Cryo-ET basics & applications

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Lecture outline

1. Why cryo-ET?

2. Sample preparation

3. Image analysis & examples

Recommended readings

Perspectives of Molecular and Cellular Electron Tomography
Koster (1997), J. Struct Biol 120, 276

The potential and limitations of neutrons, electrons and X-rays for atomic resolution microscopy of unstained biological molecules
Henderson (1995), Quart Rev Biophys 28, 171

Electron tomography of cells (recent examples highlighted)
Gan and Jensen (2012), Quart Rev Biophys 45, 27

Electron Tomography: Methods for Three-Dimensional Visualize of Structures in the Cell
Frank (2006), London, New York: Springer

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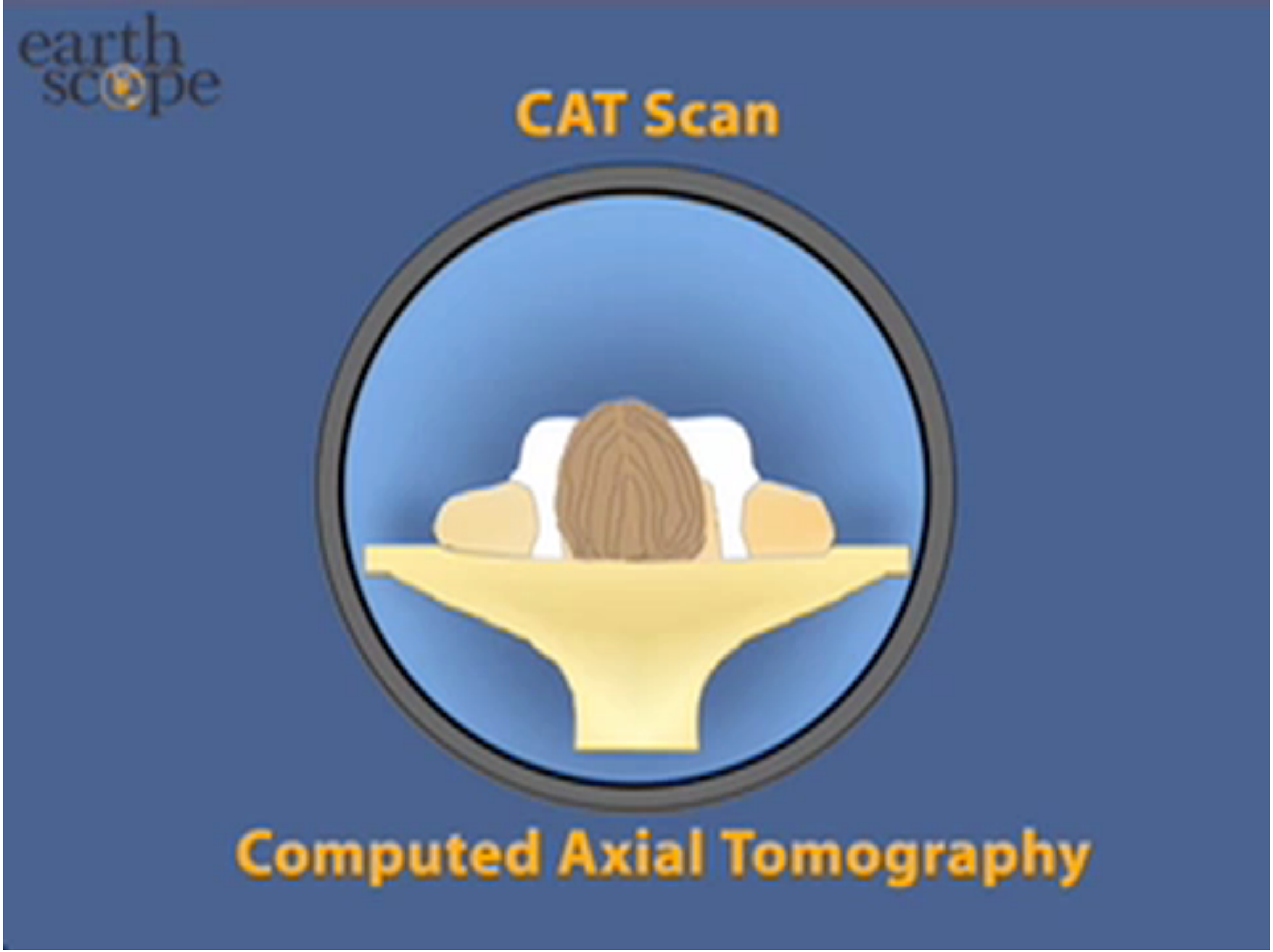
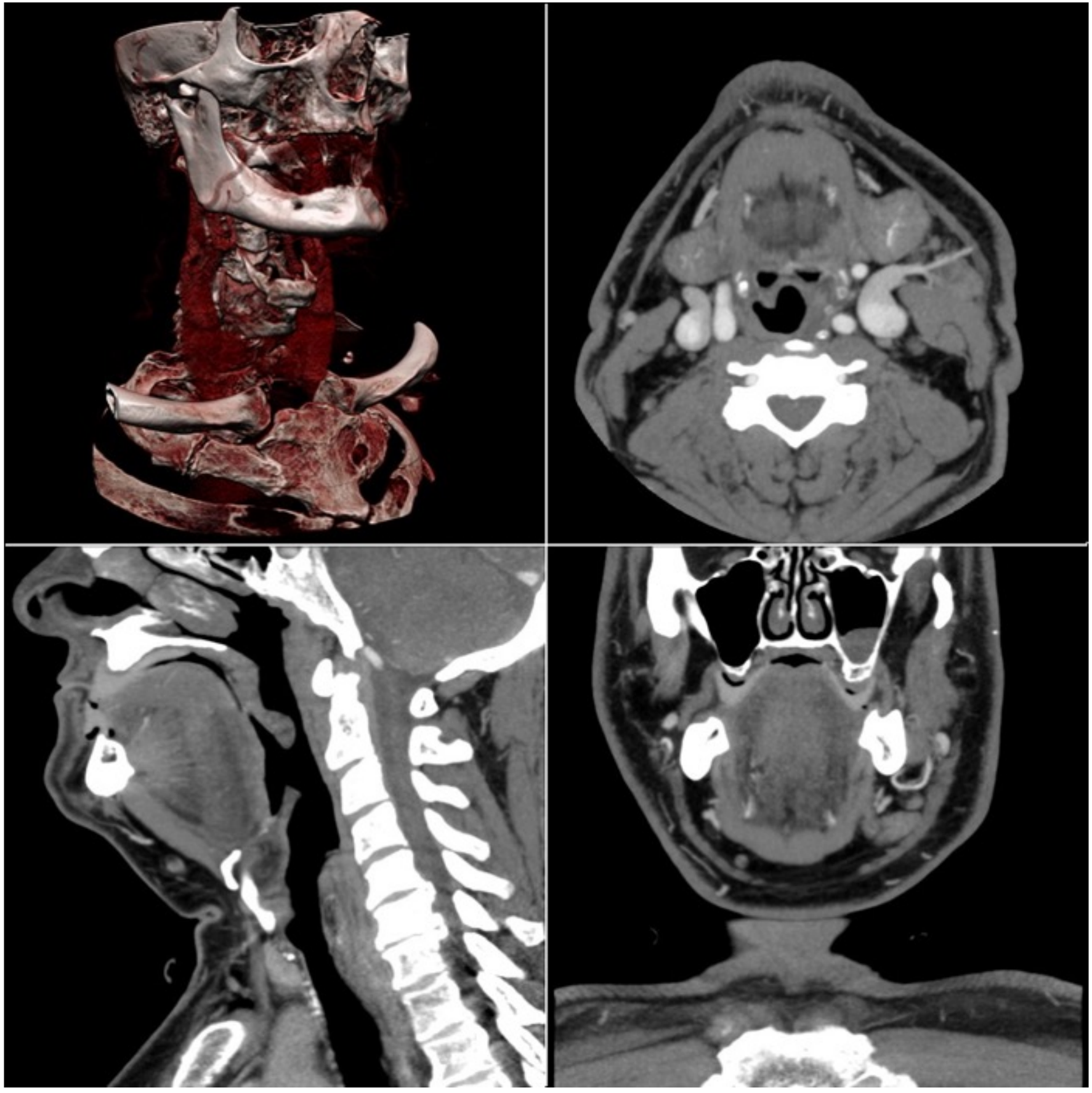
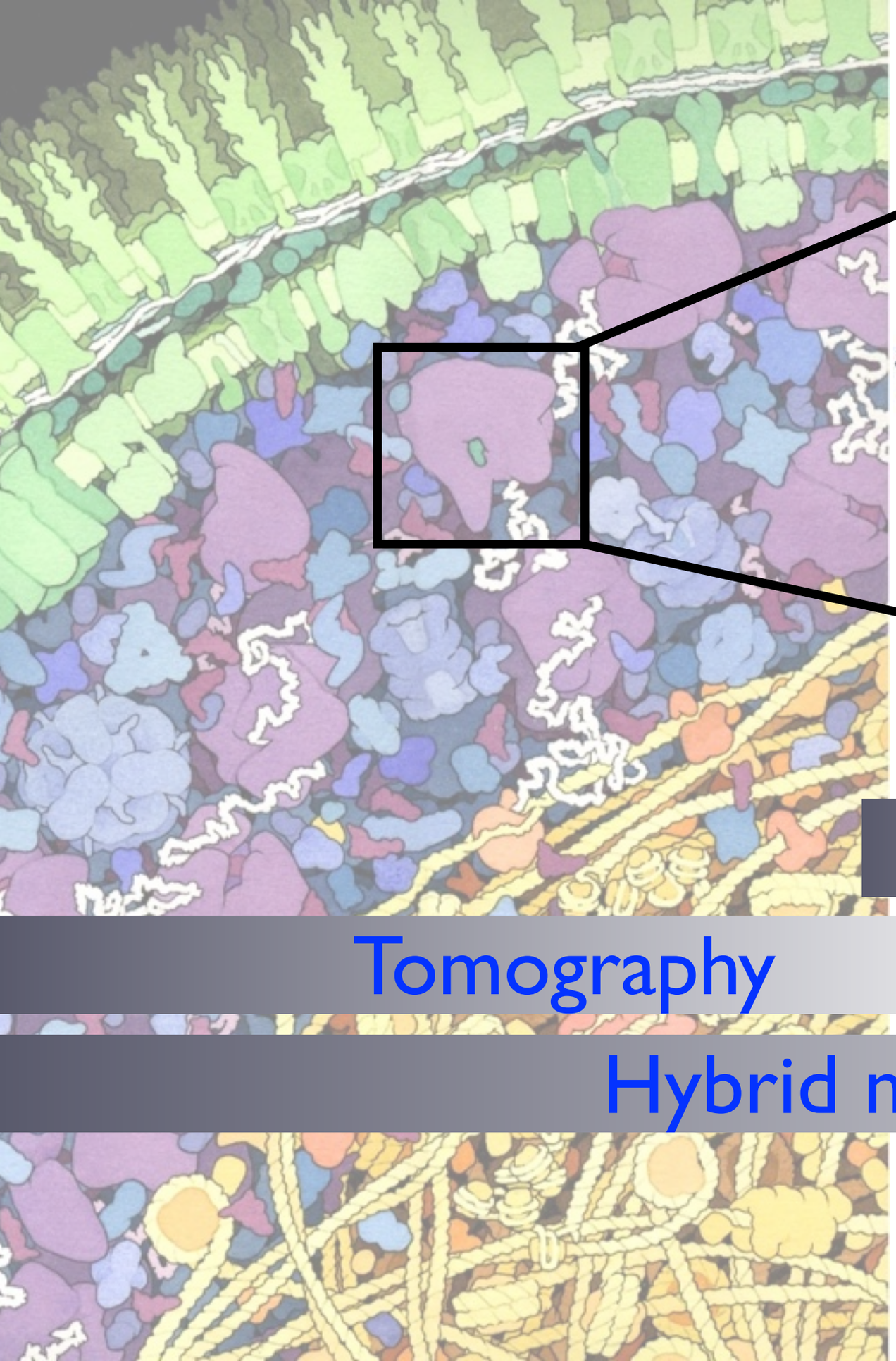
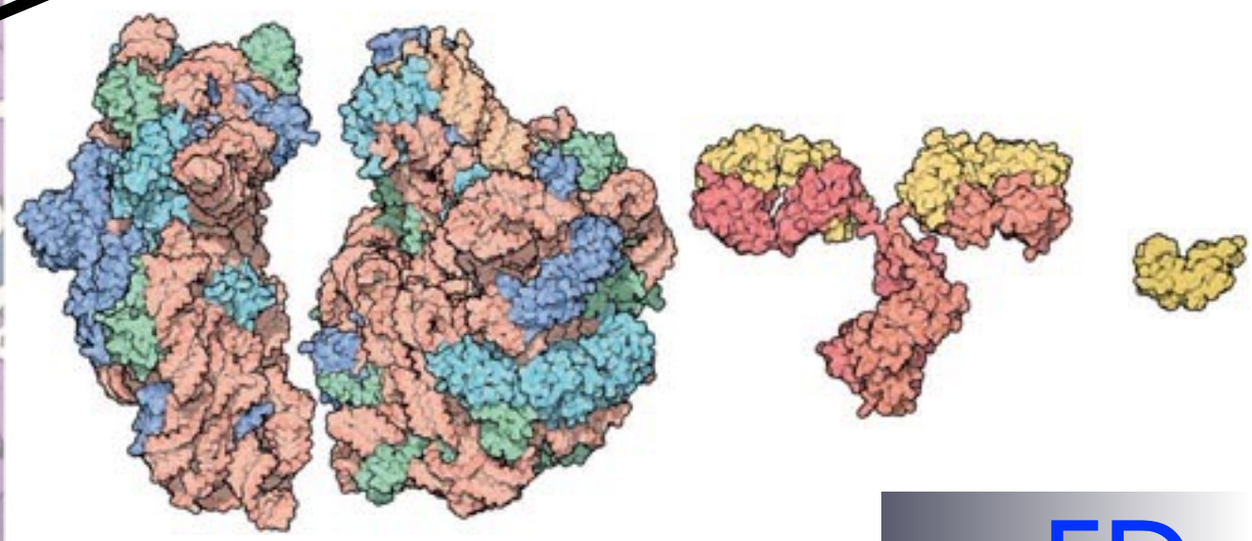


http://www.iris.edu/hq/programs/education_and_outreach/animations/7



Wikipedia





μ-ED

Crystallography

250 Å

Single-particle EM

Tomography

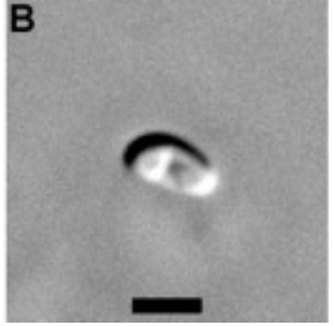
Hybrid methods

David Goodsell, PDB

What is quantifiable using EM?

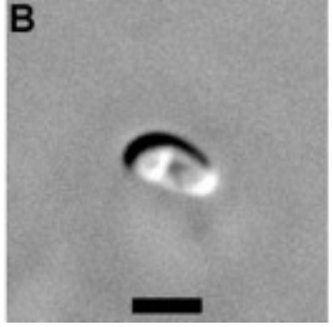
Quantity	Example
Distances	Diameter of an E.R. tubule
Volumes	Enlargement of lipid body
Counts	Envelope spikes on a virion
Positions	Distribution of ribosomes in stressed cells
"Structure"	3-D reconstruction of ribosome

a



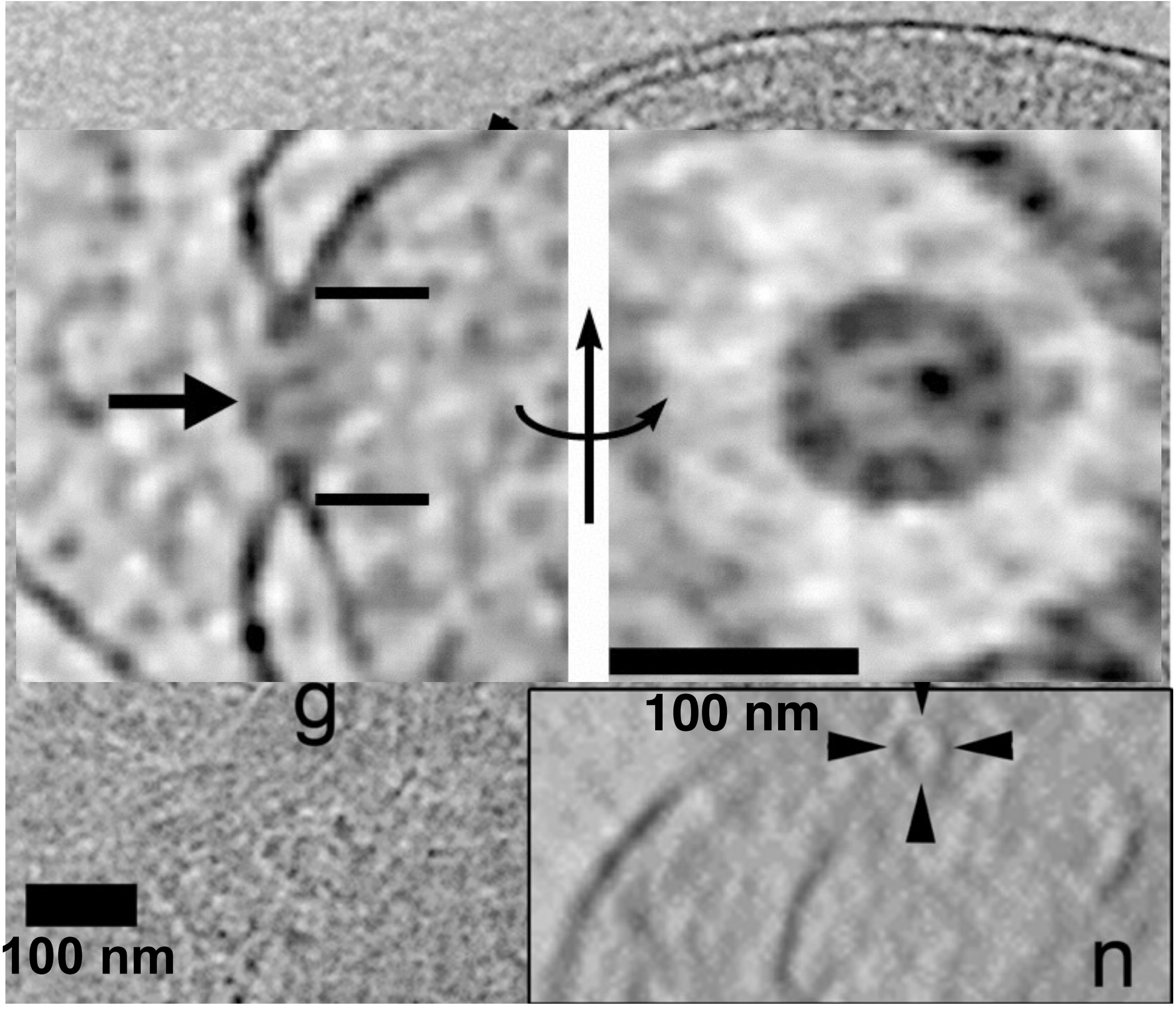
2 μm

Chen, unpublished; Gan 2011, QRB



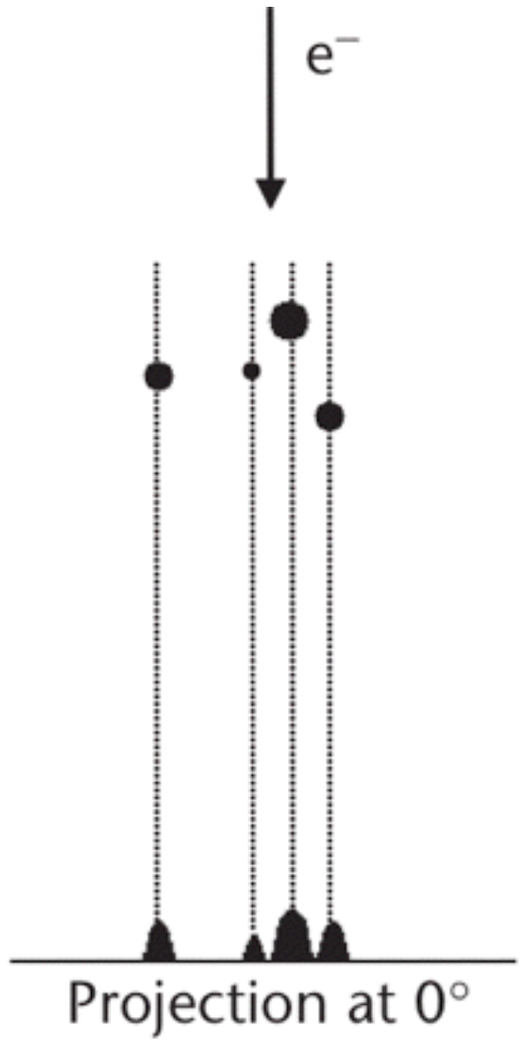
2 μm

Chen, unpublished



Henderson, 2007

A cryo-EM image is a “projection”



He, 2010

An ideal EM image is a “projection”

Projection along ***z*** *axis:*

$$p(x,y) = \int_{-\infty}^{\infty} f(x,y,z)\, dz$$

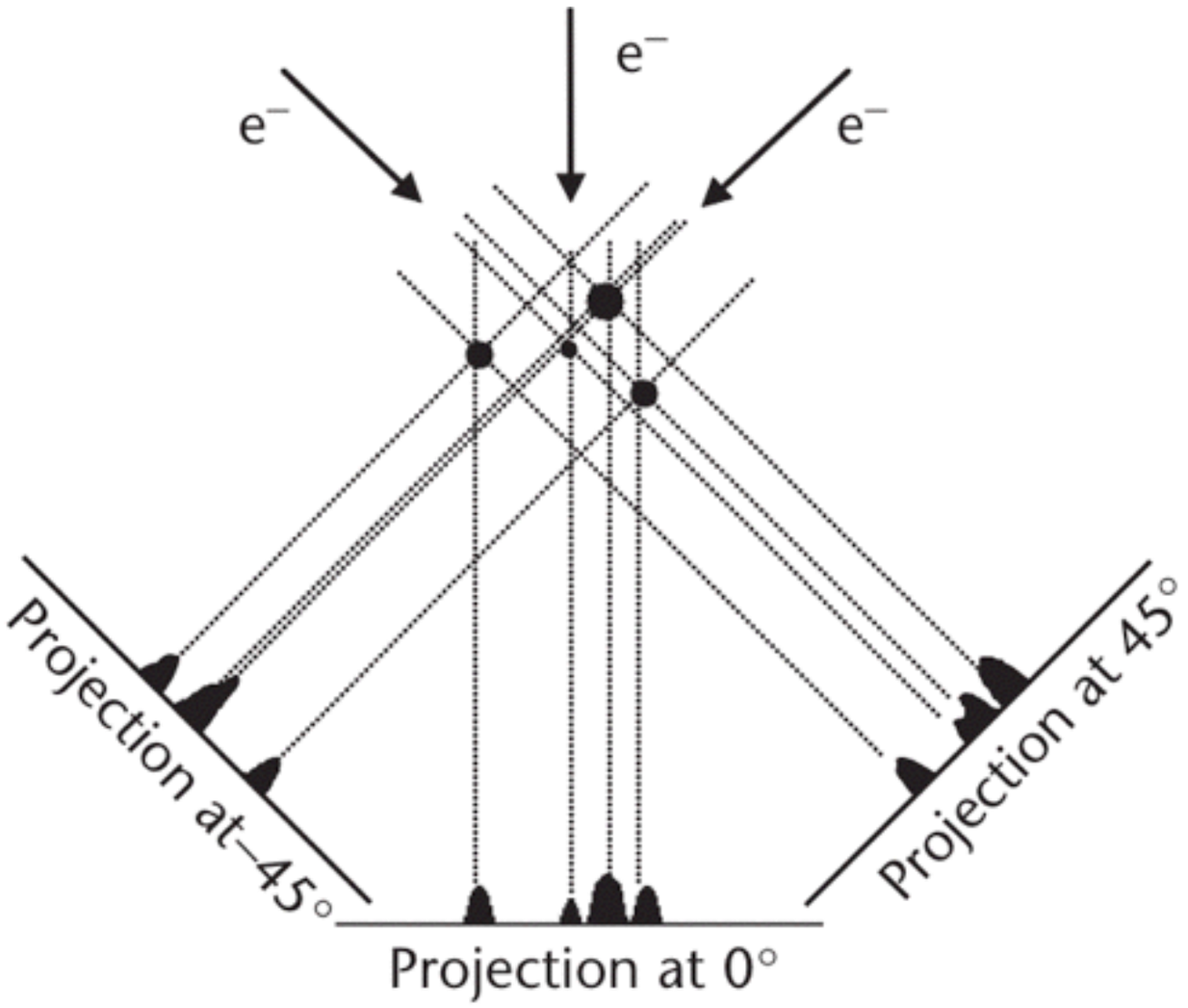
Image (recorded)

Unknown (desired)

For 3-D object

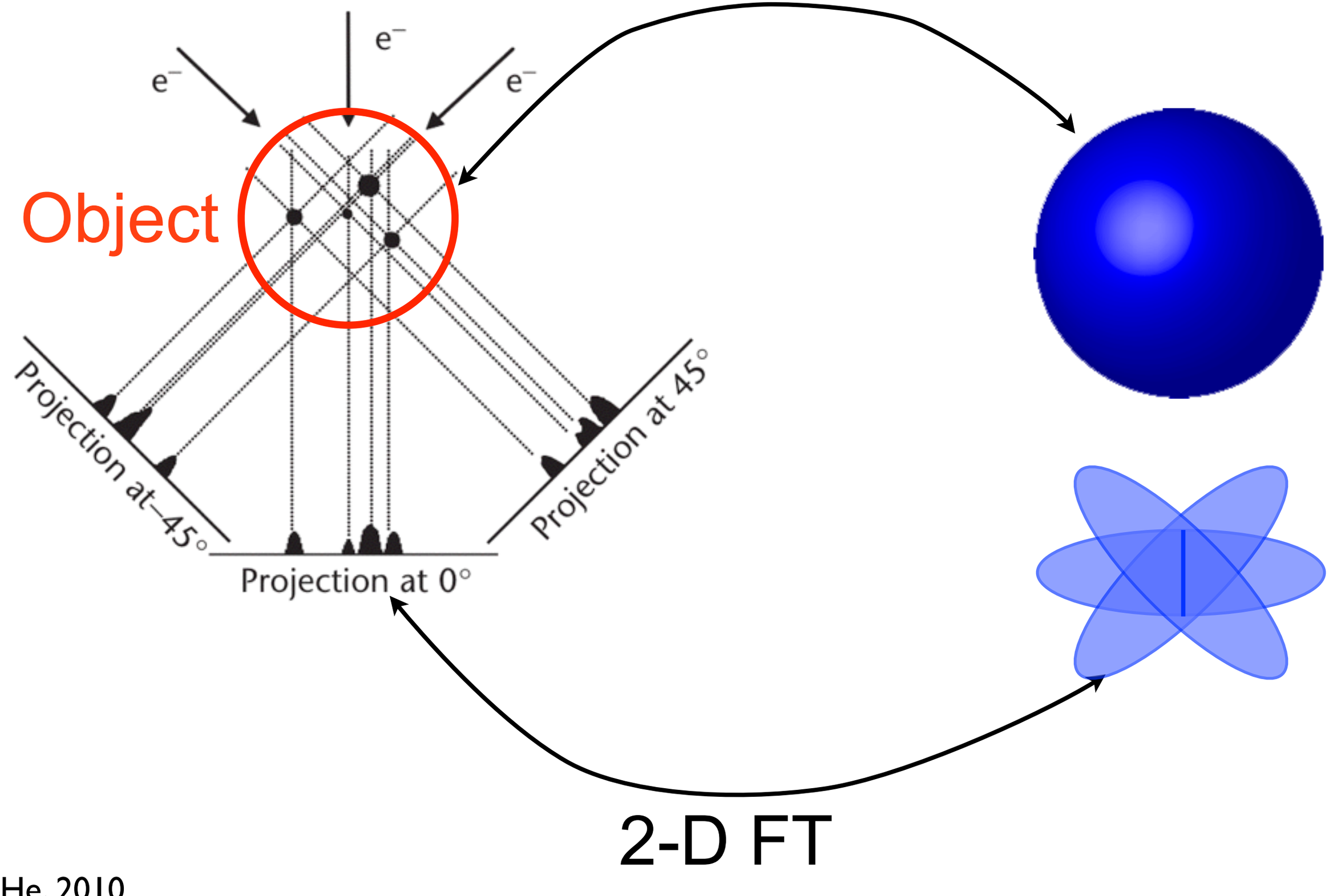
- Record projections along electron-optical (beam) axis
- Goal is to obtain **f(x,y,z)**, i.e., reconstruction/tomogram
- If object is ***n*** dimensional, projection is ***n - 1*** dimensional
- Cryo-EM images are therefore 2-dimensional

3-D reconstruction: multiple “views” are needed



He, 2010

Projection = 2-D slice from 3-D Fourier transform



He, 2010

	Single particle	Tomography
# micrographs	10 to 10,000	40 - 120
# particles	1,000 to 2,000,000+	**ONE**
dose / image	~20 $e^-/Å^2$	*1 $e^-/Å^2$*
final product	“reconstruction” / density map	“tomogram” / density map
resolution	**2.x** - 10 Å	40 - 80 Å

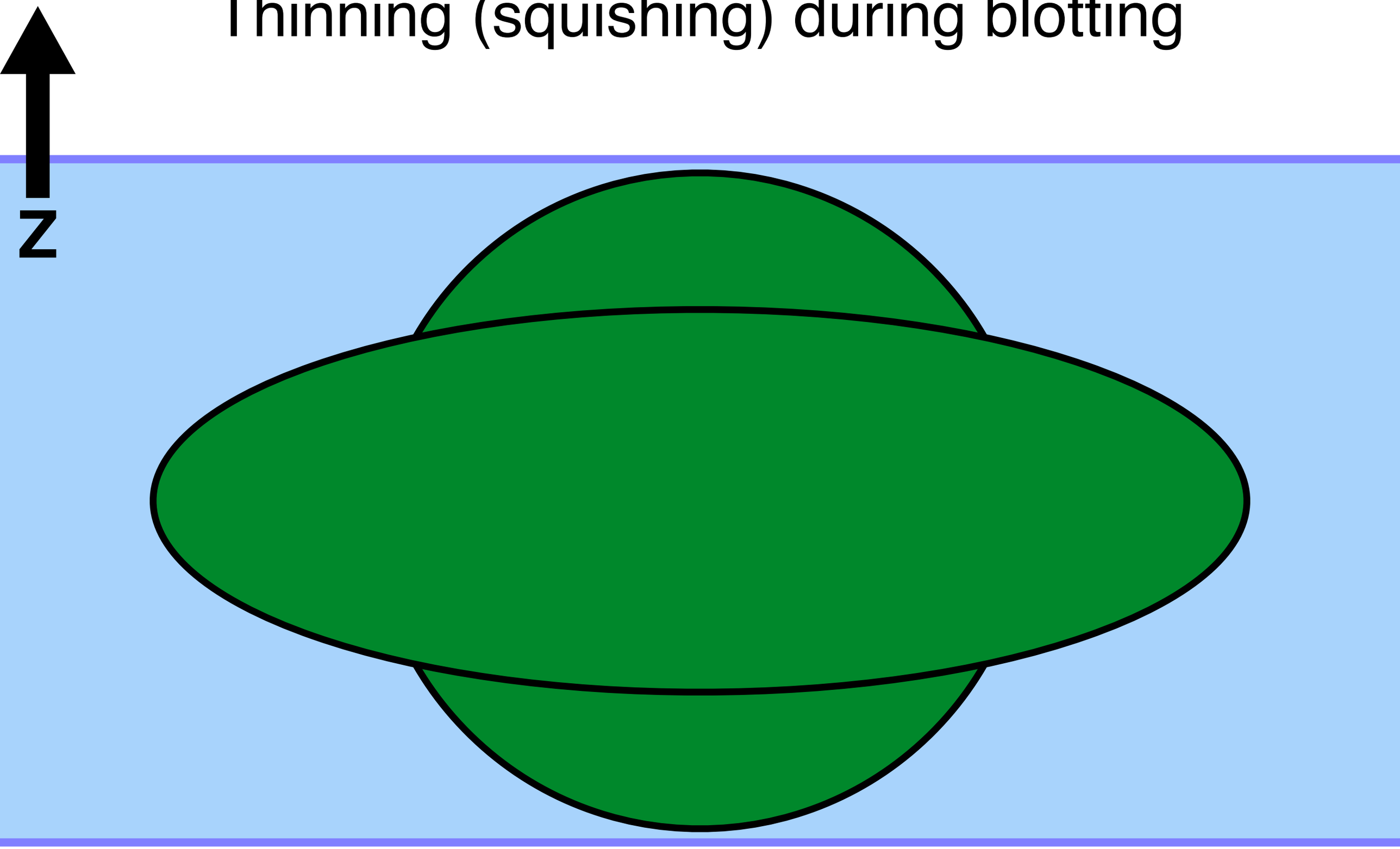
* cumulative dose: >100 $e^-/Å^2$

1. Why cryo-ET?

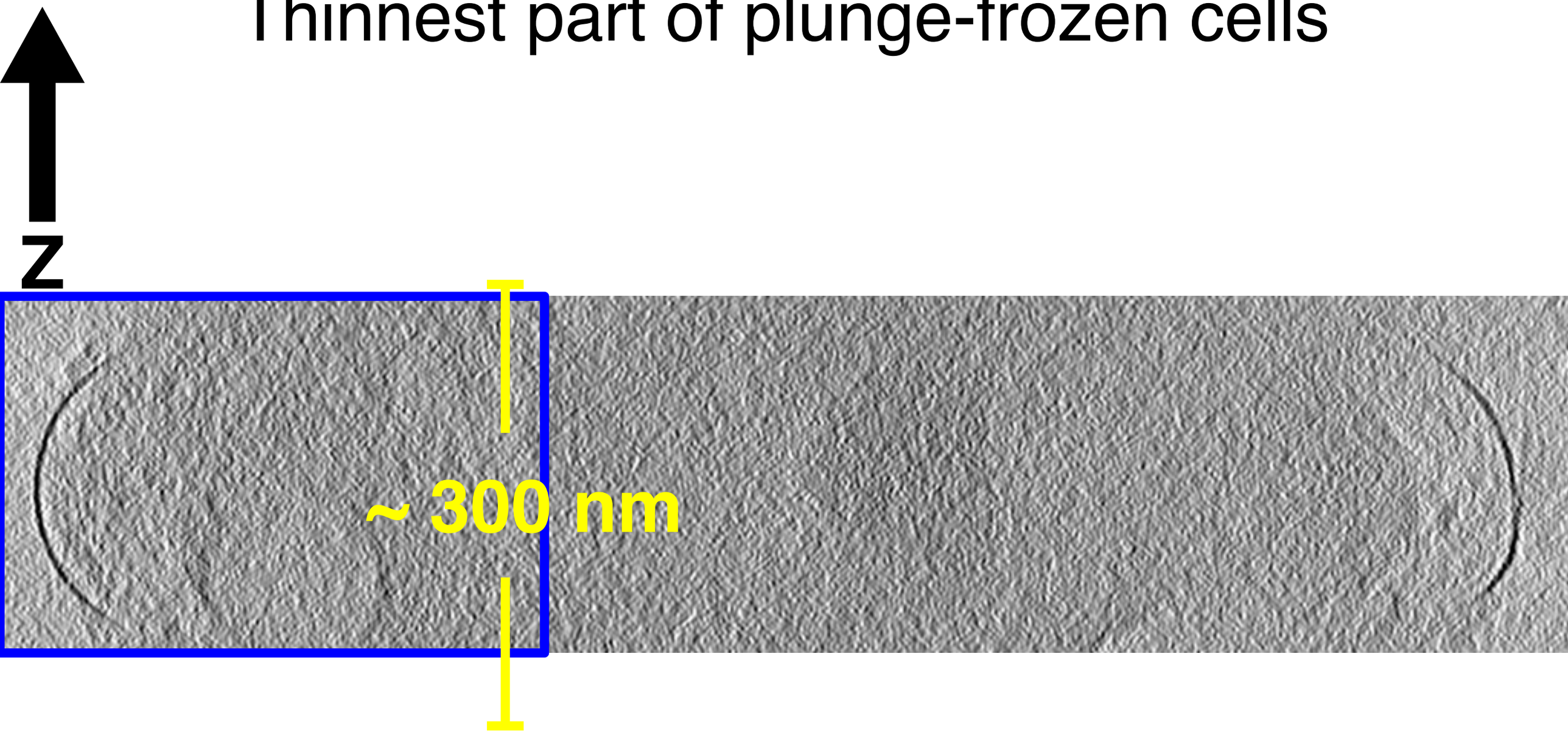
2. Sample preparation

3. Image analysis & examples

Thinning (squishing) during blotting



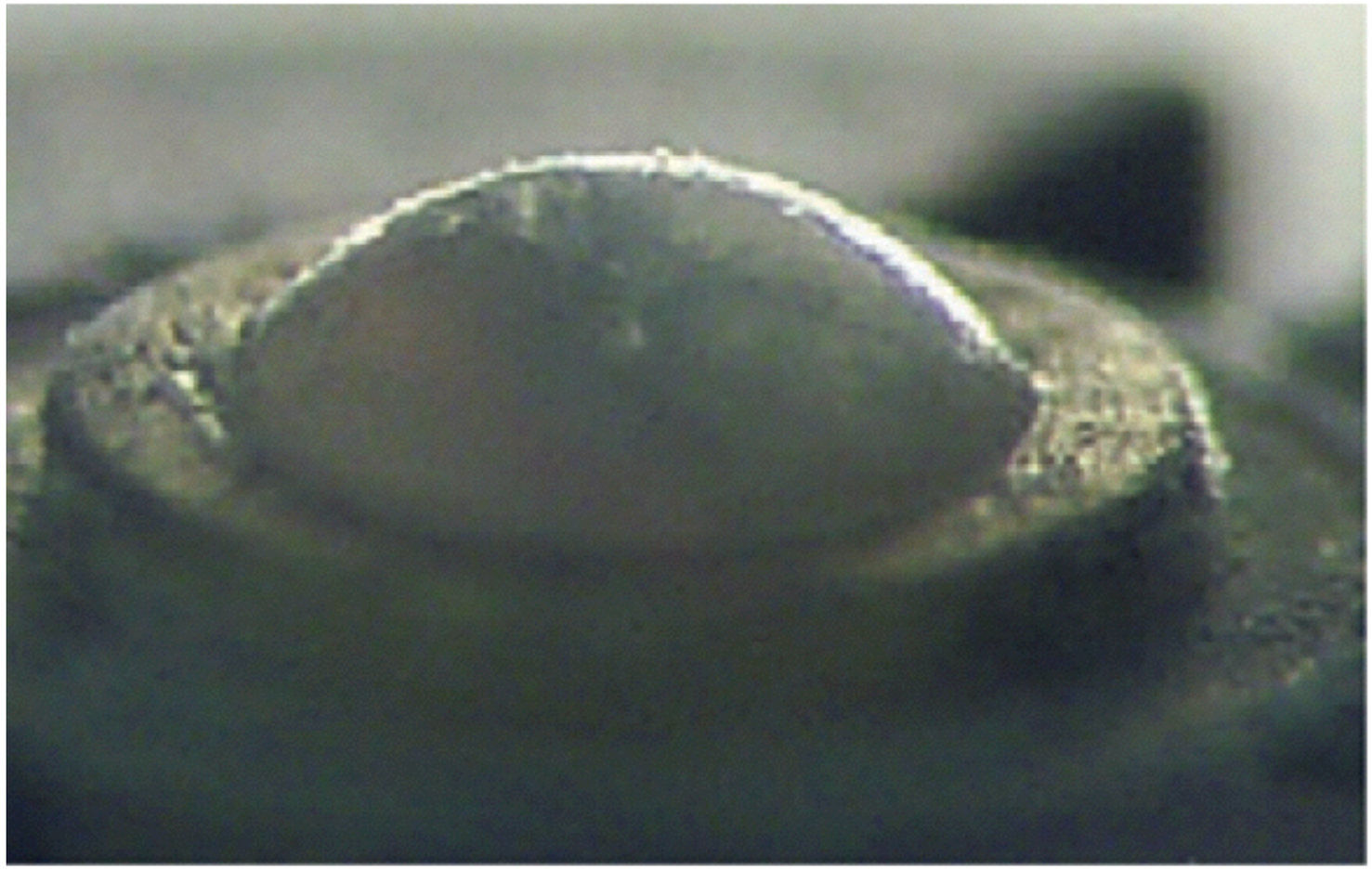
Thinnest part of plunge-frozen cells

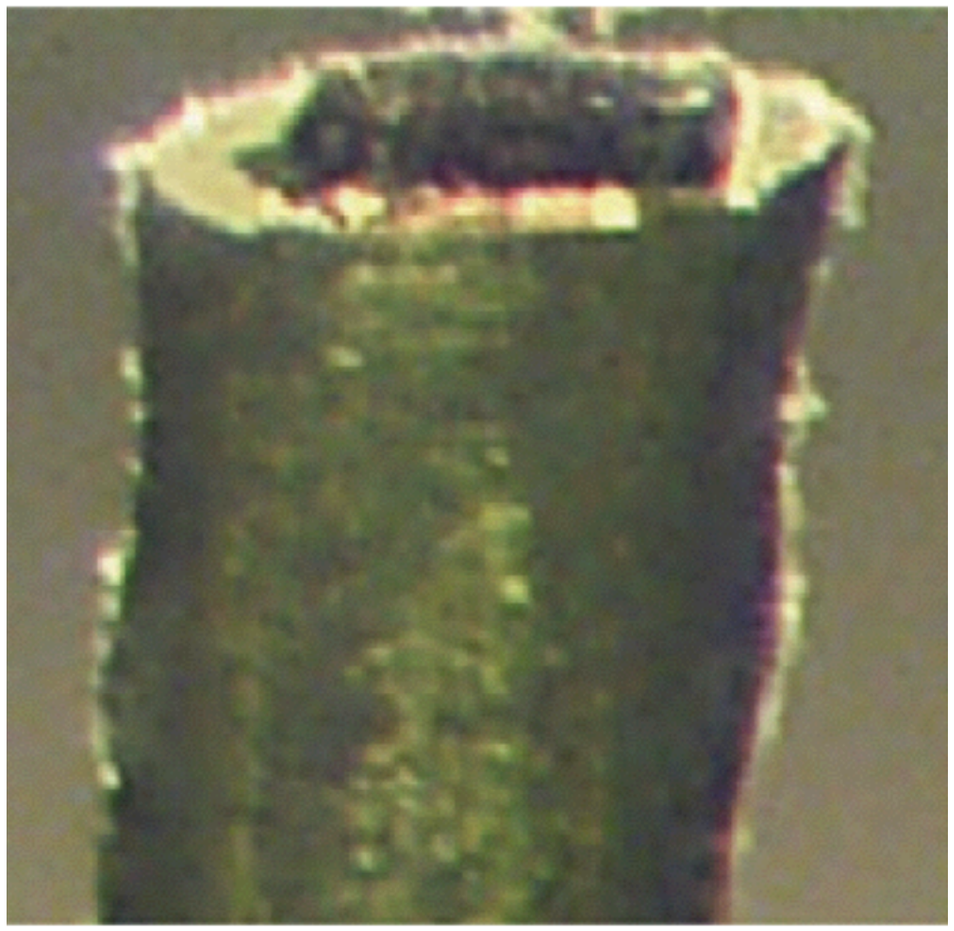


Chen, unpublished

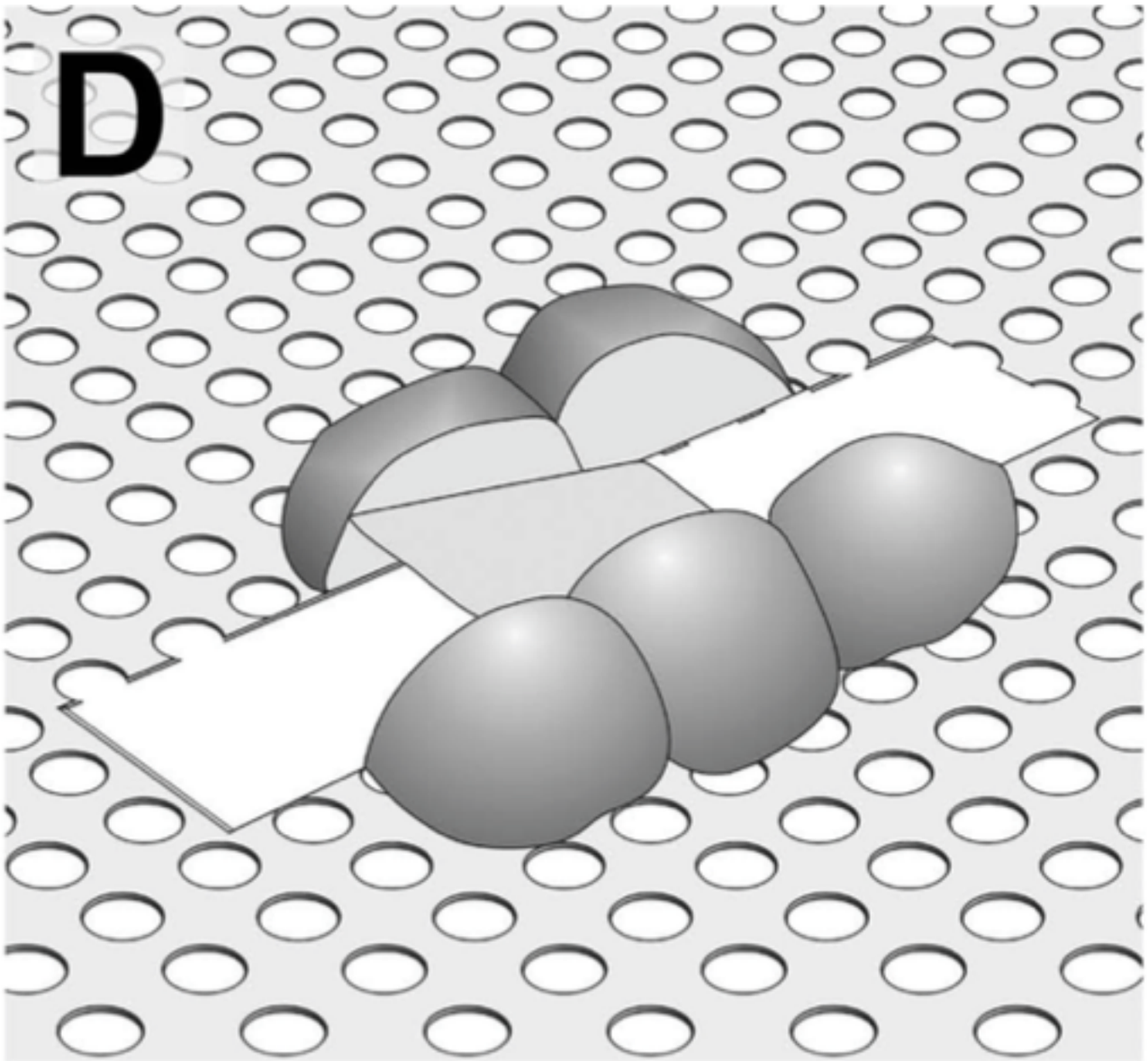
< 200 nm

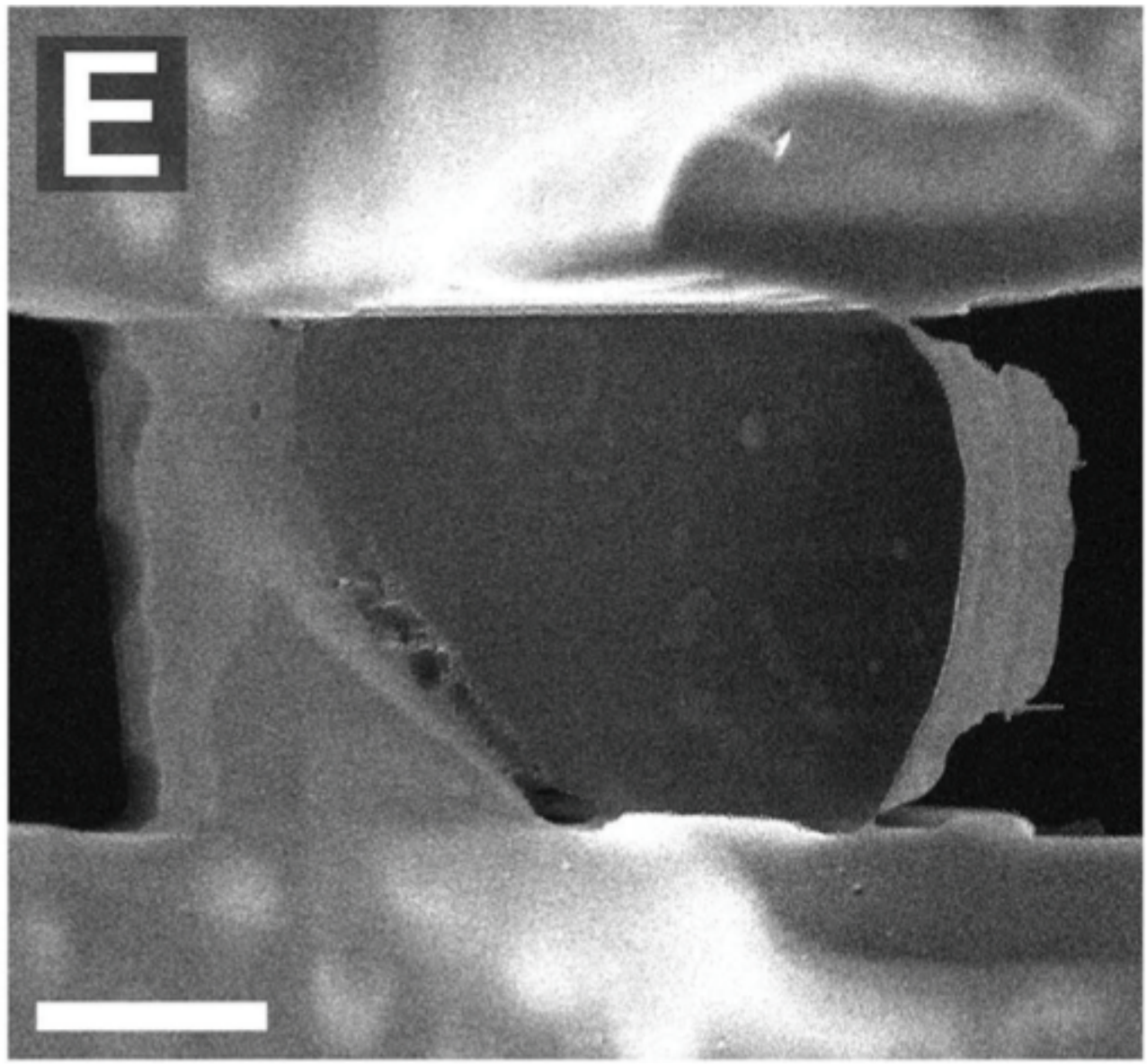
Frozen-hydrated sample, > 100μm thick



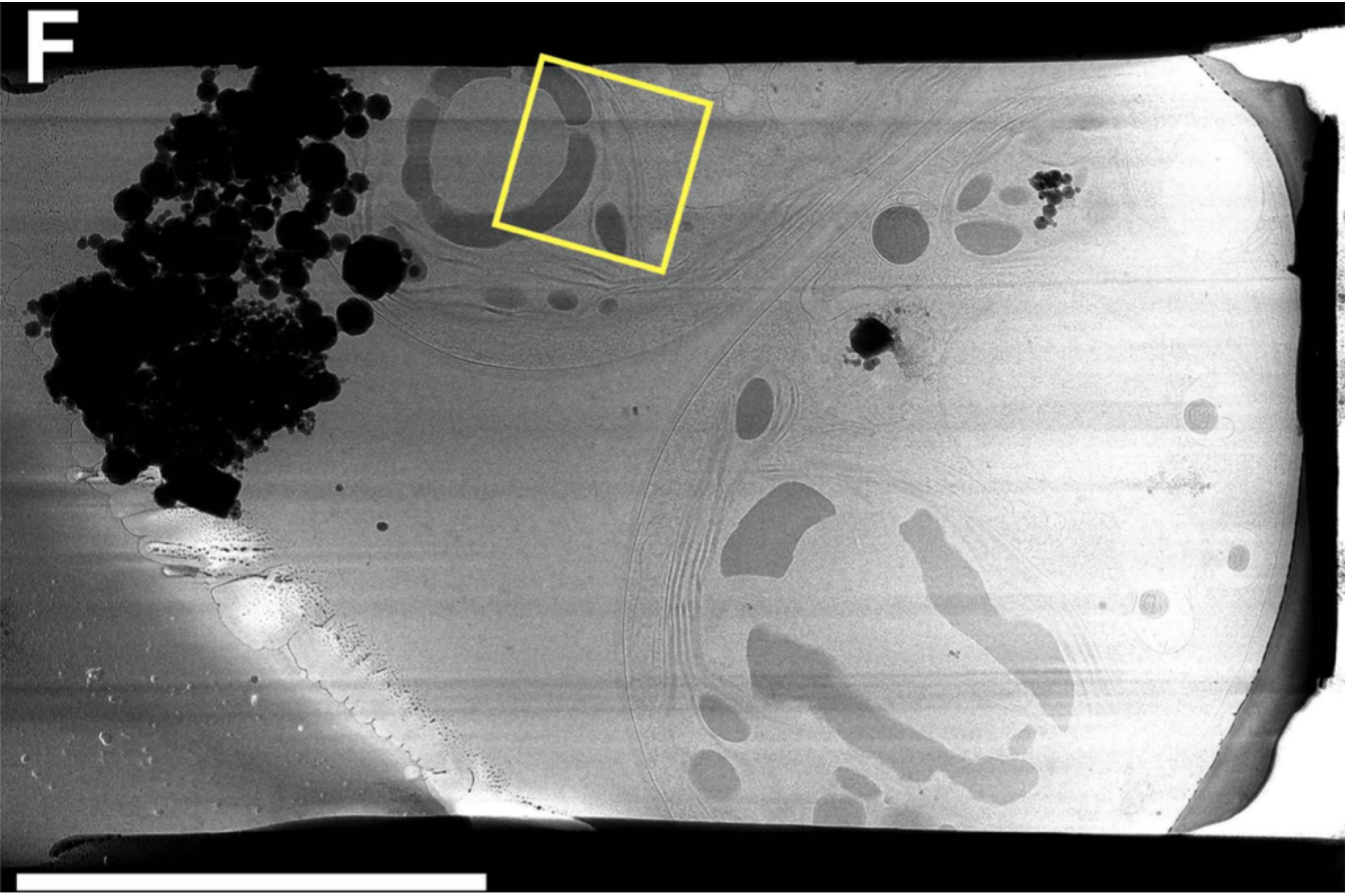


Ladinsky 2010, Meth Enz 481, p165 Fig2

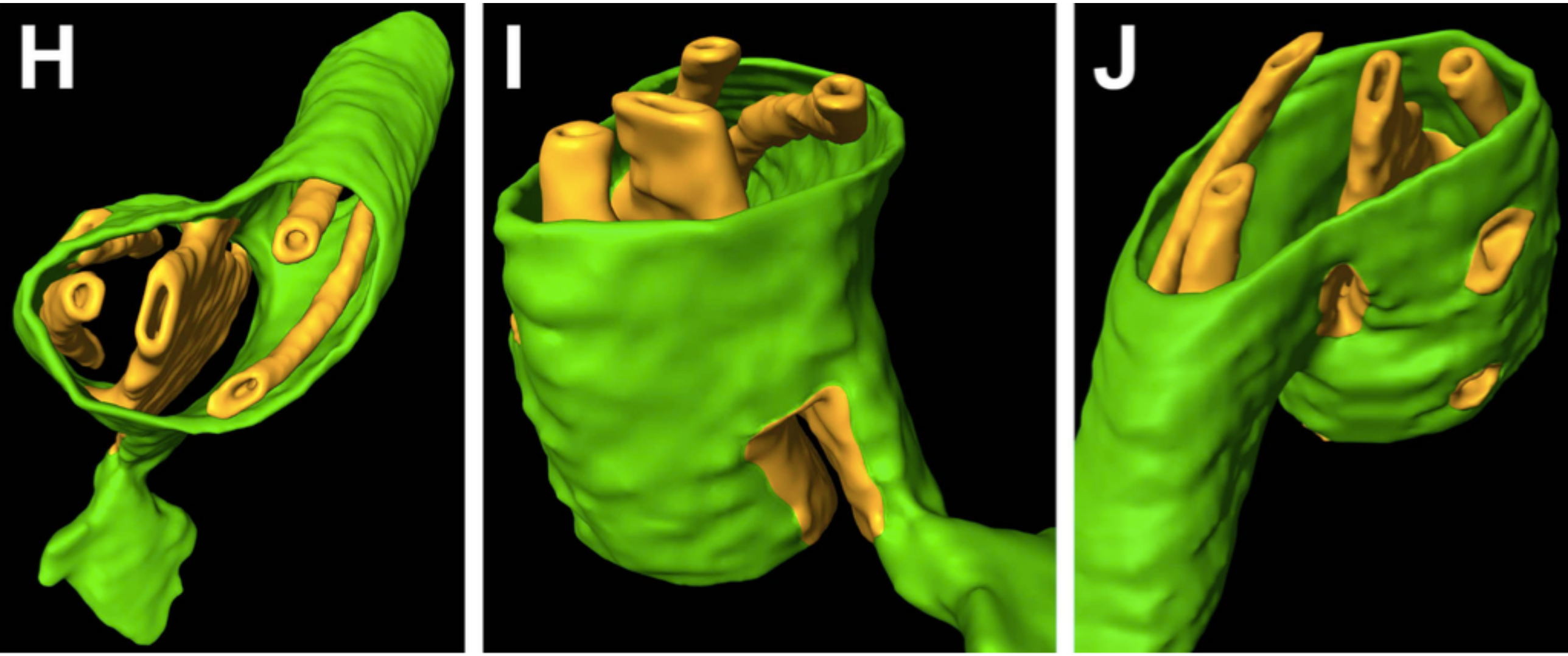




Engel, eLife 2015;4:e04889 Fig 1



Engel, eLife 2015;4:e04889 Fig 1



Engel, eLife 2015;4:e04889 Fig 11

Challenge: lots of images are needed for one tomo

The Crowther criterion:

$$m \sim \pi * D / d$$

m = number of images

D = object's diameter

d = resolution

Crowther 1970, Proc R Soc Lon A 317, p319

How to use the Crowther criterion

A bacterial cell is ~ 500 nm thick
Desired (realistic resolution) ~ 10nm
m ~ 157 images, **distributed over 180°**

∴ the tilt *increment* should be ~ 0.9°

In practice, microscopists don't follow this rule exactly. They determine imaging parameters empirically for each sample.

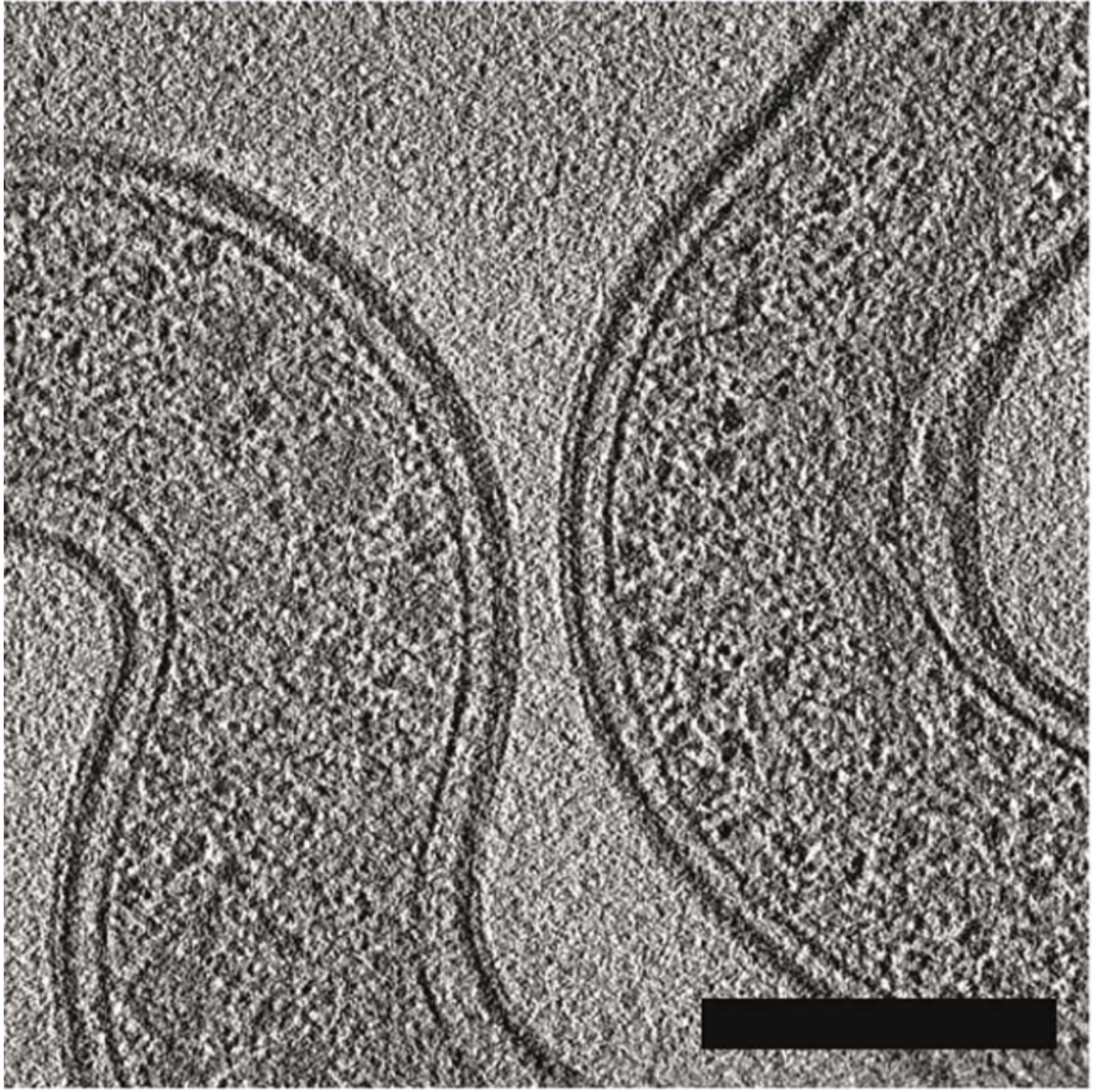
Crowther 1970, Proc R Soc Lon A 317, p319

Lecture outline

1. What is ET?

2. Sample considerations

3. Example studies



Beck 2009, Nat. Meth. 6, p817 Fig 4

ATP synthase

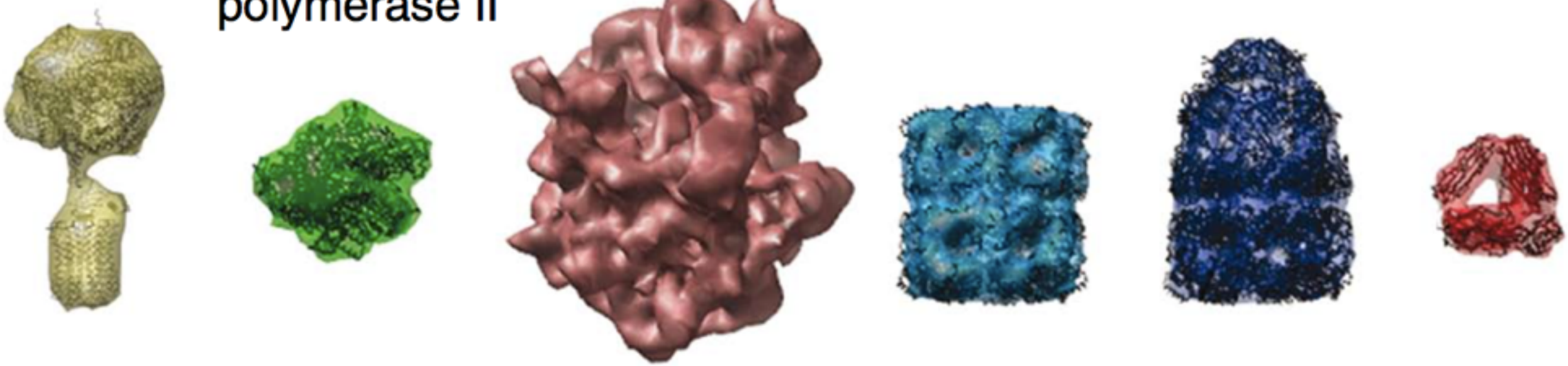
RNA polymerase II

Ribosome

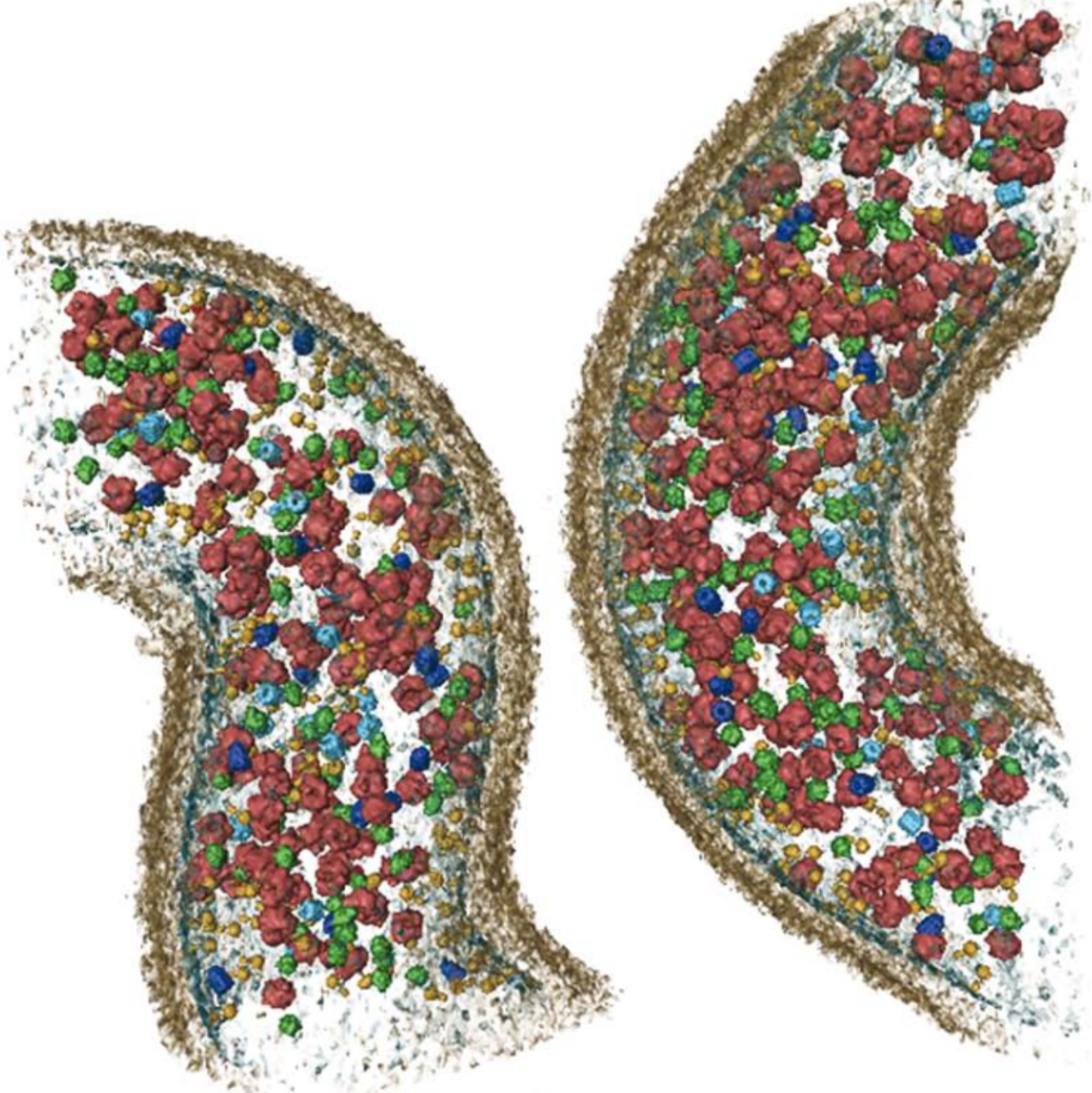
GroEL

GroEL-GroES

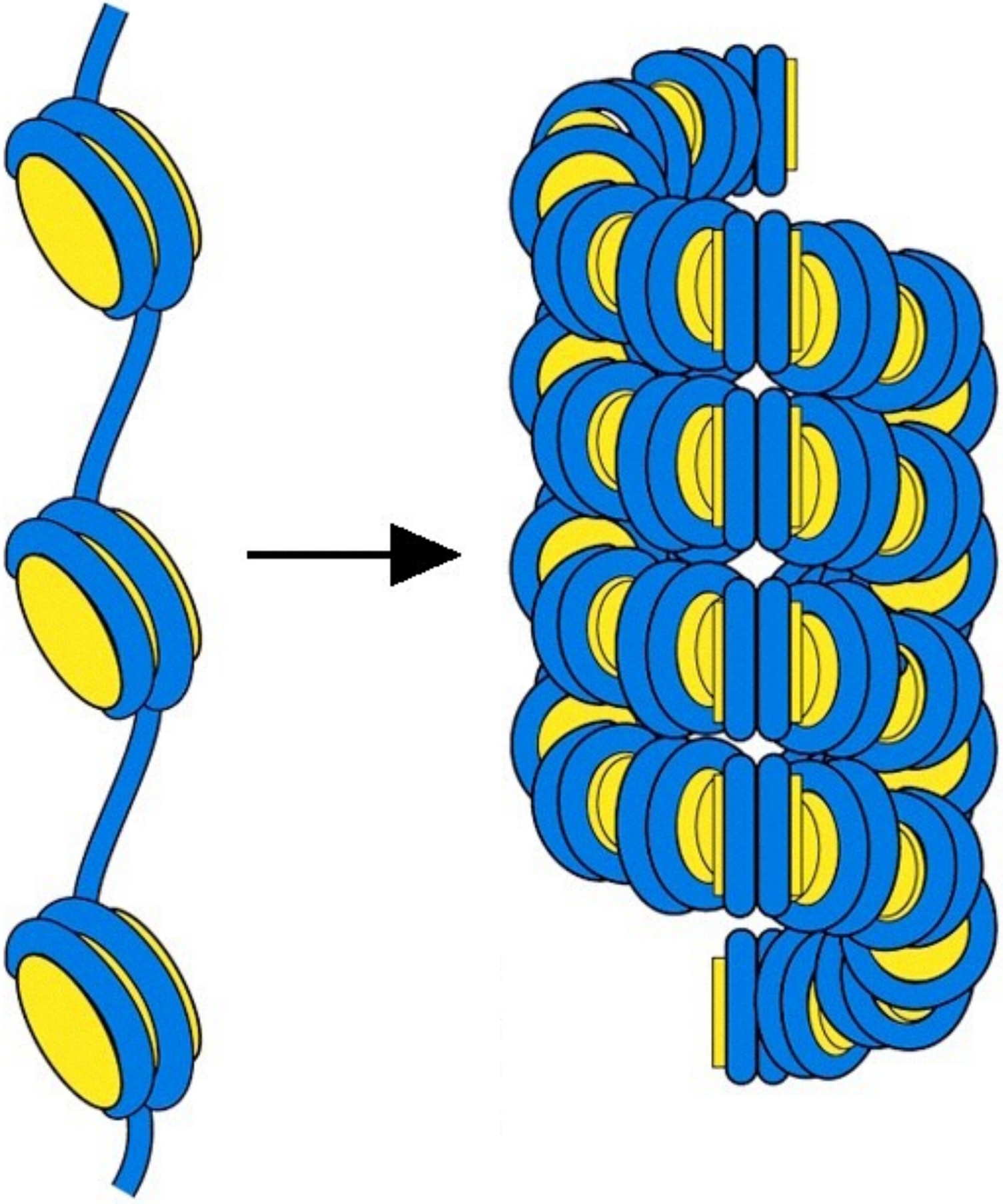
Hsp15



Beck 2009, Nat. Meth. 6, p817 Fig 4

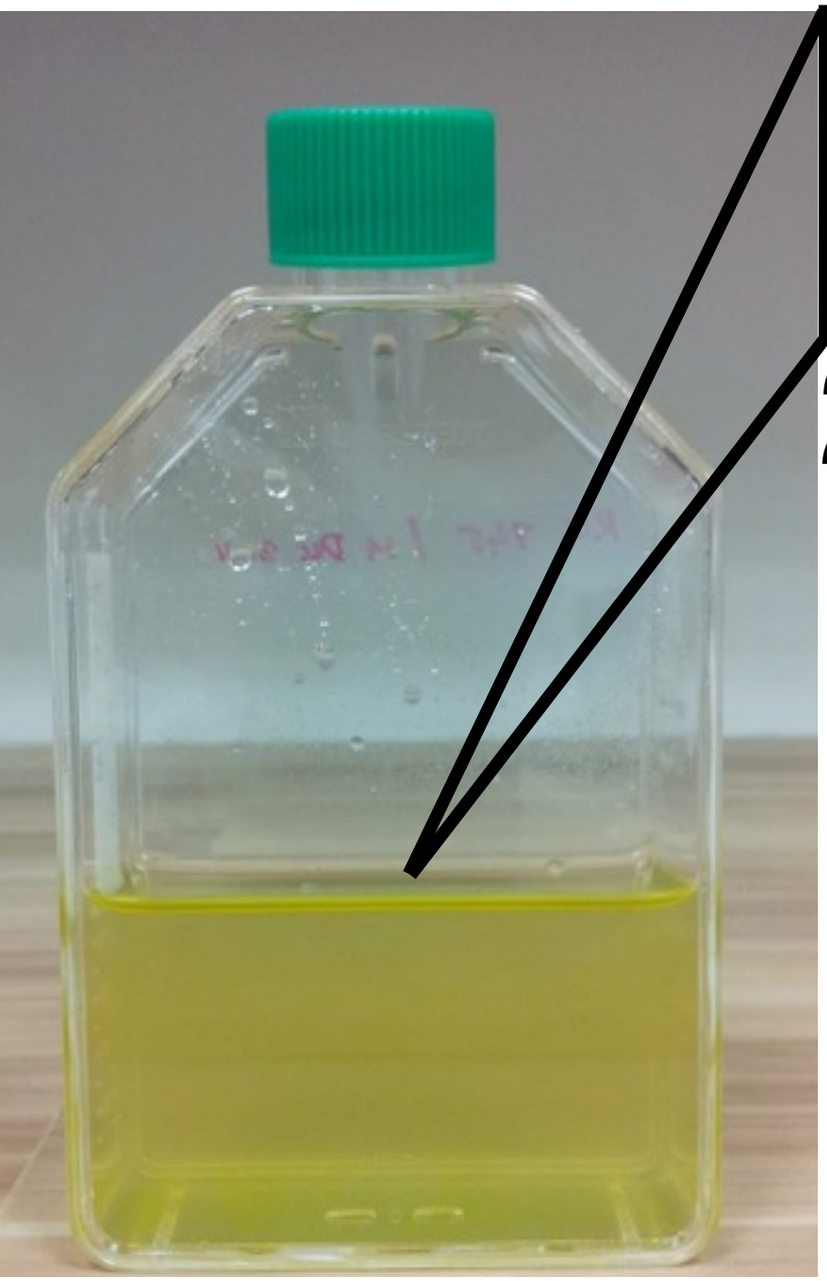


Beck 2009, Nat. Meth. 6, p817 Fig 4



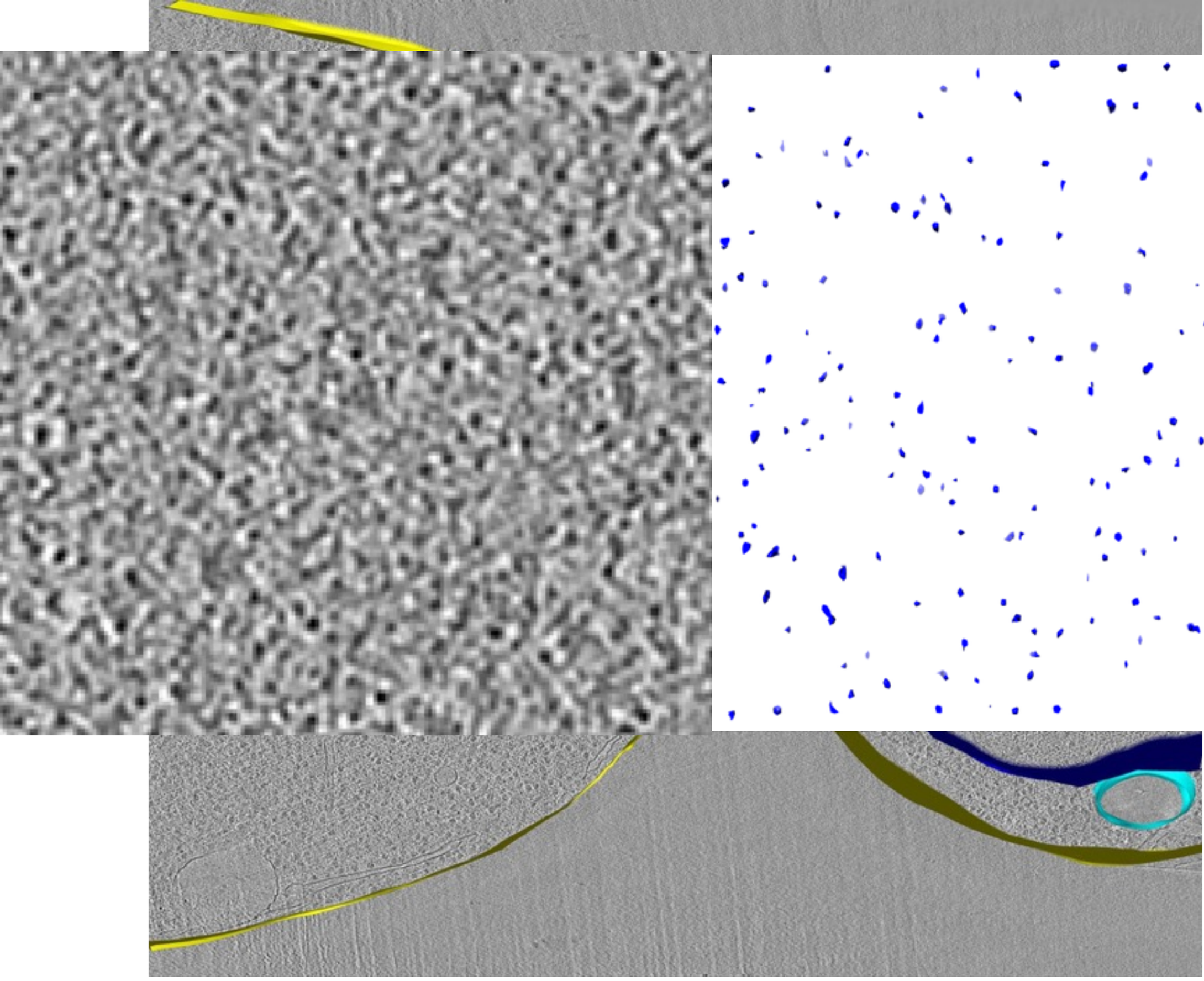
Maeshima, 2010

Ostreococcus



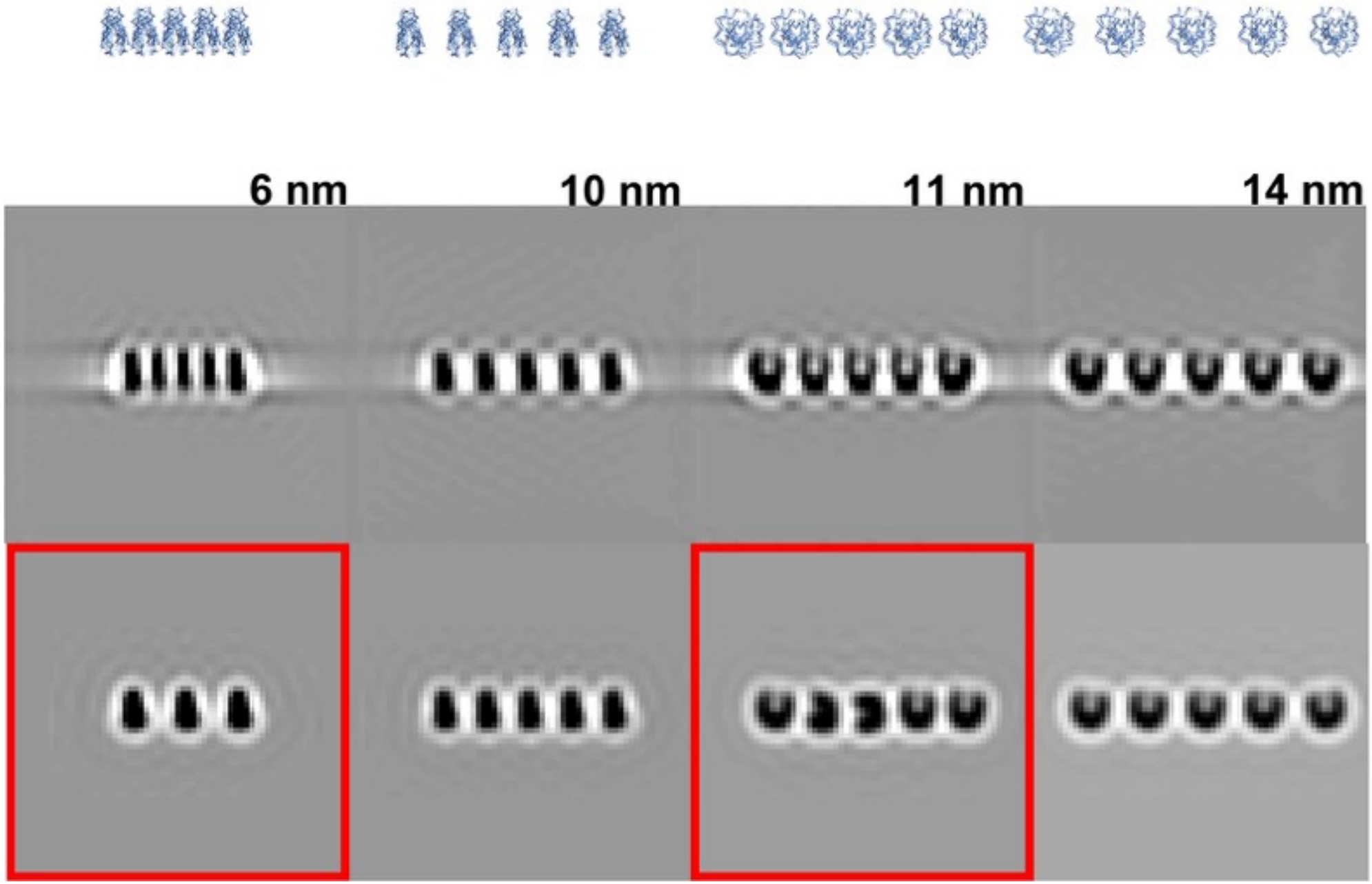
Saccharomyces

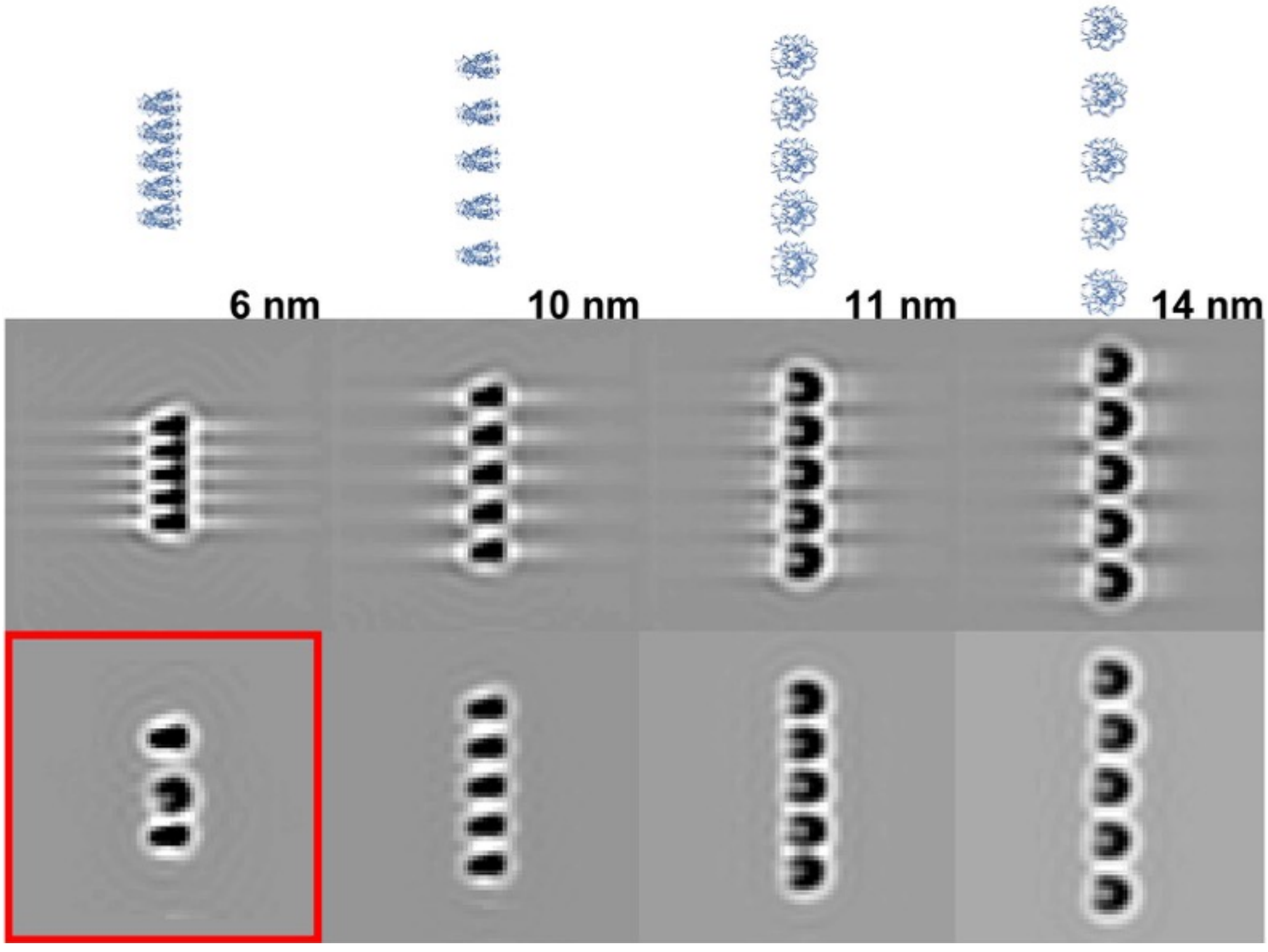
System	Fiber	Paper
Sperm	Yes	Woodcock, 1994
Erythrocyte	Yes	Scheffer, 2011
Frog *in vitro*	Yes	Konig, 2007
'601' (artificial)	Yes	Robinson, 2006
CHO	No	McDowall, 1986
HeLa	No	Eltsov, 2008
Mouse somatic	No	Fussner, 2012
Picoplankton	No	Gan, 2013

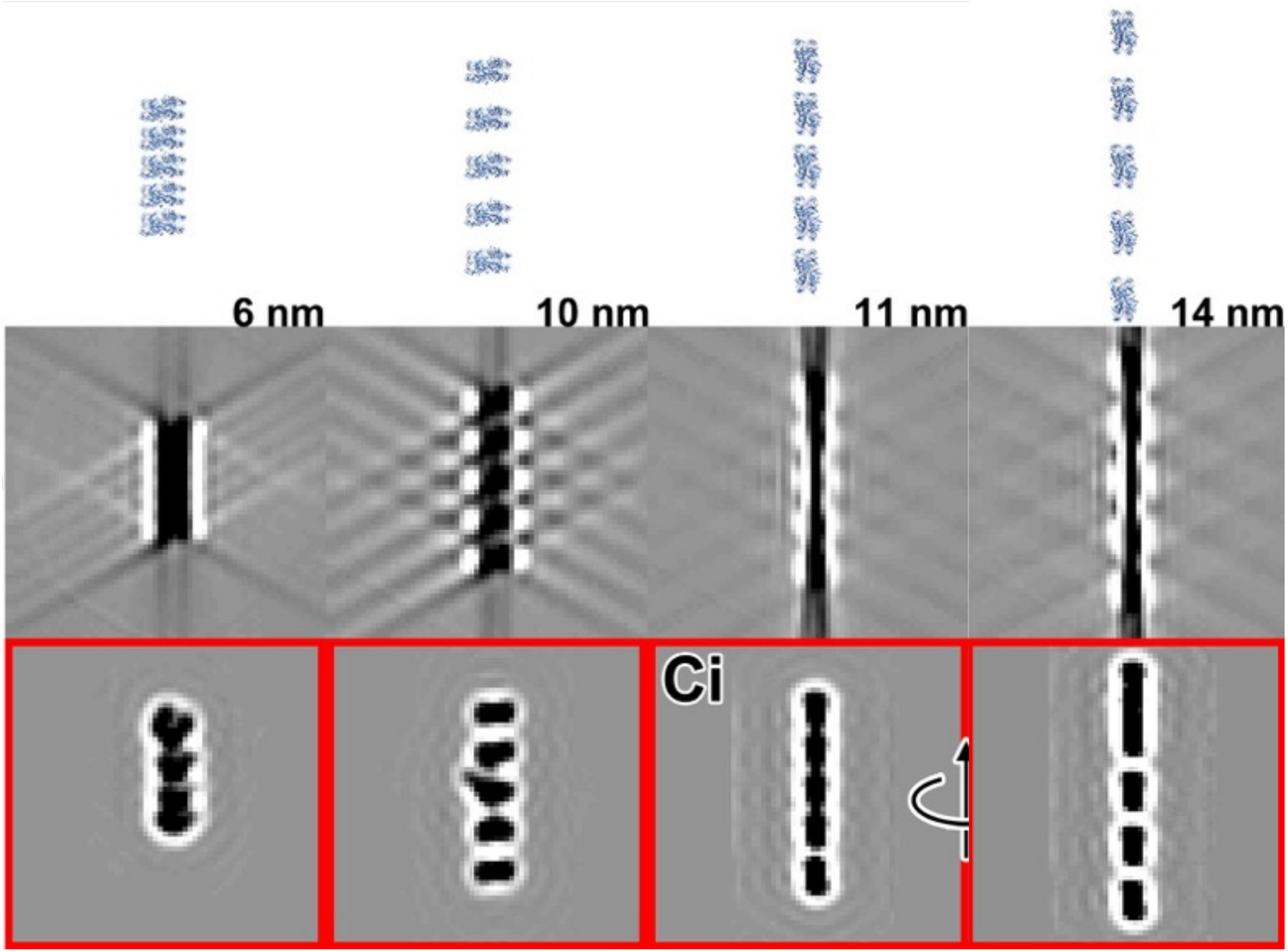


Chen, unpublished

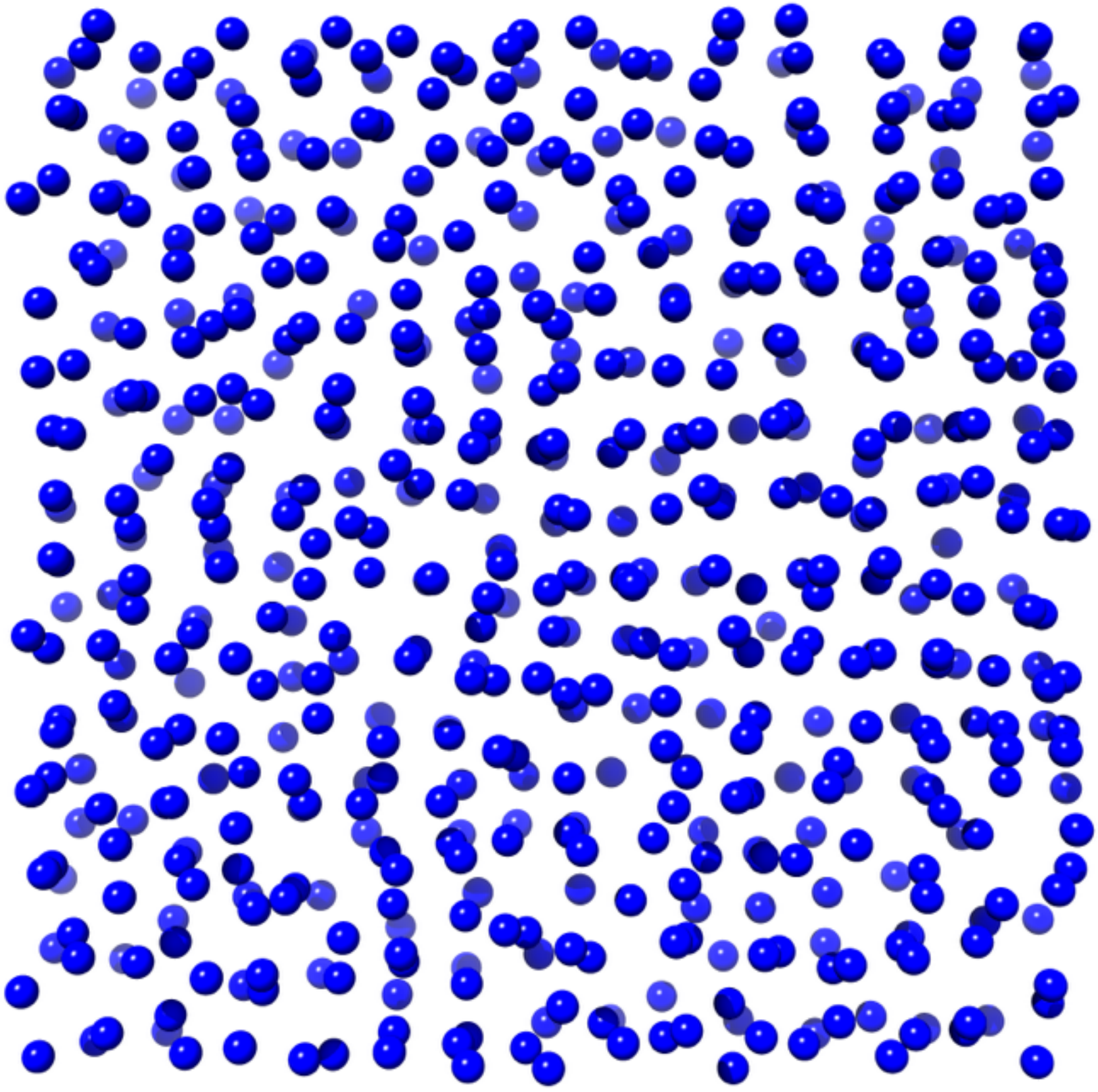
First, a control




6 nm
10 nm
11 nm
14 nm


6 nm
10 nm
11 nm
14 nm
Ci

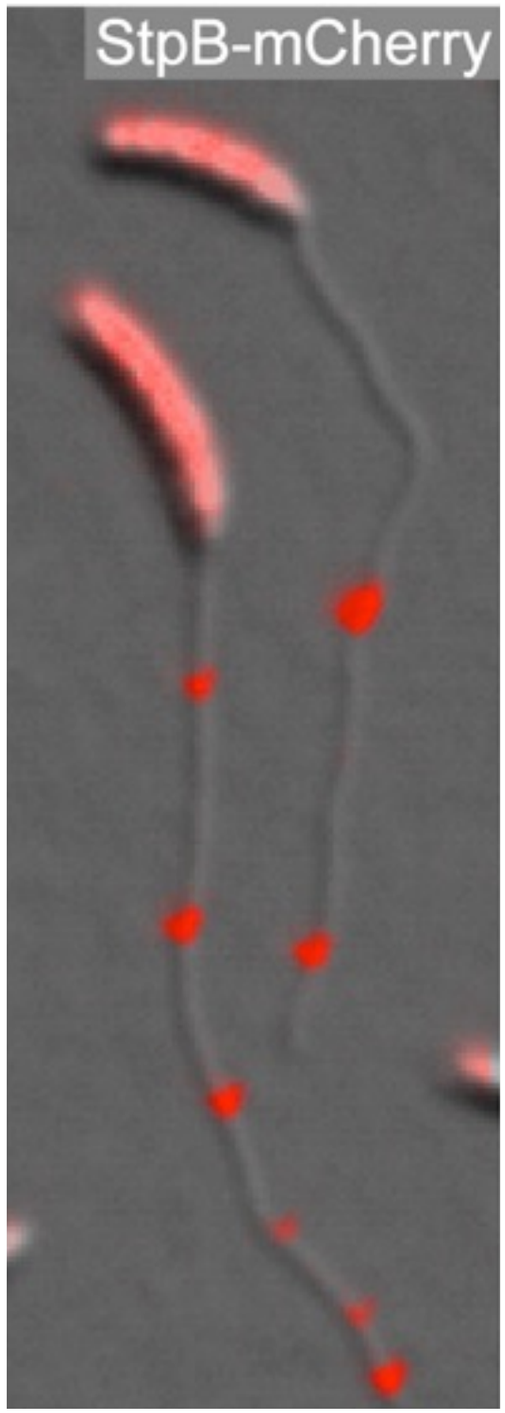
Raw data will be released on EMPIAR

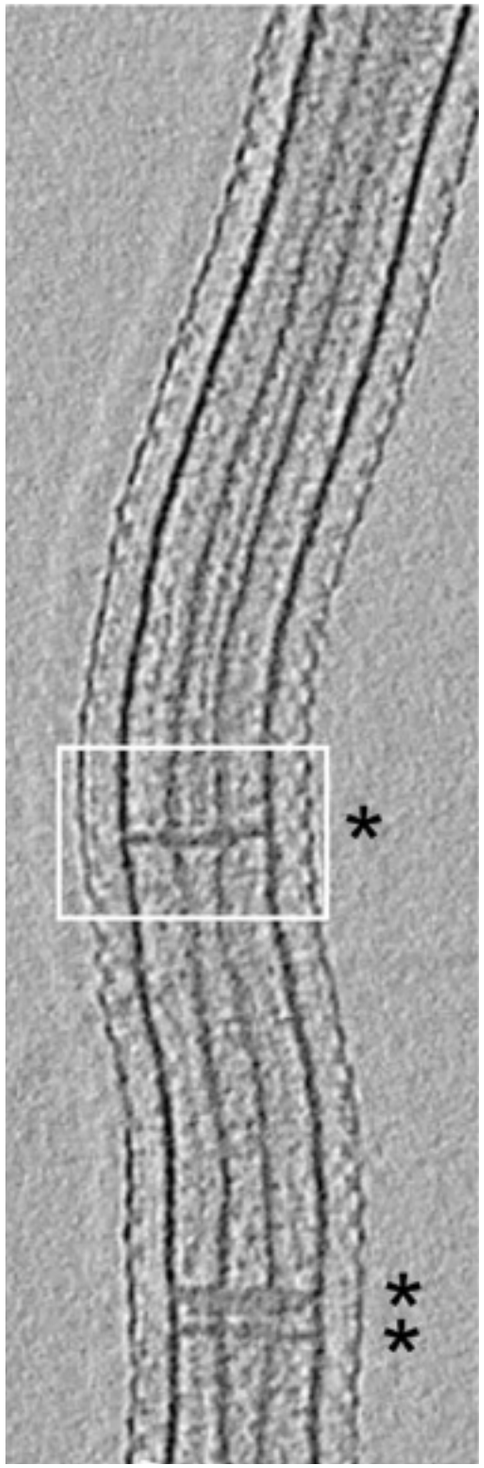


Chen, unpublished

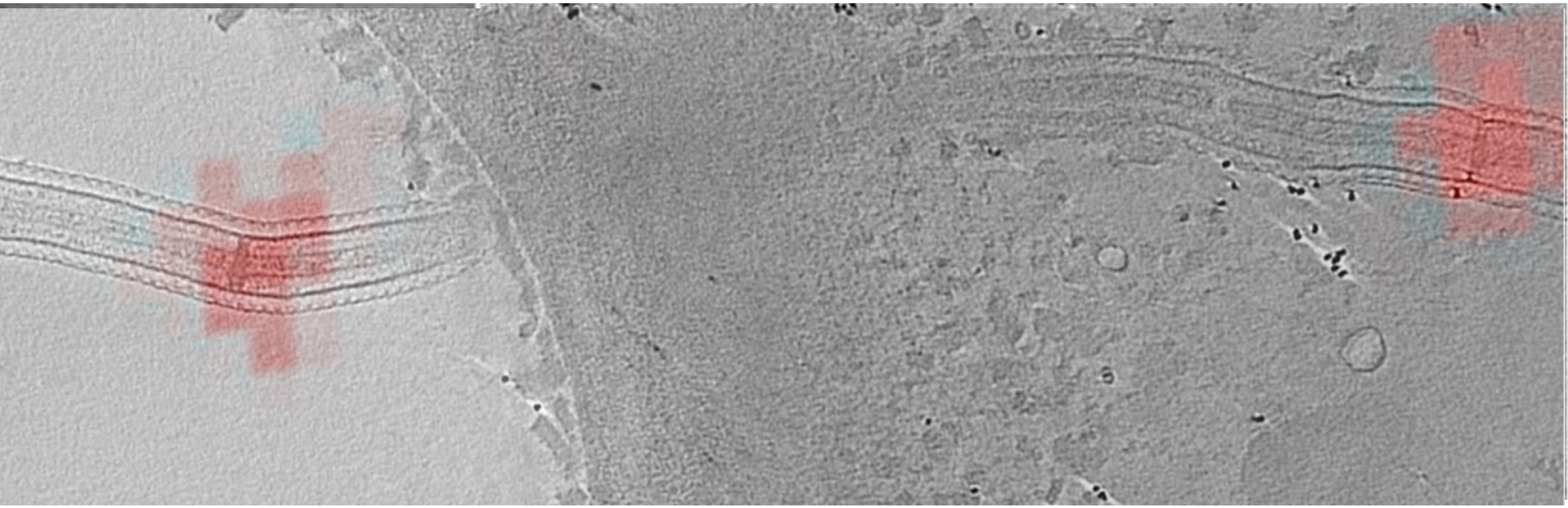
No evidence of 30-nm fibers in budding yeast

Example: periplasmic + membrane diffusion barrier

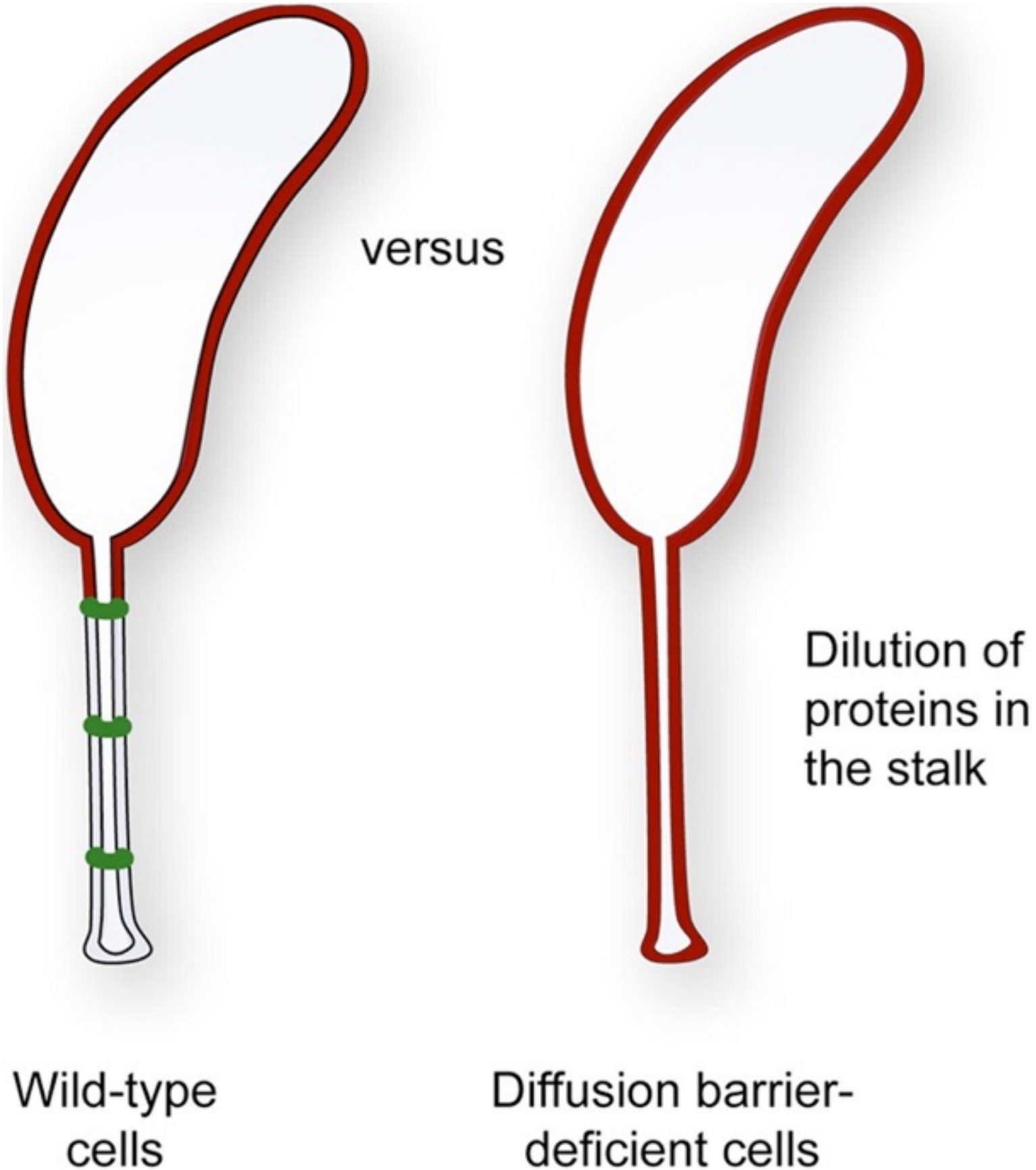




Schlimpert 2012, Cell 151, p1270 Fig3



Schlimpert 2012, Cell 151, p1270 Fig3



Schlimpert 2012, Cell 151, p1270 Fig3

Data collection:

- FEI Tomo
- JADAS
- Leginon
- TOM^2 Toolbox
- UCSF Tomo

Data processing:

- BSoft
- EMAN
- IMOD
- ProTomo
- SPIDER
- TOM^2 Toolbox

For a complete list:
en.wikibooks.org/wiki/Software_Tools_For_Molecular_Microscopy

Advice for EM students

- Learn Linux; do NOT rely on Windows/Mac
- Learn how to script in shell and code in python
- New software: work through tutorial first
- Understand the theory as best as you can
- When possible, do “wet” and/or “dry” controls
- Ask questions in ccpem/3dem/IMOD mailing list