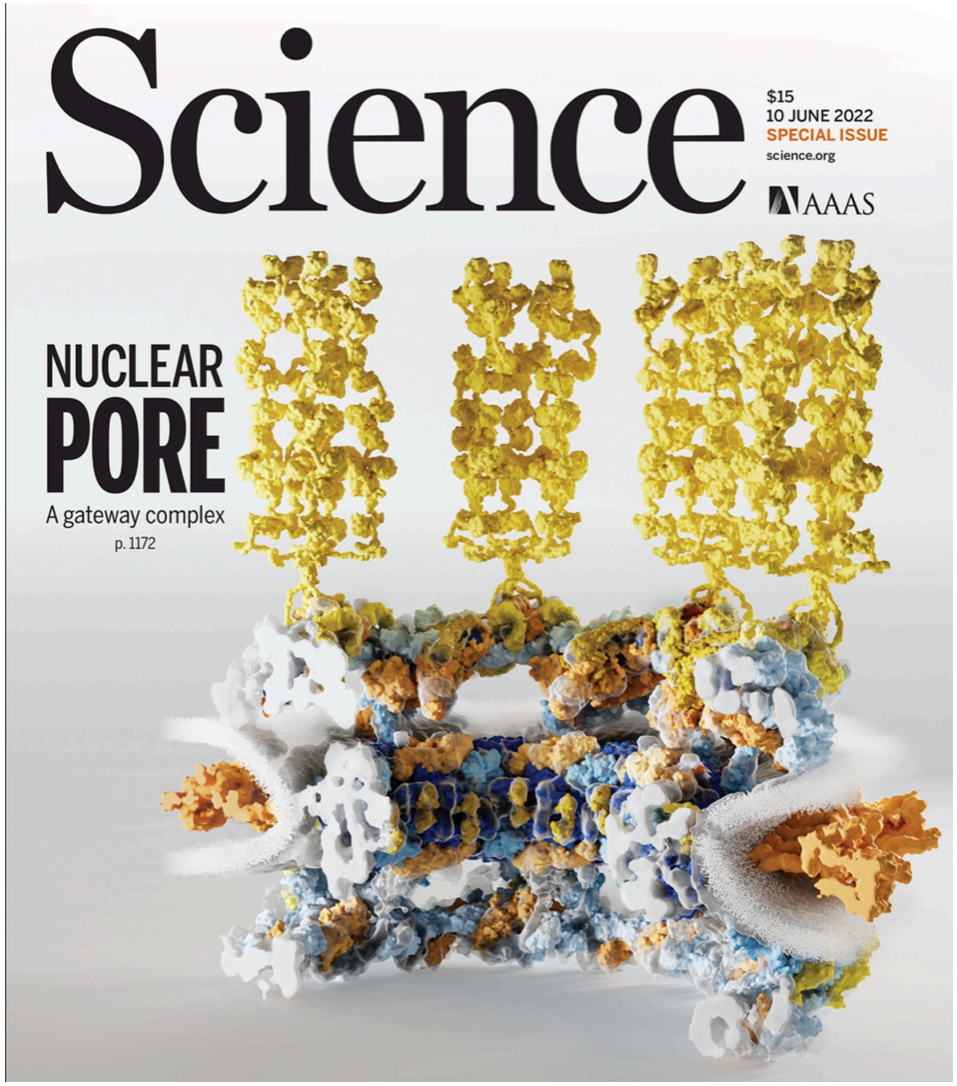


O'Reilly et al. Science 2020



Tegunov et al. Nature Methods 2021

NPC



"build on decades of painstaking work of biochemical reconstitution, x-ray crystallography, mass spectroscopy, mutagenesis, and cell biology; **use substantially improved cryo–electron tomography reconstructions of the entire human NPC;** and leverage artificial intelligence to accurately model components."

The future of cryo-ET is bright!