Cryo-EM Course
at the Laboratory for BioMolecular Structure (LBMS)
June 14-17th, 2022
Brookhaven National Laboratory

Cryo-Electron Tomography: Introduction

Jun Liu, Ph.D

Department of Microbial Pathogenesis
Microbial Sciences Institute
Yale University

Overview of cryo-ET tutorials

Day 3 - Thursday 16 June 2022

Time (EDT)	Speaker	Topic
10:00-11:00	Jun Liu (Yale)	Introduction to Cryo-electron tomography
11:00-12:00	Digvijay Singh (UCSD)	cryo-FIB to prepare cryo-FIB samples
12:00-13:00		Lunch break
13:00-14:50	Jianfeng Lin (Yale)	Cryo-ET sample preparation tutorial and demonstration
14:50-15:00		Coffee break
15:00-17:00	Jun Liu (Yale)	Cryo-ET data collection and reconstruction tutorial and demonstration

Day 4 - Friday 17 June 2022

Time (EDT)	Speaker	Topic
10:00-11:00	Raphael Park (Yale)	Tomographic data segmentation tutorial and demonstration.
11:00-13:00	Muyan Chen (BCM)	Subtomography averaging tutorial and demonstration in EMAN2

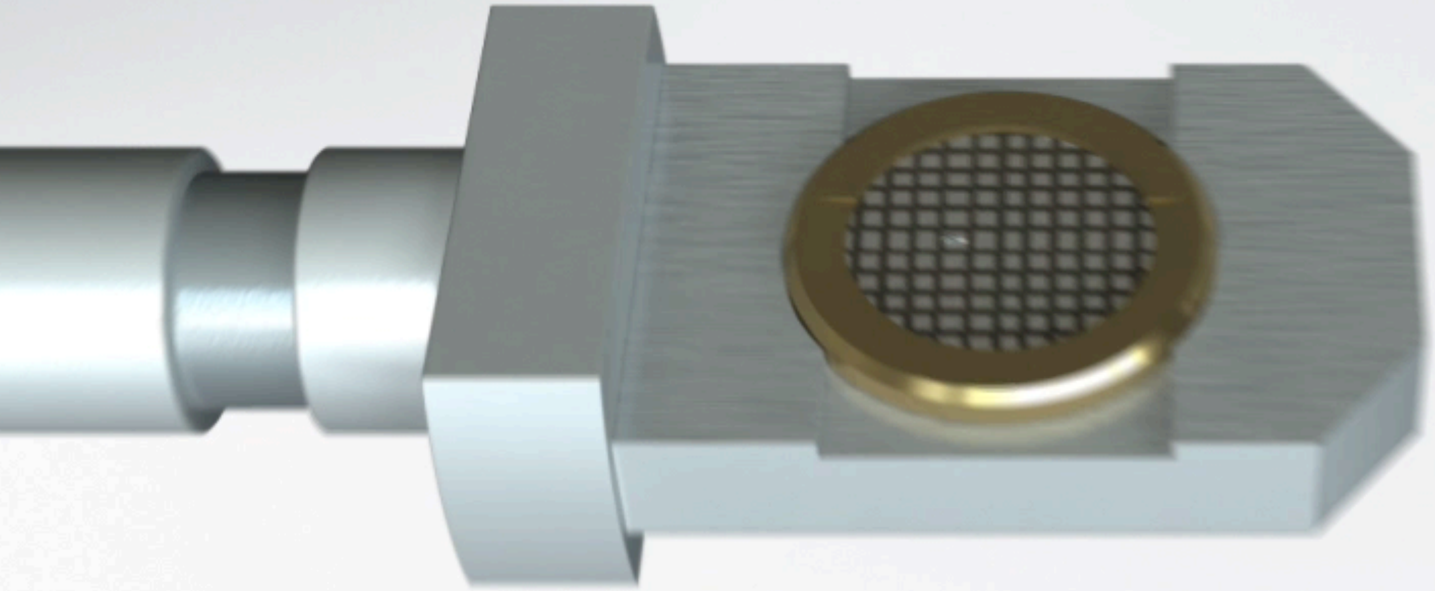
Do you have any questions about cryo-ET?

- What's cryo-electron tomography (cryo-ET)?
- What's the difference between cryo-ET and single particle cryo-EM?
- How should I prepare samples for cryo-ET?
- How should I collect cryo-ET data?
- How should I analyze cryo-ET data?
- How could I use cryo-ET effectively?

Outline

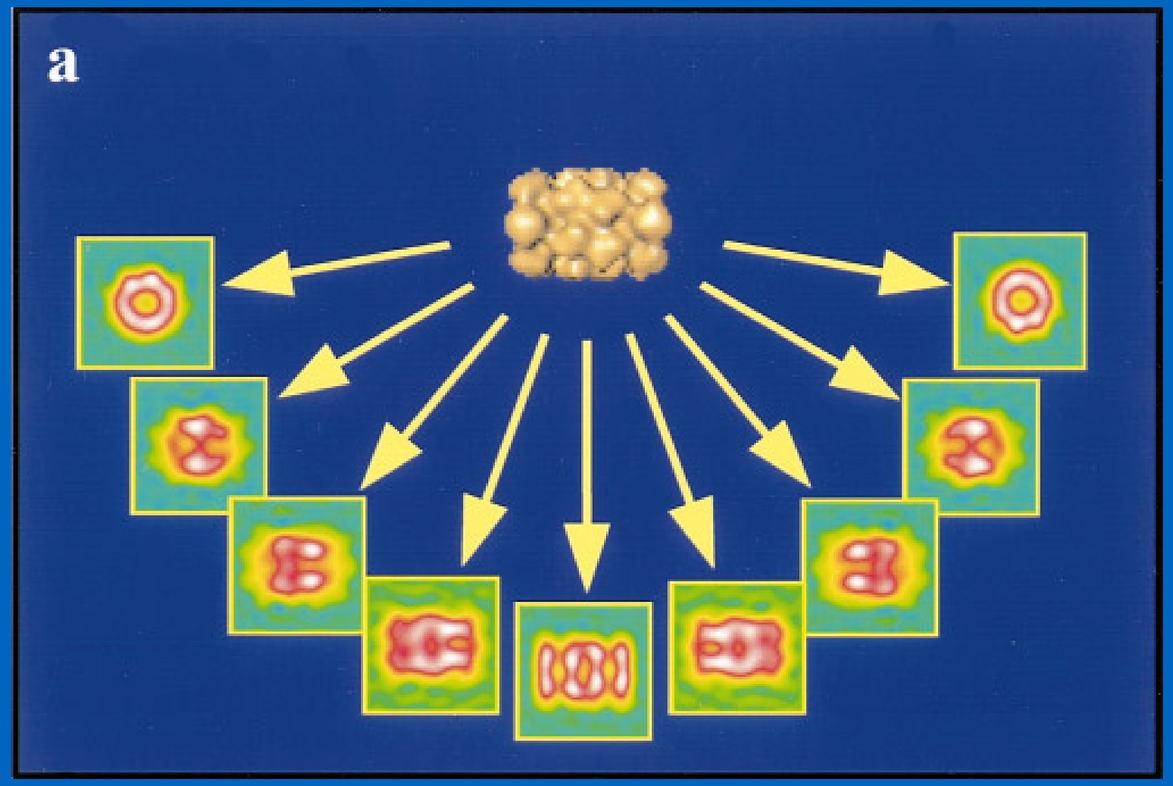
- What's electron tomography
- The power of cryo-ET
- Key steps in cryo-ET:
  - Sample preparation (Digvijay Singh & Jianfeng Lin)
  - Data collection (Jun Liu)
  - Image analysis (Raphael Park & Muyuan Chen)
- Some classic cryo-ET applications

What's electron tomography?

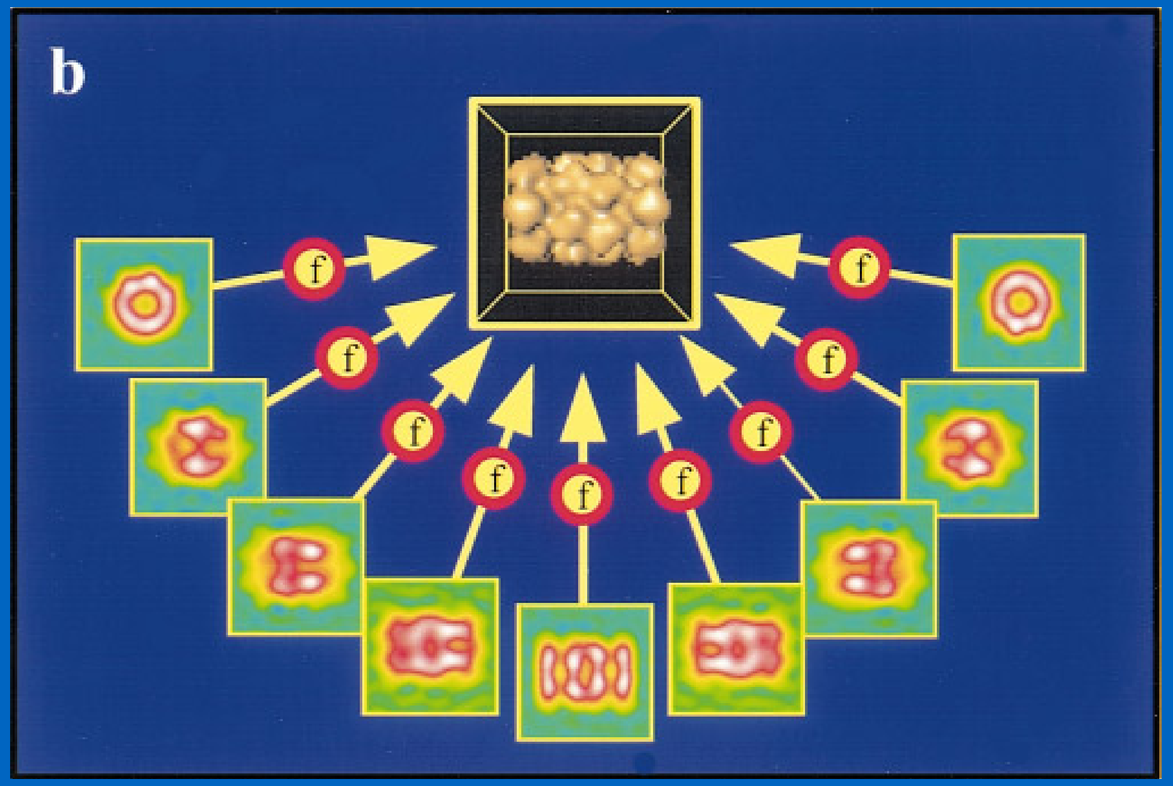


Thermo Fisher

Principle of Electron Tomography



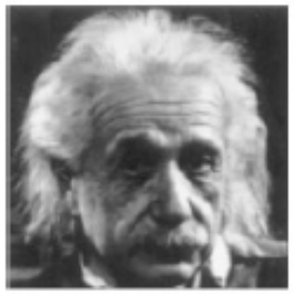
2D projection images from different views



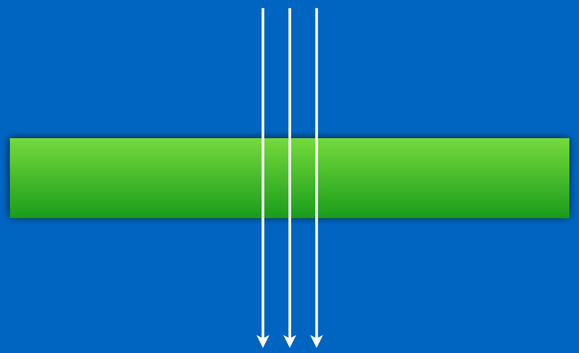
Back-projection to generate 3D reconstruction

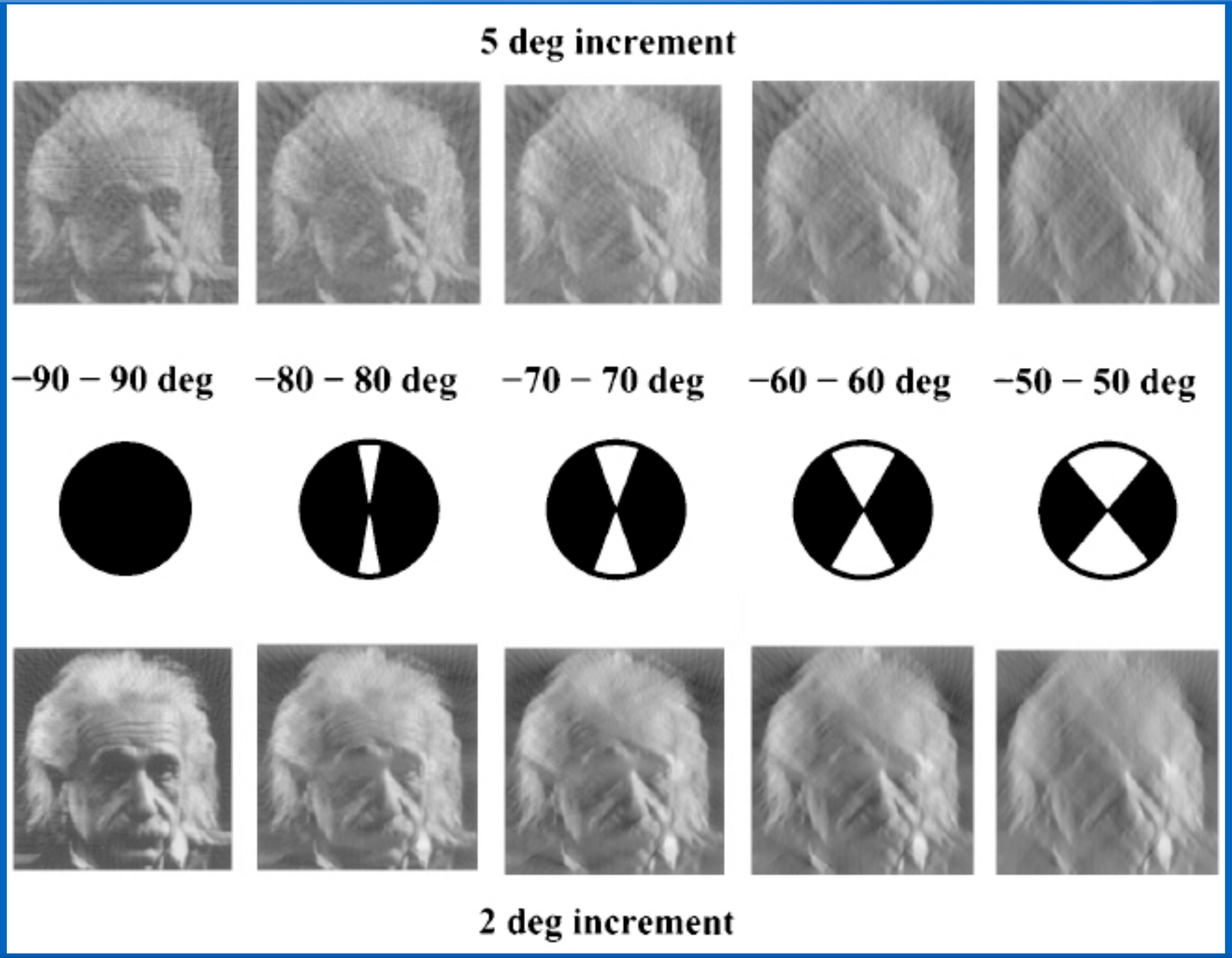
Koster et al., JSB 1997

Key parameters to consider



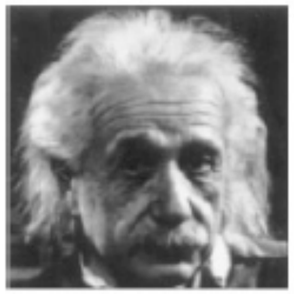
original image





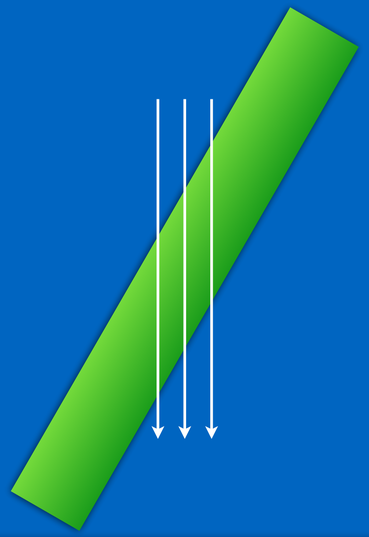
Koster et al. 1997

Key parameters to consider



original image

1. Sample thickness (< 300nm)
2. Total doses (< 100e/Å2)
3. Tilt increment
4. Tilt range



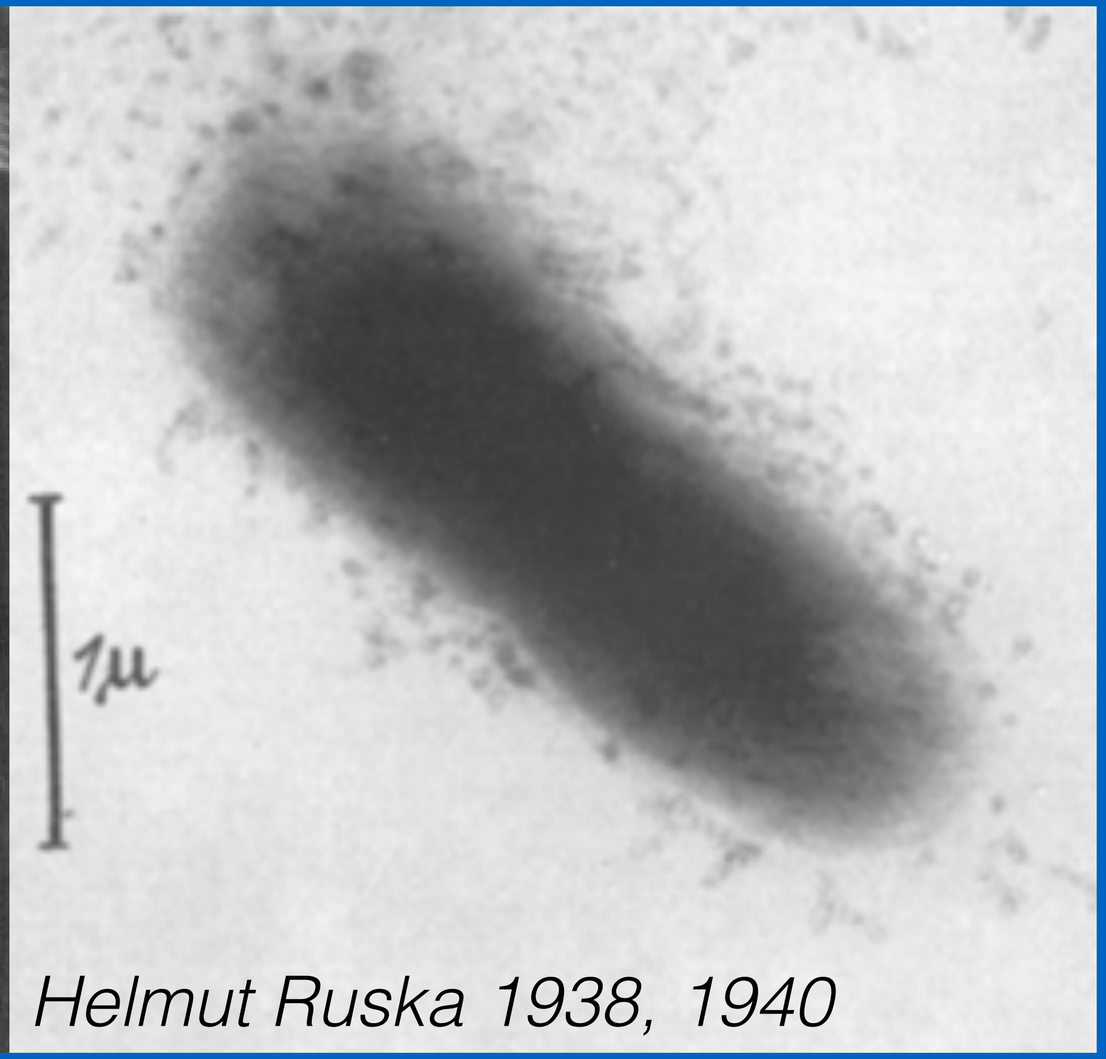
2 deg increment

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).

Ruska and his first electron microscope

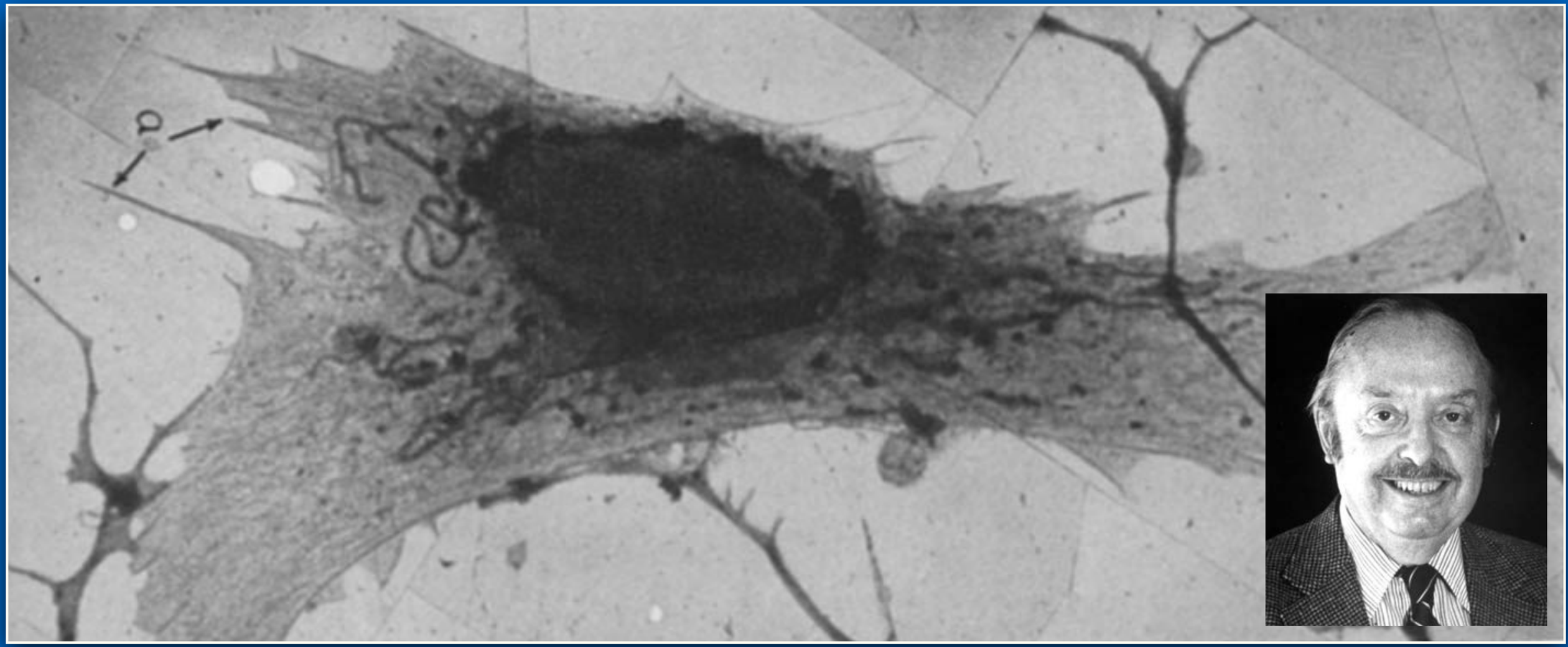
Ernst Ruska & Max Knoll 1931



Helmut Ruska 1938, 1940

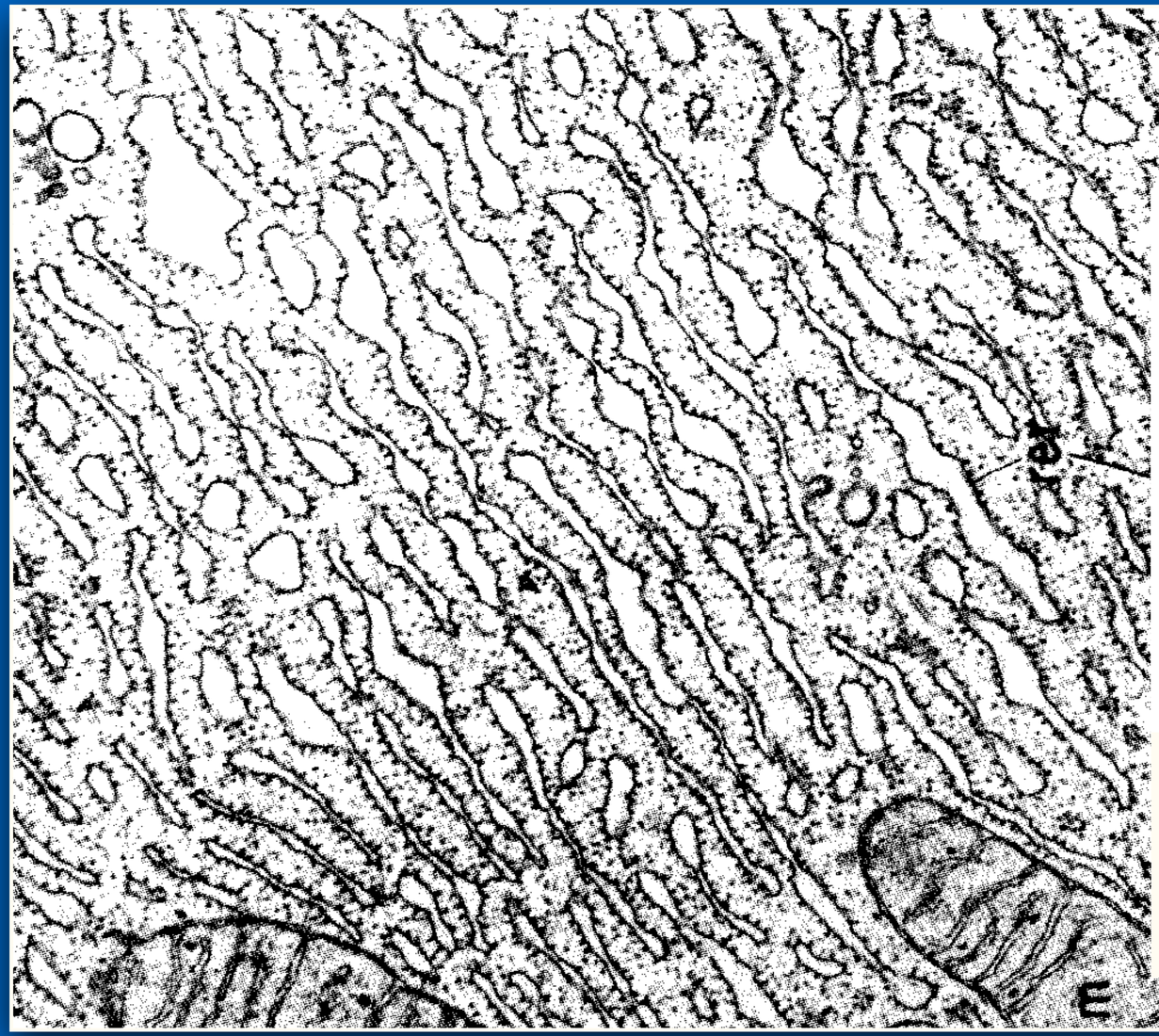
The Nobel Prize in 1986 awarded to Ruska for designing electron microscope

Electron microscopy in Cell Biology

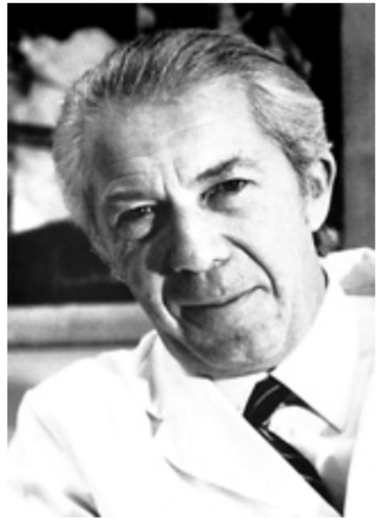


Keith Porter et al. 1944

Electron microscopy in Cell Biology



Albert Claude

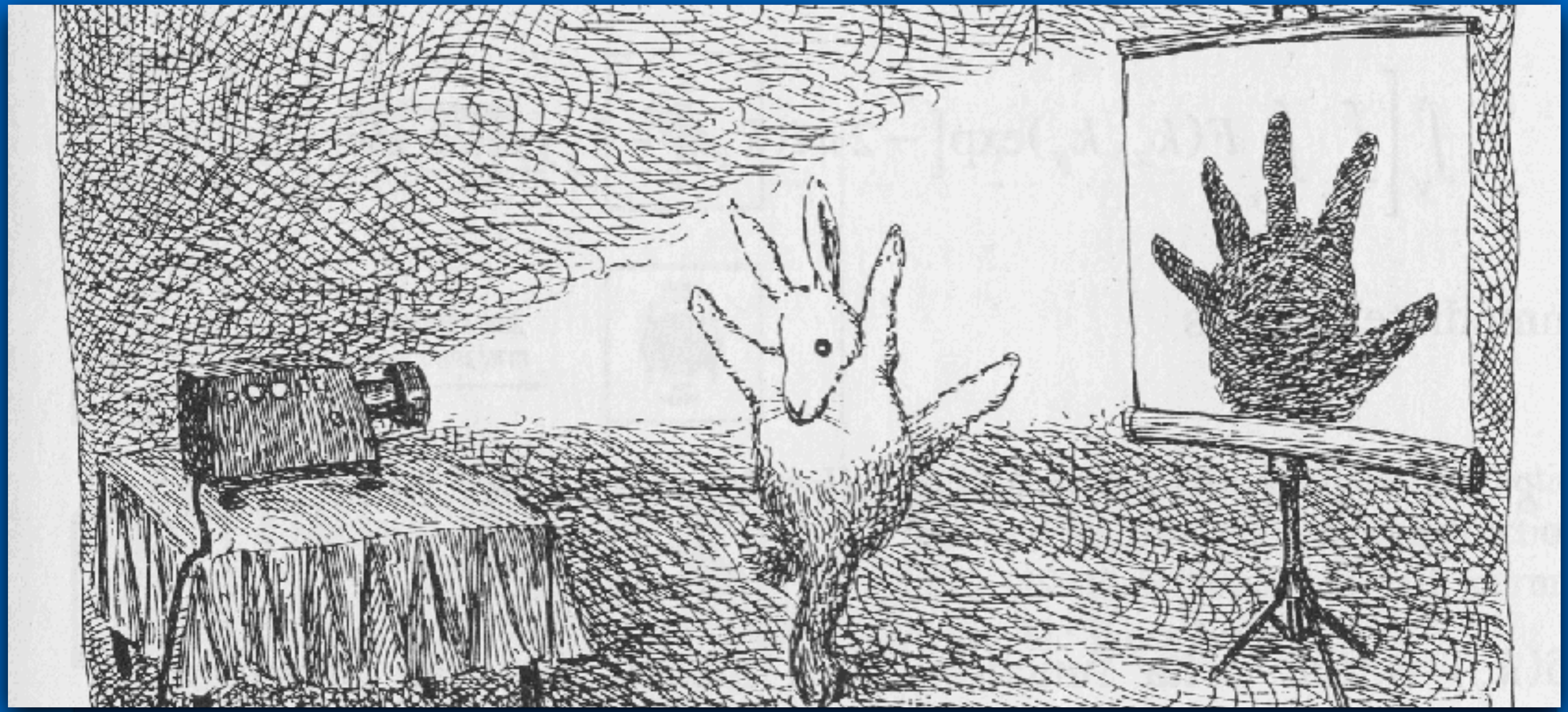


Christian de Duve

George E. Palade

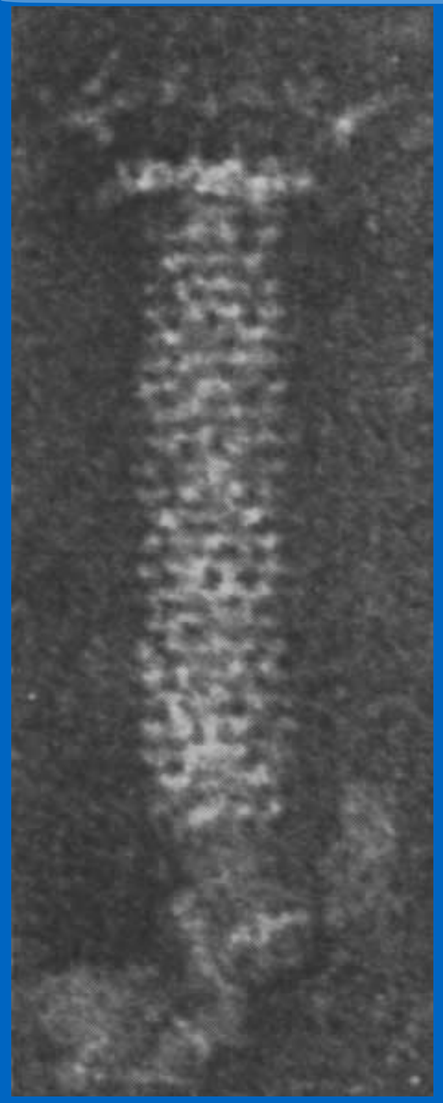
won the nobel prize in 1974 for their discoveries concerning the structural and functional organization of the cell

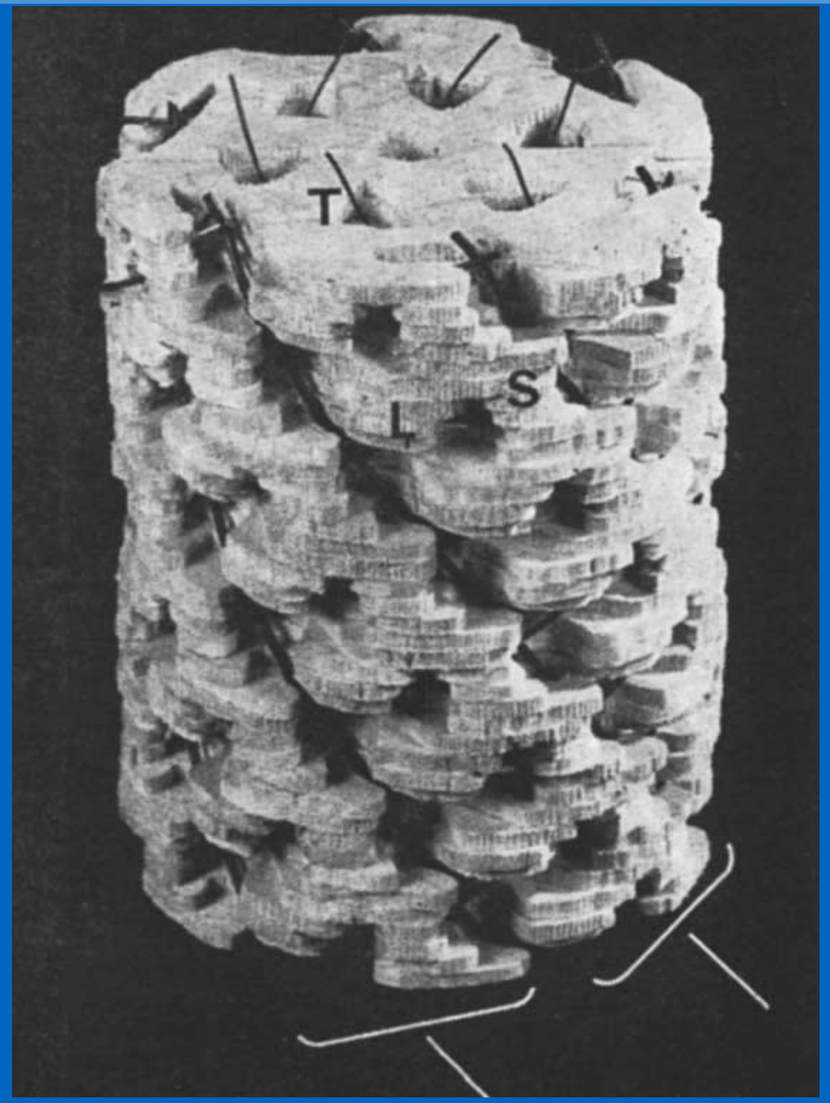
A single 2D image is not sufficient



John O'brien 1991

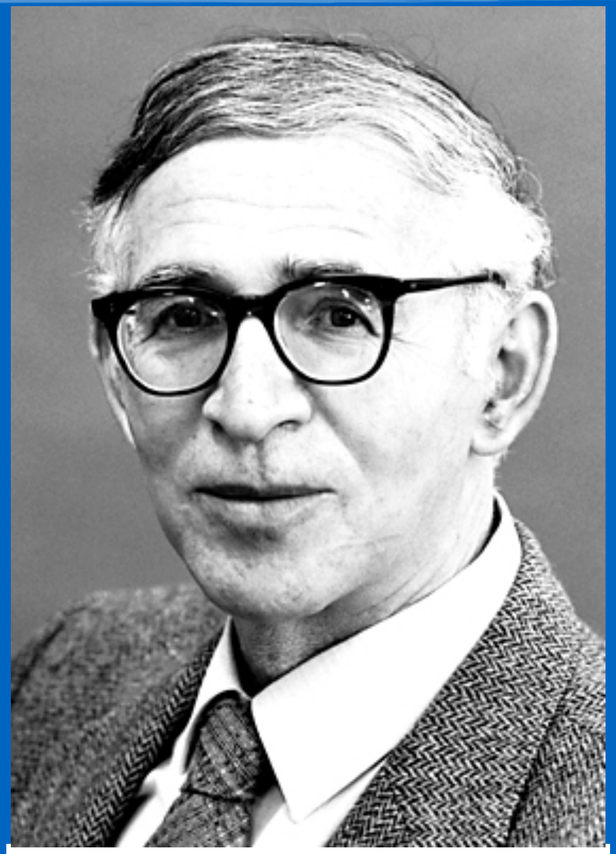
3D reconstruction from 2D images





David DeRosier

Brandeis University

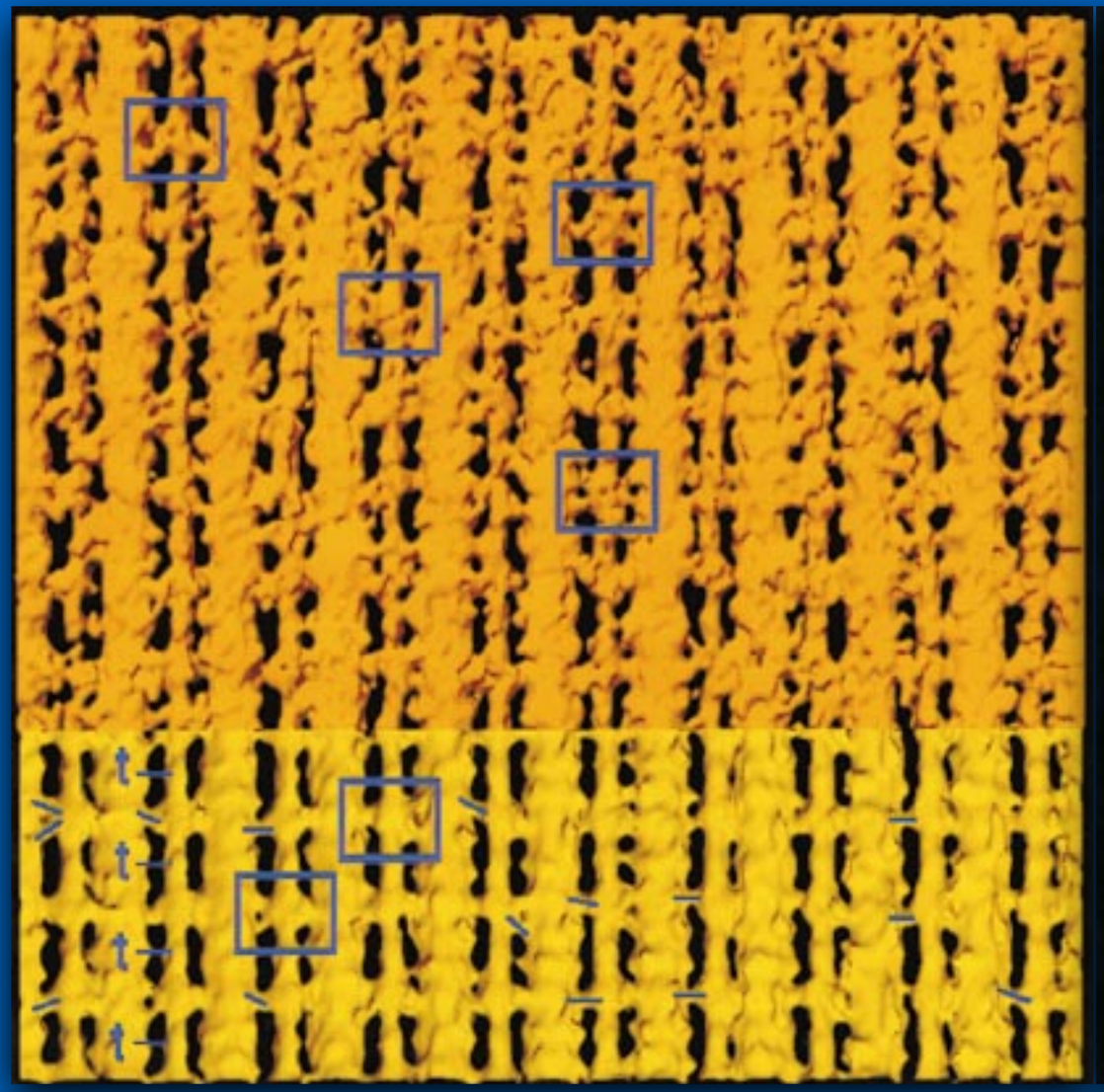


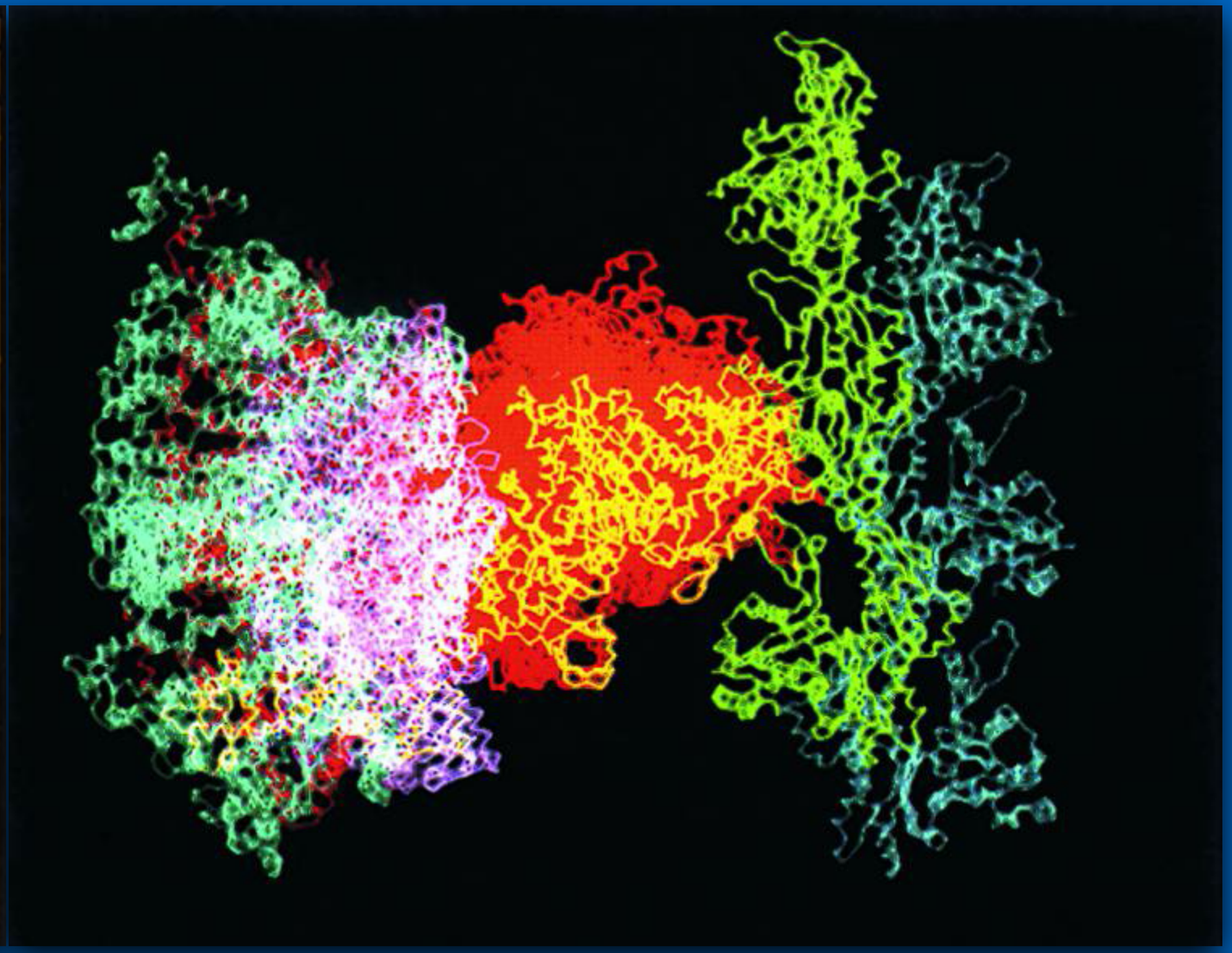
Aaron Klug

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

De Rosier & Klug, Nature1968

ET provides unique 3D information





Taylor et al., Cell 1999

CryoET — bridging the information gap

The Nobel Prize in Chemistry 2017

© Nobel Media. Ill. N. Elmehed
Jacques Dubochet
Prize share: 1/3

© Nobel Media. Ill. N. Elmehed
Joachim Frank
Prize share: 1/3

© Nobel Media. Ill. N. Elmehed
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"*.

The Nobel Prize in Chemistry 2014

© Nobel Media AB. Photo: A. Mahmoud
Eric Betzig
Prize share: 1/3

© Nobel Media AB. Photo: A. Mahmoud
Stefan W. Hell
Prize share: 1/3

© Nobel Media AB. Photo: A. Mahmoud
William E. Moerner
Prize share: 1/3

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner "for the development of super-resolved fluorescence microscopy."

CryoET — 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

Huang et al., Science 2008

Outline

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- The power of cryo-ET
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  - Sample preparation (Digvijay Singh & Jianfeng Lin)
  - Data collection (Jun Liu)
  - Image analysis (Raphael Park & Muyuan Chen)
- Some classic cryo-ET applications

Sample preparation is the key for cryo-ET

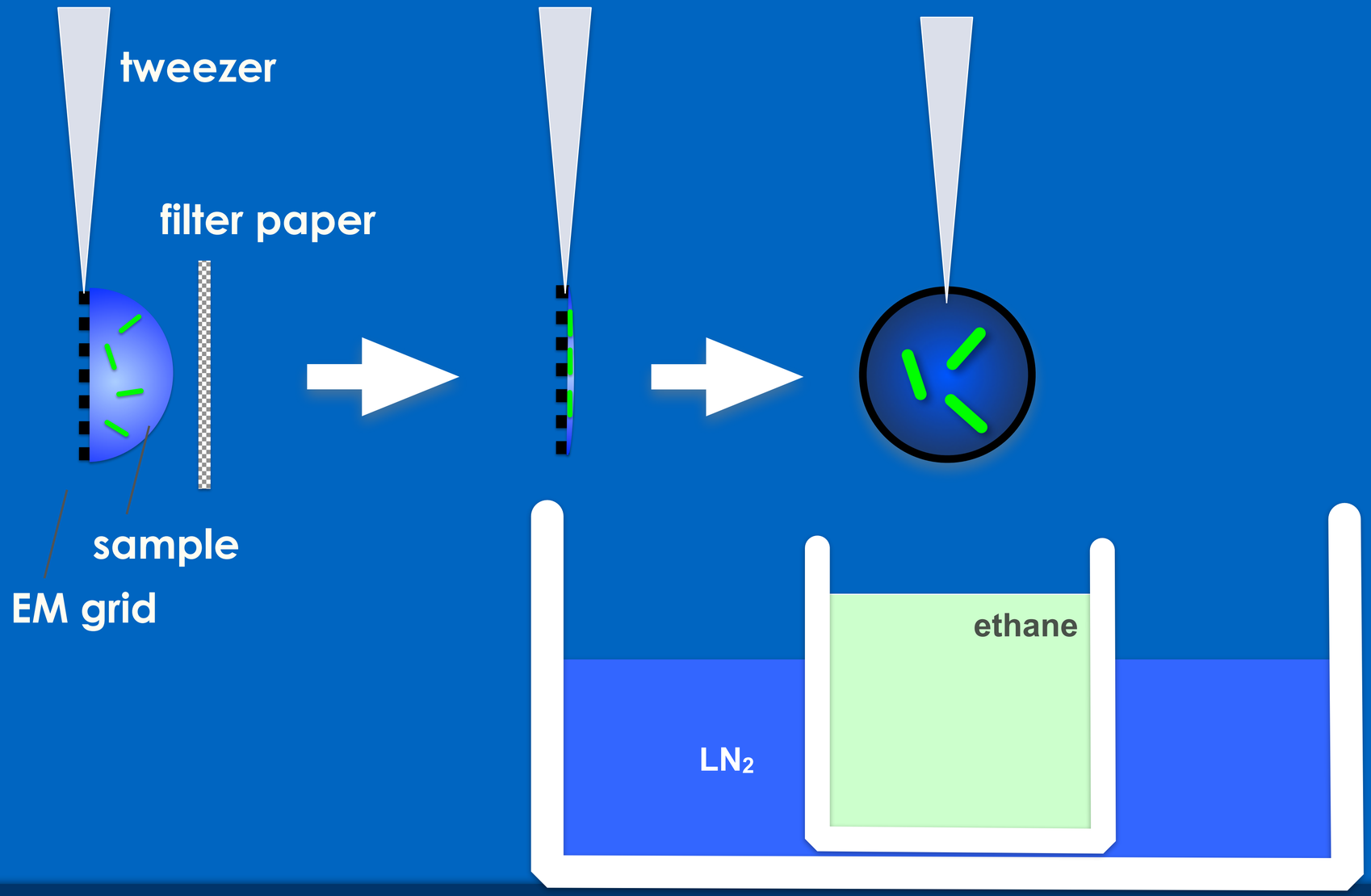
Thin specimen (less than 0.3μm) is required for high-resolution cryo-ET imaging.

Protein, virus, thin cell (< 10 *μ*m)
Fast freezing to achieve a frozen hydrated state

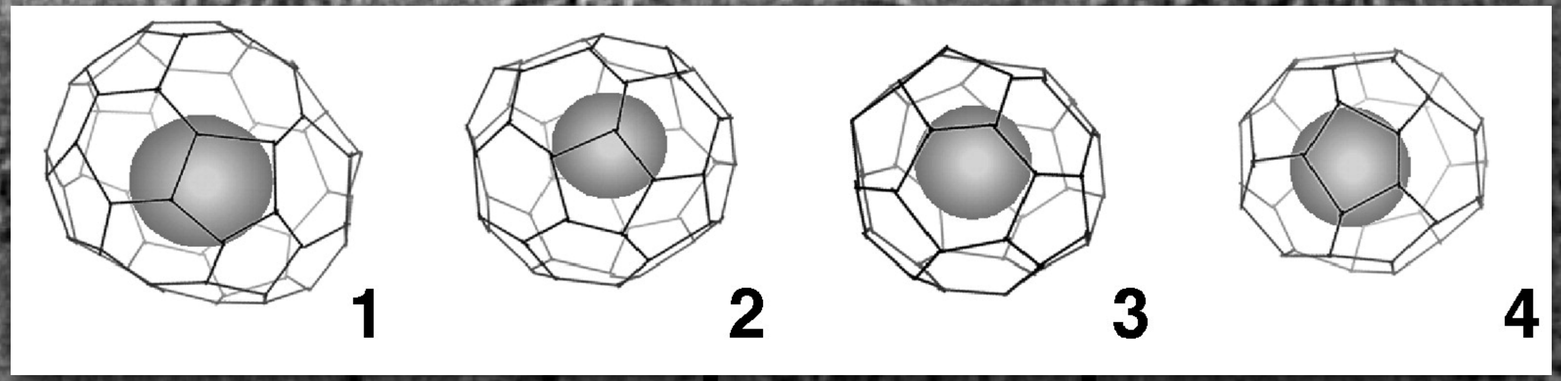
Large cell, tissue (> 10 *μ*m)
High-pressure freezing

Cryo-section & Focused ion beam milling

Frozen hydrated specimen preparation

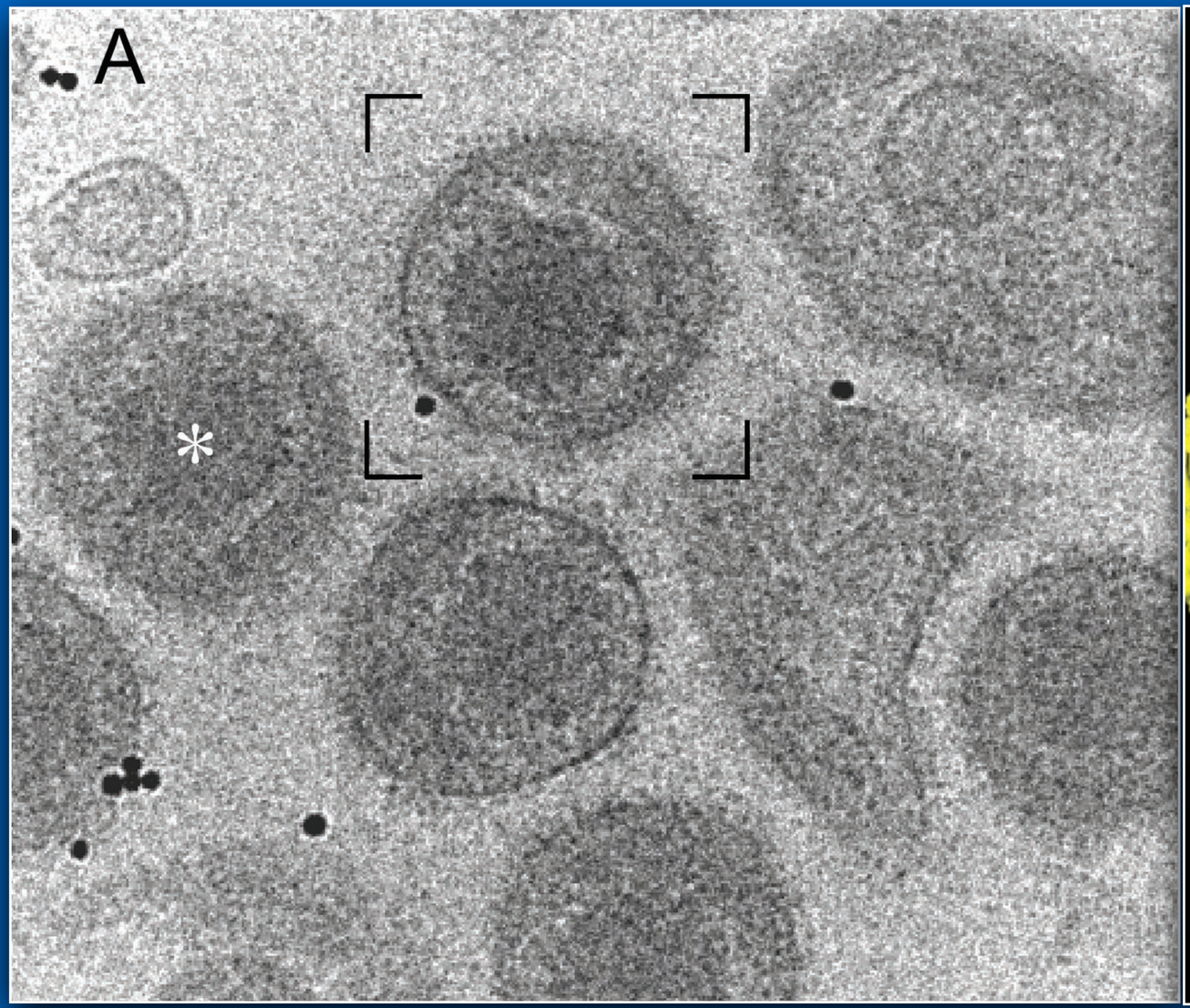


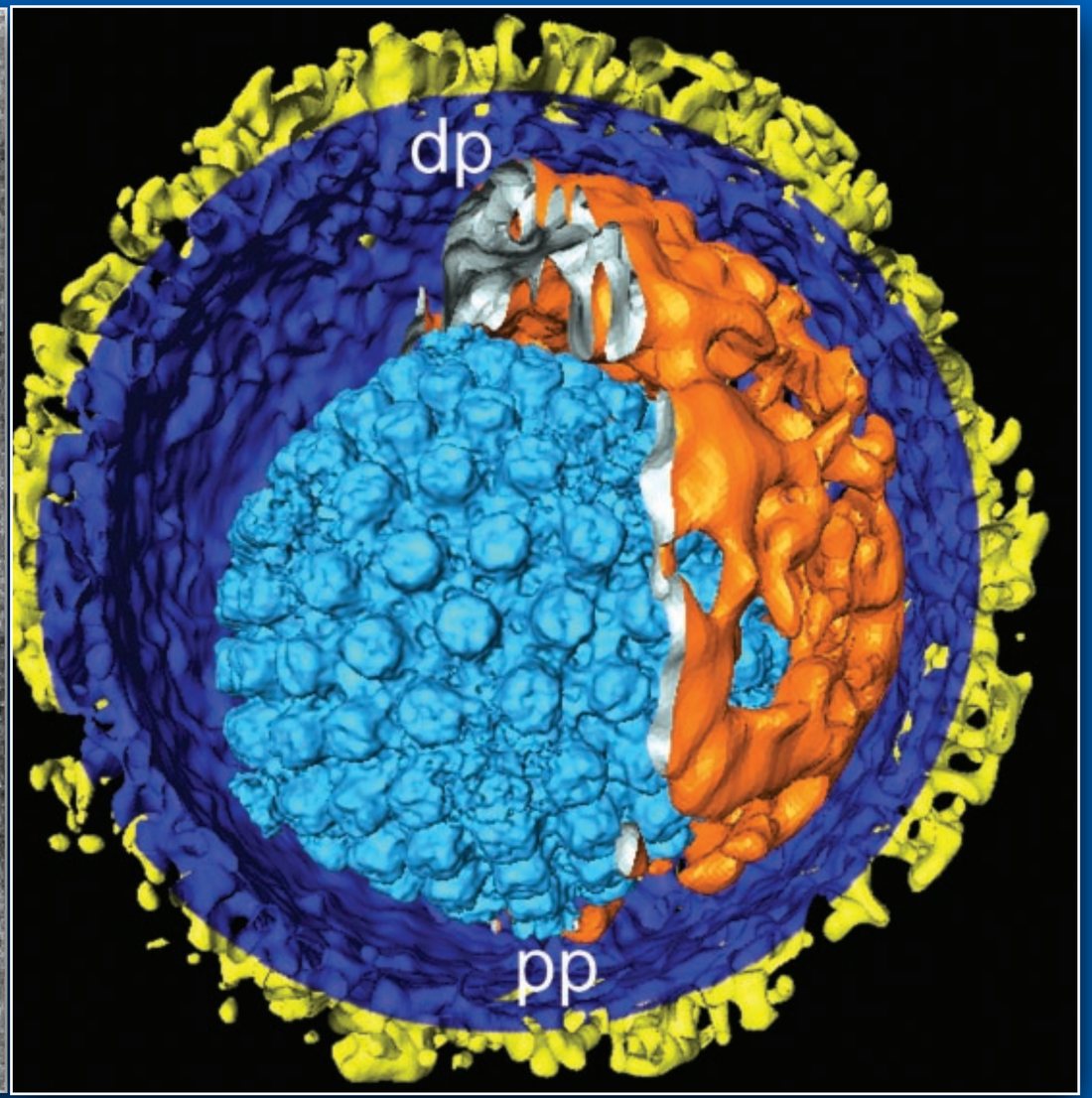
Large protein complexes



Cheng et al., JMB 2007

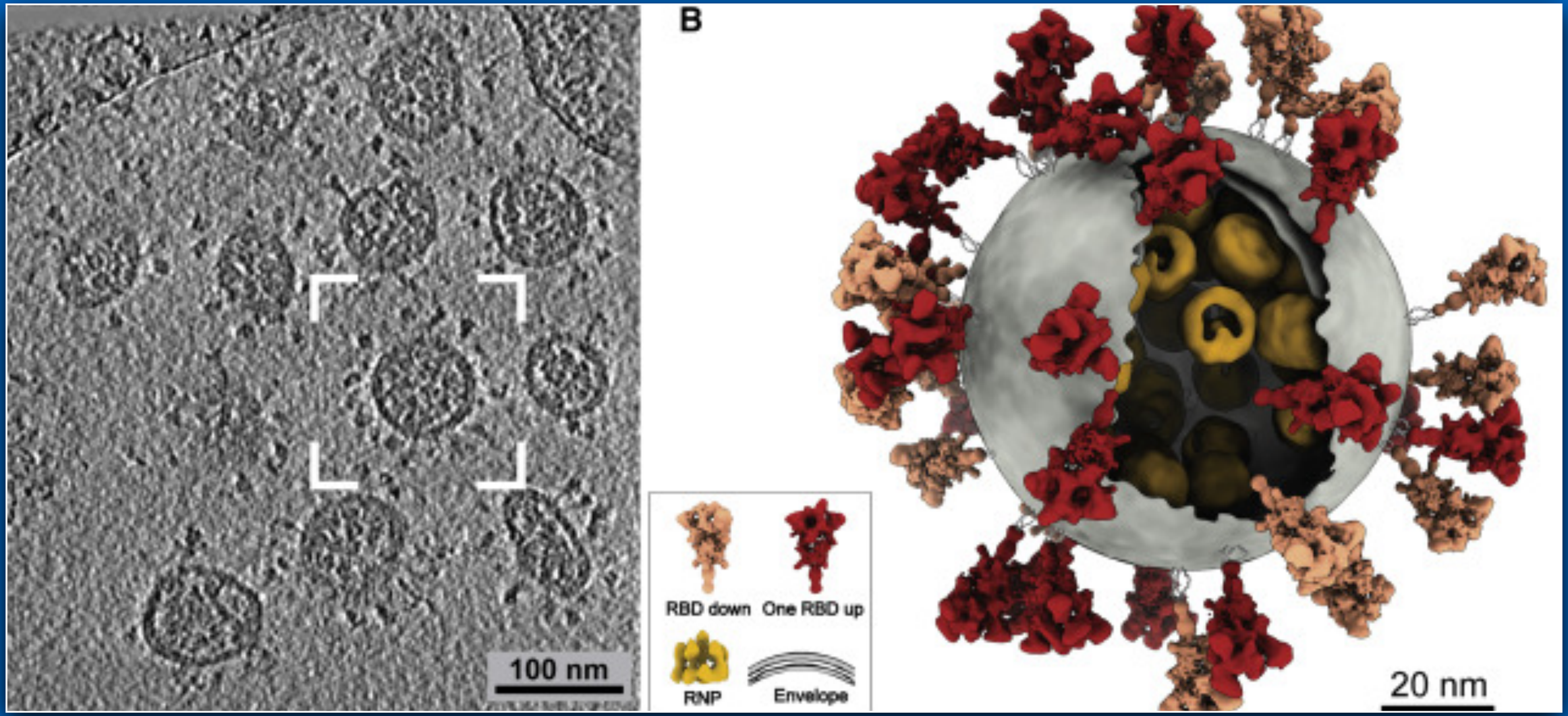
Herpes Simplex Virus





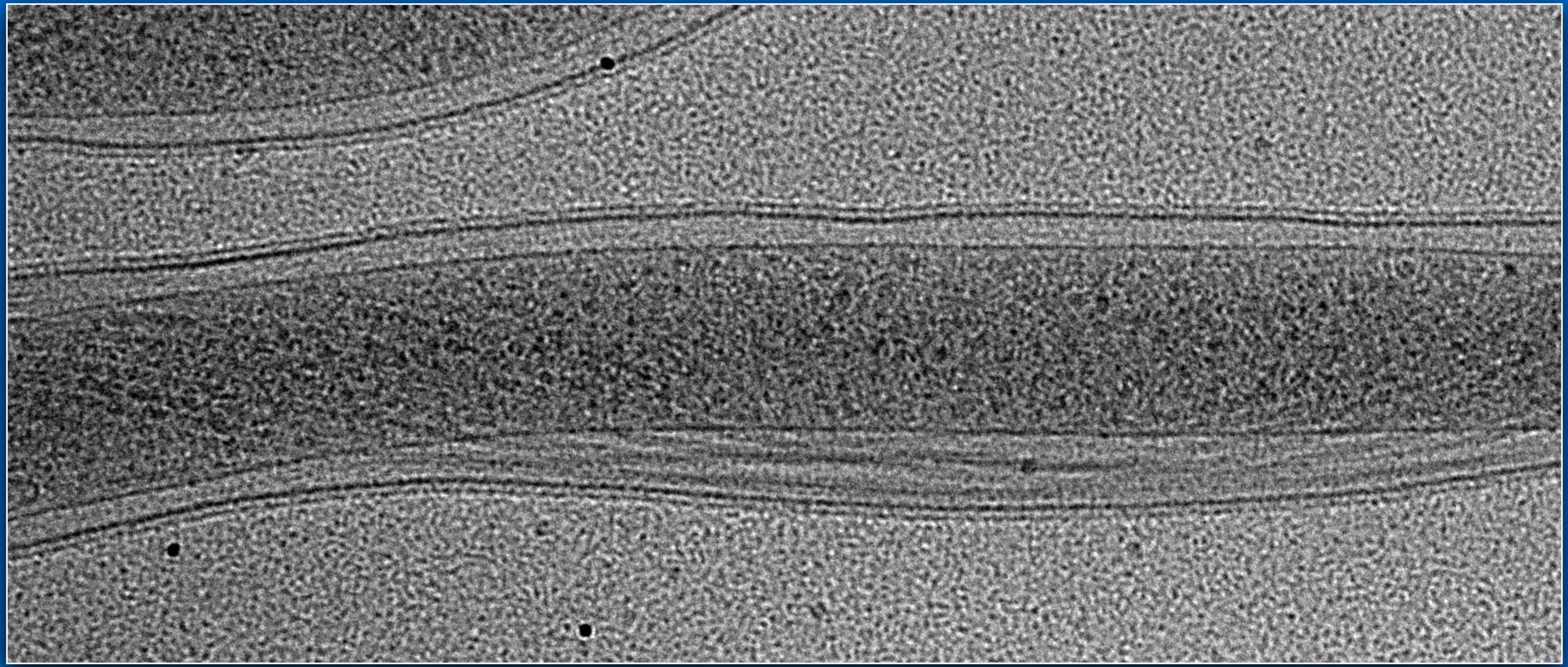
Grünewald et al. Science 2003

Viruses



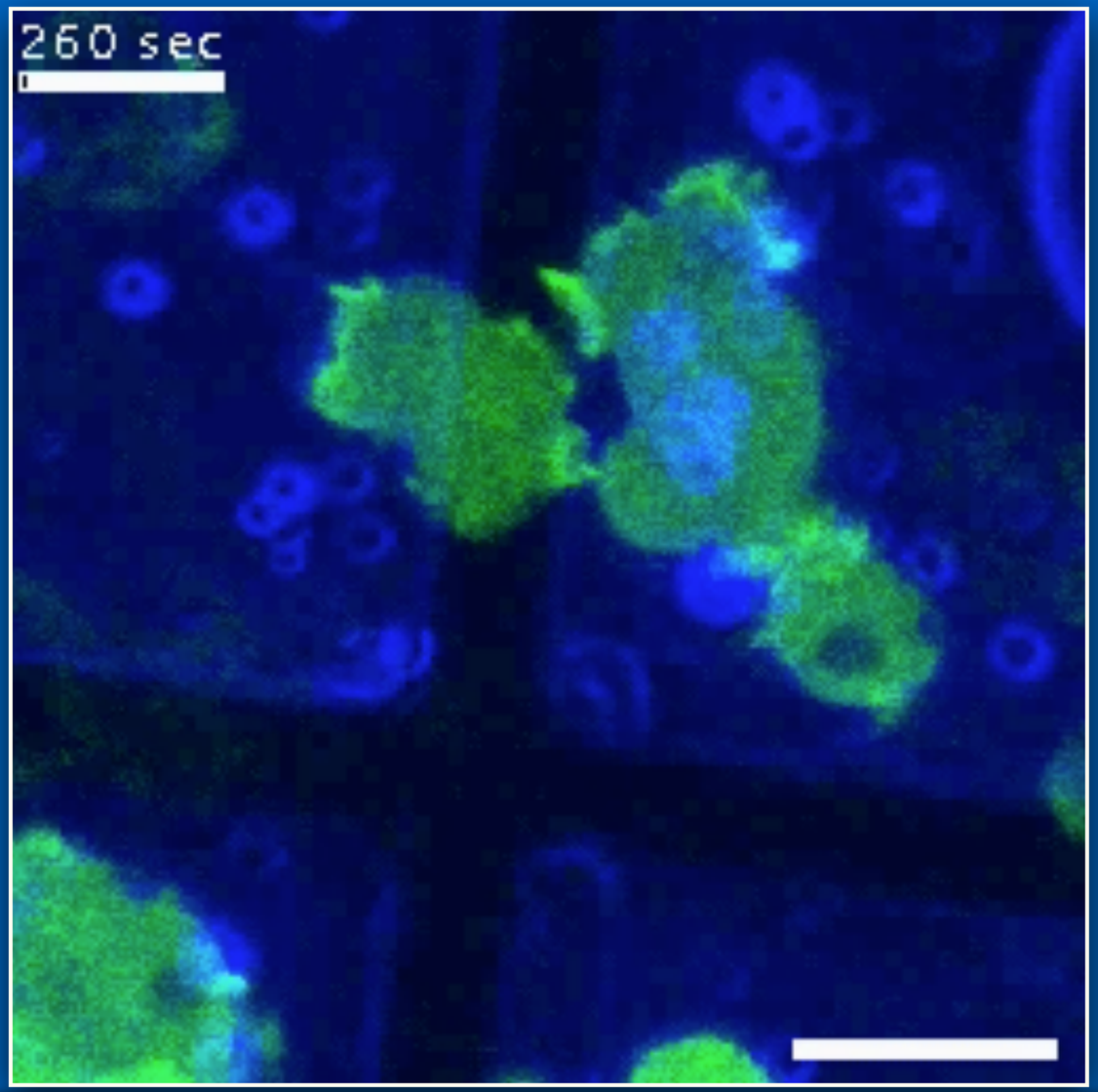
Yao et al., Cell 2020

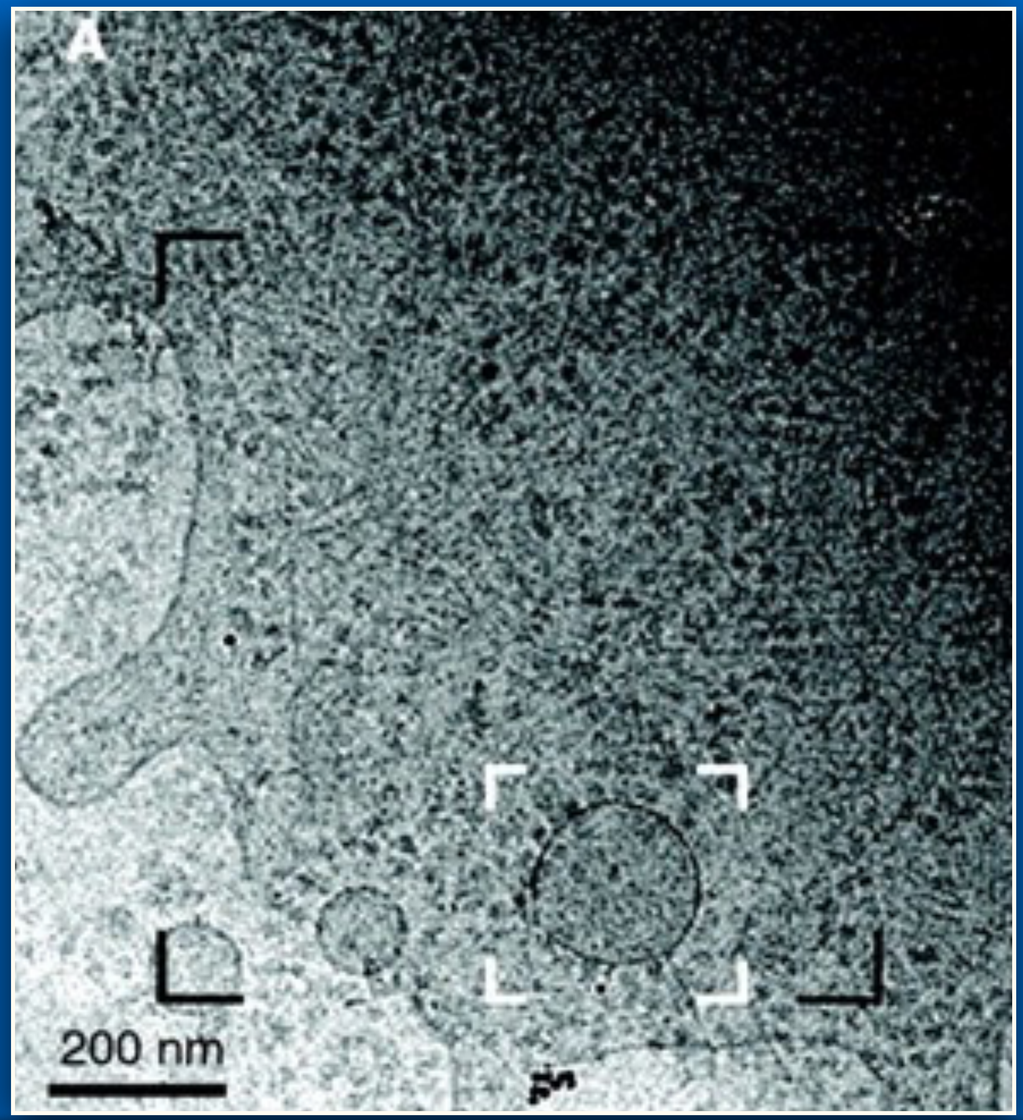
Bacteria



Liu et al., JB 2009

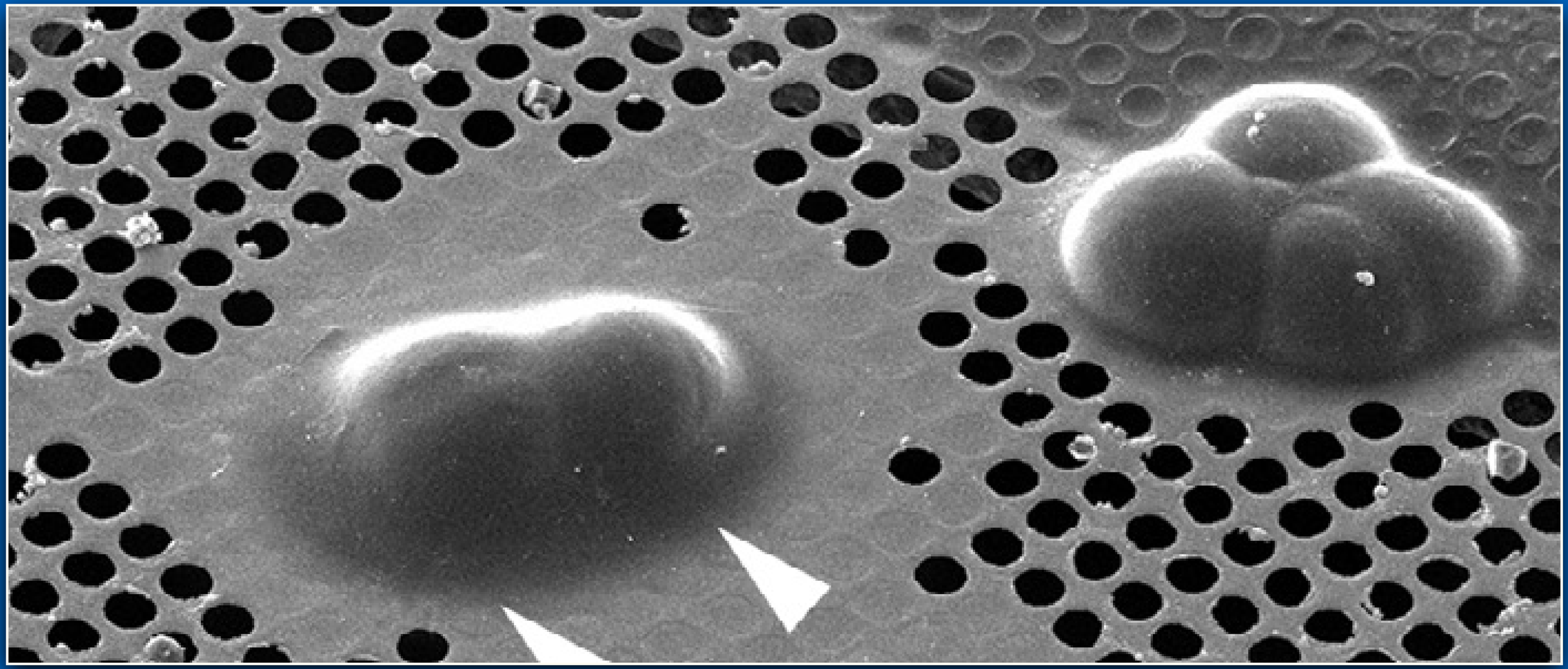
Thin periphery of large cells



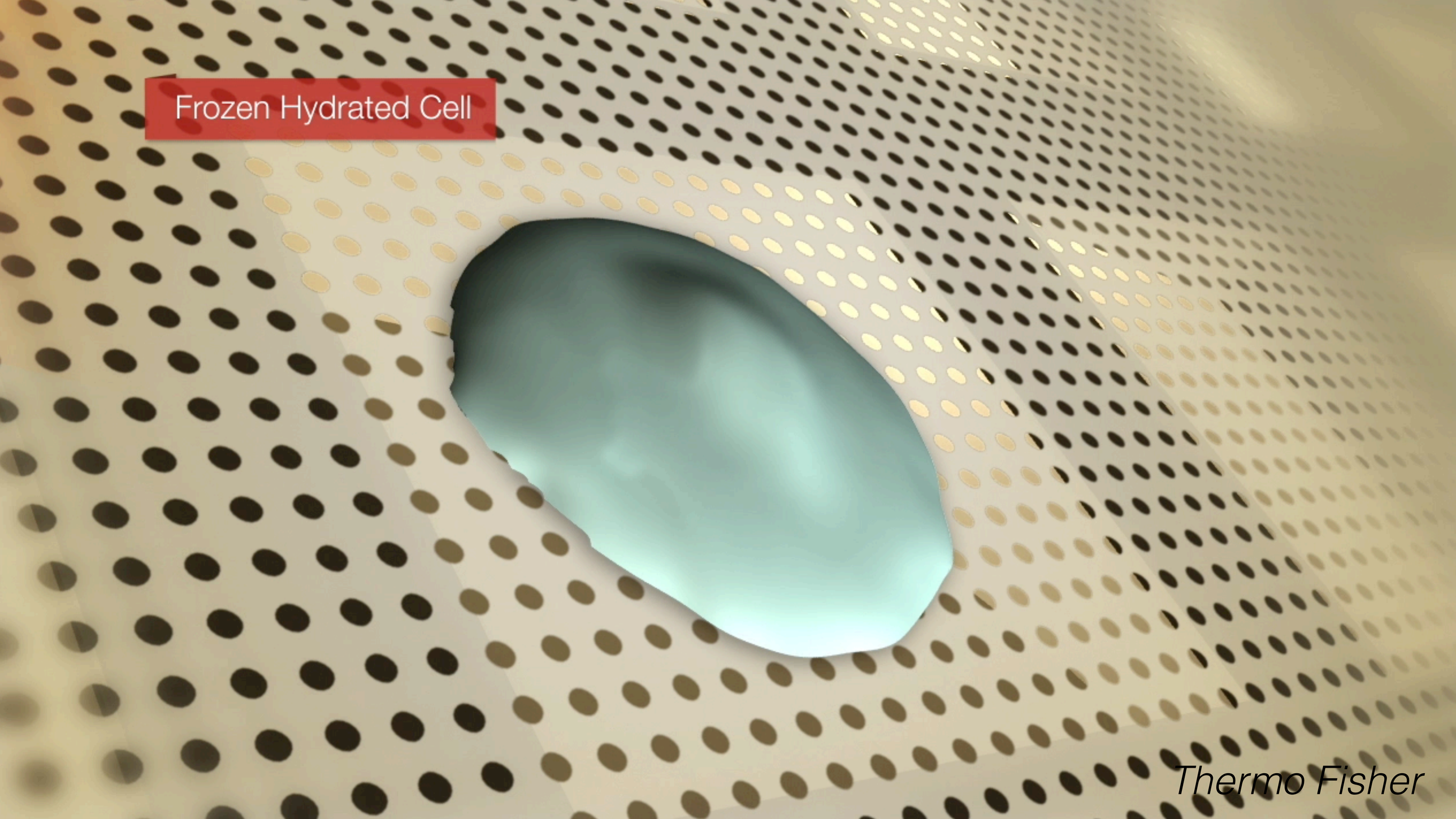


Medalia et al., Science 2002

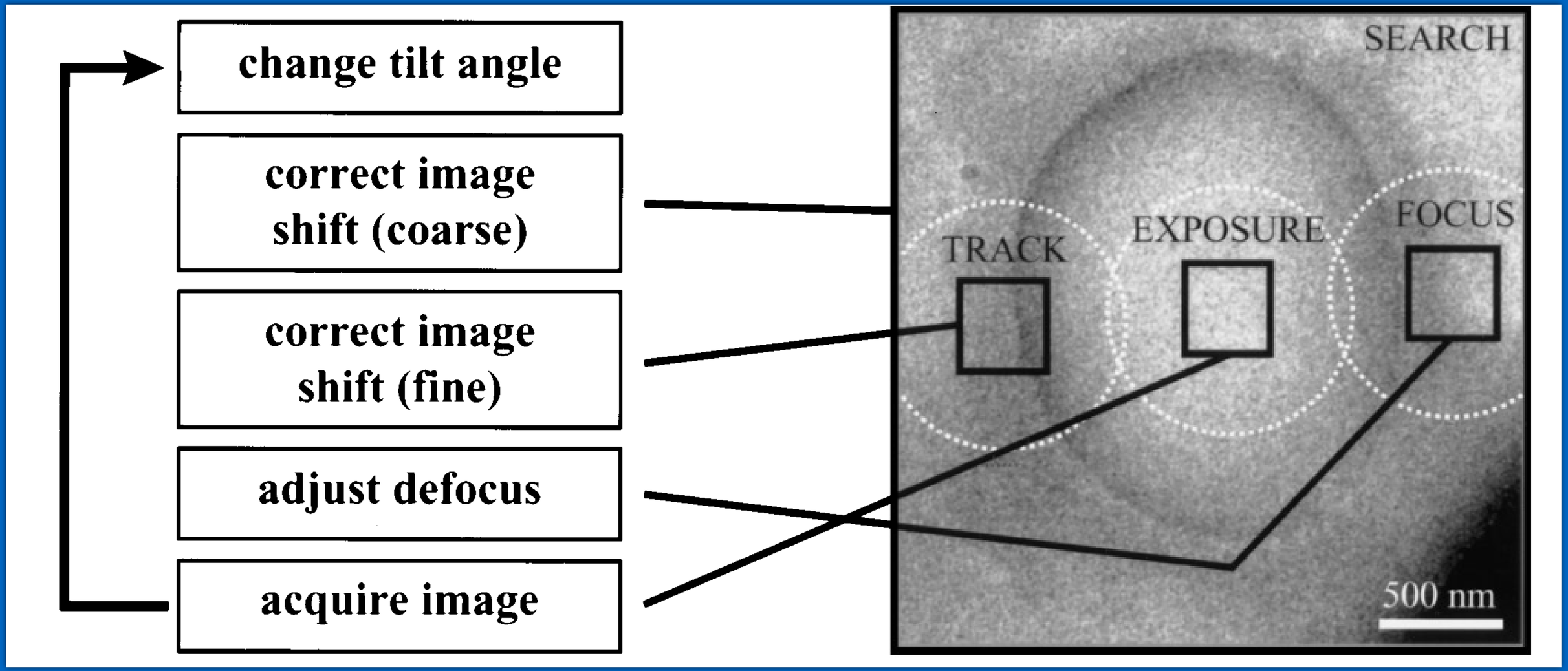
Most cells are too large for cryo-ET imaging



Schaffer et al., 2016

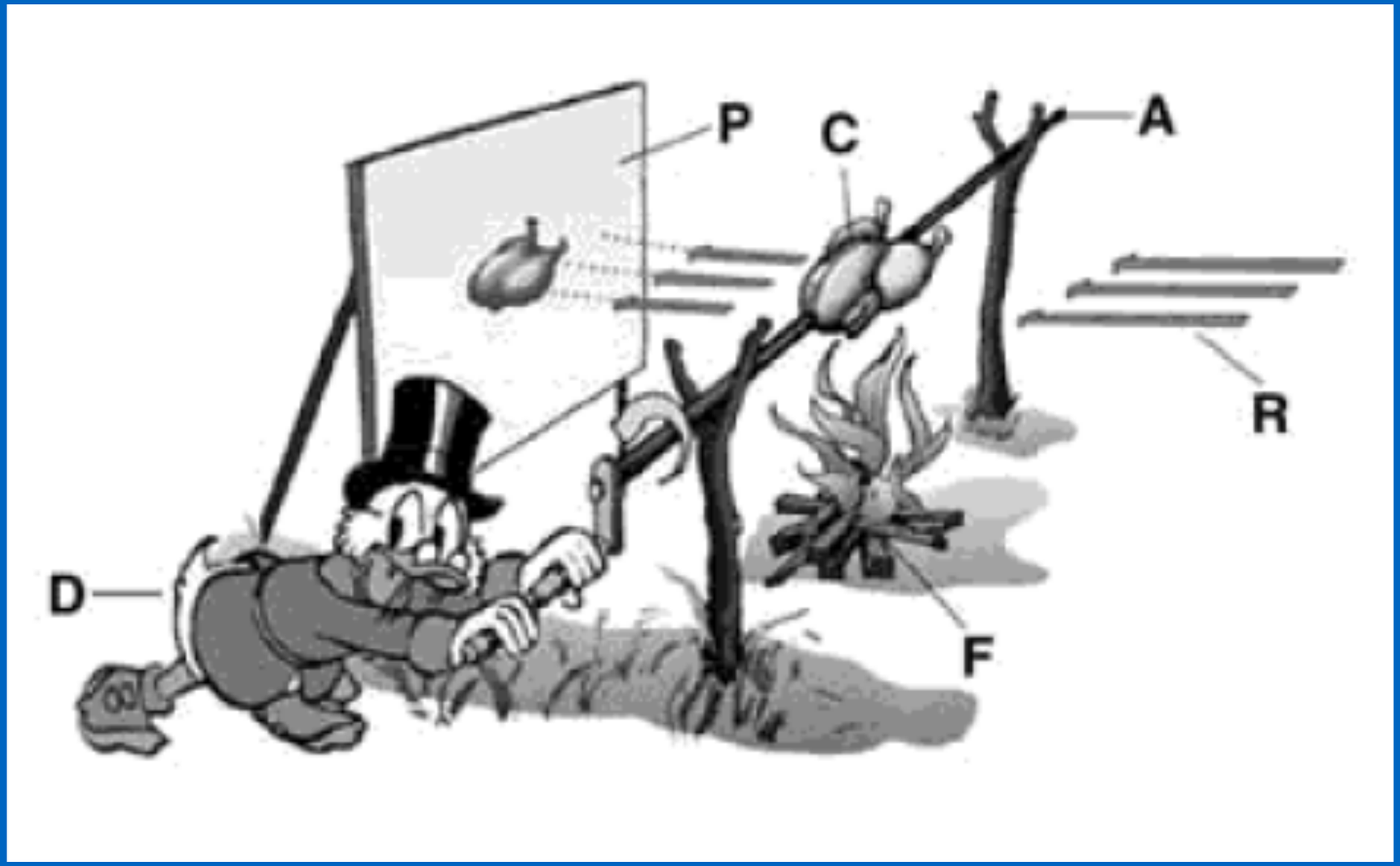

Frozen Hydrated Cell
Thermo Fisher

Cryo-ET data collection

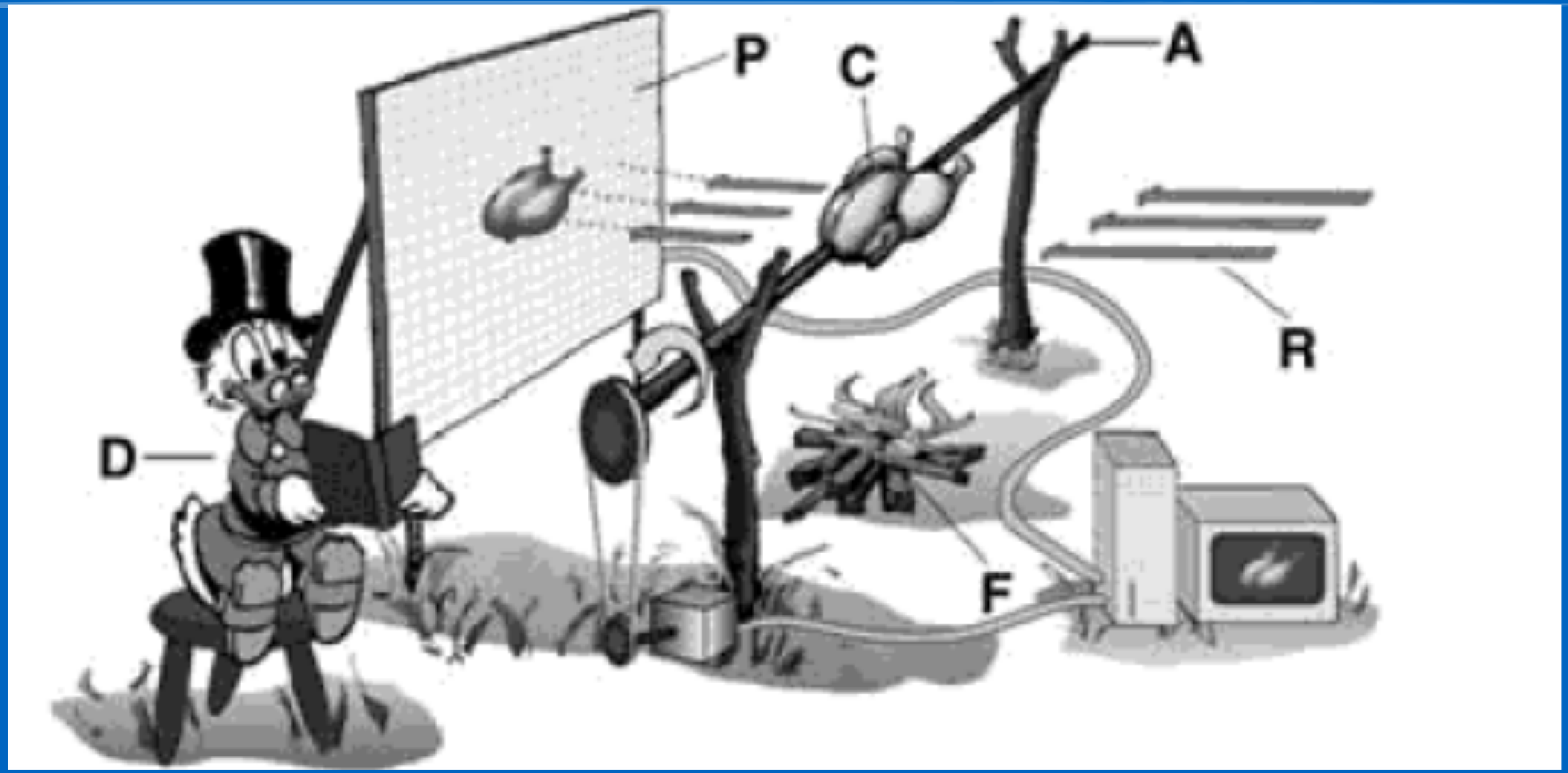


Koster et al., JSB 1997

Cryo-ET data collection is tedious



Automation is the key



Frank: Electron Tomography

Many cryo-ET data acquisition packages

SerialEM

UCSF Tomo
Leginon
FEI tomography
EM-Manu

SerialEM

Automated electron microscope tomography using robust prediction of specimen movements

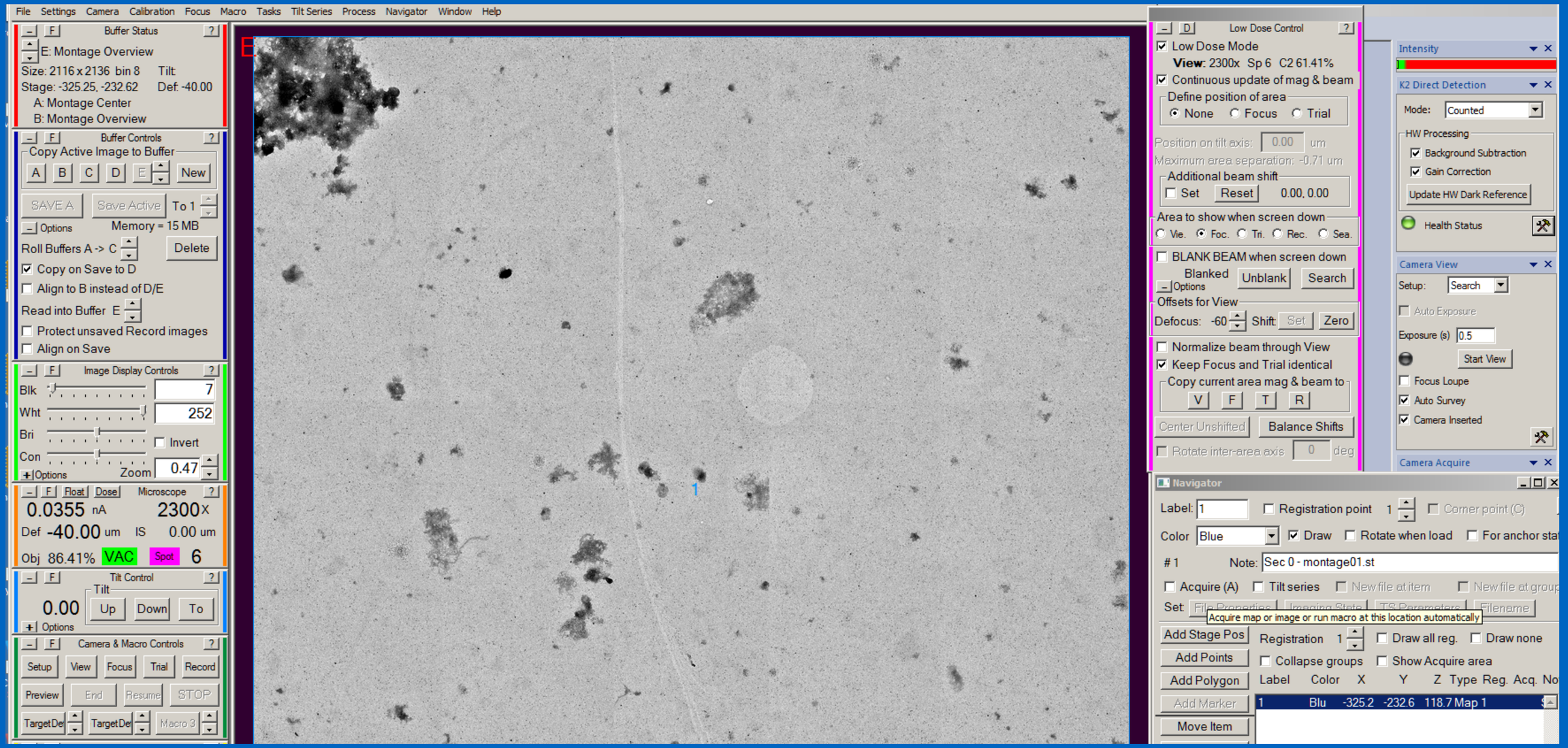
2005

David N. Mastronarde *

Boulder Laboratory for Three-Dimensional Electron Microscopy of Cells, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309, USA

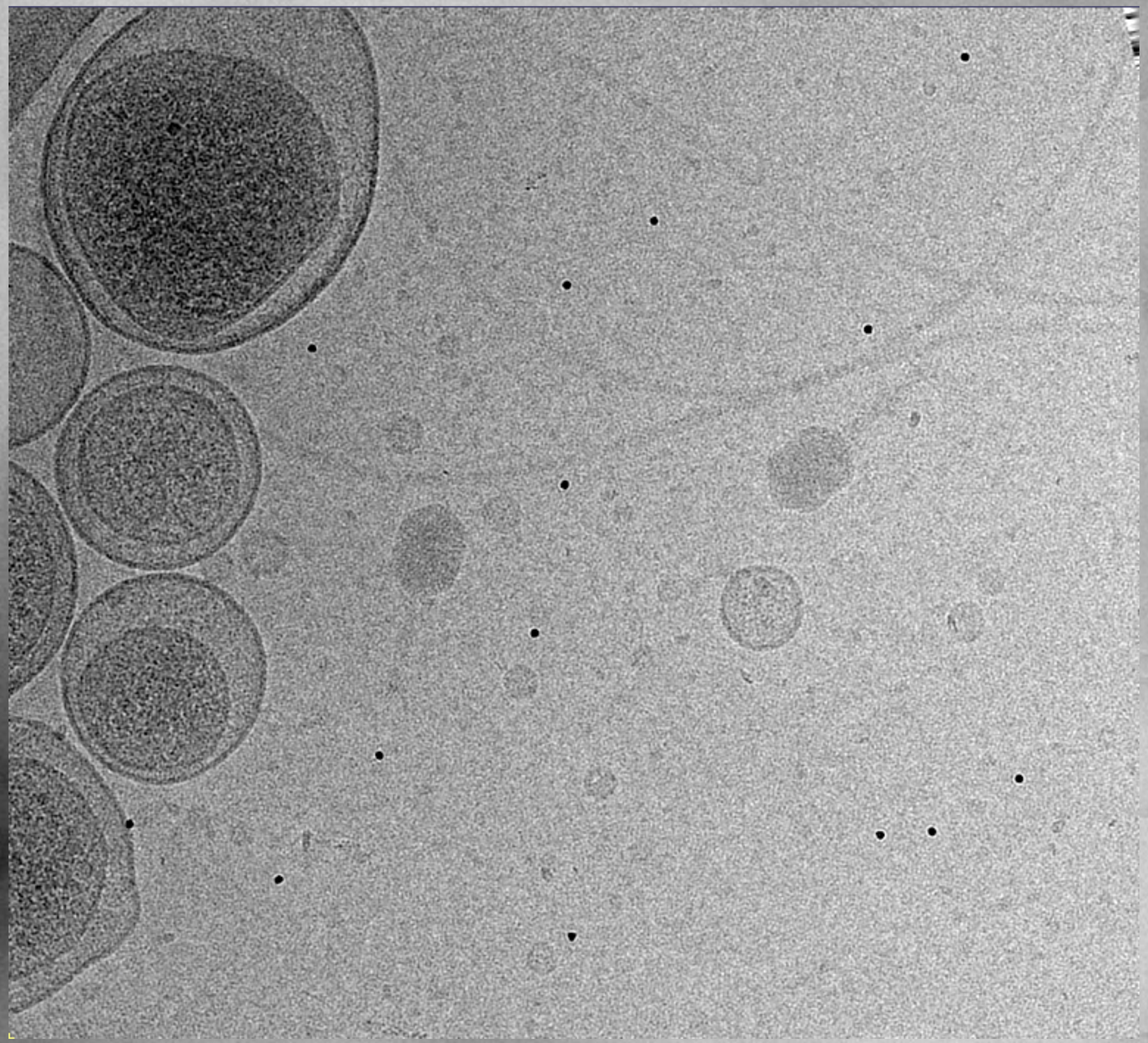
SerialEM provides a flexible interface. The script capability provides a relatively easy way to add commands requested by users.

SerialEM (David Mastronarde)



Selecting targets (SerialEM)

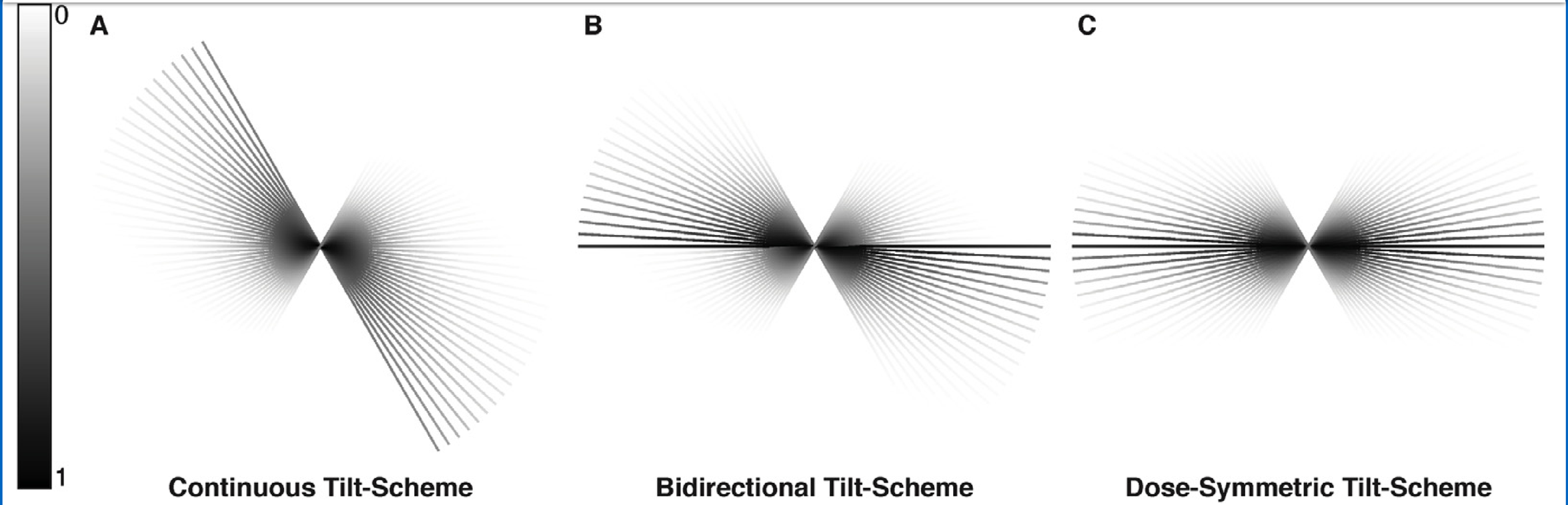
Collecting tilt series (SerialEM)



Advanced cryo-ET tilt scheme

Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging

Wim J.H. Hagen, William Wan, John A.G. Briggs *



FastTomo

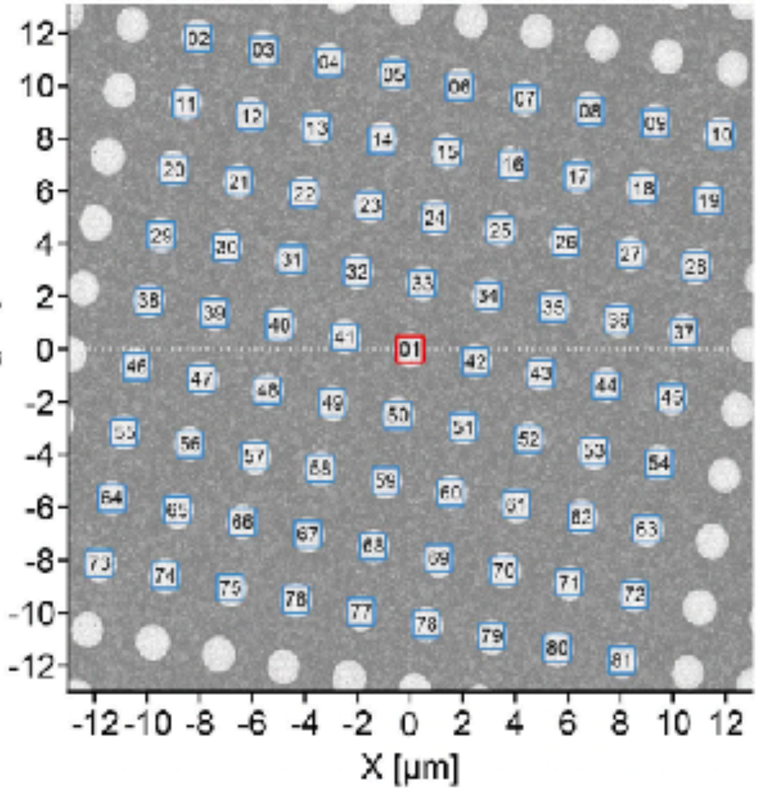
FastTomo: A SerialEM Script for Collecting Electron Tomography Data

Albert Xu, Chen Xu†
Department of Biochemistry and Molecular Pharmacology & Cryo-EM Core Facility
University of Massachusetts Medical School
Email: albert.t.xu@gmail.com, †Chen.Xu@umassmed.edu

"It achieves a speedup over conventional tracking methods by minimizing the usage of off-target tracking shots, and instead applies proportional control to the specimen images."

Parallel cryo electron tomography on *in situ* lamellae

Fabian Eisenstein[a], Haruaki Yanagisawa[a], Hiroka Kashihara[b], Masahide Kikkawa[a], Sachiko Tsukita[b], Radostin Danev[a,*]



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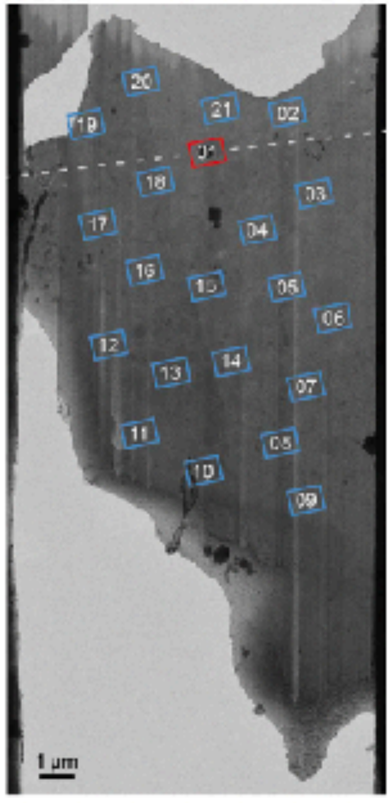

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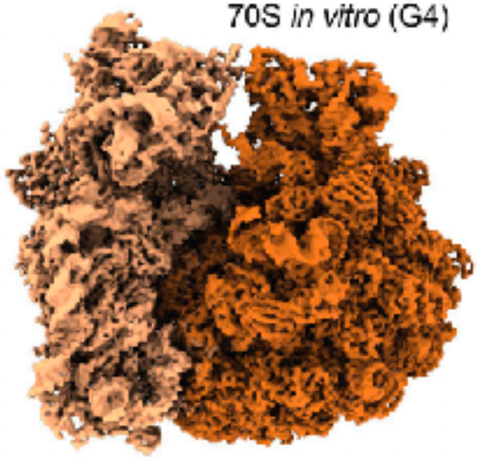

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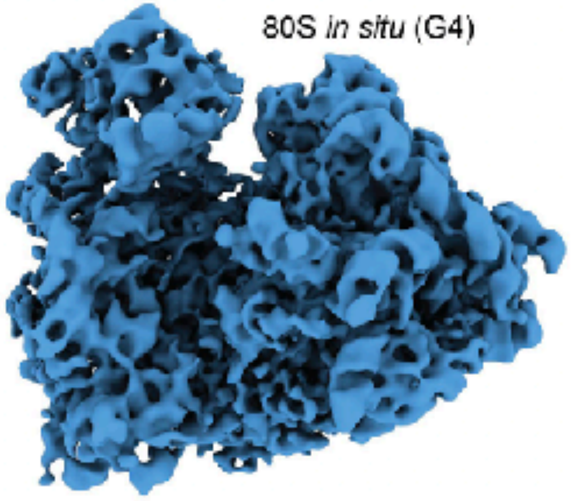

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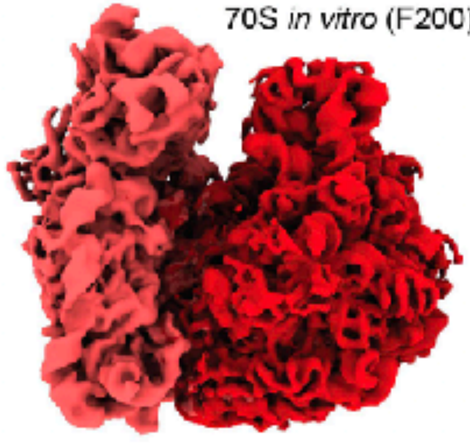

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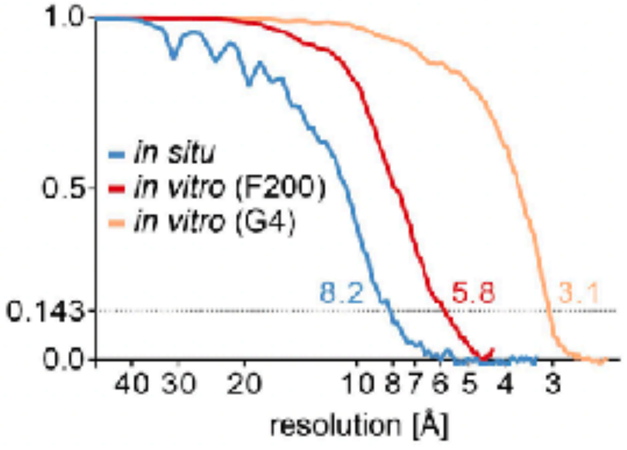


Image analysis — Alignment & Reconstruction

MotionCorr2

IMOD

EMAN2
Appion-Protomo
AreTomo

…

IMOD

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

David N. Mastronarde *, Susannah R. Held

Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309, United States

Alignment and reconstruction (IMOD)

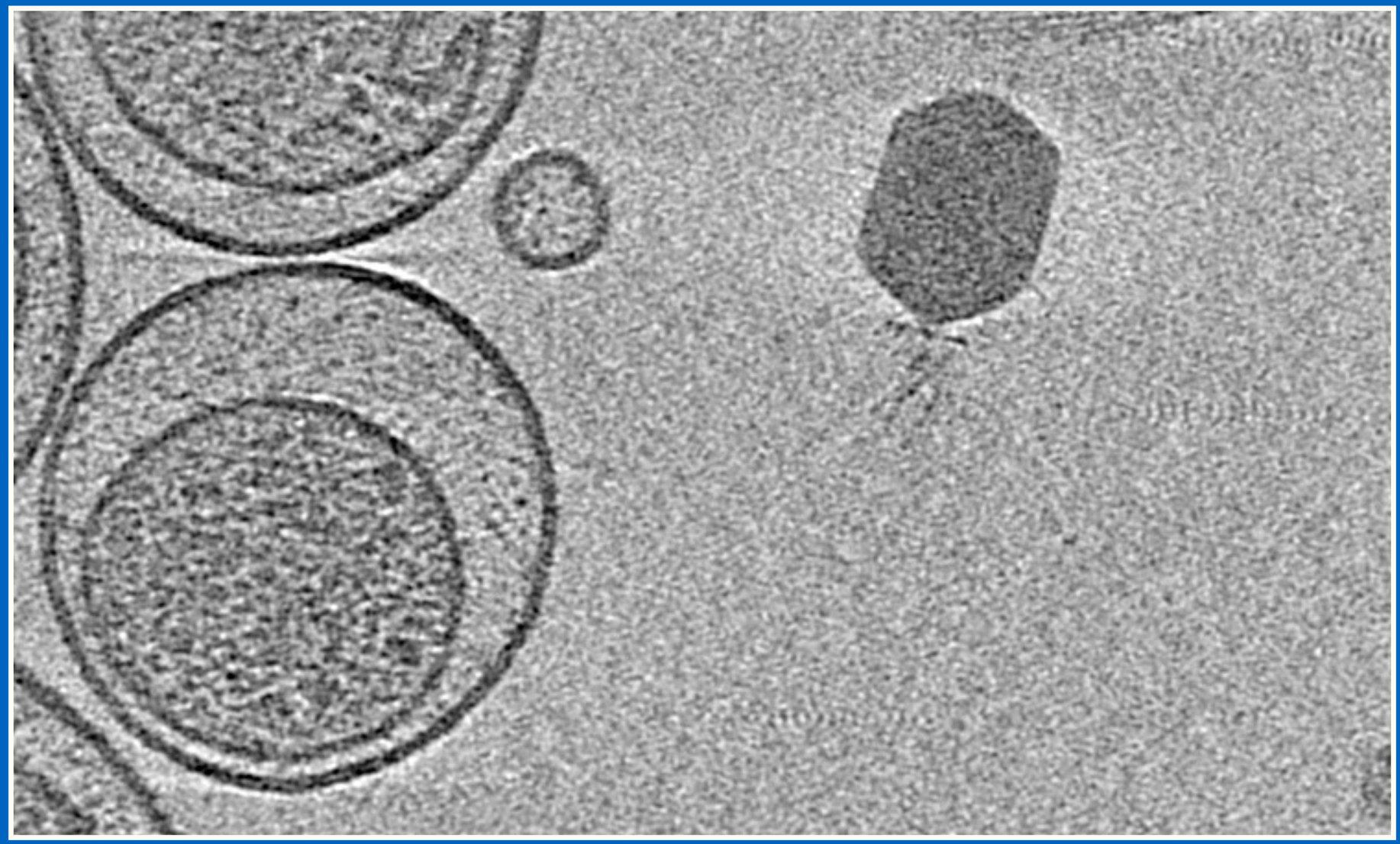


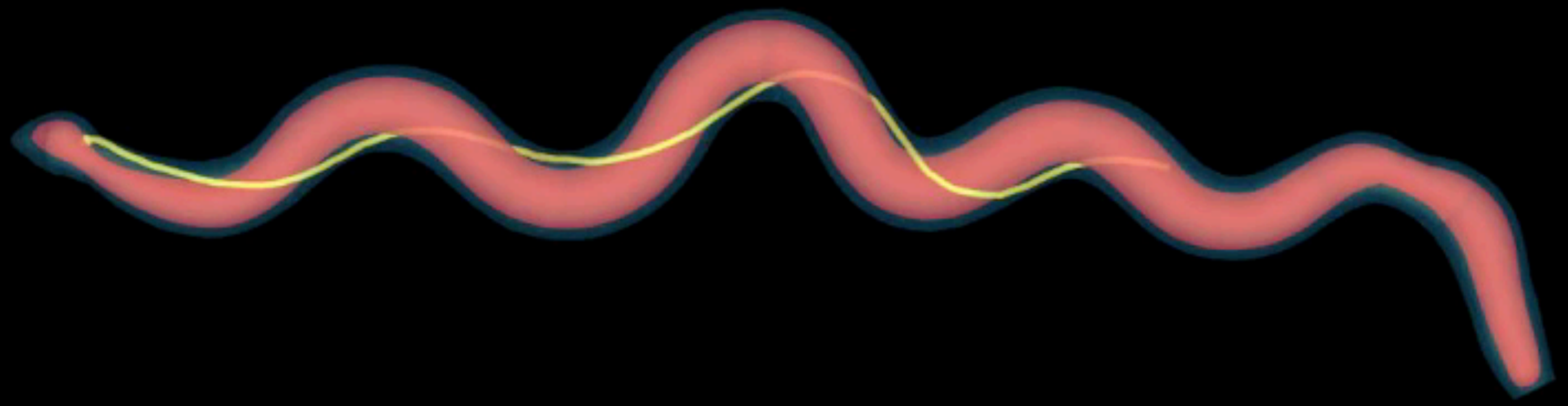
Image analysis — Segmentation

IMOD
EMAN2

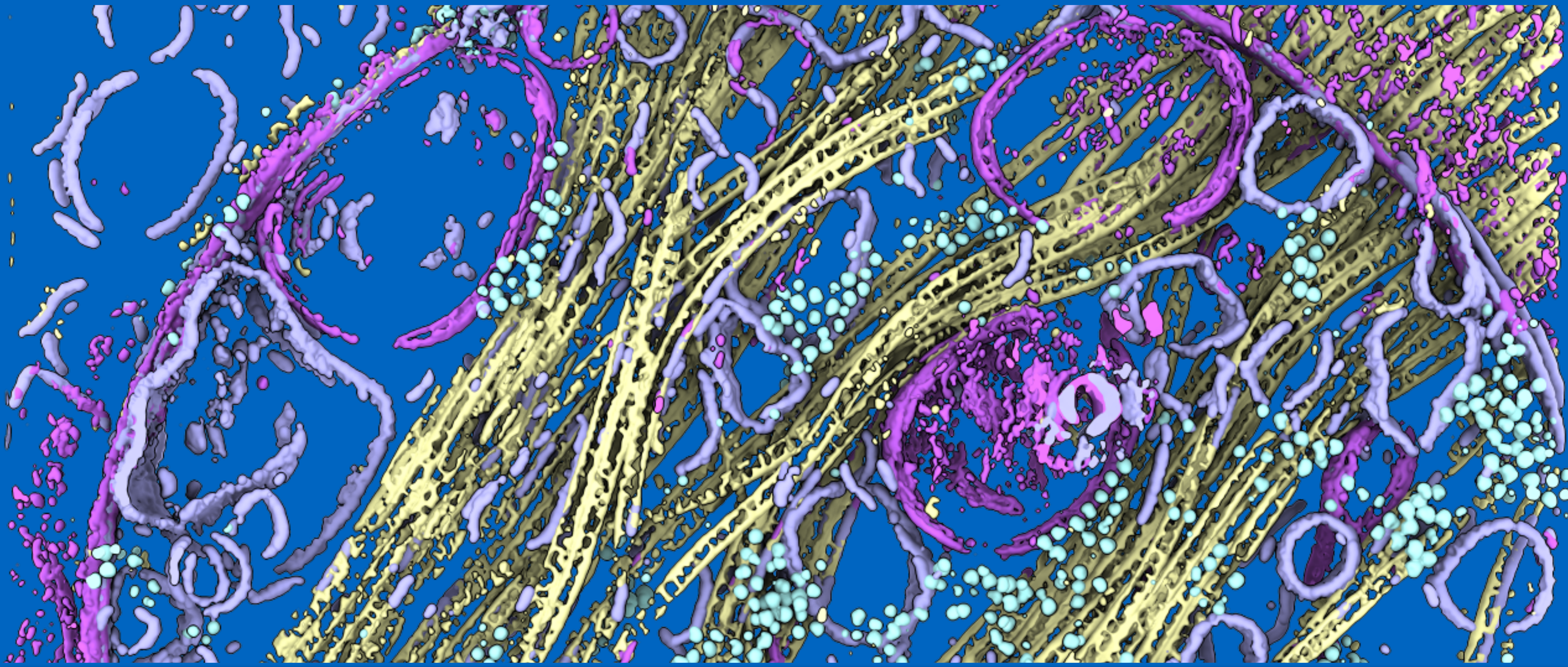
Amira

…

Segmentation (IMOD)



EMAN2 — Raphael & Muyuan

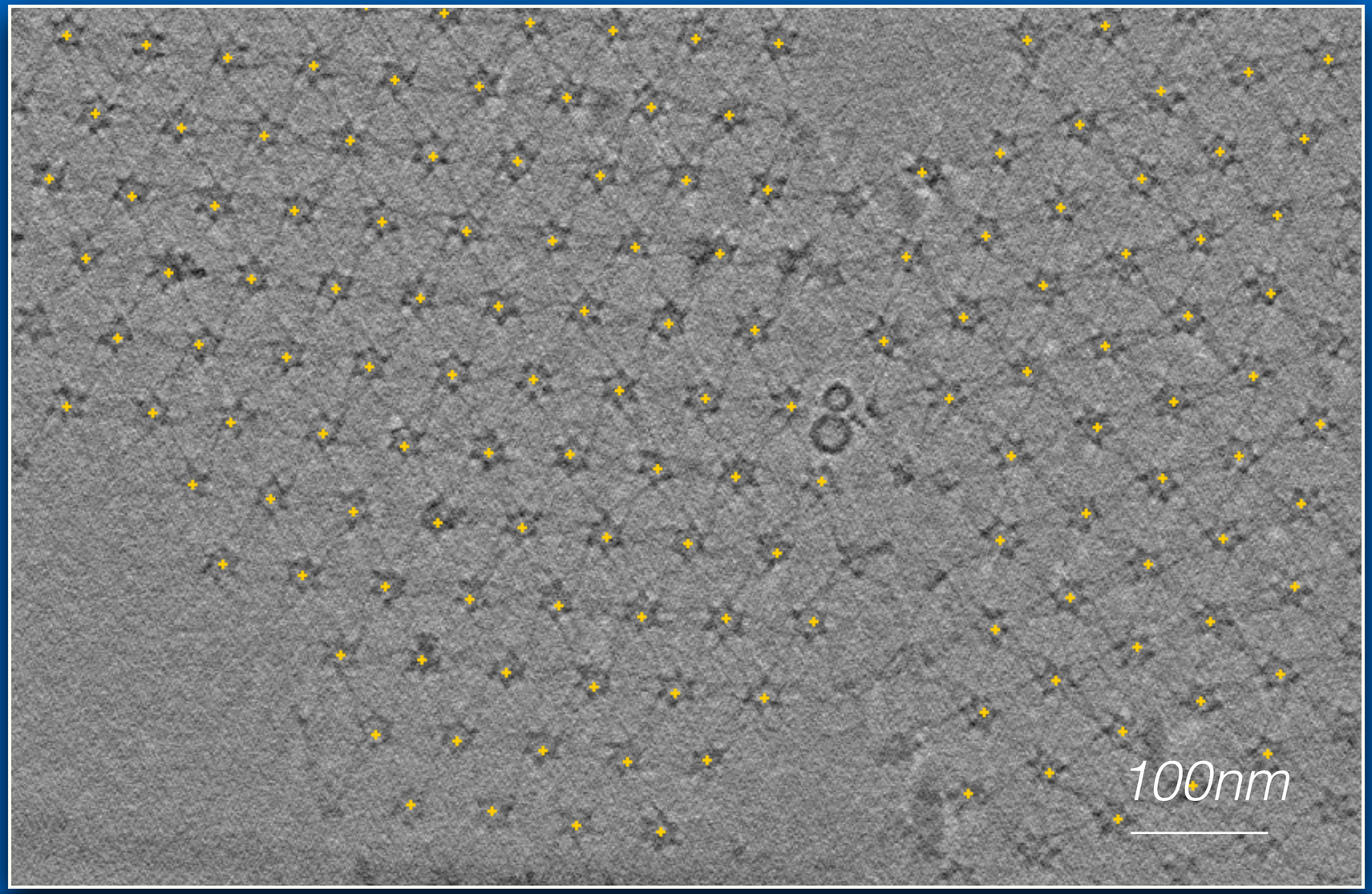


Chen et al., Nat Methods 2017, 2019, 2021

Image analysis — Sub-tomogram averaging

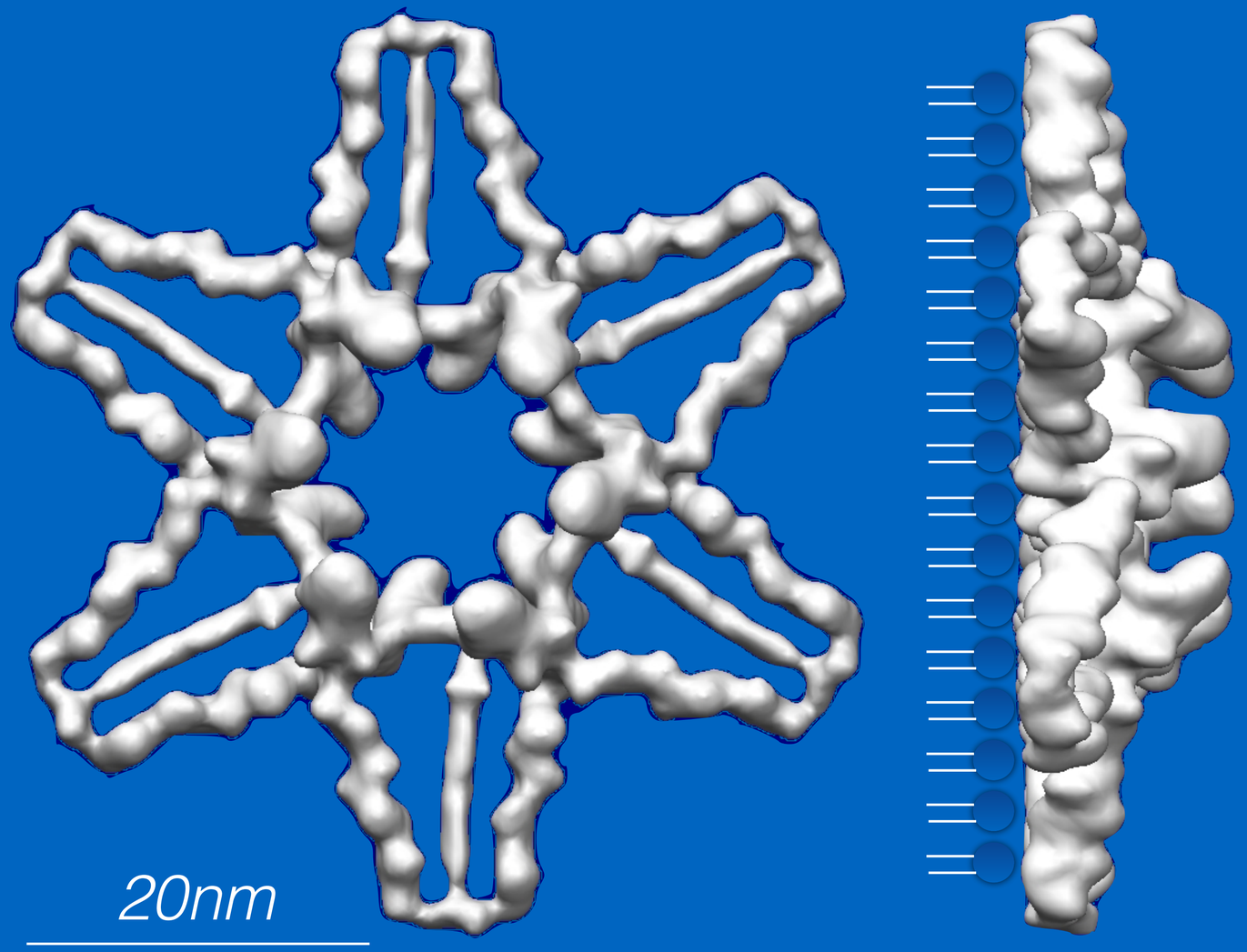
EMAN2
I3
Peet
Dynamo
Relion
EmClarity
…

Picking 3-D sub-tomograms



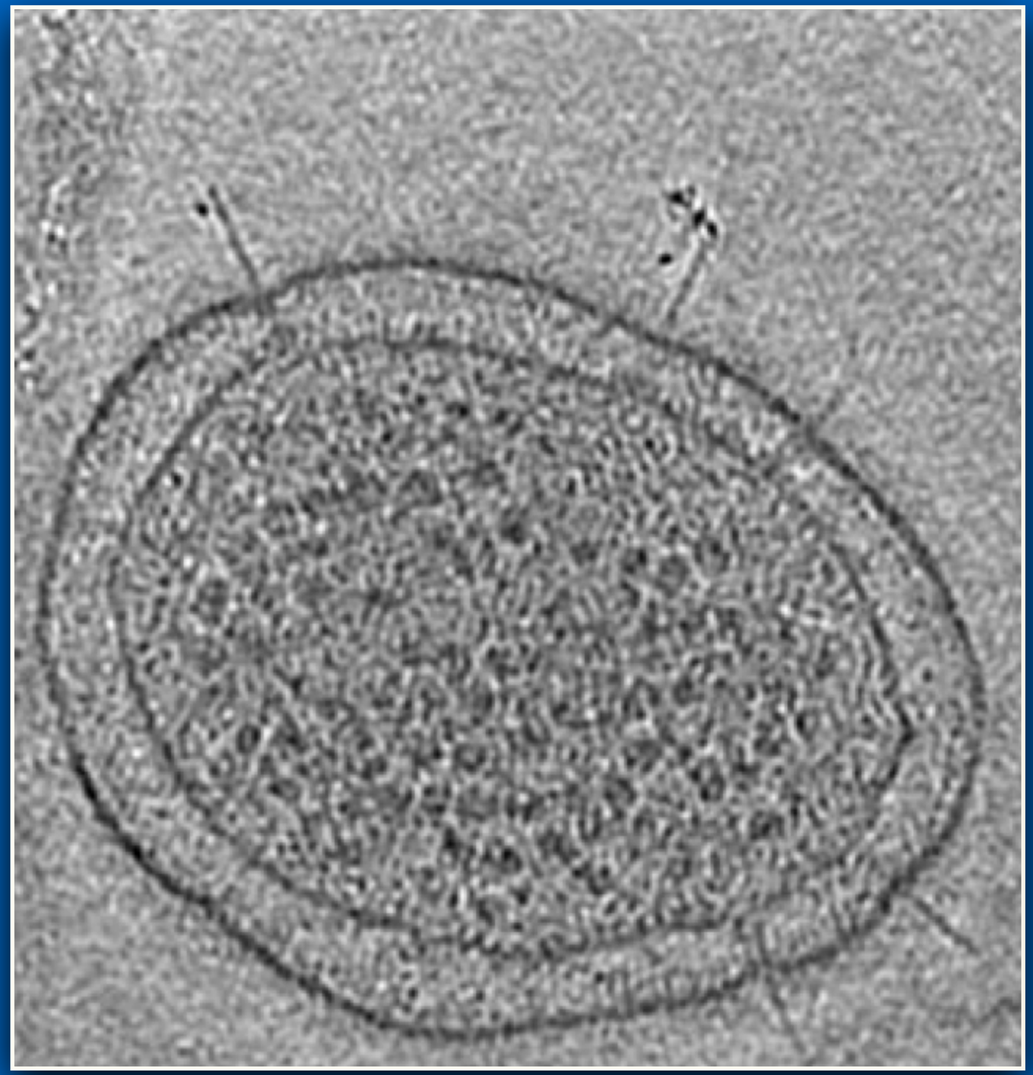
Liu et al. Nature 2006

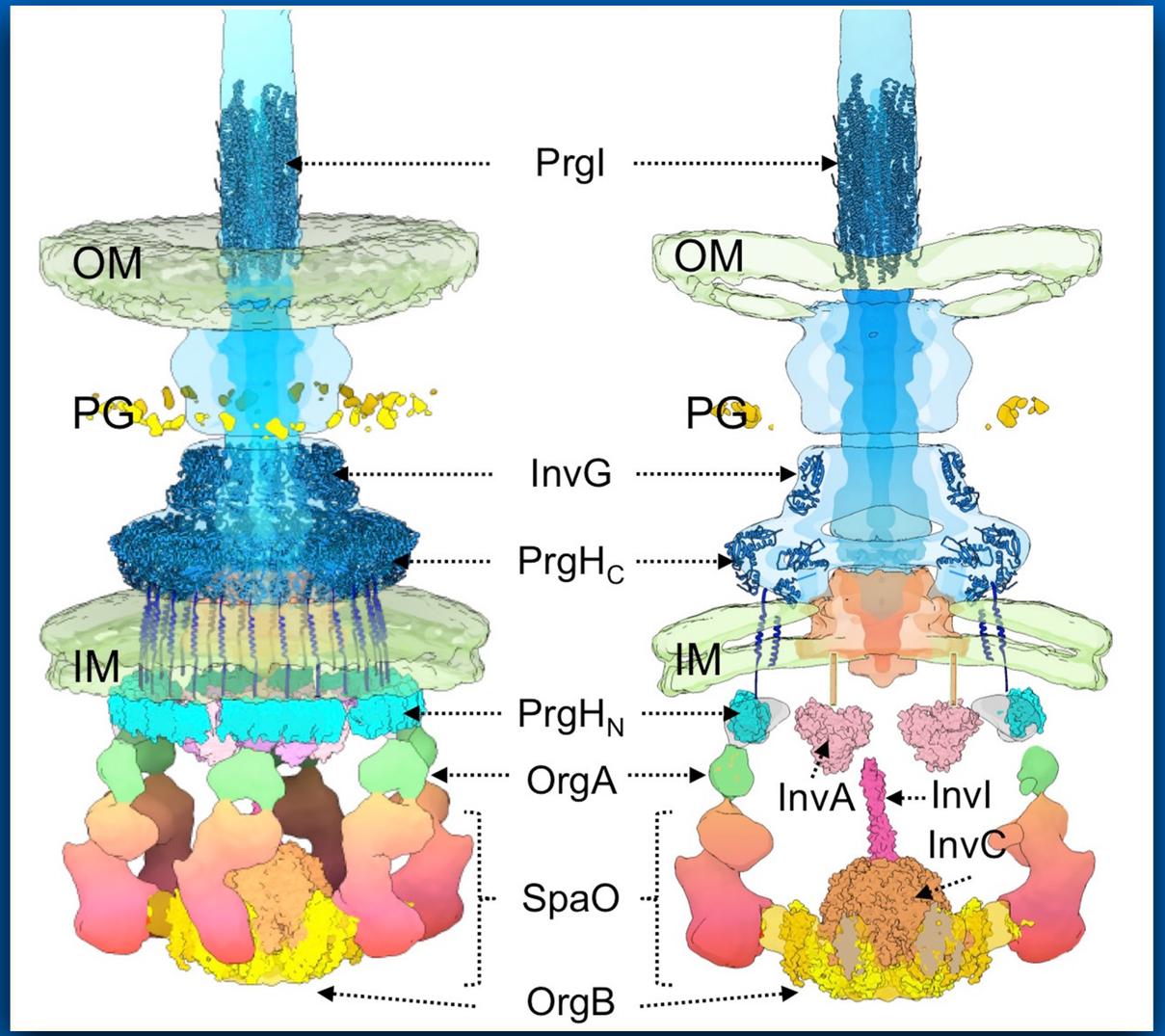
Sub-tomogram averaging



Liu et al. Nature 2006

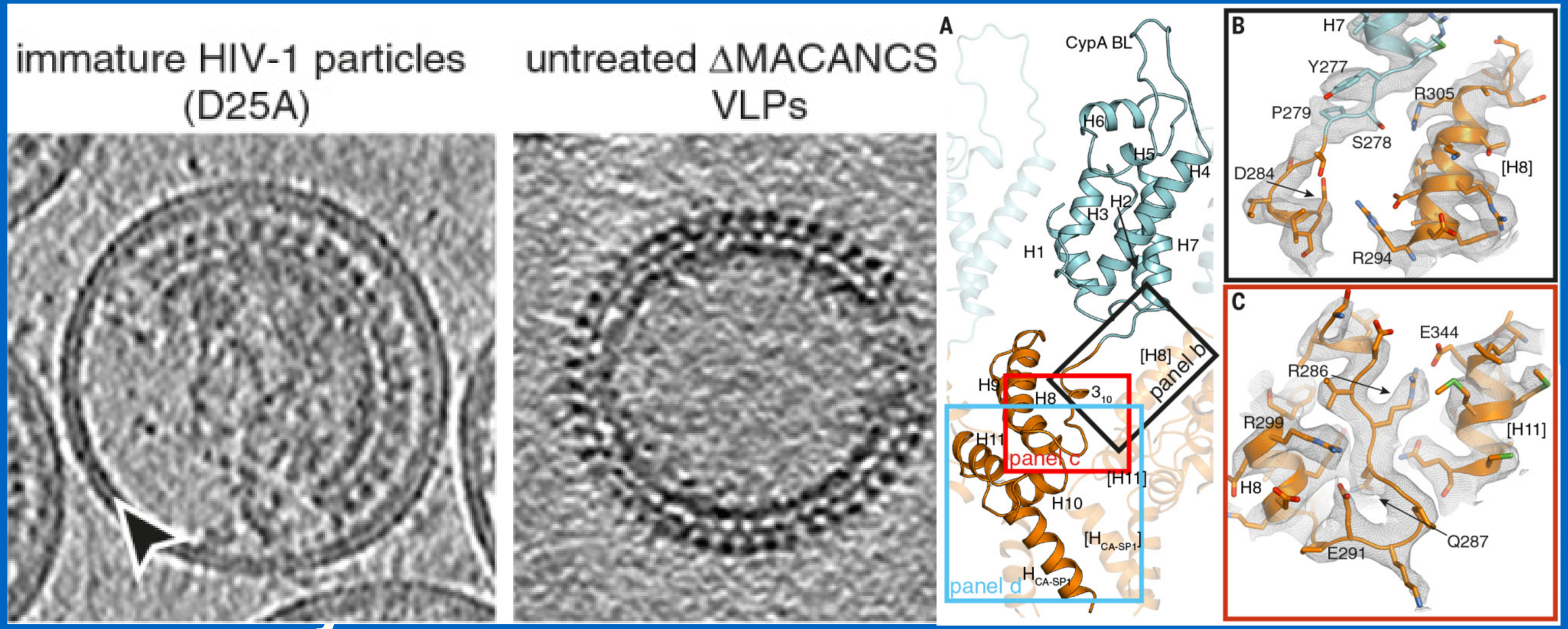
Salmonella Secretion Machine





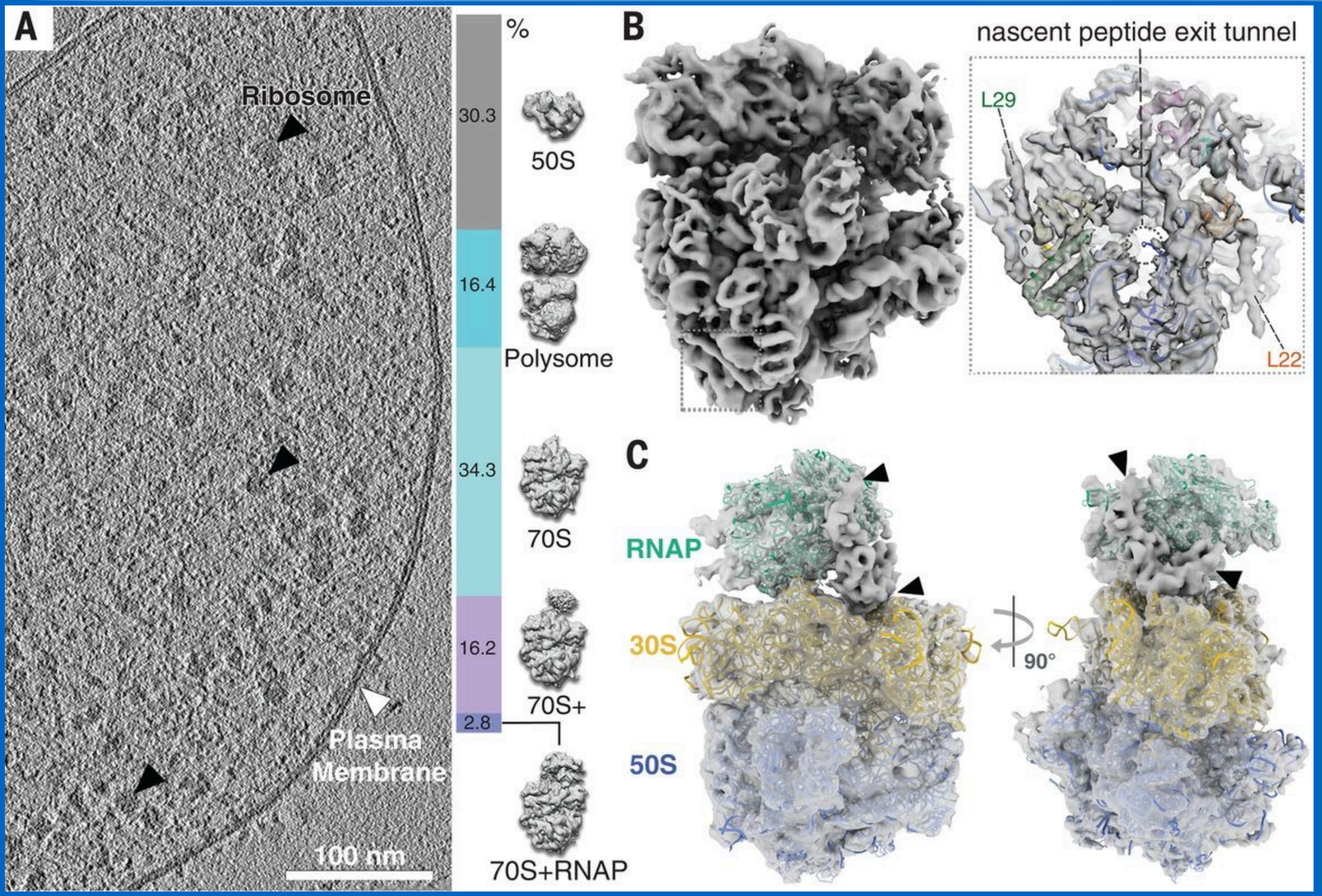
Hu et al. Cell 2017

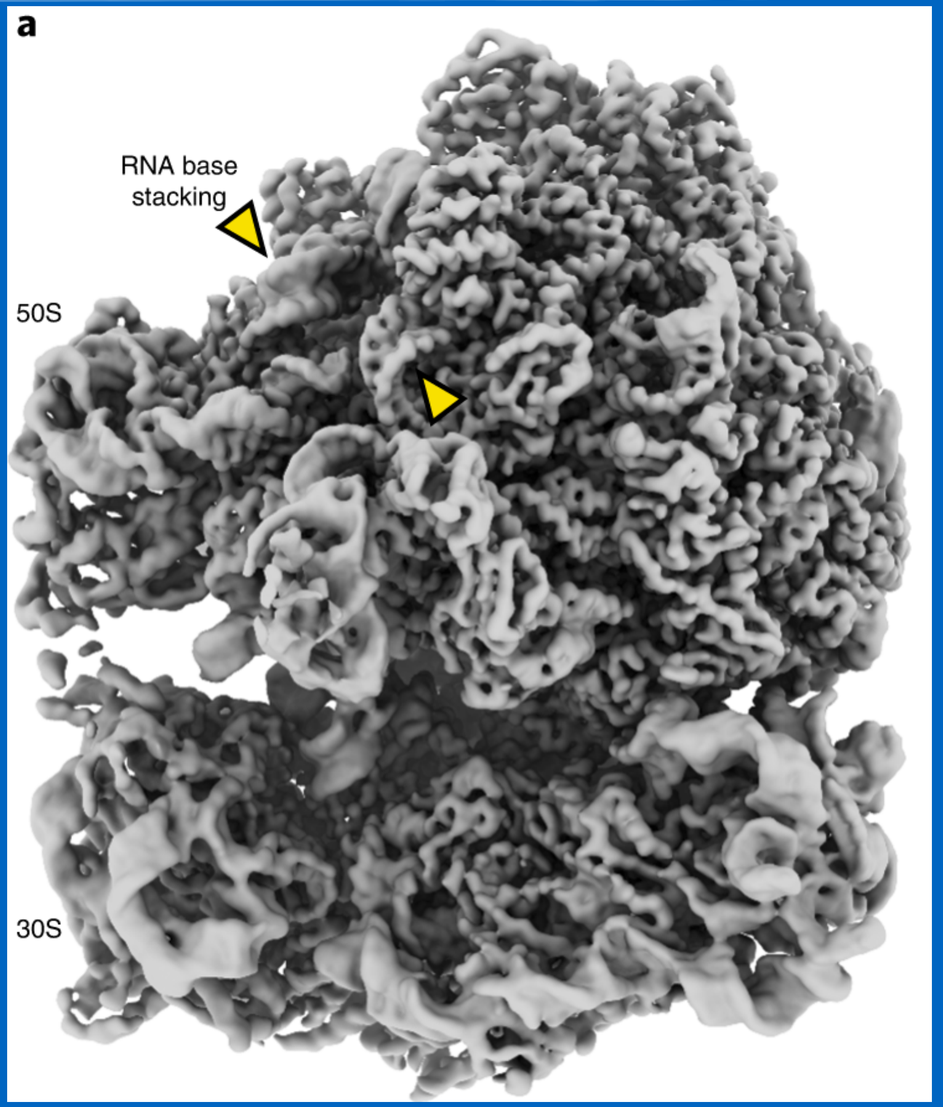
HIV Capsid at 3.9Å Resolution



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

In-cell atomic architecture of large complexes





O'Reilly et al. Science 2020 Tegunov et al. Nature Methods 2021

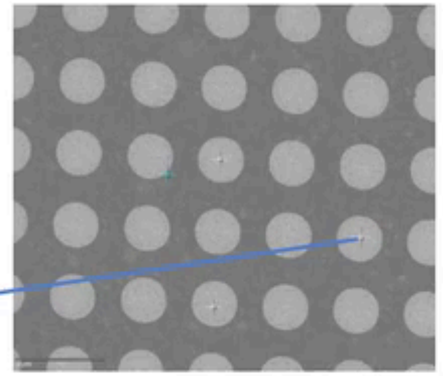
Tomography is increasingly useful

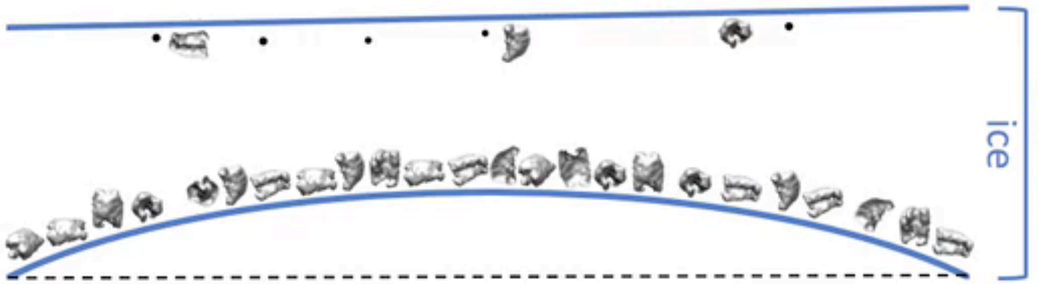
Routine single particle CryoEM sample and grid characterization by tomography

Alex J Noble[1], Venkata P Dandey[1†], Hui Wei[1†], Julia Brasch[1,2], Jillian Chase[3,4], Priyamvada Acharya[1,5], Yong Zi Tan[1,2], Zhening Zhang[1], Laura Y Kim[1], Giovanna Scapin[1,6], Micah Rapp[1,2], Edward T Eng[1], William J Rice[1], Anchi Cheng[1], Carl J Negro[1], Lawrence Shapiro[2], Peter D Kwong[5], David Jeruzalmi[3,4,7,8], Amedee des Georges[3,4,8,9], Clinton S Potter[1,2], Bridget Carragher[1,2*]

Sample #33

DNAB Helicase-helicase Loader

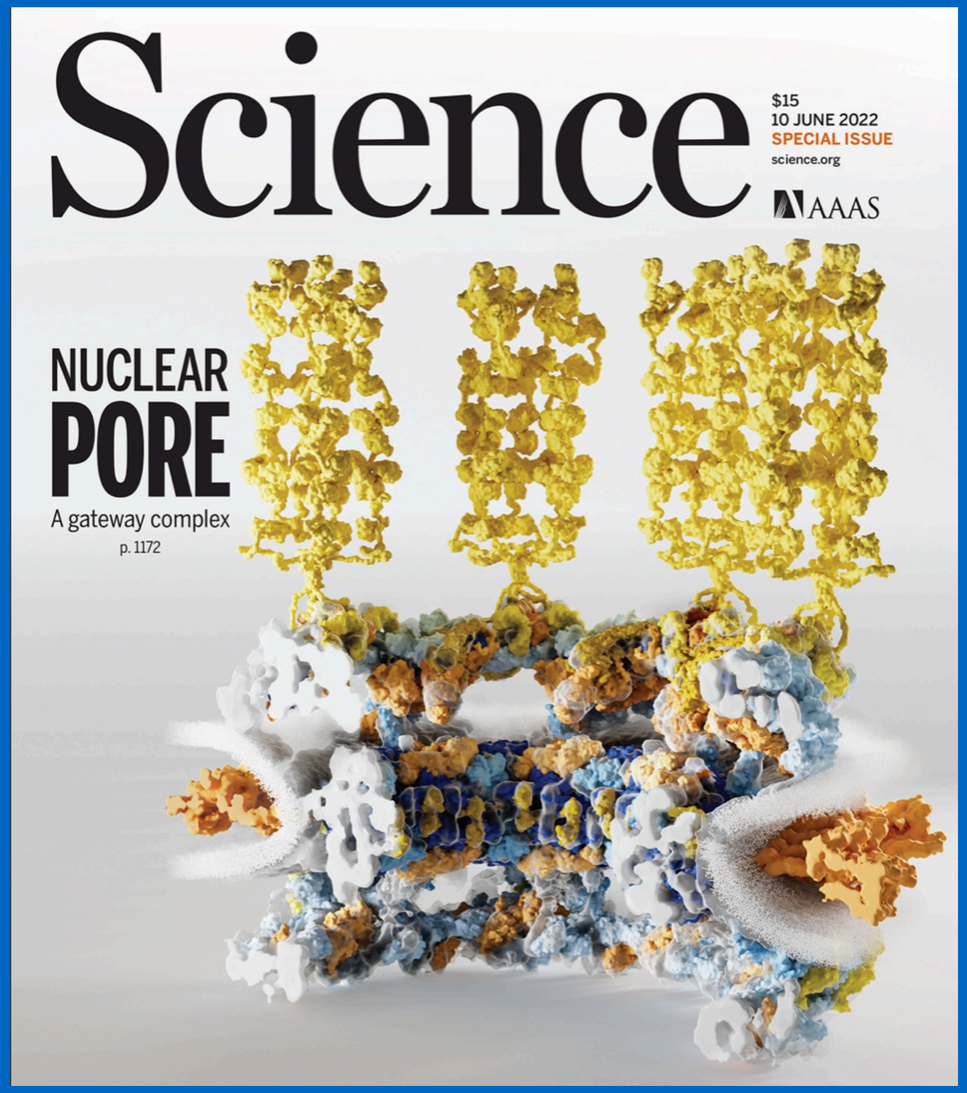




250 nm

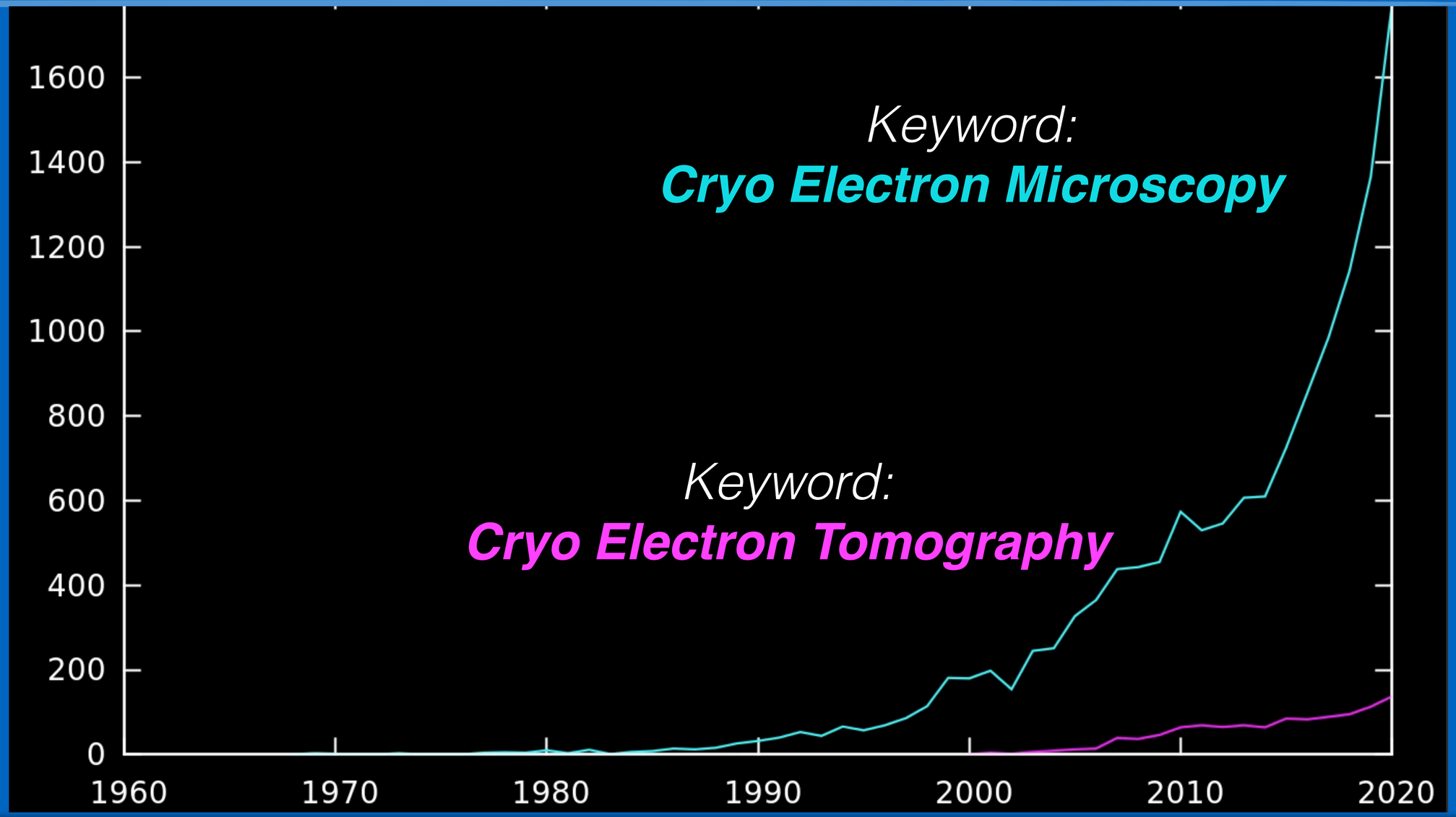
-36:21:3°, Gold Quantifoil, Krios, K2

NPC



"*Science* presents three papers that piece together this giant jig-saw puzzle, **revealing a near-atomic picture of the massive human NPC.** These studies 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!



Do you have any questions about cryo-ET?

- What's cryo-electron tomography (cryo-ET)?
- What's the difference between cryo-ET and single particle cryo-EM?
- How should I prepare samples for cryo-ET?
- How should I collect cryo-ET data?
- How should I analyze cryo-ET data?
- How could I use cryo-ET effectively?