Cryo-ET Basics

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Lecture outline

1. What is tomography?

2. Sample preparation (what kind?)

3. Principles of reconstruction

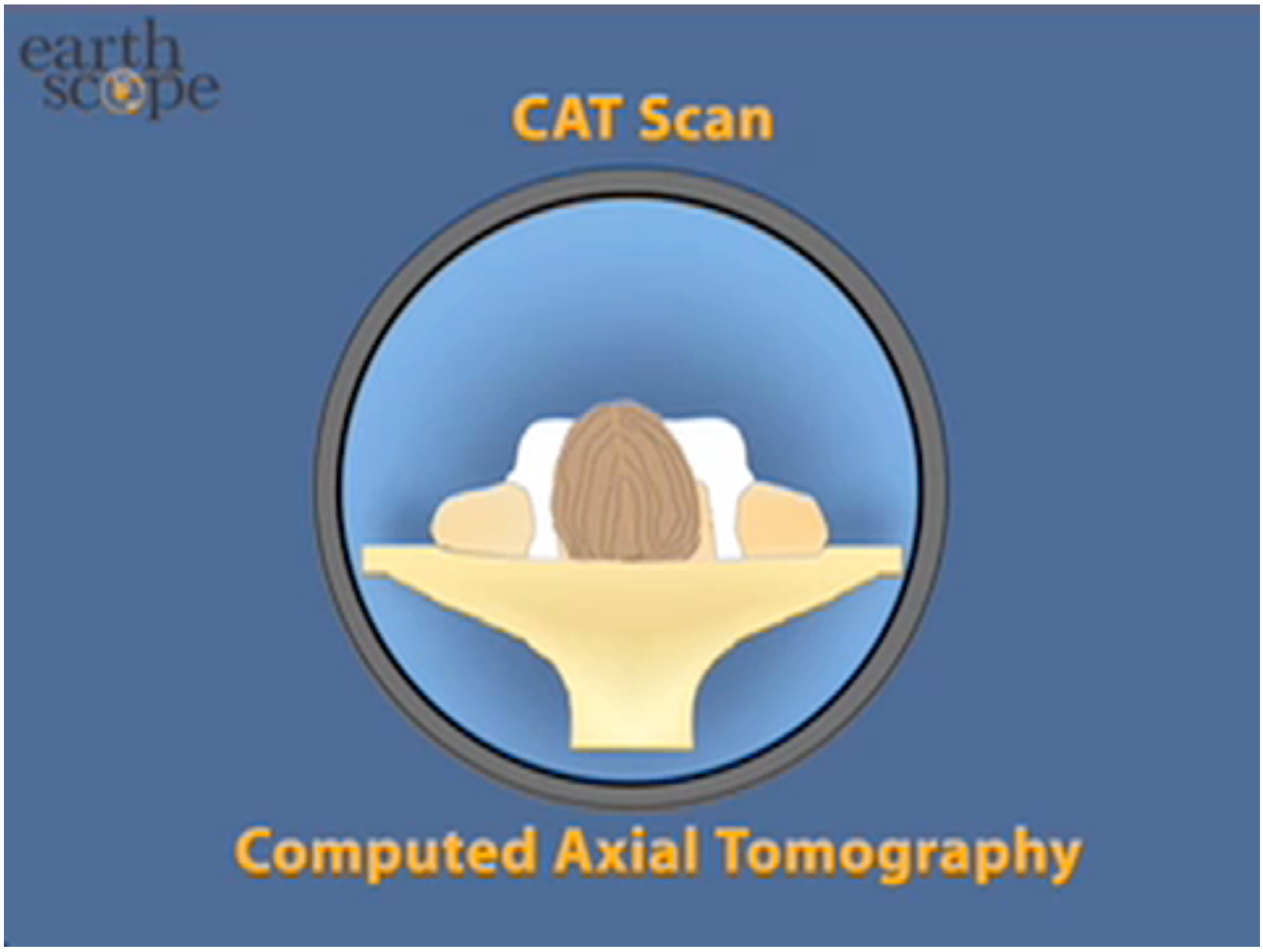
4. Beware of artifacts

5. Example studies

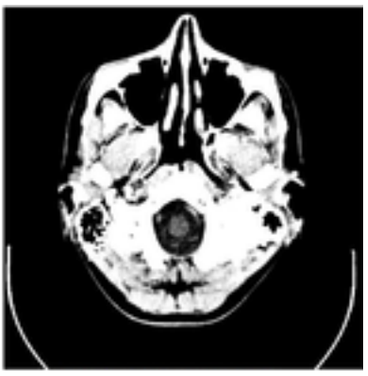
What is tomography?

τέμνειν: to cut (Greek)

Cut, in the virtual sense

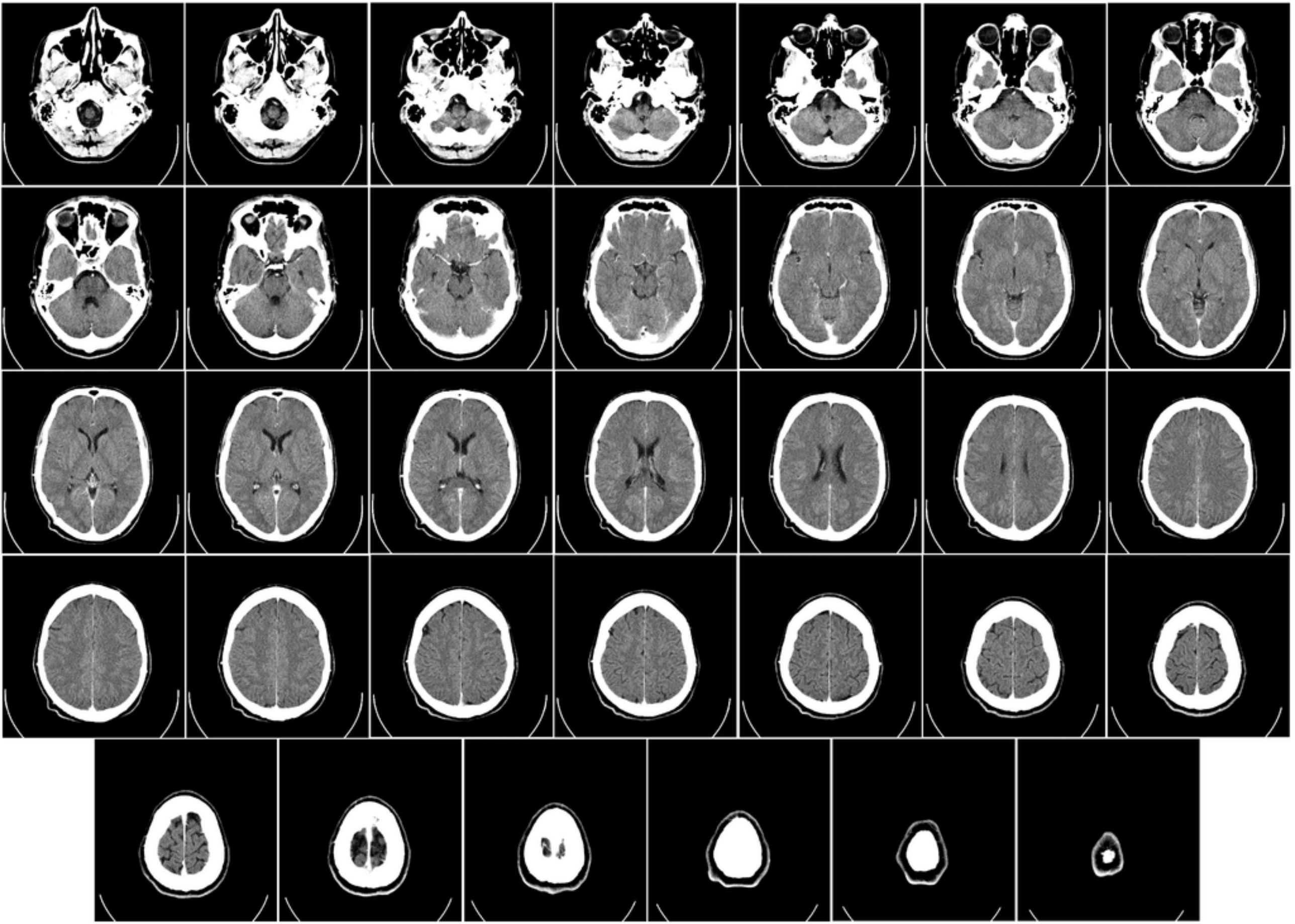


http://www.iris.edu/hq/programs/education_and_outreach/animations/7



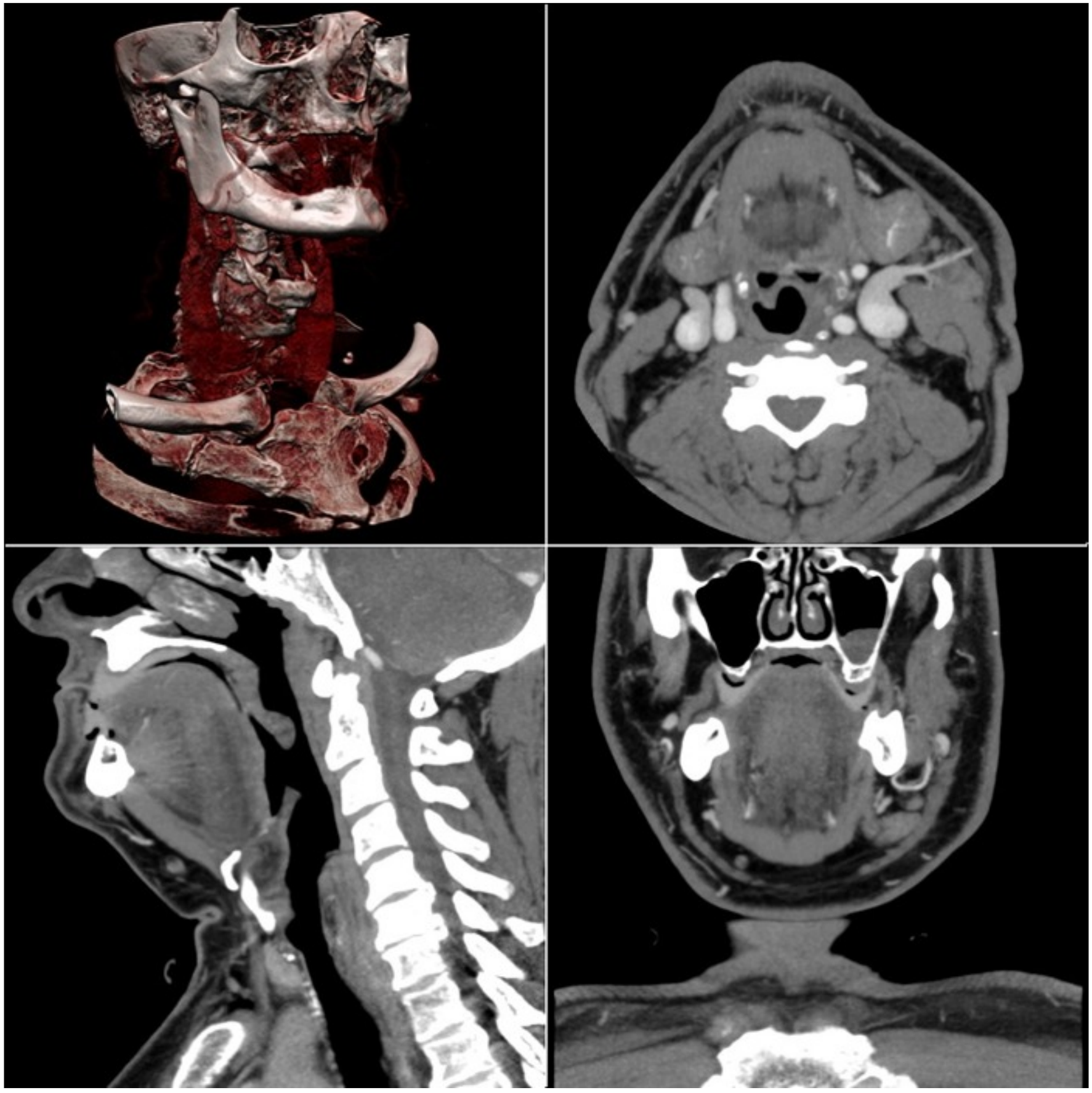
Wikipedia

Tuesday, July 10, 12



Wikipedia

Tuesday, July 10, 12



Wikipedia

Tuesday, July 10, 12

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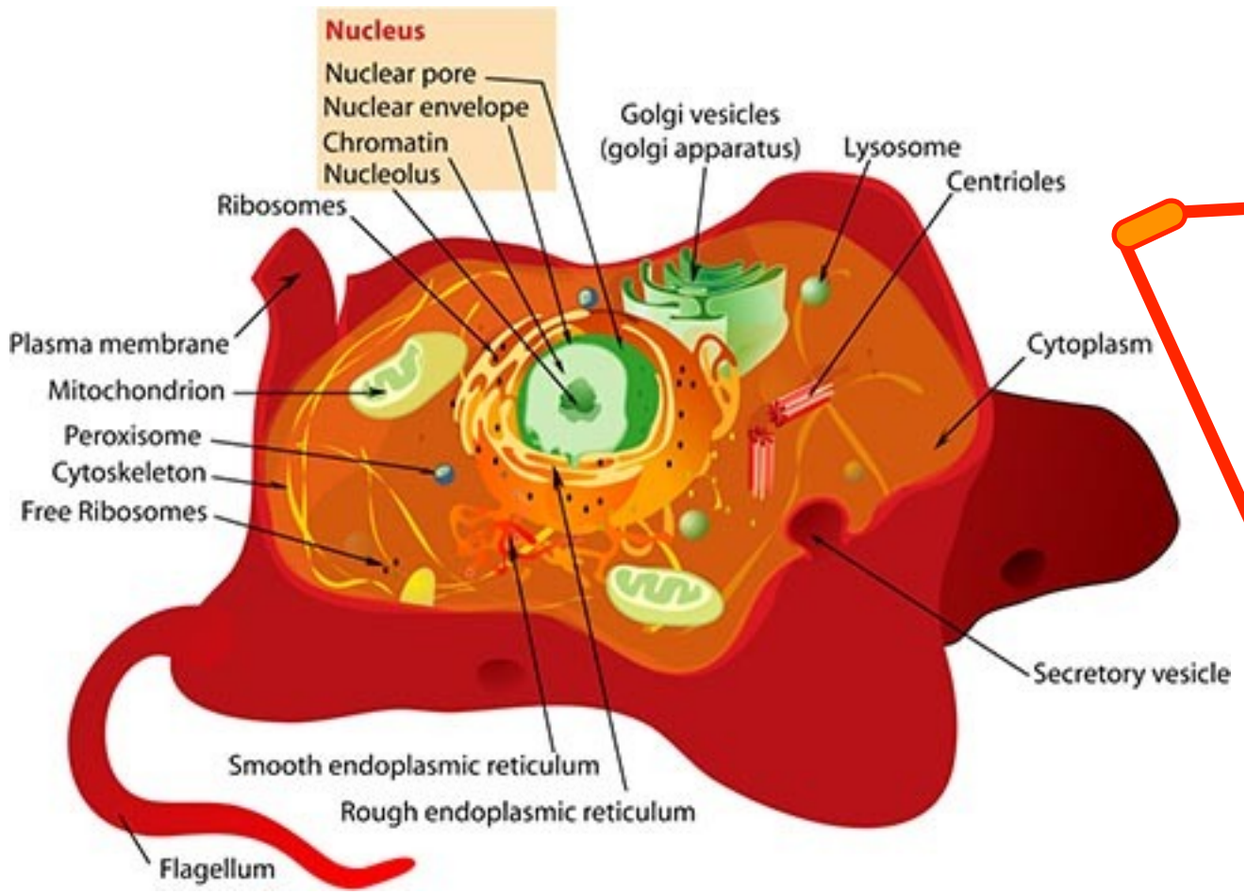
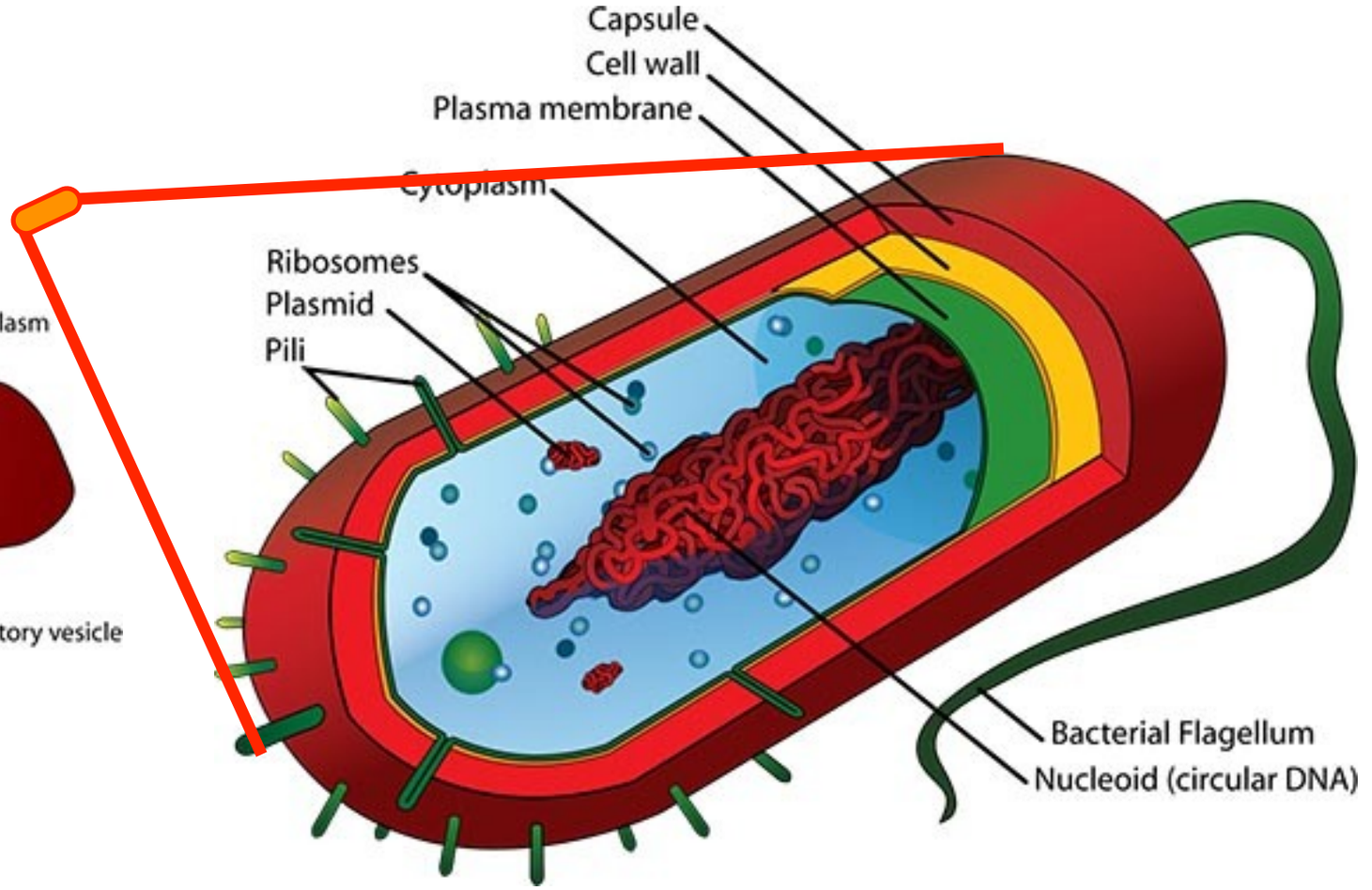
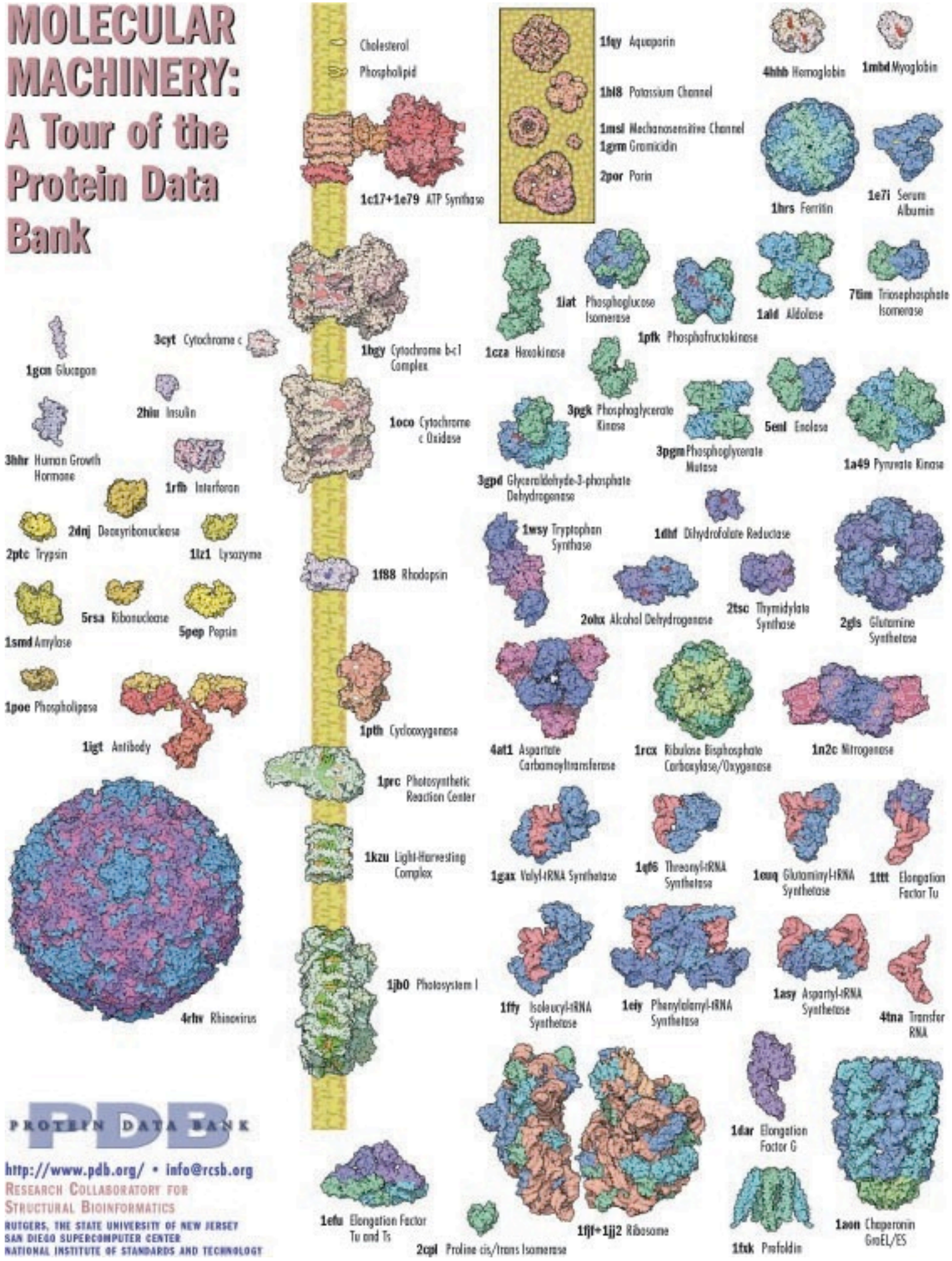


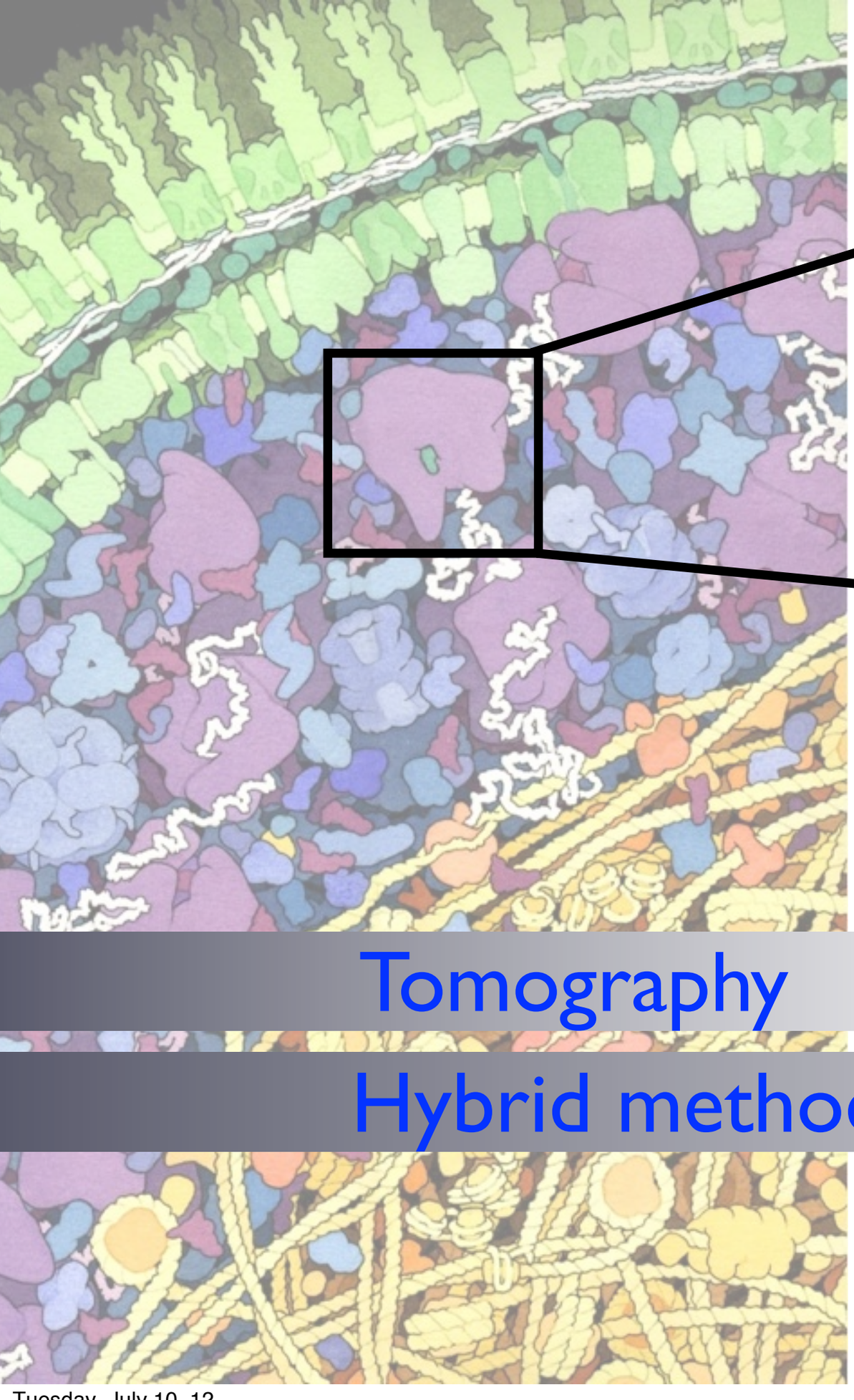


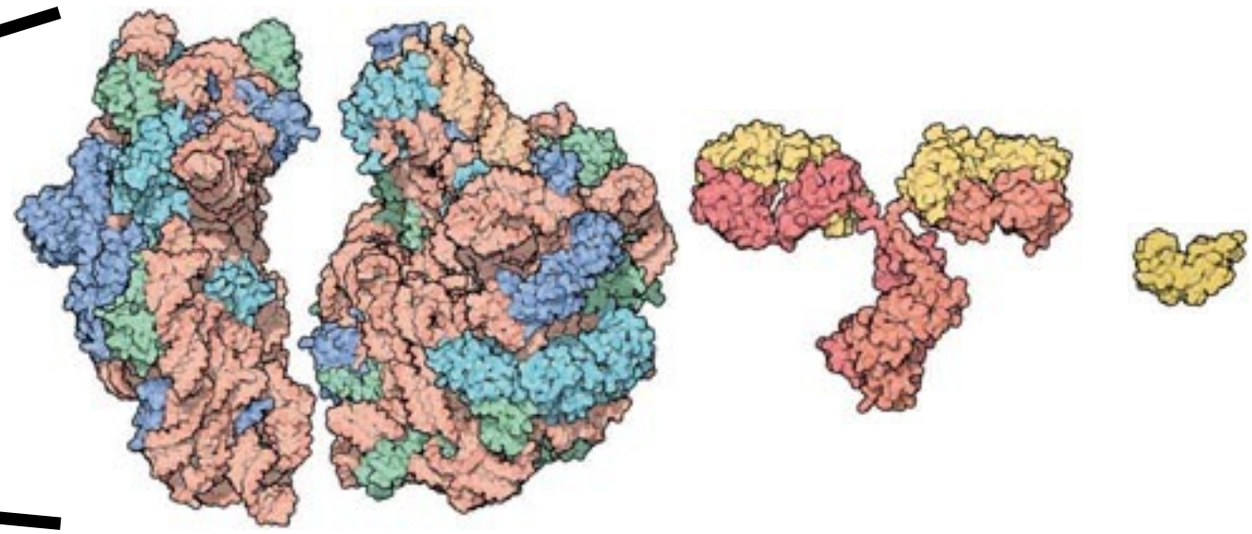
≥ 10μm

~ 1μm

wikipedia


MOLECULAR MACHINERY: A Tour of the Protein Data Bank
Cholesterol
Phospholipid
1c17+1e79 ATP Synthase
1fgy Aquaporin
1hl8 Potassium Channel
1msi Mechanosensitive Channel
1grm Gramicidin
2por Porin
4hhb Hemoglobin
1mbd Myoglobin
1hrs Ferritin
1e7i Serum Albumin
1gcn Glucagon
3cyt Cytochrome c
2hiu Insulin
1hgy Cytochrome bc1 Complex
1iat Phosphoglucose Isomerase
1pfk Phosphofructokinase
1ald Aldolase
7tim Triosephosphate Isomerase
1cza Hexokinase
3hhr Human Growth Hormone
1rfb Interferon
1oco Cytochrome c Oxidase
3pgk Phosphoglycerate Kinase
3pgm Phosphoglycerate Mutase
5enl Enolase
1a49 Pyruvate Kinase
3gpd Glyceraldehyde-3-phosphate Dehydrogenase
2dnj Deoxyribonuclease
2ptc Trypsin
1lz1 Lysozyme
1wsy Tryptophan Synthase
1dhf Dihydrofolate Reductase
1f88 Rhodopsin
2ohx Alcohol Dehydrogenase
2tsc Thymidylate Synthase
2gls Glutamine Synthetase
1smd Amylase
5rsa Ribonuclease
5pep Pepsin
1poe Phospholipase
1igt Antibody
1pth Cyclooxygenase
4af1 Aspartate Carbamoyltransferase
1rcx Ribulose Bisphosphate Carboxylase/Oxygenase
1n2c Nitrogenase
1prc Photosynthetic Reaction Center
1kzu Light-Harvesting Complex
1gax Valyl-tRNA Synthetase
1qf6 Threonyl-tRNA Synthetase
1euq Glutaminyl-tRNA Synthetase
1ttt Elongation Factor Tu
4rhv Rhinovirus
1jb0 Photosystem I
1ffy Isoleucyl-tRNA Synthetase
1eiy Phenylalanyl-tRNA Synthetase
1asy Aspartyl-tRNA Synthetase
4tna Transfer RNA
PDB PROTEIN DATA BANK
http://www.pdb.org/ • info@rcsb.org
RESEARCH COLLABORATORY FOR STRUCTURAL BIOINFORMATICS
RUTGERS, THE STATE UNIVERSITY OF NEW JERSEY
SAN DIEGO SUPERCOMPUTER CENTER
NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
1efu Elongation Factor Tu and Ts
1fjf+1jj2 Ribosome
1dar Elongation Factor G
1fxk Prefoldin
1aon Chaperonin GroEL/ES
2cpl Proline cis/trans Isomerase





Crystallography

250 Å

Single-particle EM

Tomography

Hybrid methods (Mike Schmid)

Why it's not easy to get to near-atomic resolution

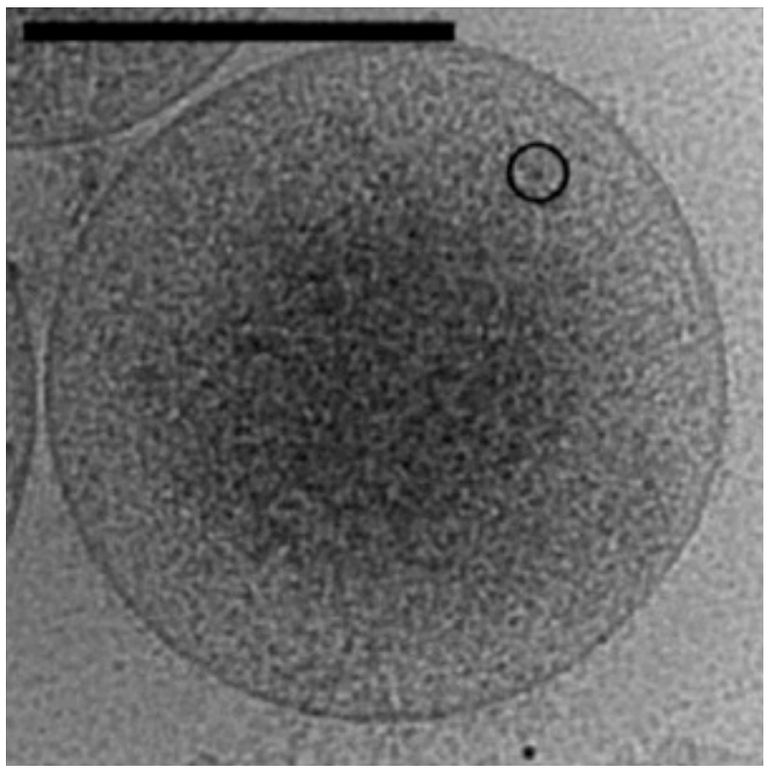
	Biological	Graphene	"Materials"
flux	10 pA/cm^2	1A/cm^2	>>10 pA/cm^2
dose	10 e$^-$/Å^2	>10^{17} e$^-$/Å^2	>>10 e$^-$/Å^2

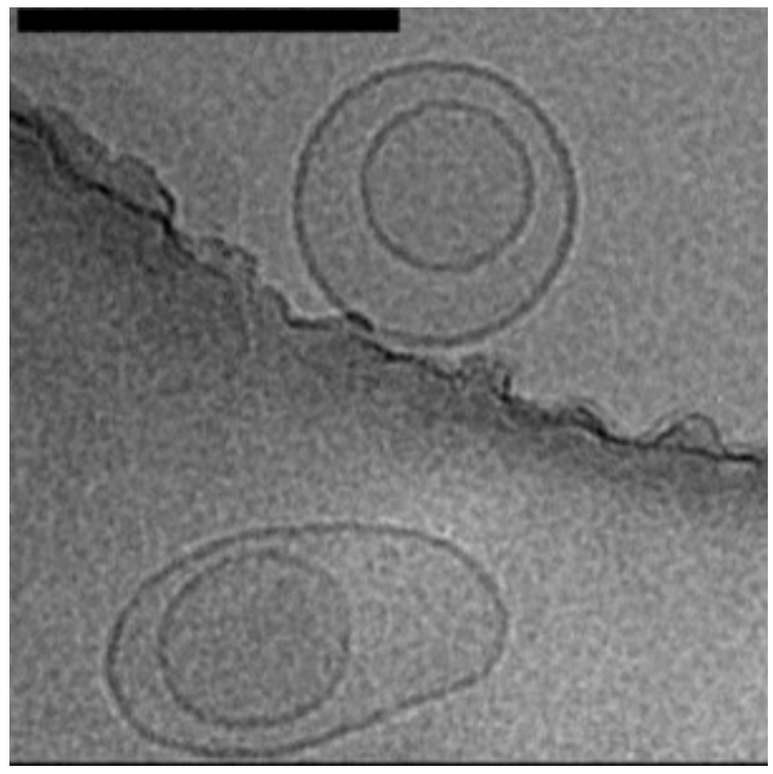
Why it's not easy to get to near-atomic resolution

	Biological	Graphene	"Materials"
atom	Z < 8	Z = 6	Z > 8
contrast	Phase	Phase	Amplitude
defcous	1 - 10 μm	~100nm	<< 1μm

What does this mean for your sample?

10 $e^-/Å^2$

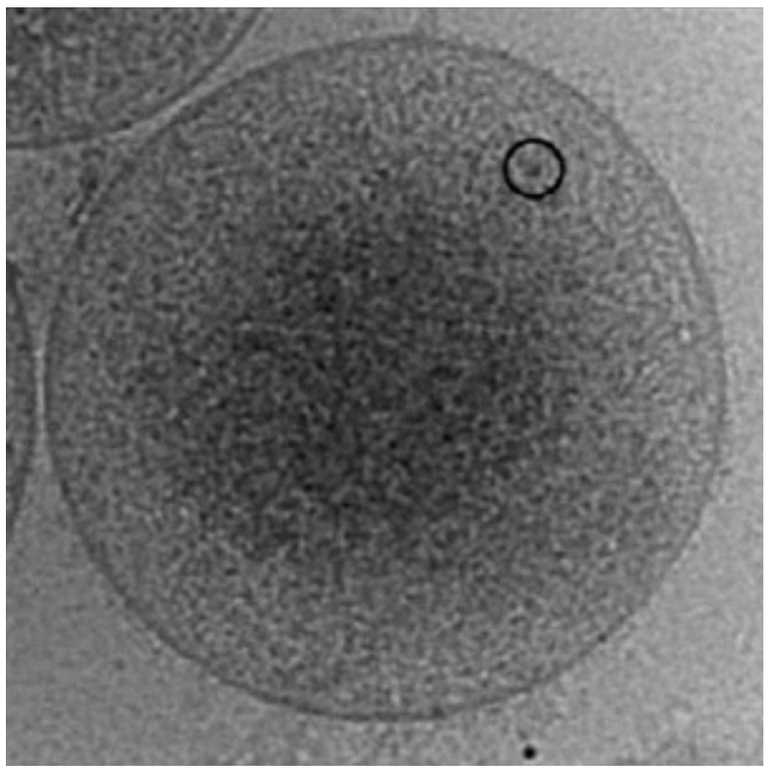


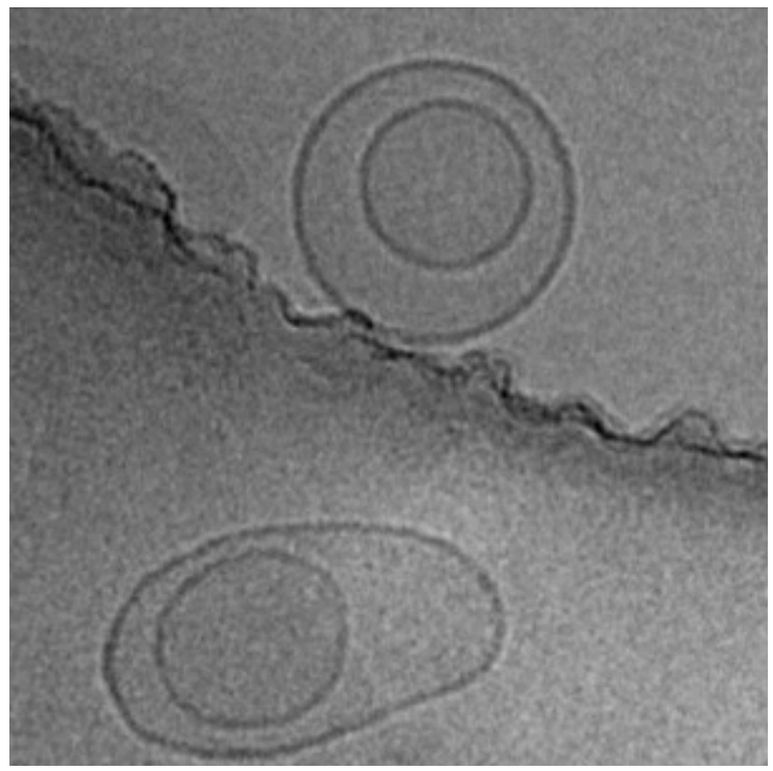


Iancu, 2006

What does this mean for your sample?

40 $e^-/Å^2$

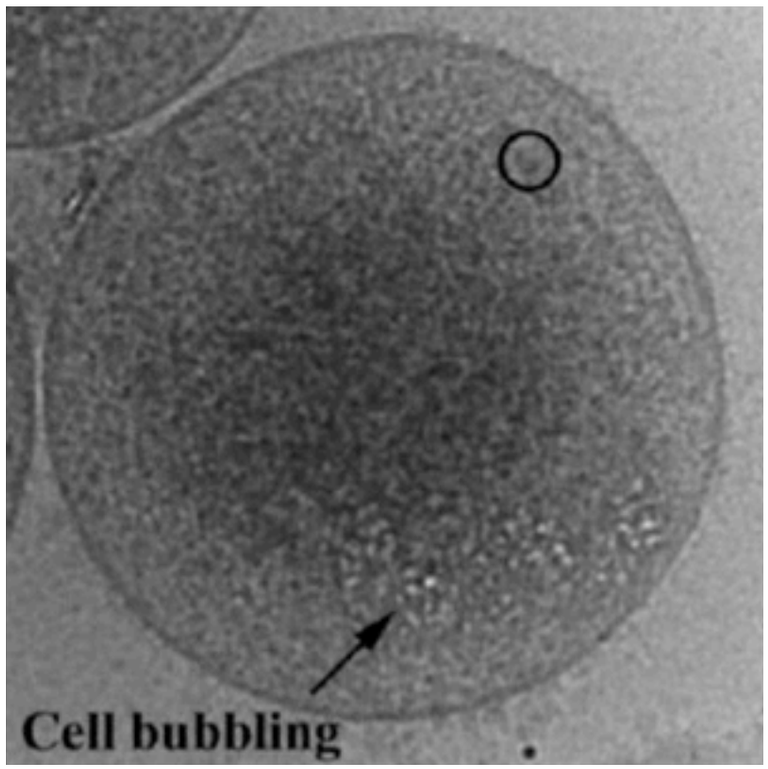


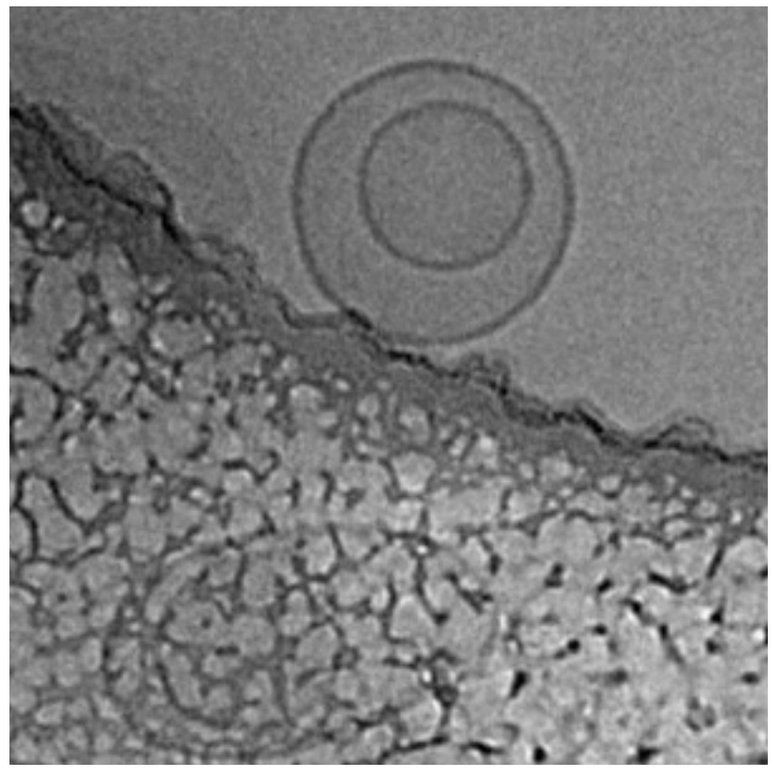


Iancu, 2006

What does this mean for your sample?

140 $e^-/Å^2$





When starting with a new type of sample, do a dose series to find out the acceptable dose.

Iancu, 2006

	Single particle	Tomography
Sample	Protein complexes, viruses	viruses, small cells
max thickness	500 nm	500 nm
preparation	plunge freeze, neg. stain	plunge freeze, high-pressure freeze, embedding, sectioning (plastic + cryo)
fiducials (alignment)	n/a	colloidal gold

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

	Single particle	Tomography
resolution	3.5 - 10 Å	40 - 80 Å
imaging / reconstruction	1 - 100 days	0.5 - 2 hours
reconstruction	1 - 365 days	10 minutes *
recs / paper	1 - 10	10 - 100

Your time and TEM time are valuable resources. Plan your experiments carefully.

* Post-tomographic image processing can take weeks to months.

What quantities are measurable in a cell?

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

If the structure is not resolvable, you must use subtomogram averaging (Mike Schmid's lecture).

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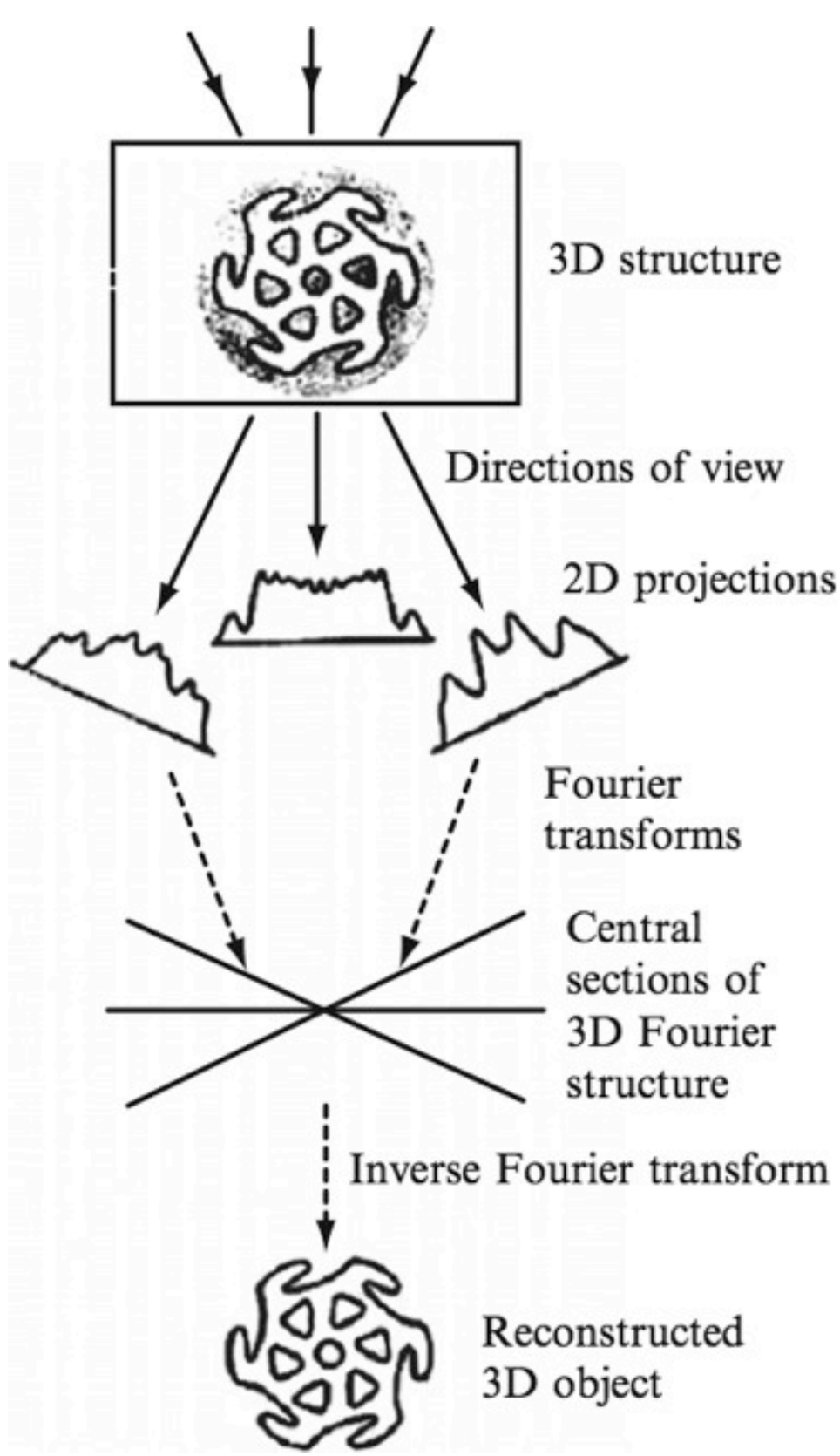
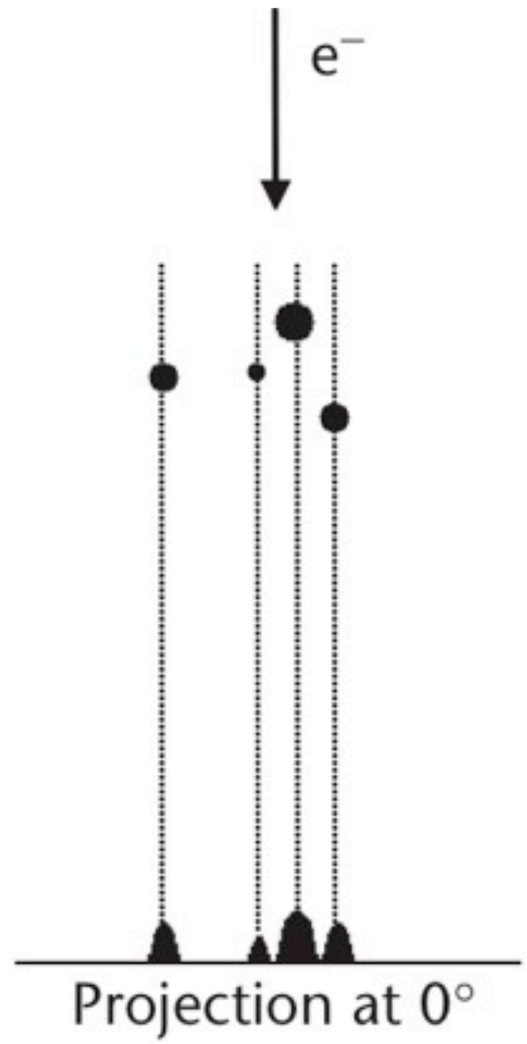


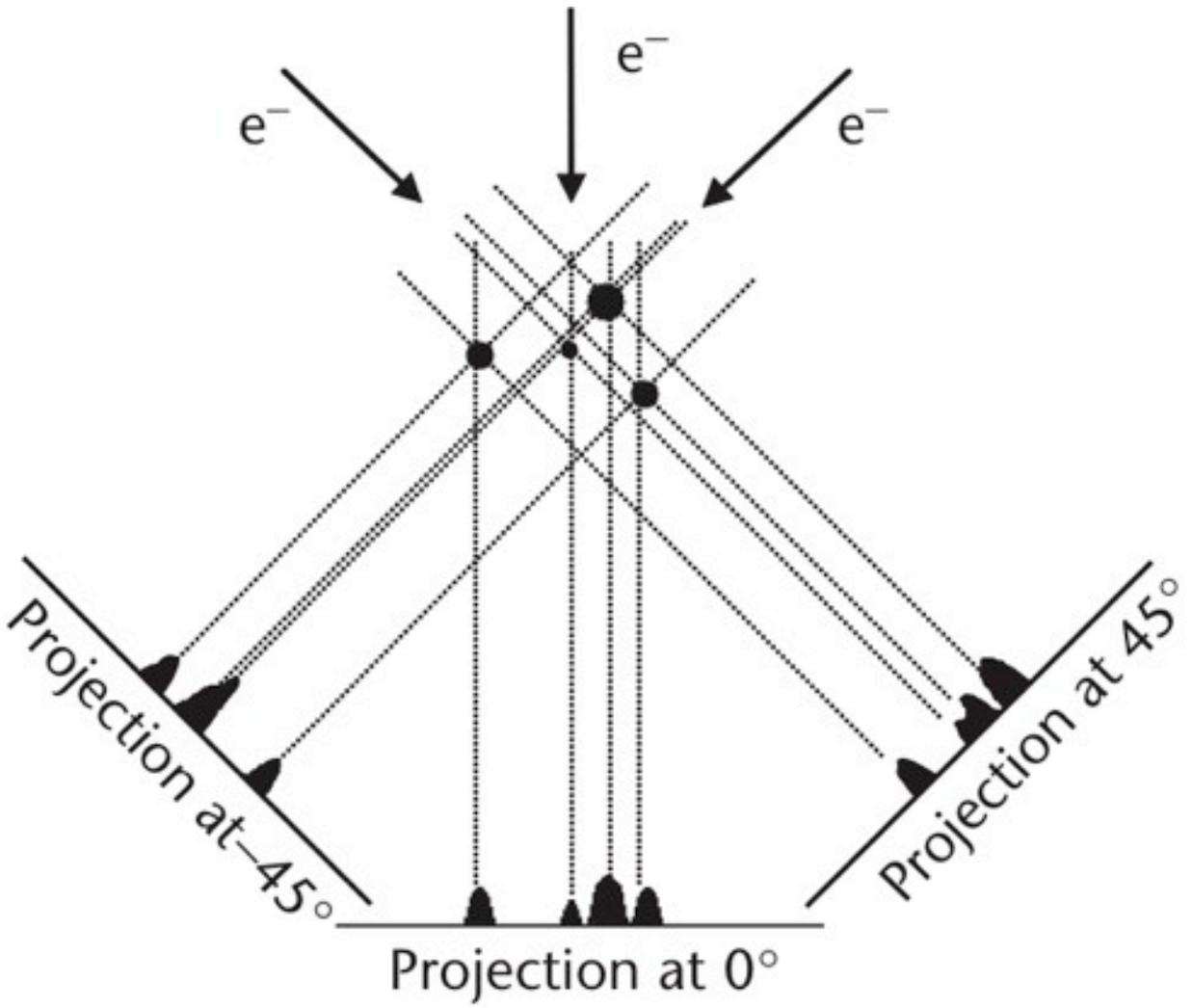
DeRosier, 1968

A cryo-EM image is a "projection"



He, 2010

Tilt series: a set of projections from <u>one</u> object



He, 2010

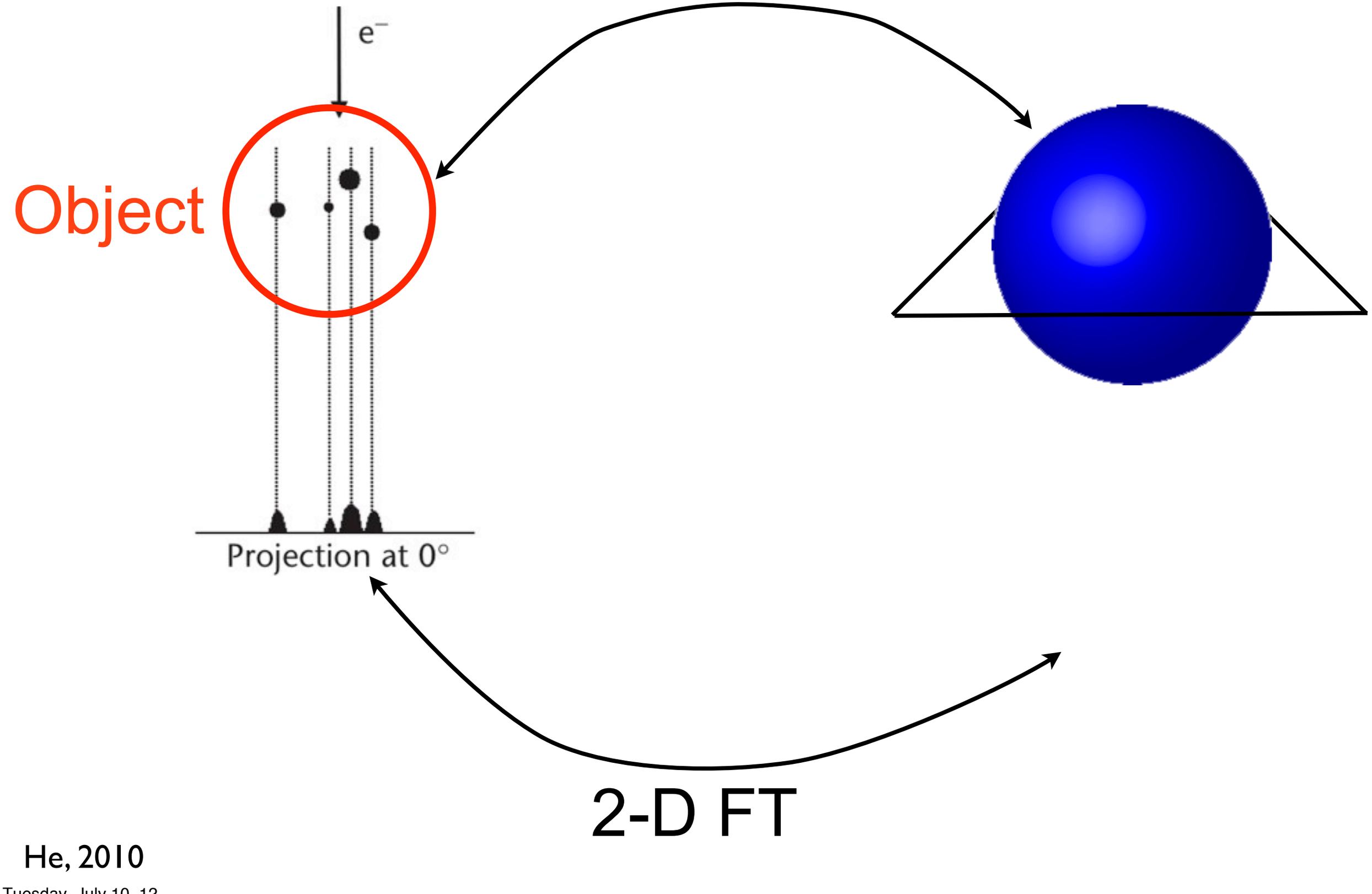
How can we understand (use) projections?

The projection theorem:

A projection (2-D) in real space corresponds to a central section (2-D) perpendicular to the projection direction in Fourier space (3-D).

Tilt series: a <u>set</u> of projections from <u>one</u> object

3-D FT



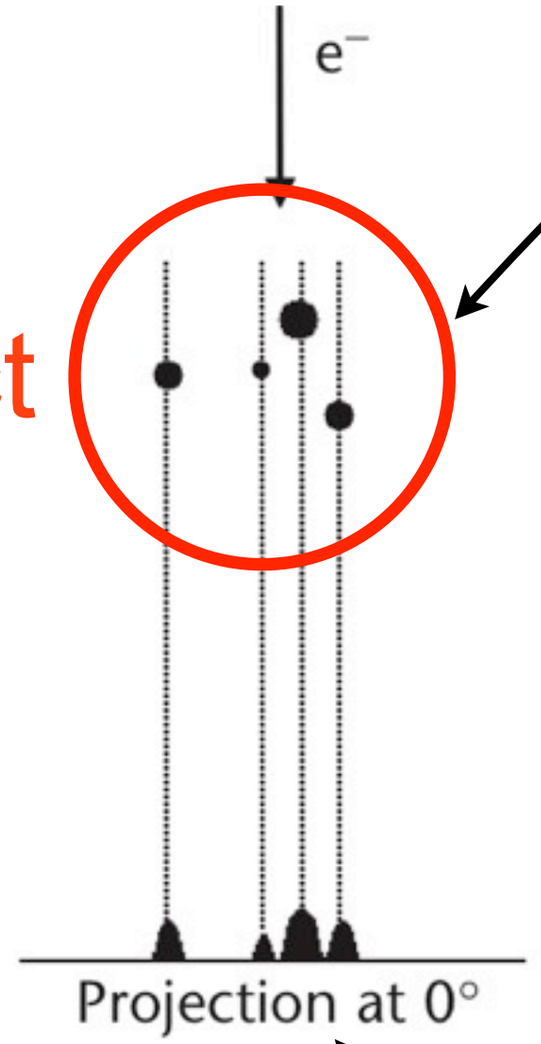
2-D FT

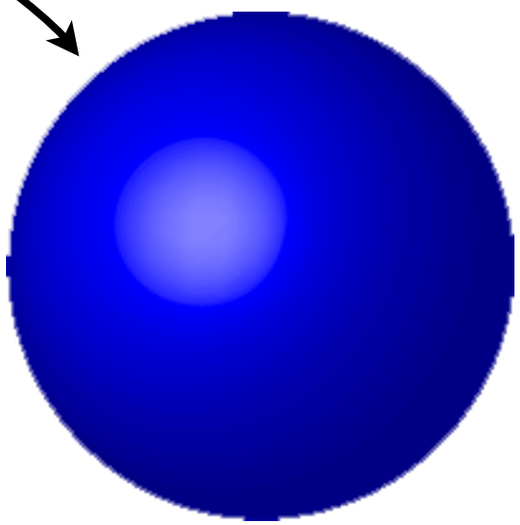
He, 2010

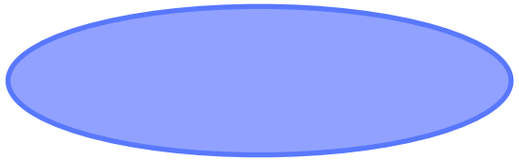
Tilt series: a set of projections from <u>one</u> object

3-D FT

Object





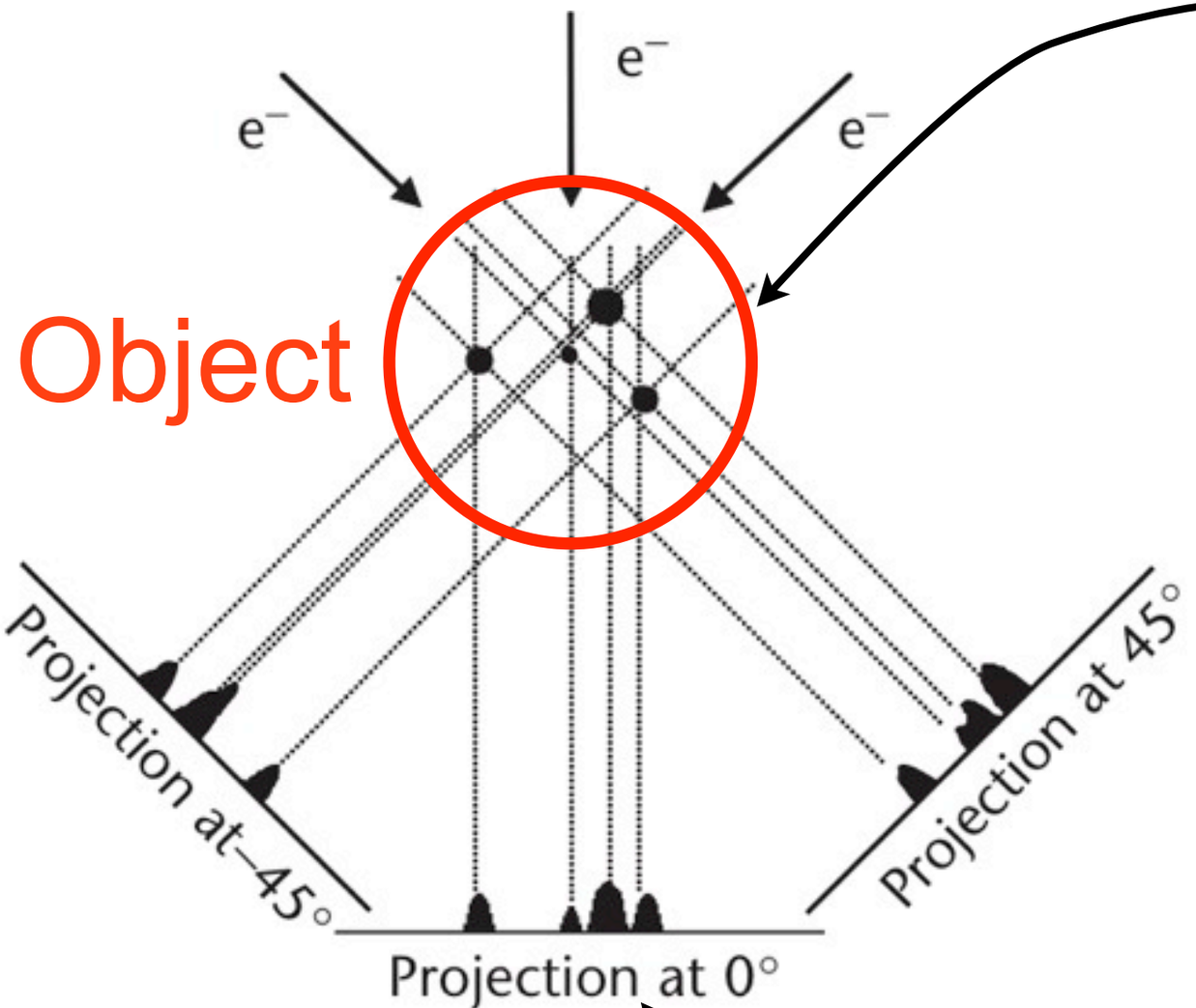


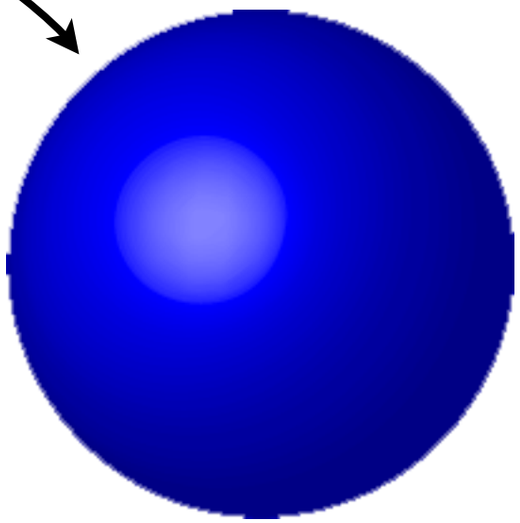
2-D FT

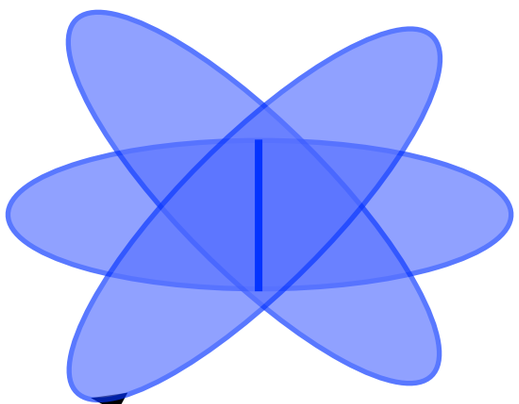
He, 2010

Tilt series: a set of projections from one object

3-D FT







2-D FT

He, 2010

3-D image from 2-D images: back projection

He, 2010

How many images are needed?

The Crowther criterion:

$m \sim \pi * D / d$

m = number of images
D = diameter of object
d = resolution

Crowther, 1970

How to use the Crowther criterion

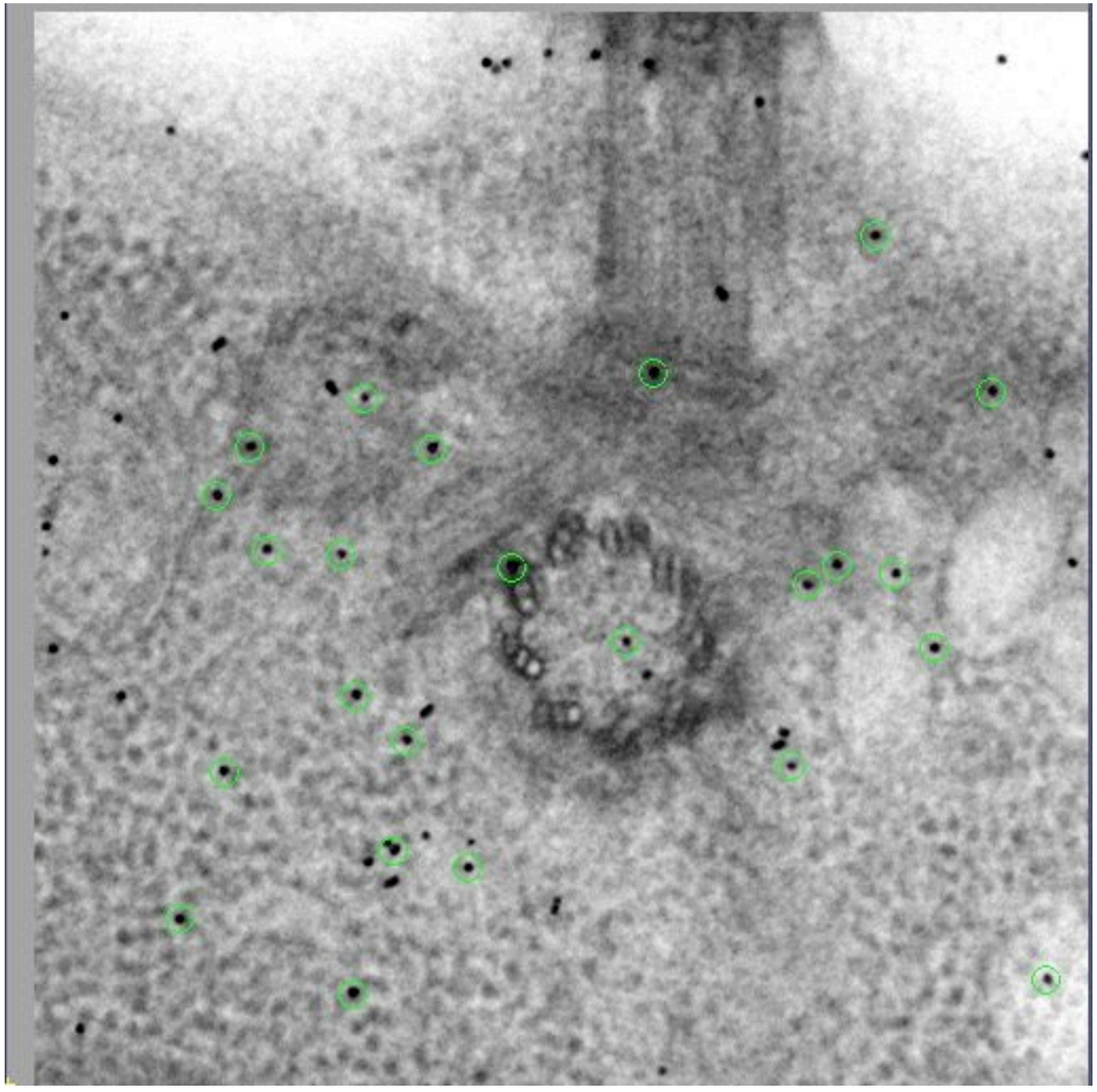
A bacterial cell is ~ 500 nm thick
Desired (realistic resolution) ~ 10nm
m ~ 157 images, **distributed over 180°**

∴ the tilt *increment* should be ~ 0.9°

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

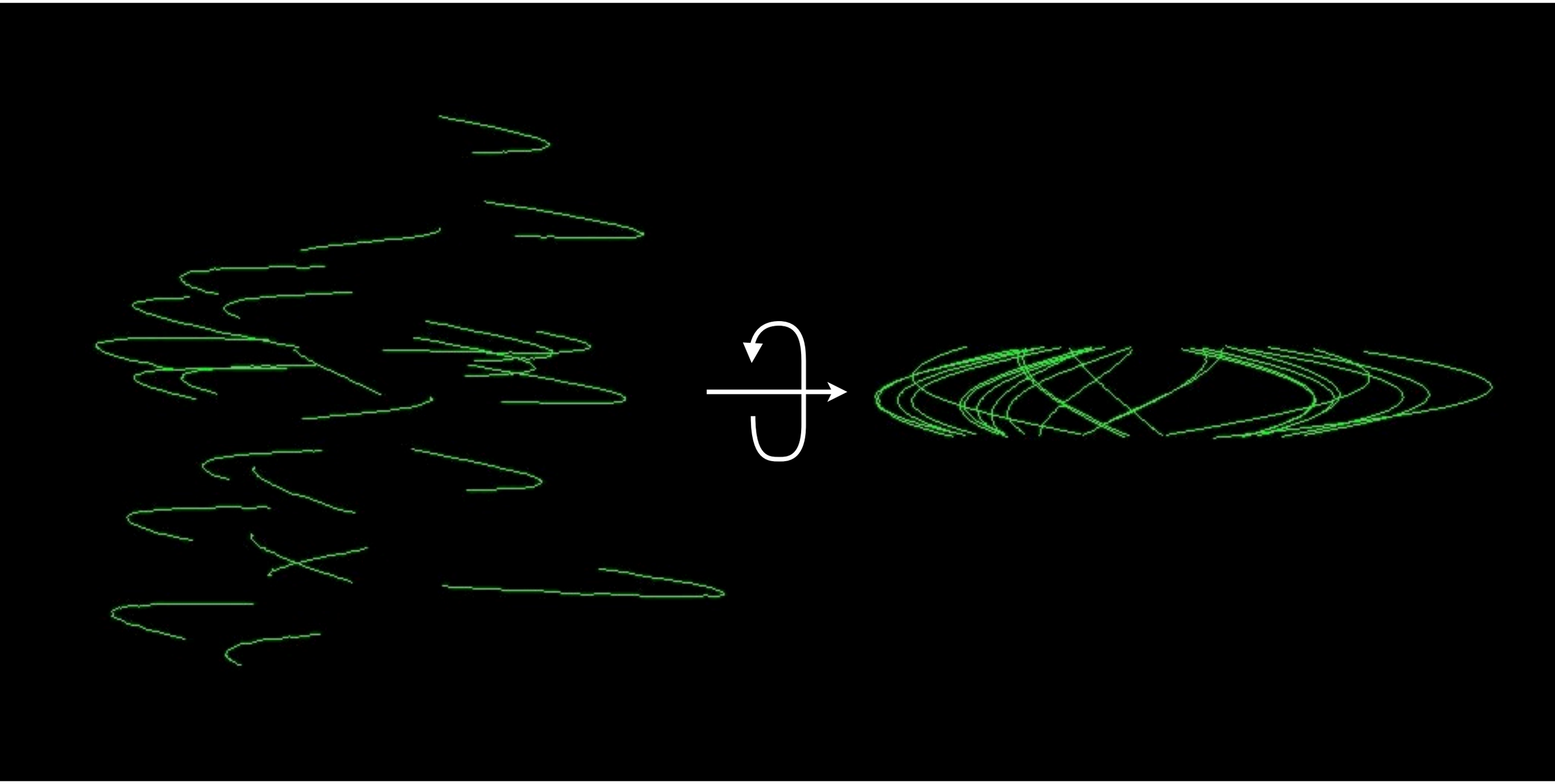
Crowther, 1970

Image alignment is assisted using gold fiducials

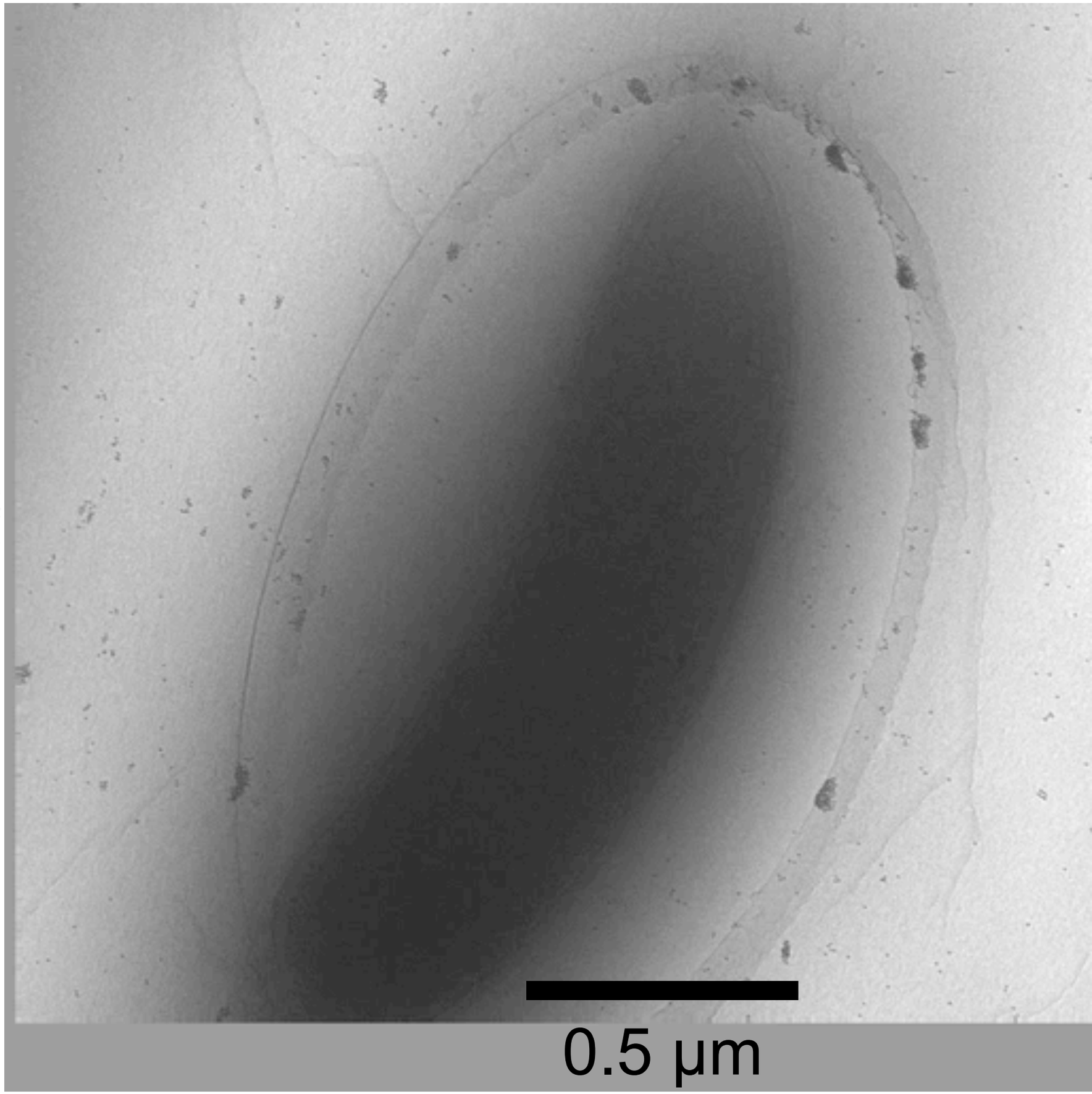


Mastronarde, 1997

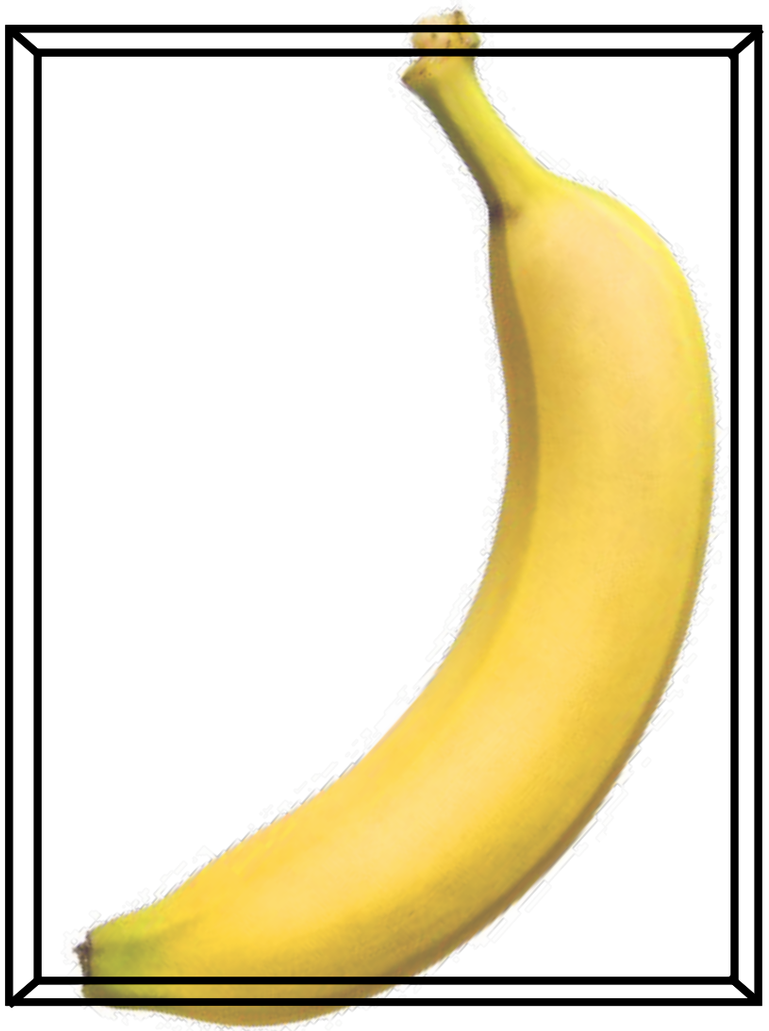
Aligned fiducials have a smooth “trajectory”

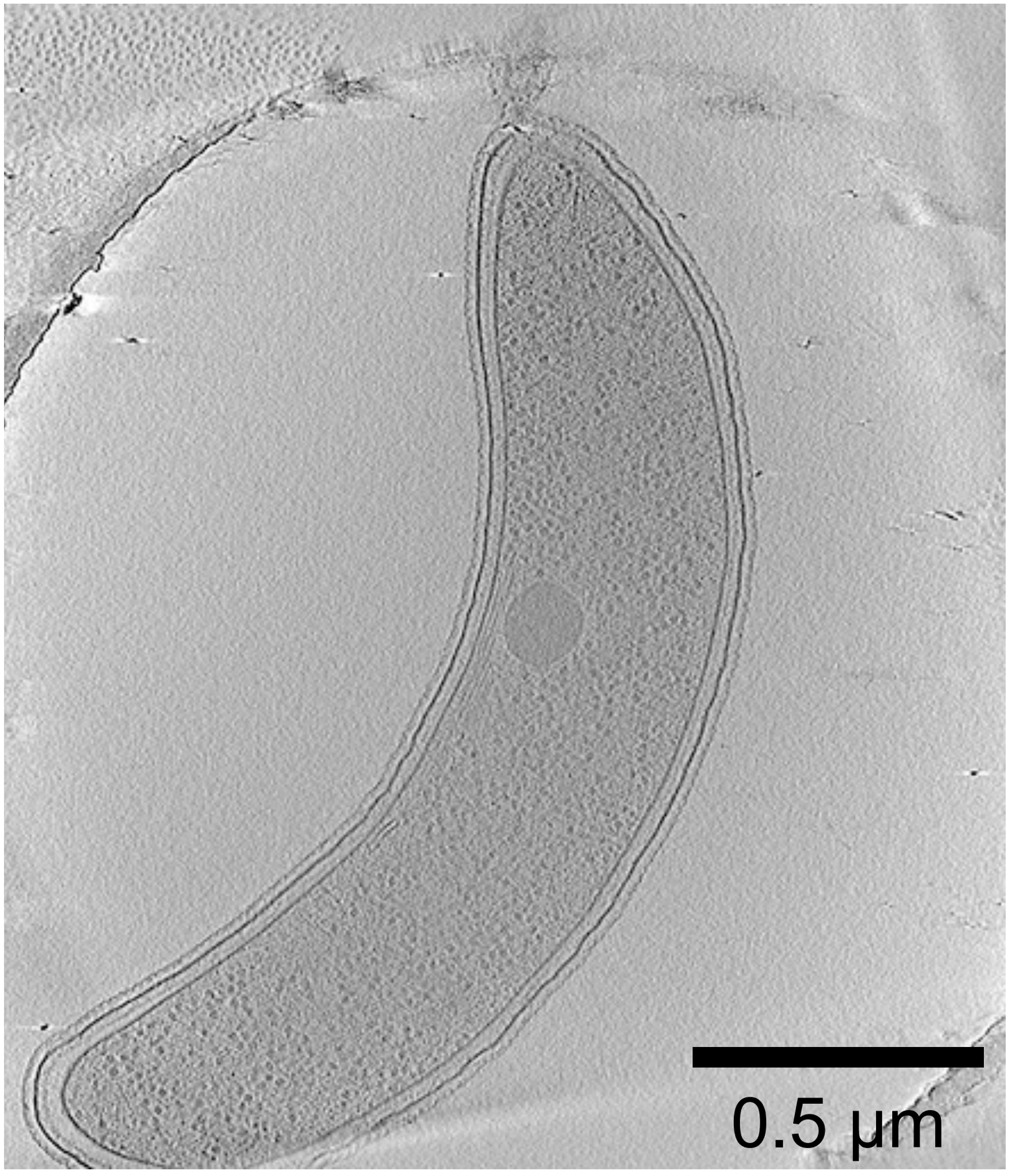


Mastronarde, 1997



Briegel, 2006





Briegel, 2006

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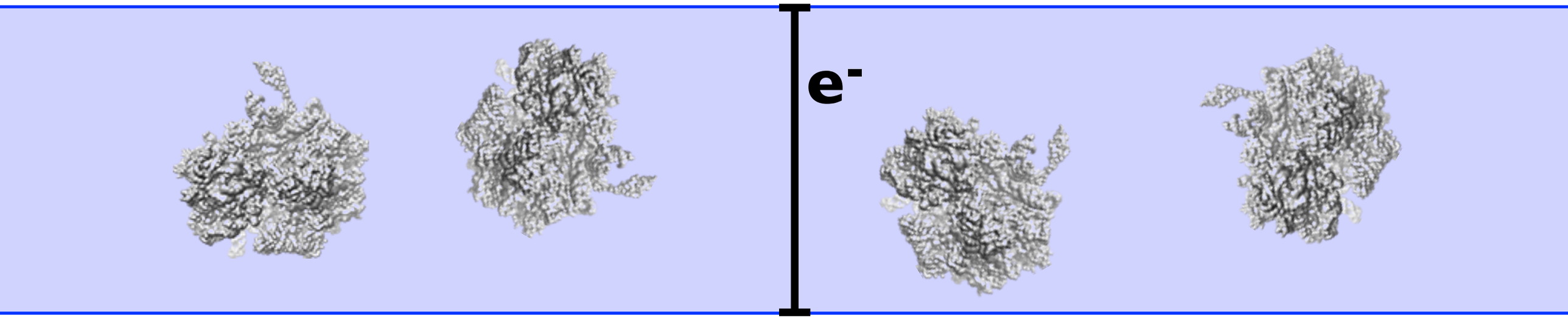
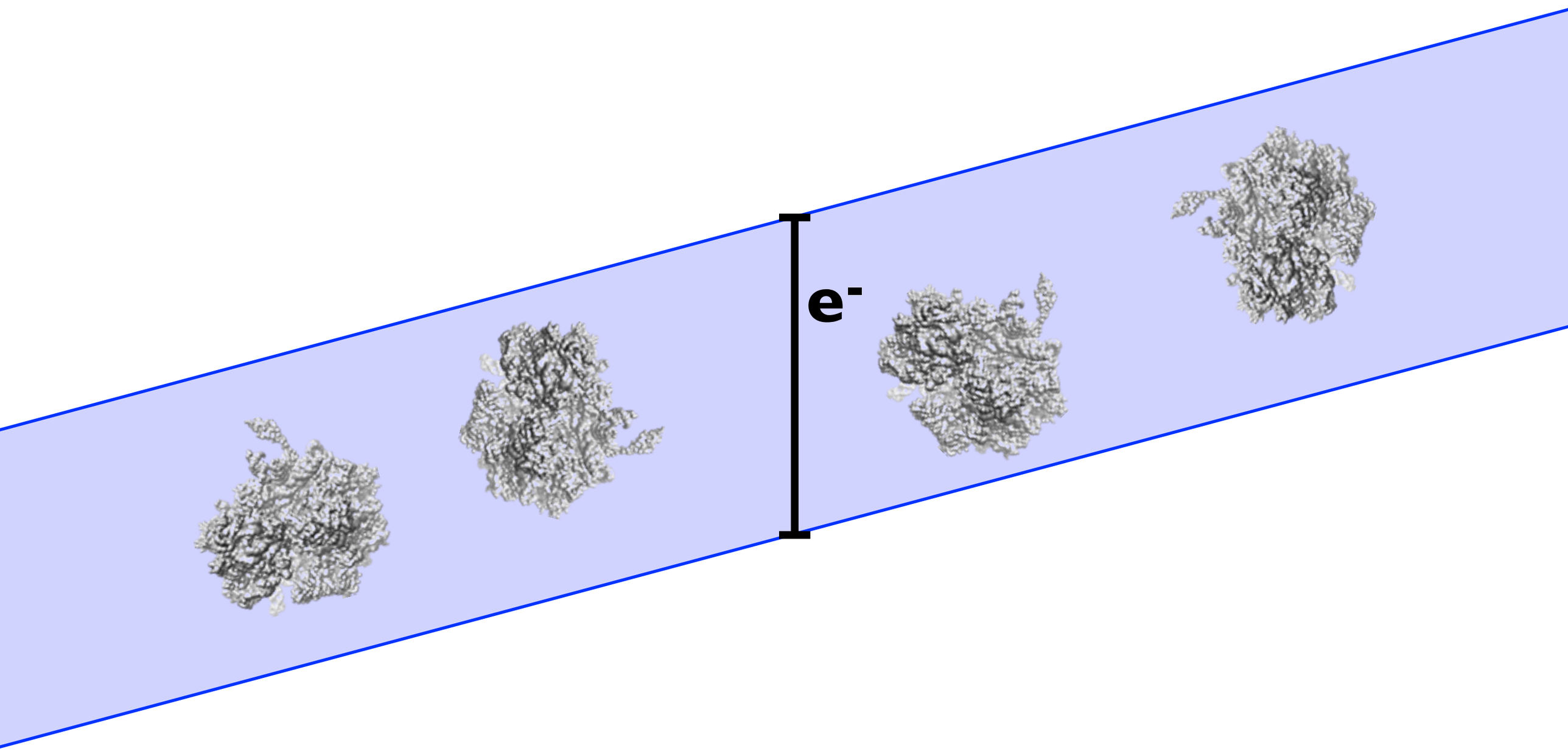
cryo-ET: missing wedge

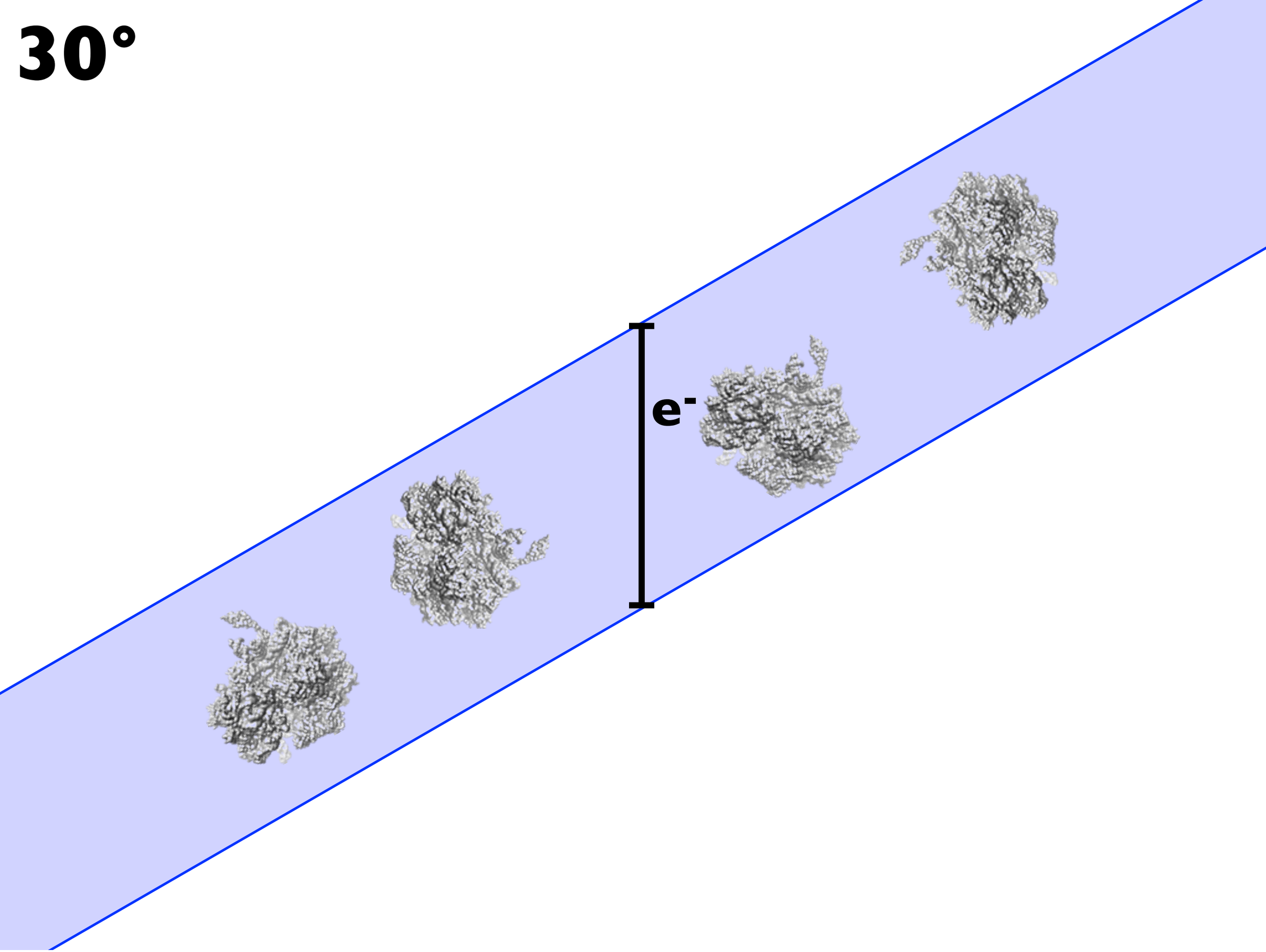
0°



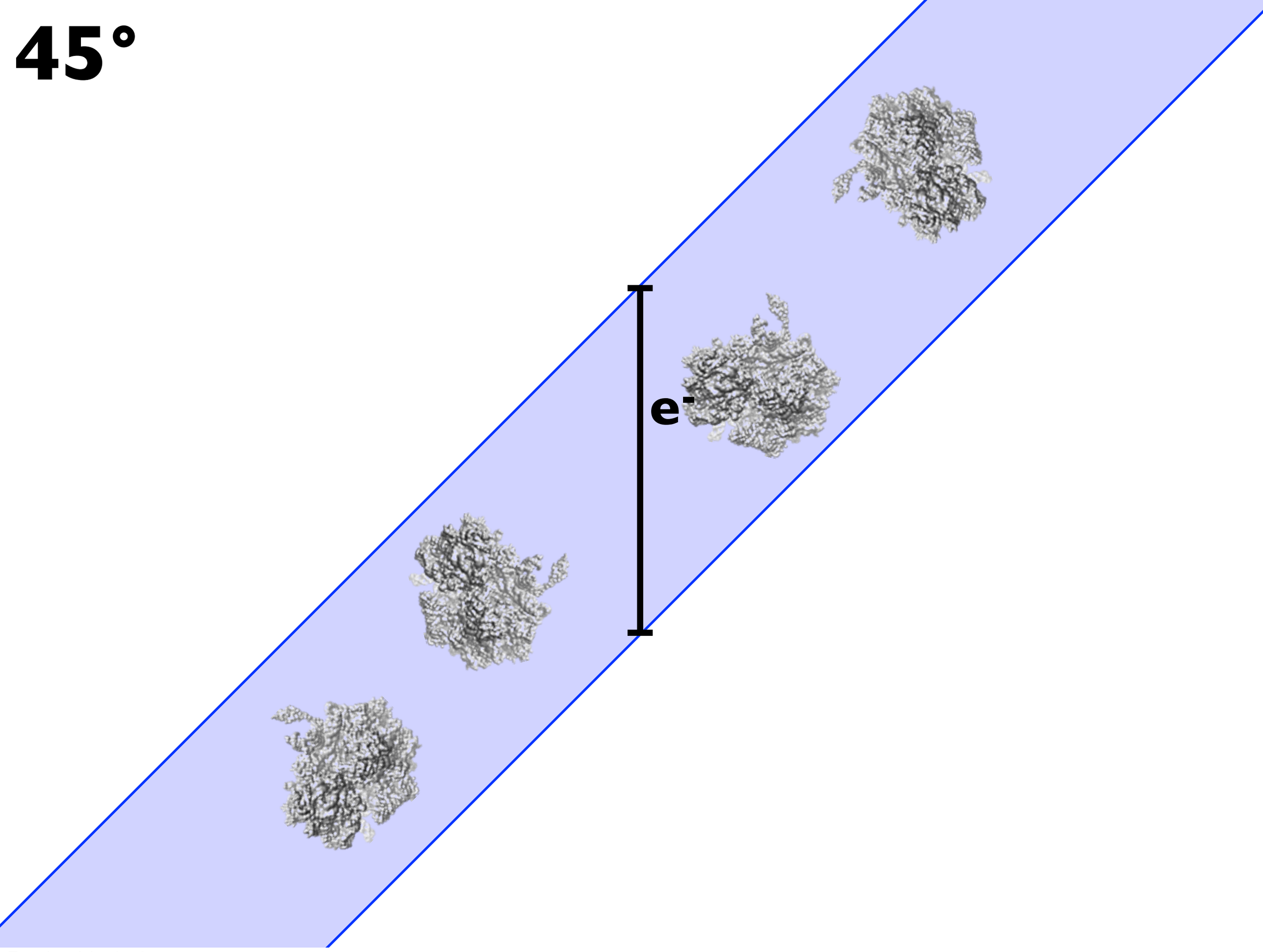
15°



30°

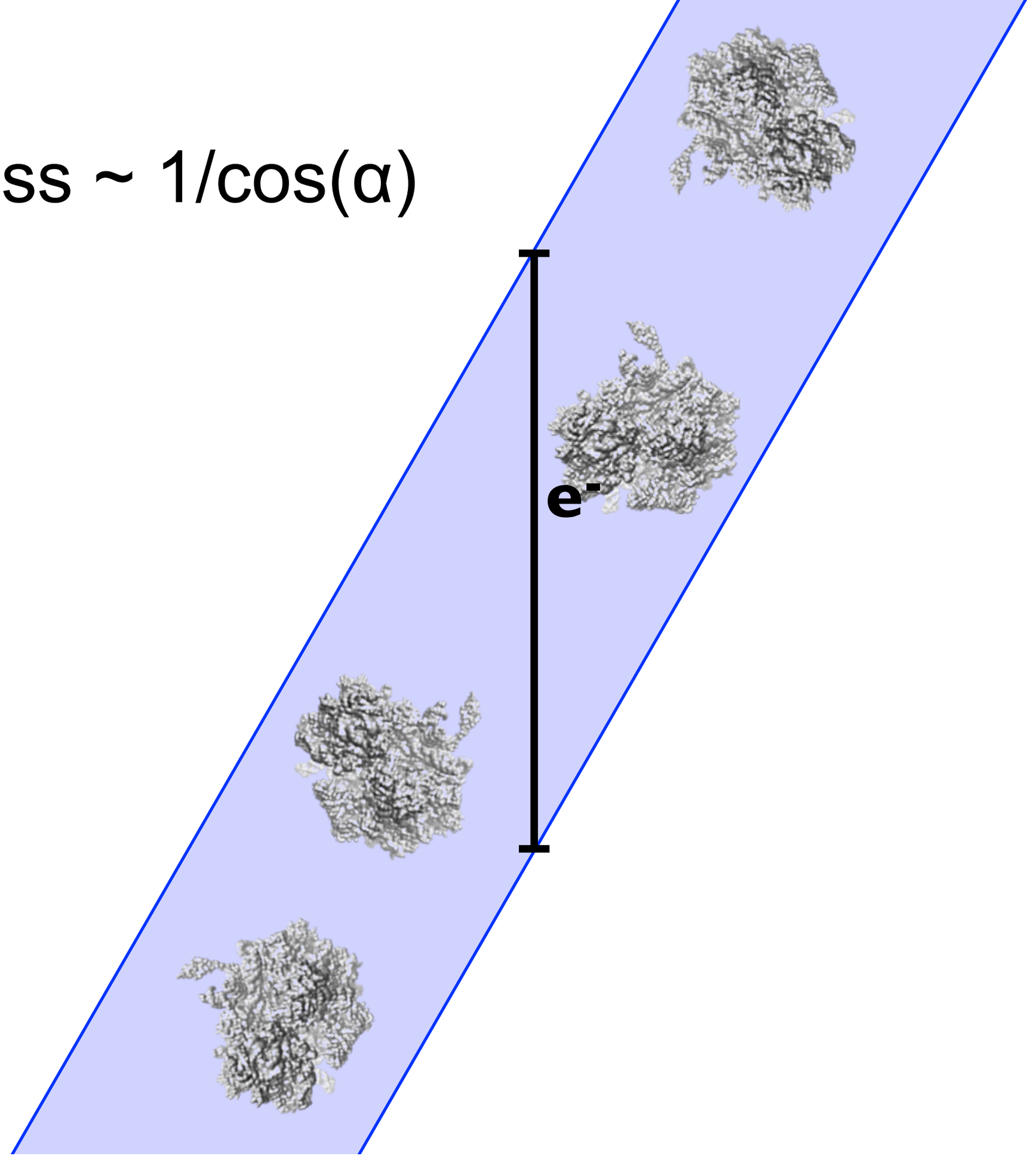


45°

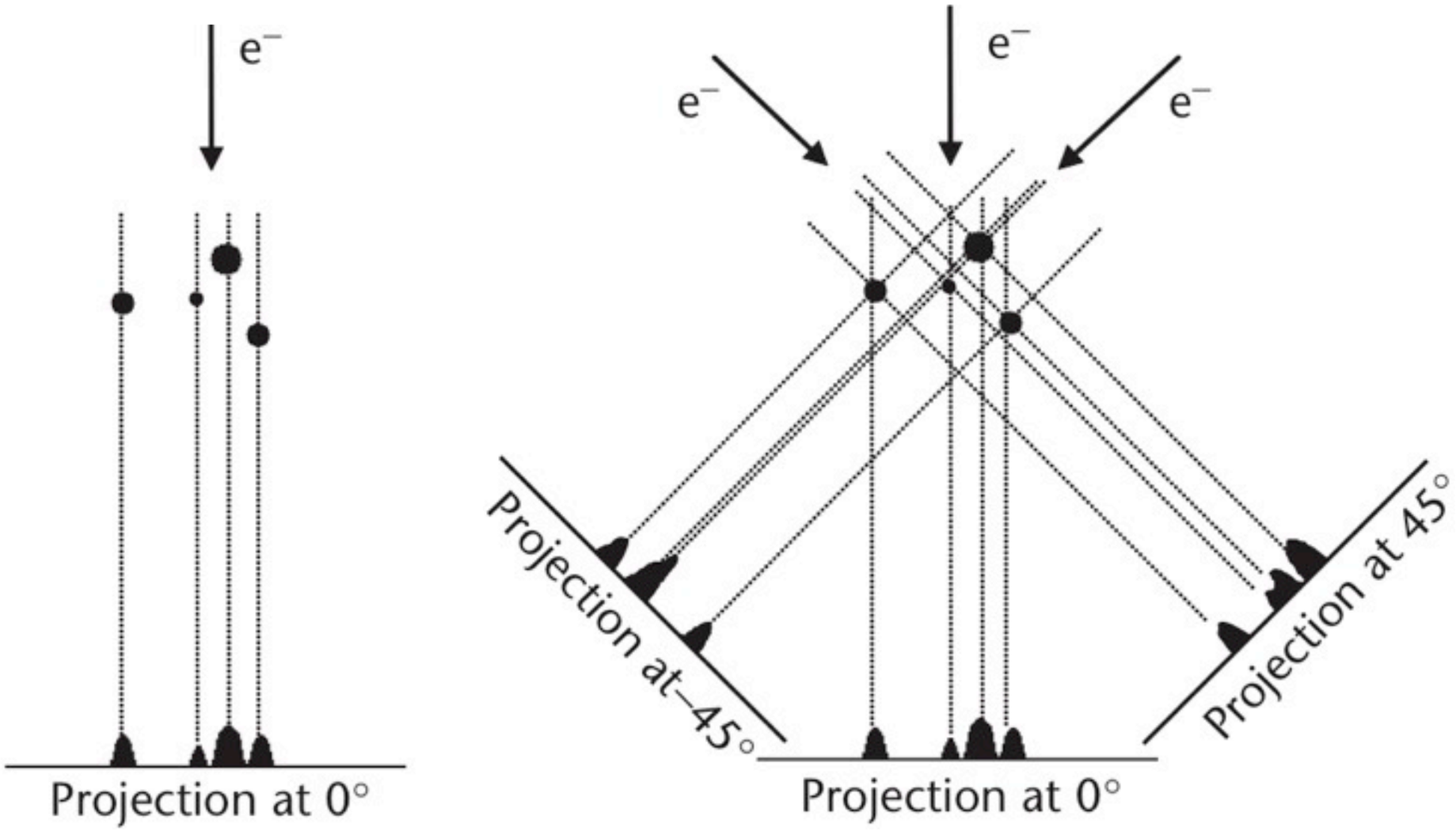


60°

thickness ~ 1/cos(α)

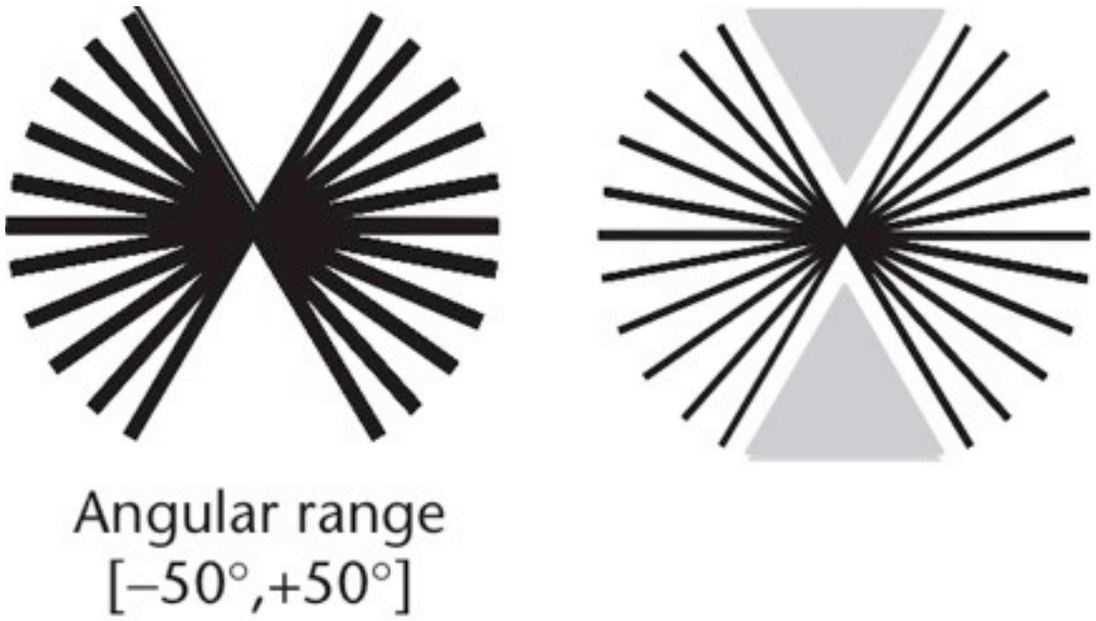


cryo-ET: missing wedge



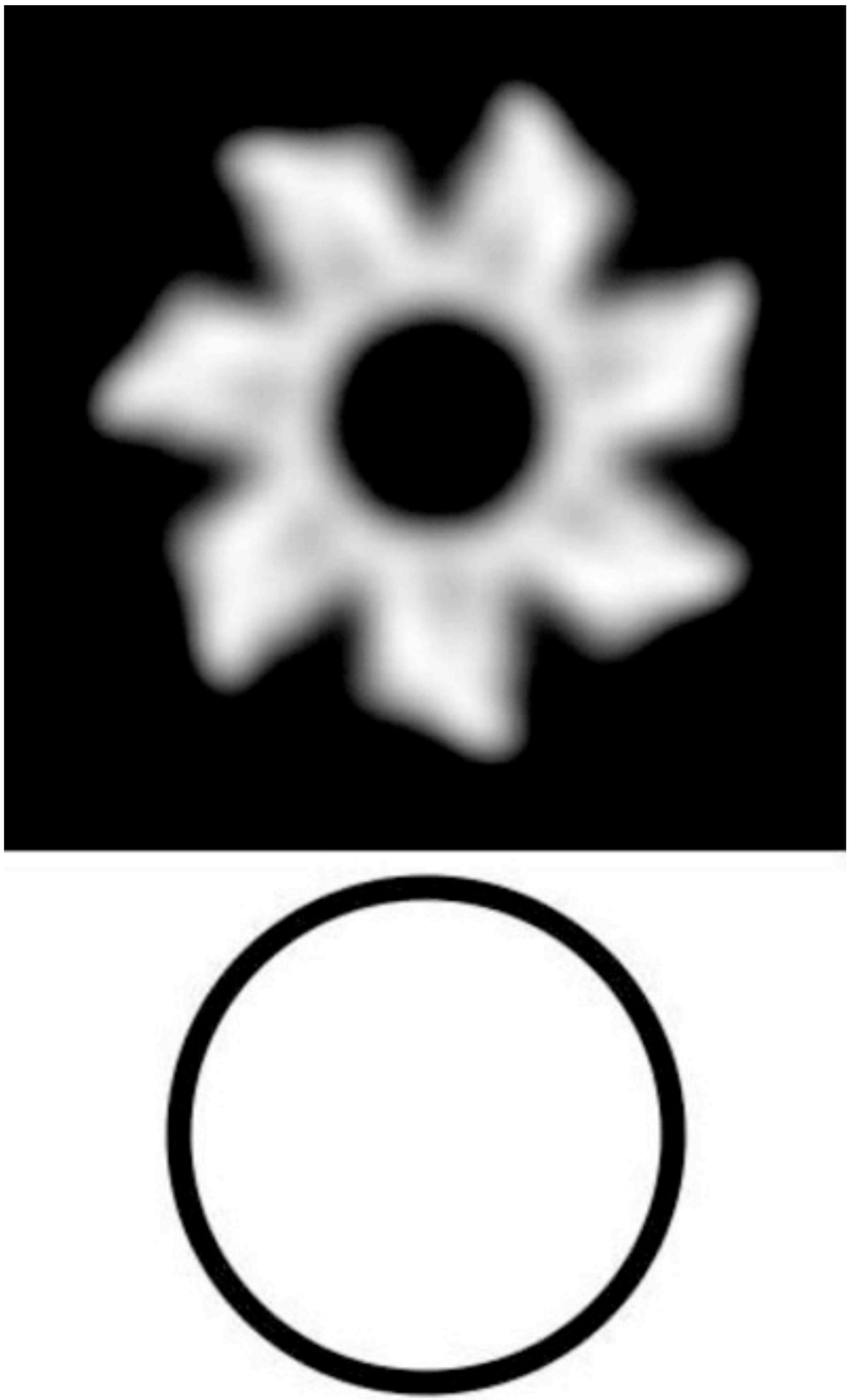
He, 2010

cryo-ET: missing wedge in reciprocal space



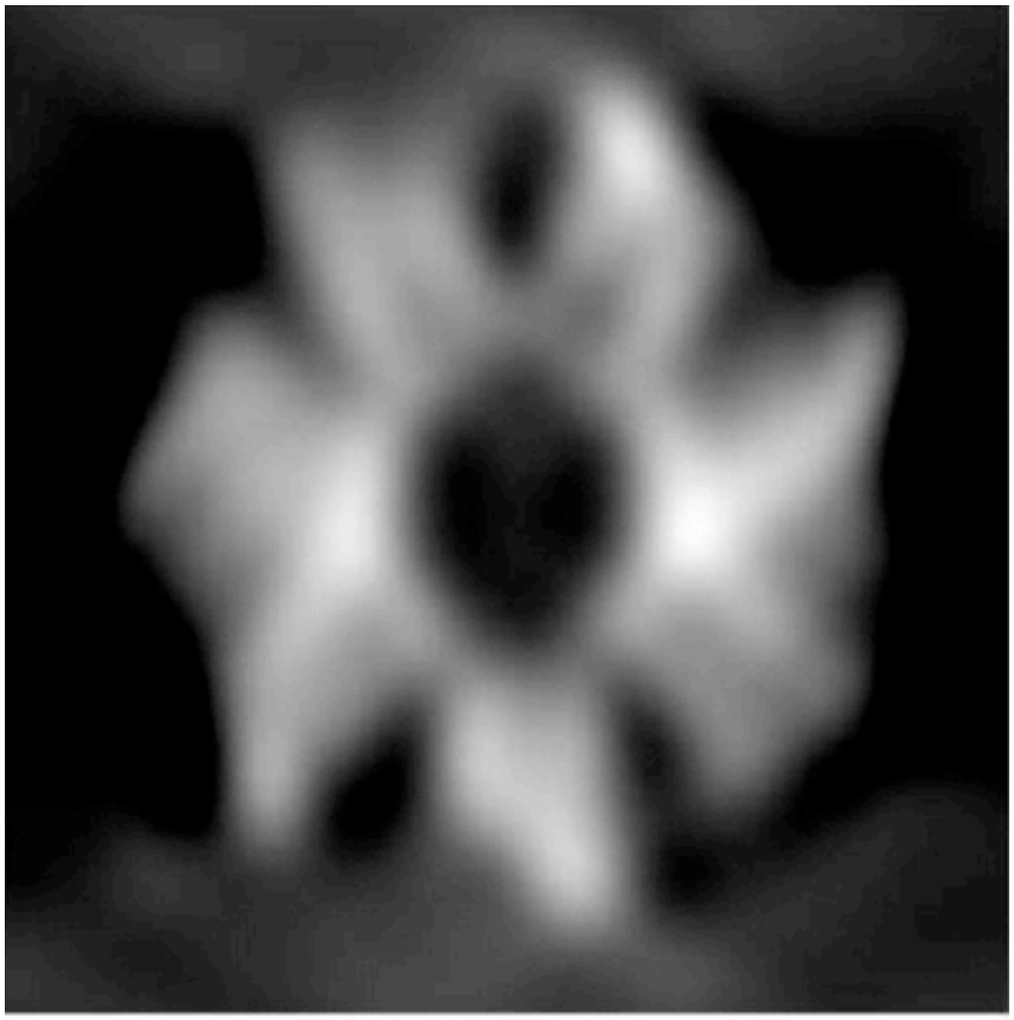
He, 2010

Cryo-ET: missing wedge effect in real space



Fernandez, 2012

Cryo-ET: missing wedge effect in real space



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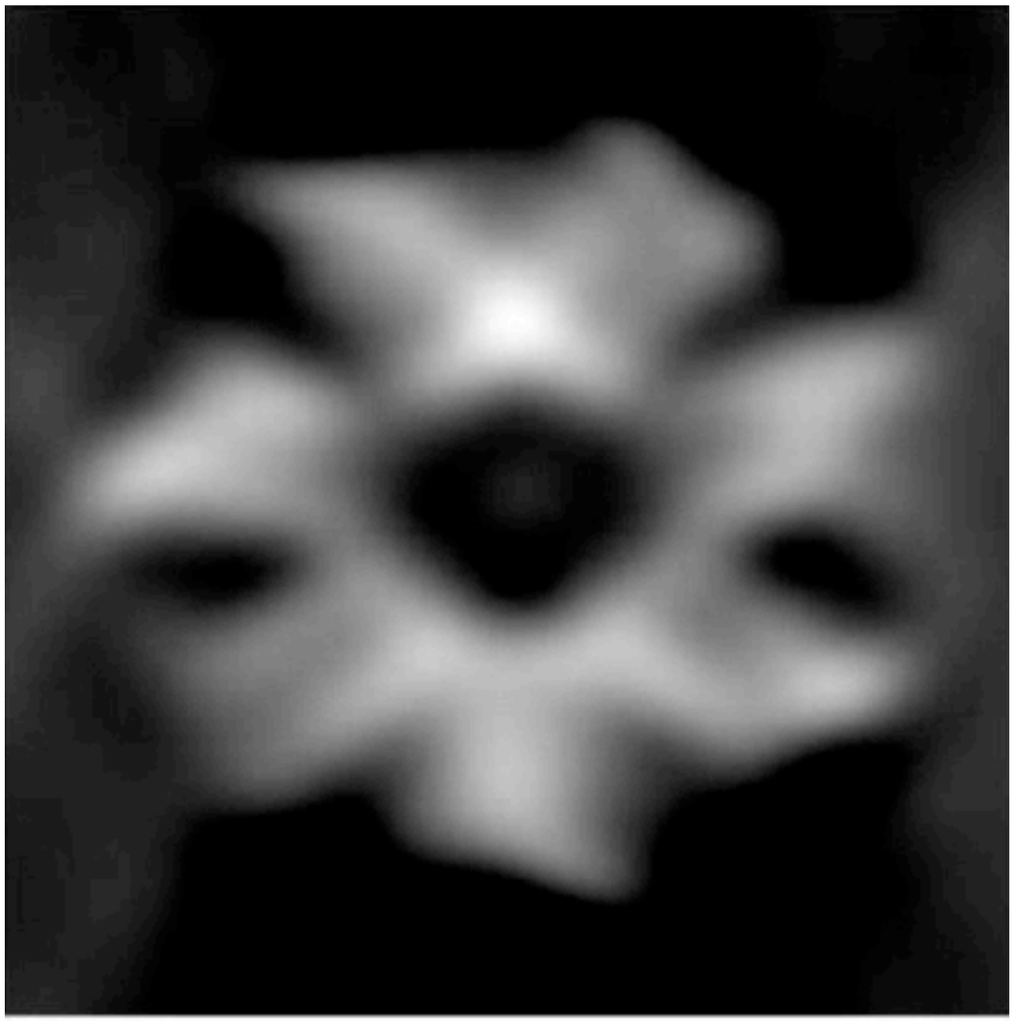

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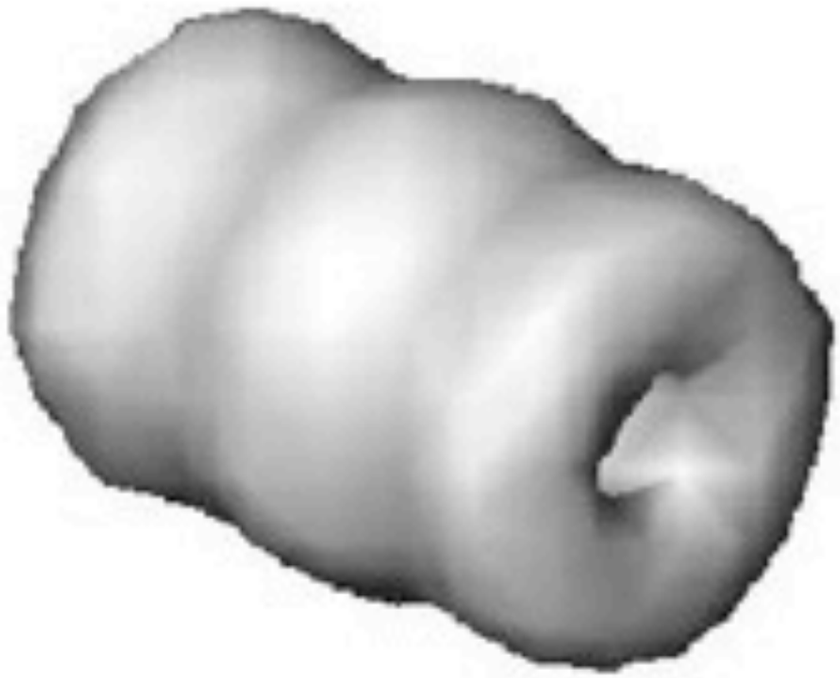
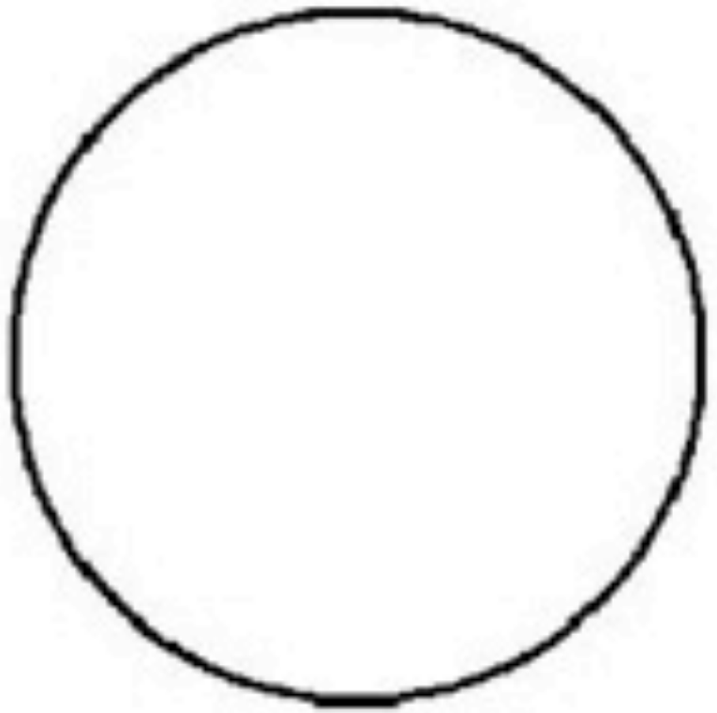
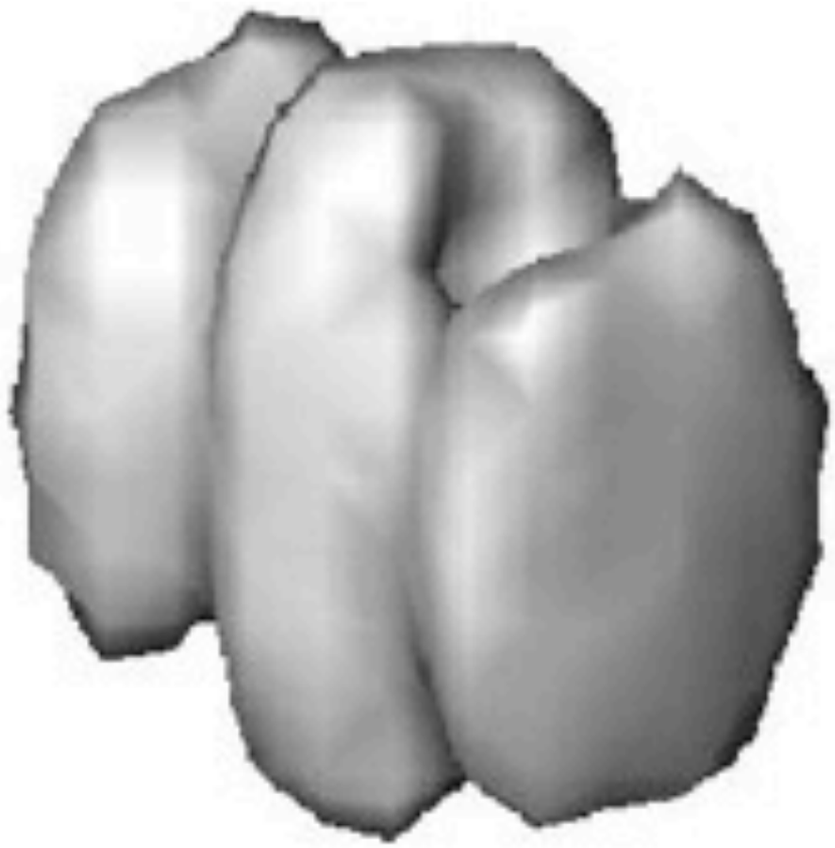
Cryo-ET: missing wedge effect in real space

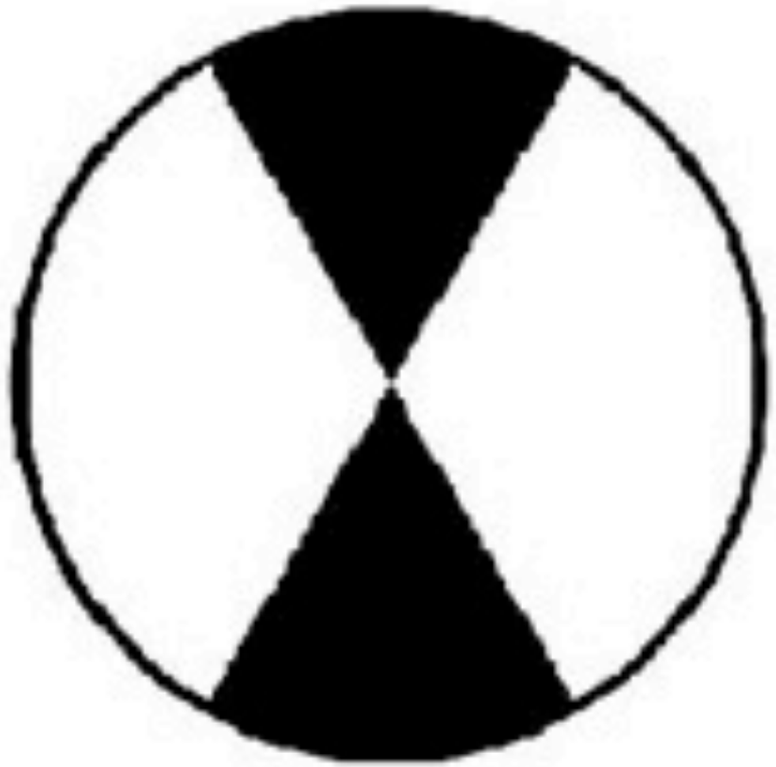




Stolken, 2011

Cryo-ET: missing wedge effect in real space





Stolken, 2011

The contrast transfer function

$CTF(s;\Delta f) \approx \sin(\gamma(s;\Delta f))$

Phase perturbation function:

$\gamma(s;\Delta f) = 2\pi(-\frac{1}{2}\,\Delta f\,\lambda\,s^2 + \frac{1}{4}C_s\,\lambda^3\,s^4)$

A = % amplitude contrast

Δf = underfocus

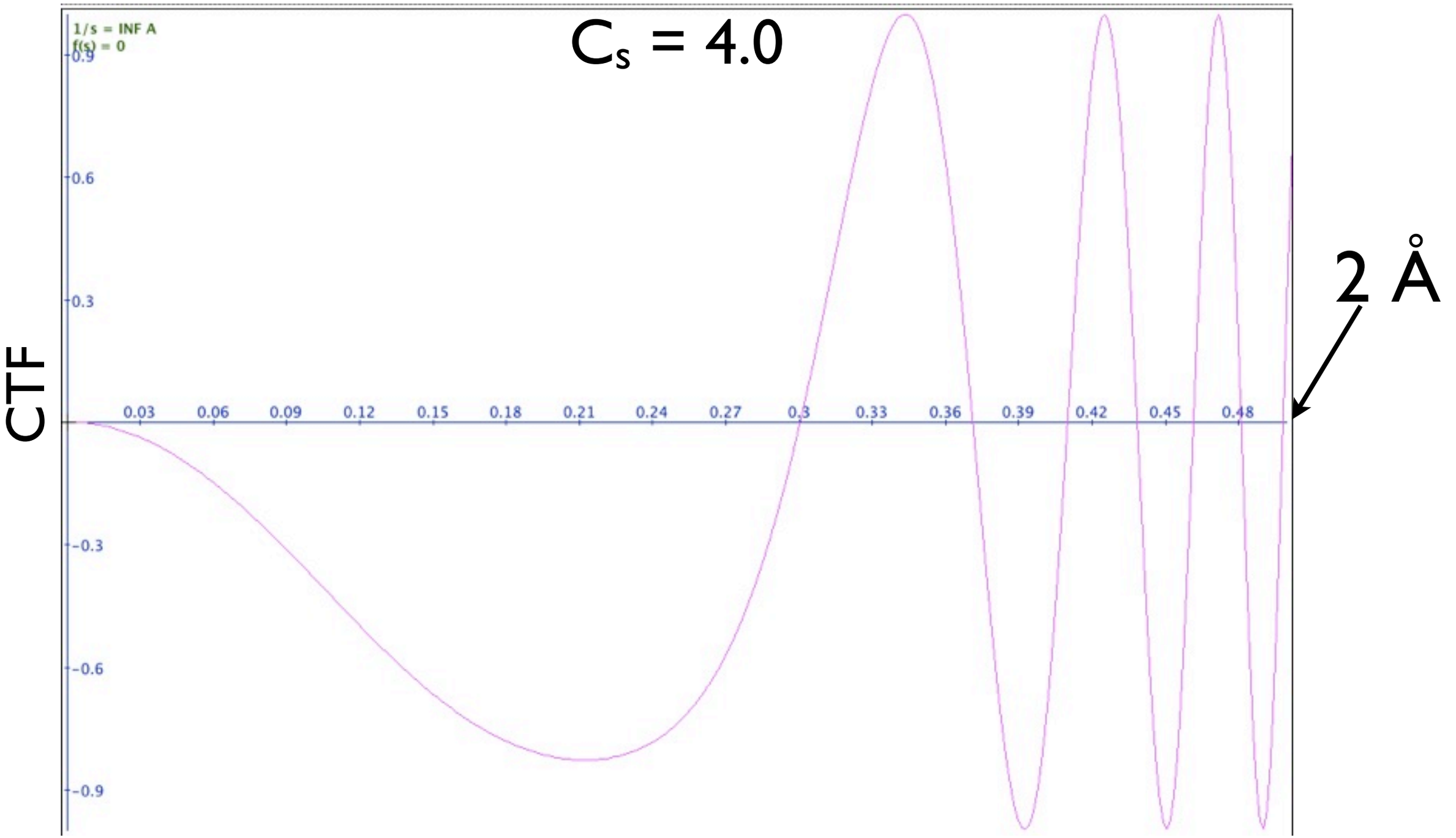
λ = electron wavelength

C_s = sph. abber. coefficient

s = spatial frequency (resolution)

Penczek, 2010

cryo-ET: underfocus matters



ncmi.bcm.tmc.edu/homes/wen/ctf/ctfapplet.html

The contrast transfer function

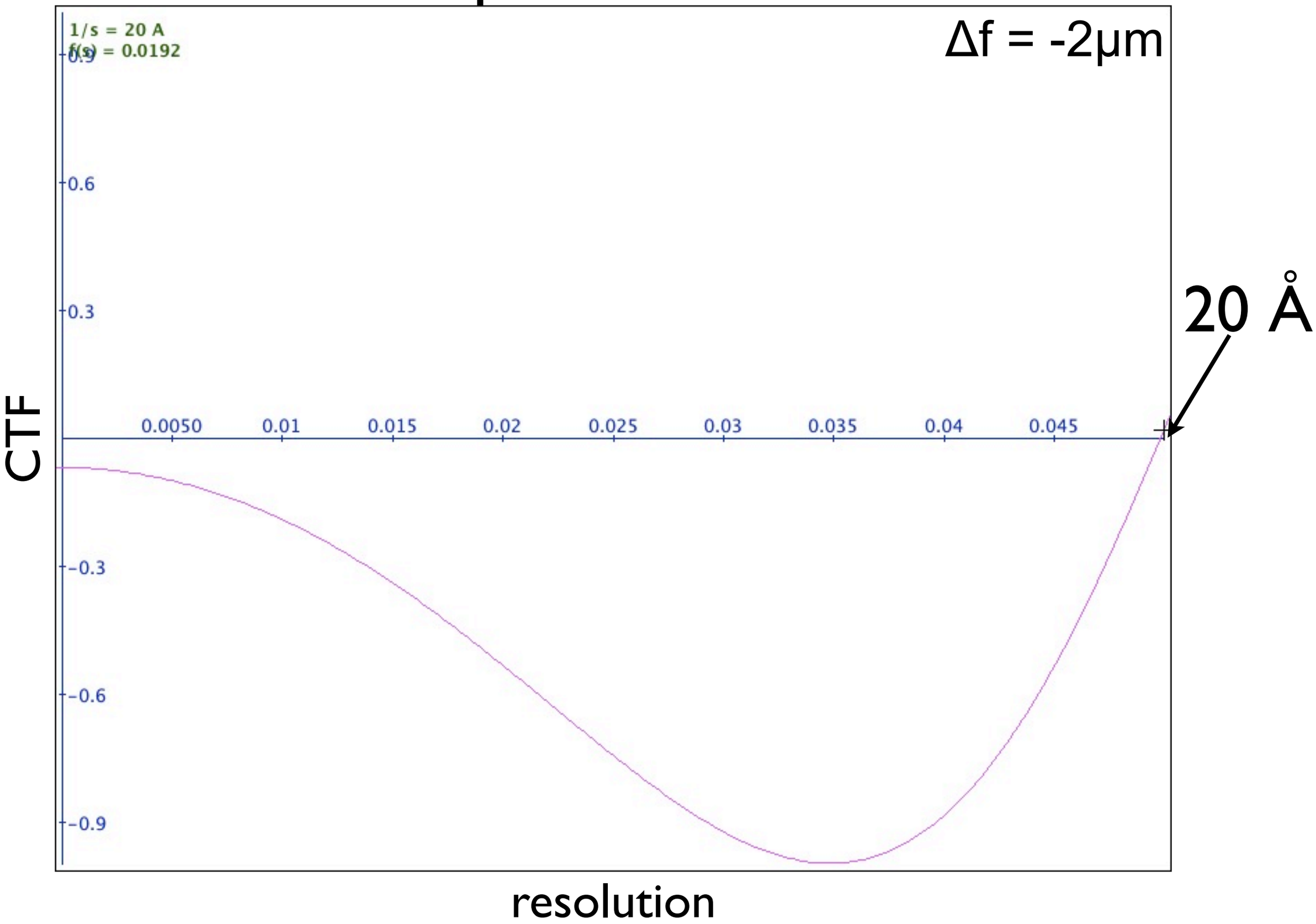
$$CTF(s; \Delta f) \approx -\sin(\pi \Delta f \lambda s^2)$$

Phase perturbation function:

$$\gamma(s;\Delta f) = 2\pi(-\tfrac{1}{2} \Delta f \lambda s^2)$$

Penczek, 2010

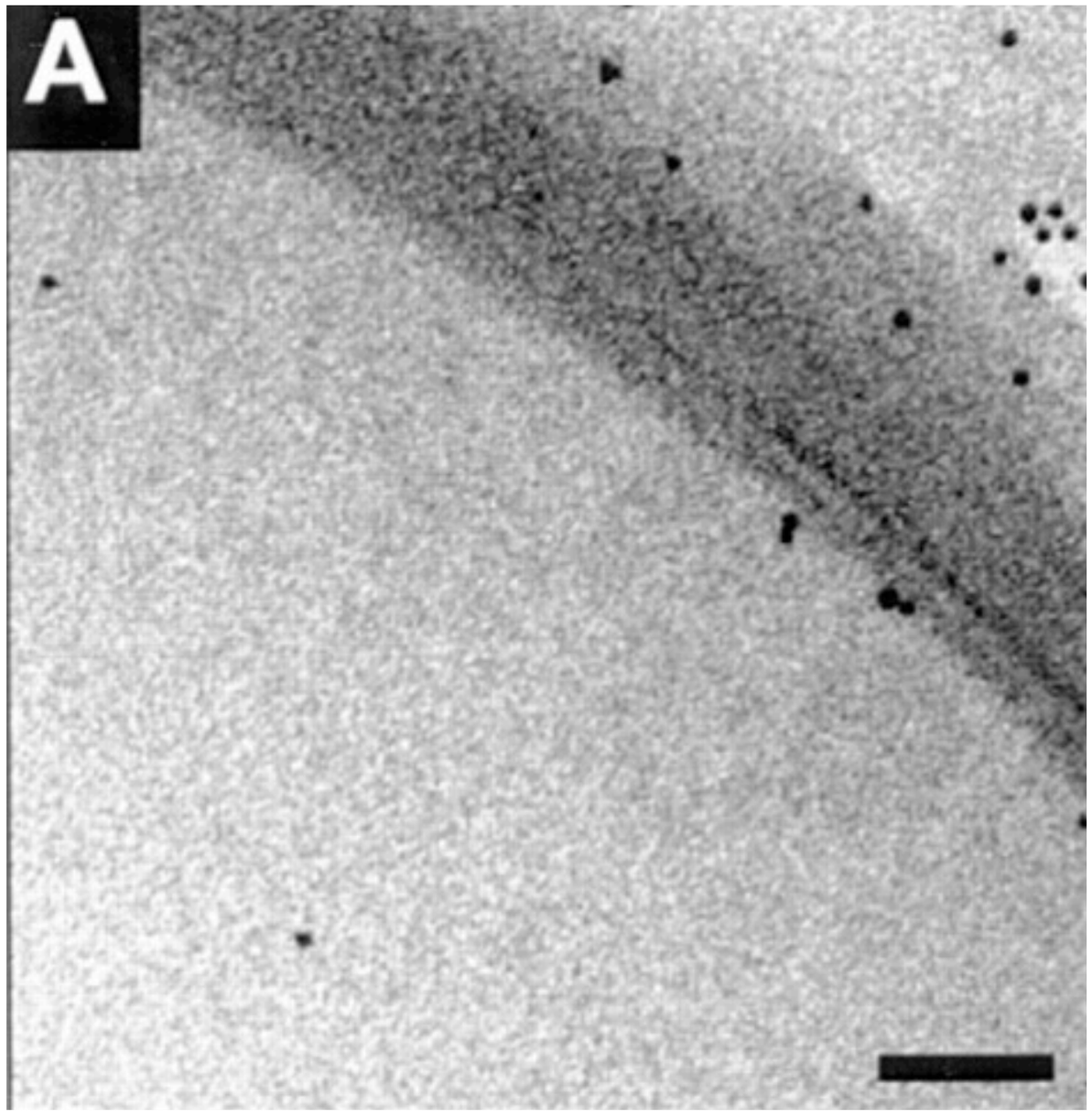
“close to focus” = poor low-resolution contrast



ncmi.bcm.tmc.edu/homes/wen/ctf/ctfapplet.html

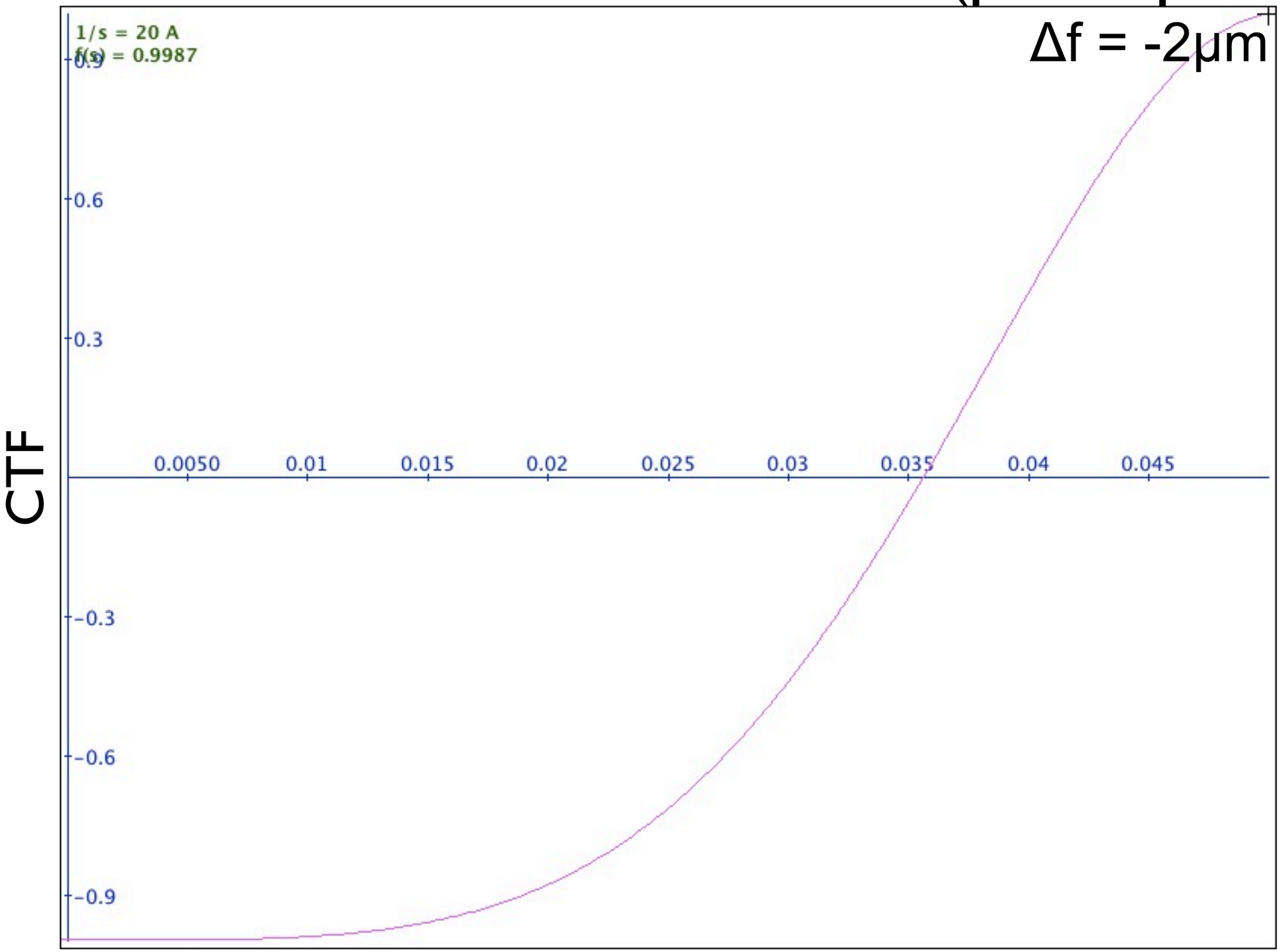
hard to detect molecules; worse in cells

Δf = -0.5μm



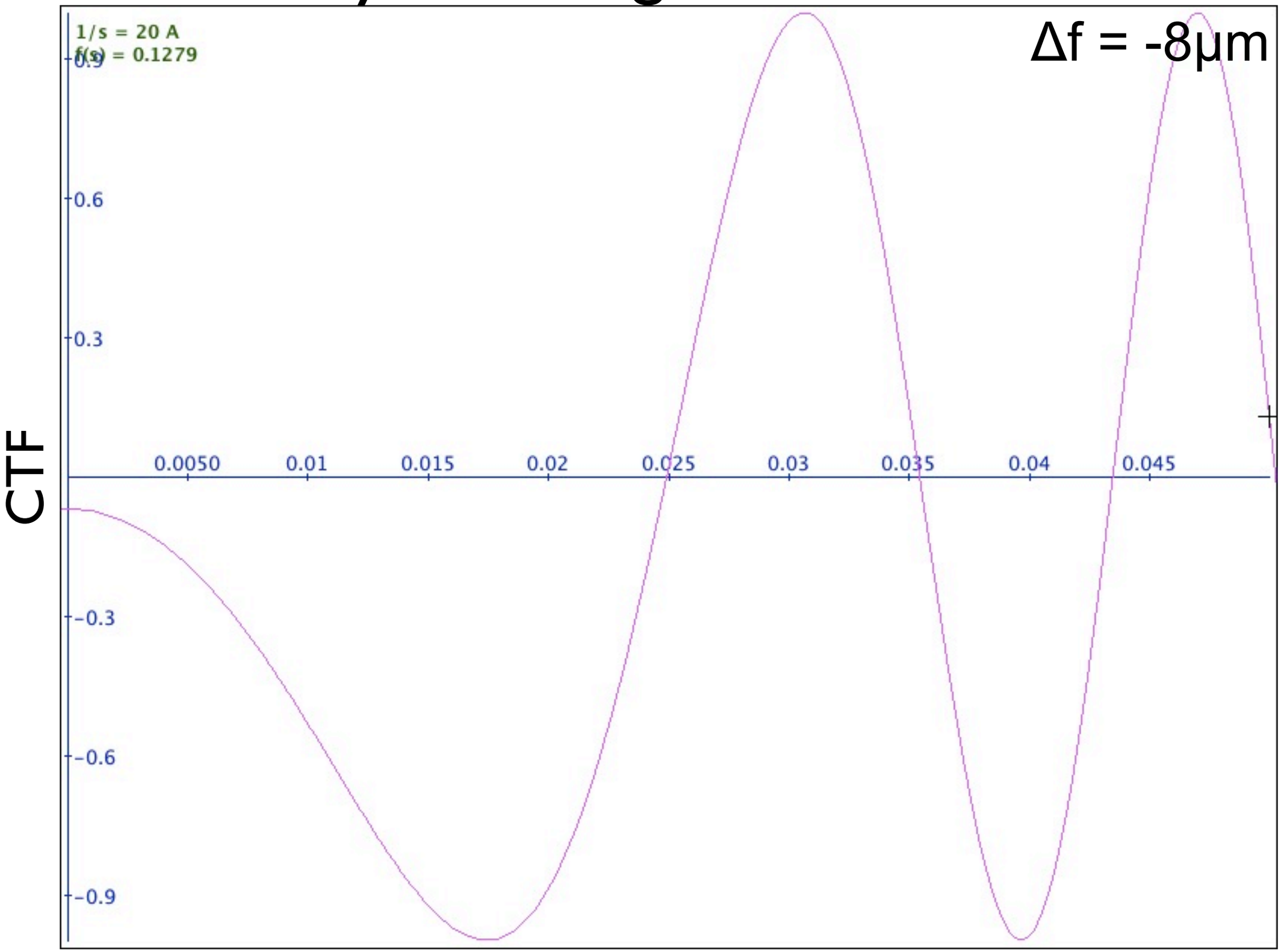
Walz, 1997

ideal contrast transfer function (phase plate)



ncmi.bcm.tmc.edu/homes/wen/ctf/ctfapplet.html

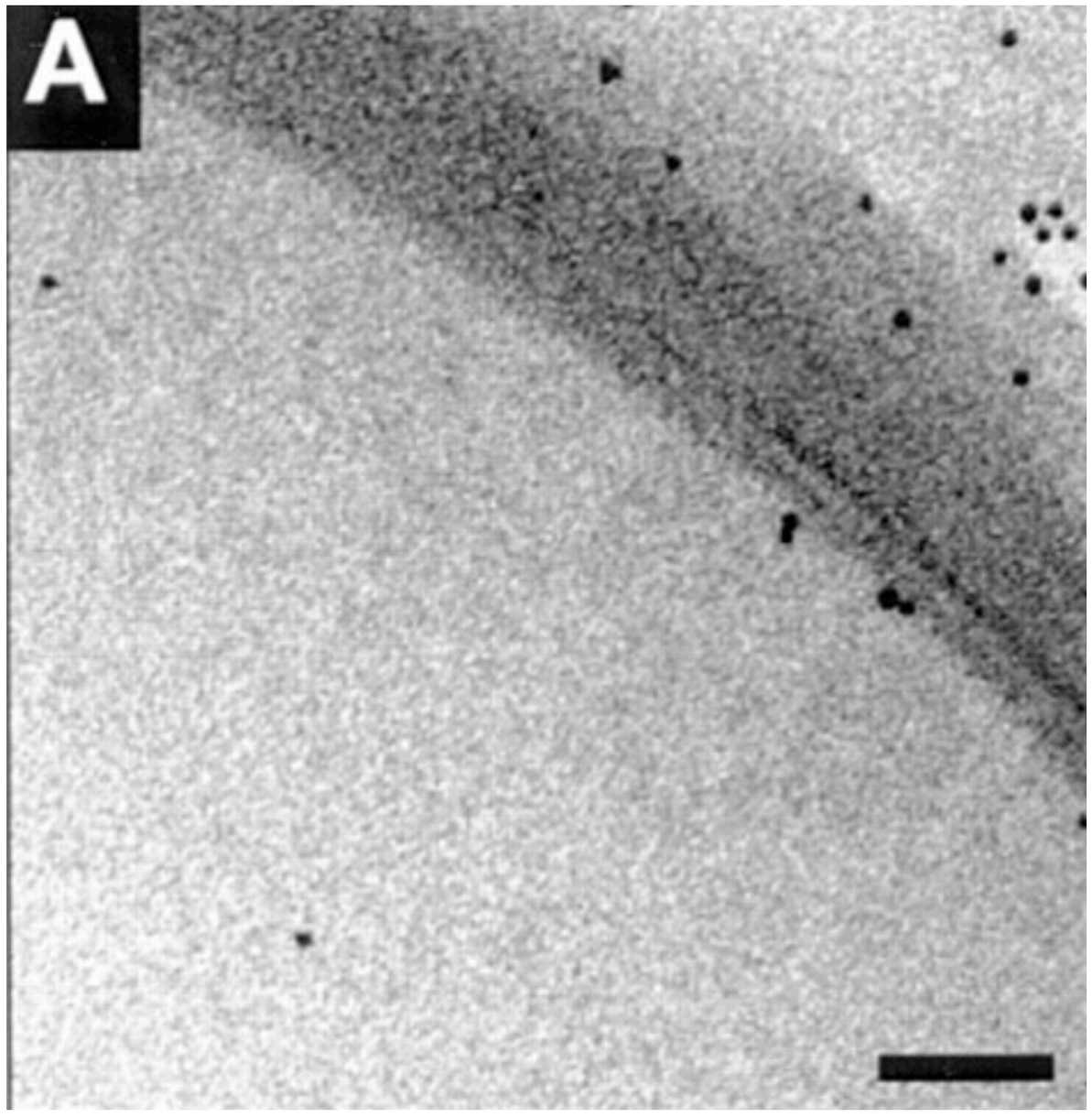
cryo-ET: large underfocus



ncmi.bcm.tmc.edu/homes/wen/ctf/ctfapplet.html

hard to detect molecules; worse in cells

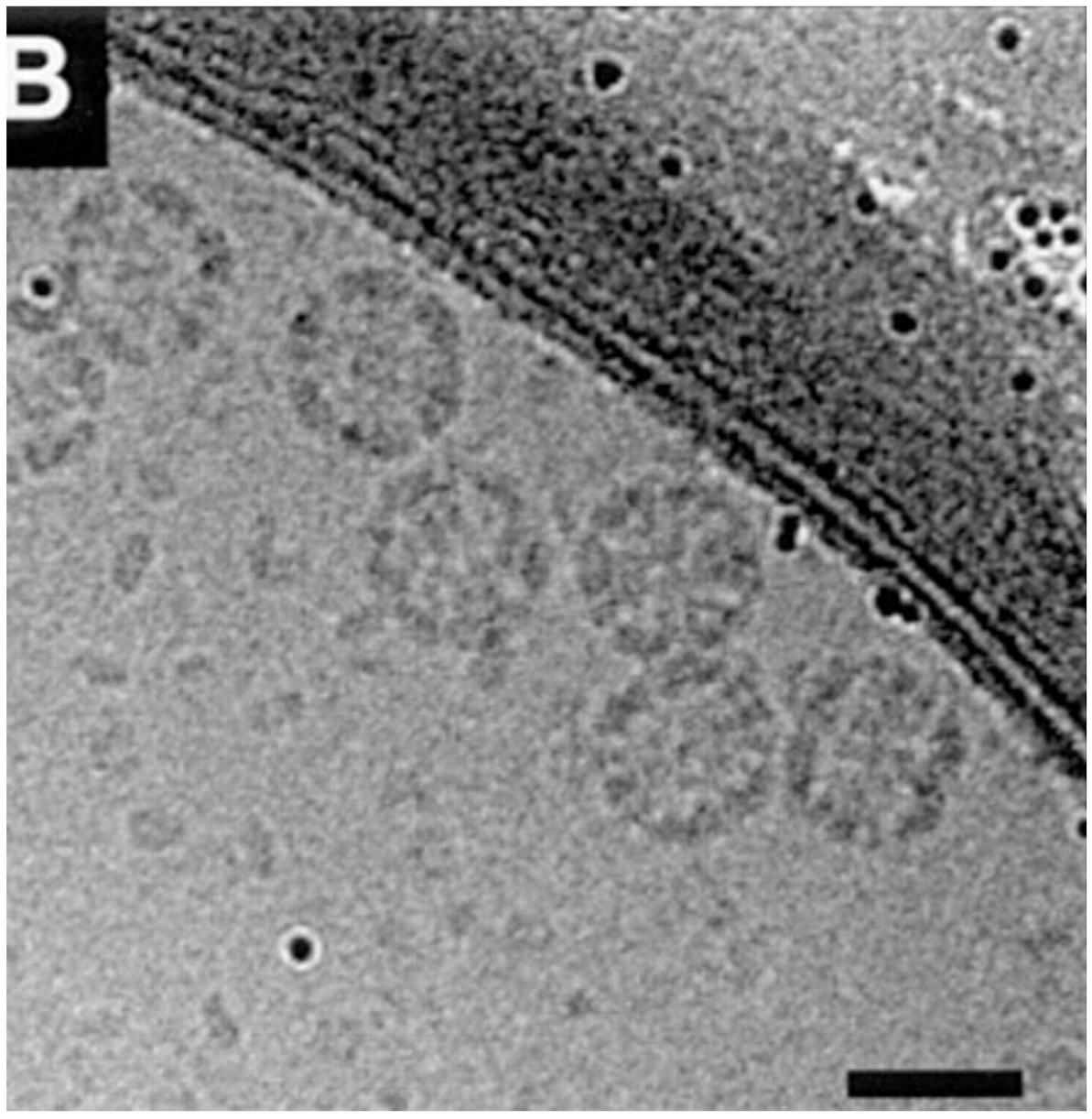
Δf = -0.5μm



Walz, 1997

without phase plate: need large underfocus

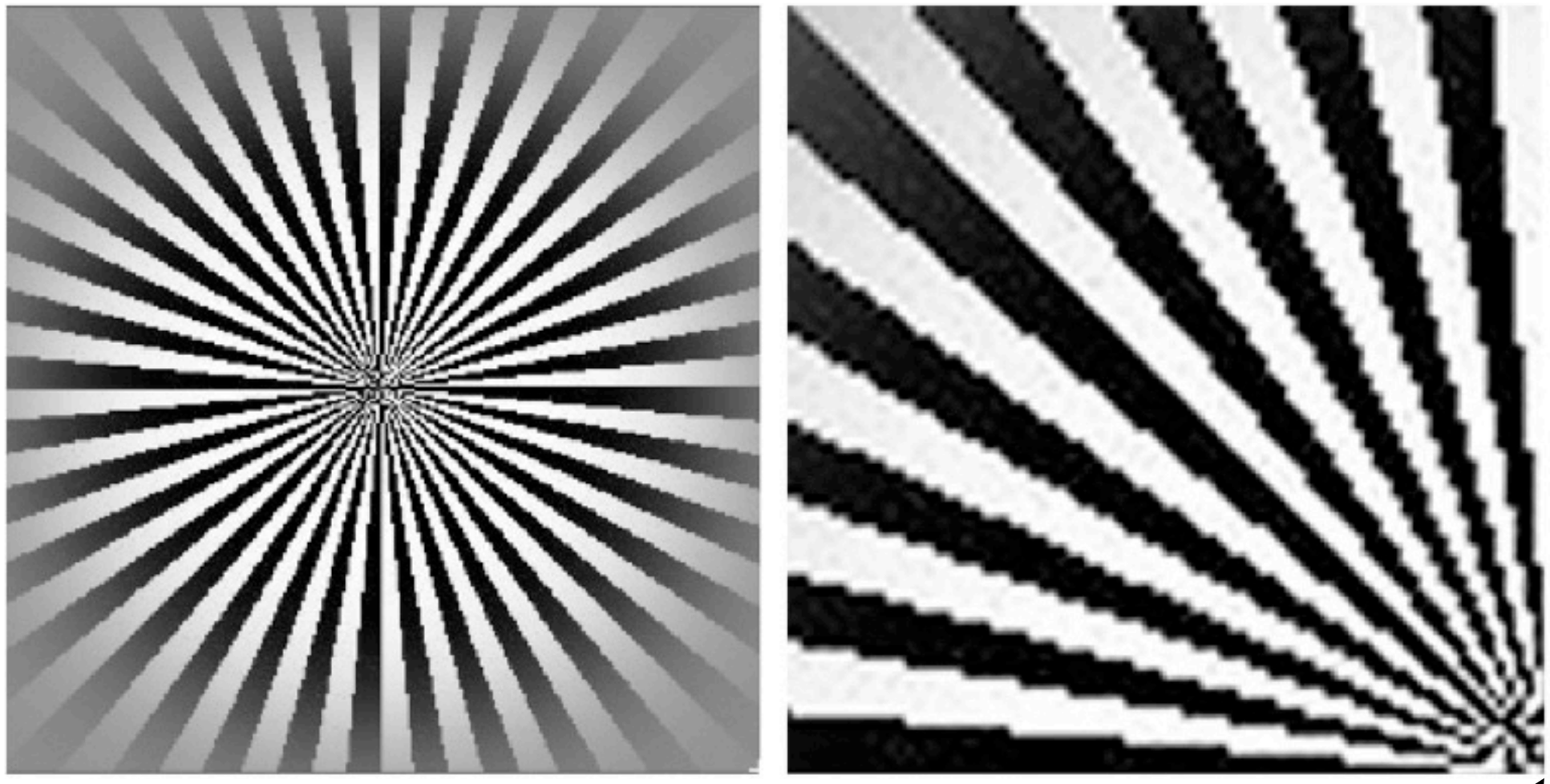
Δf = -5.0μm



Walz, 1997

cryo-ET: large defocus

original

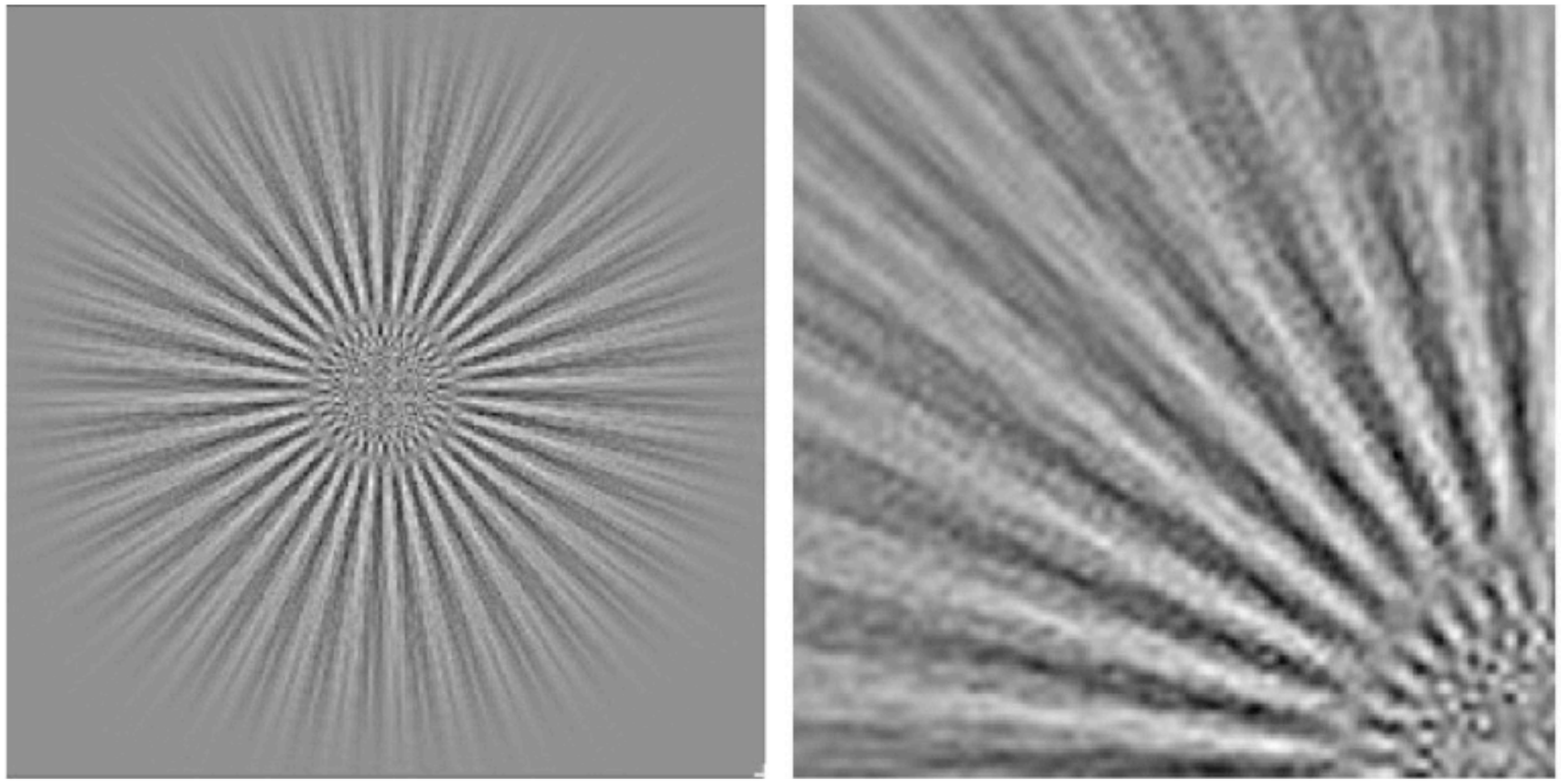


small spacing =
higher resolution

Fernandez, 2012

cryo-ET: large defocus

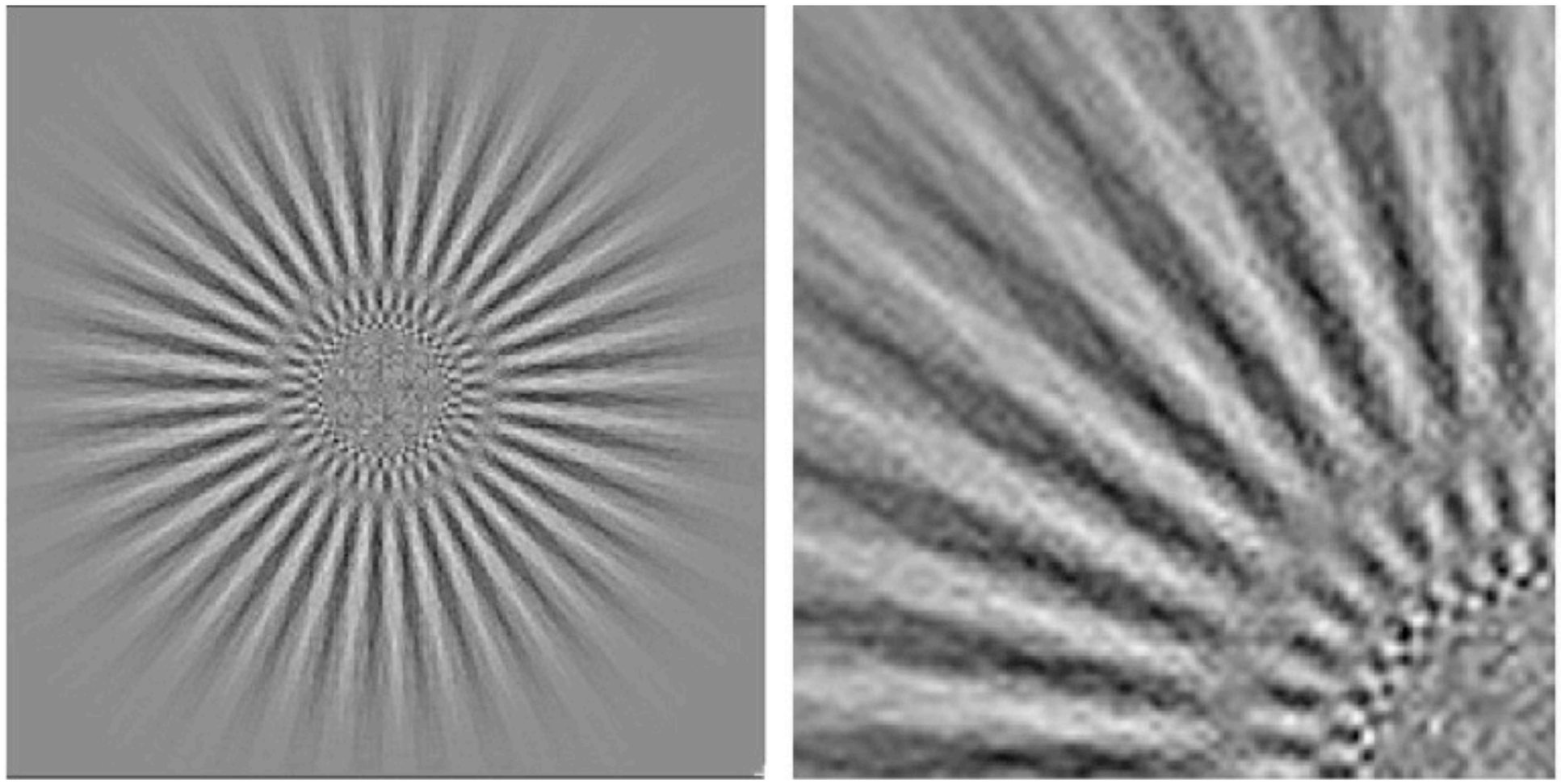
Δf = -2μm



Fernandez, 2012

cryo-ET: large defocus

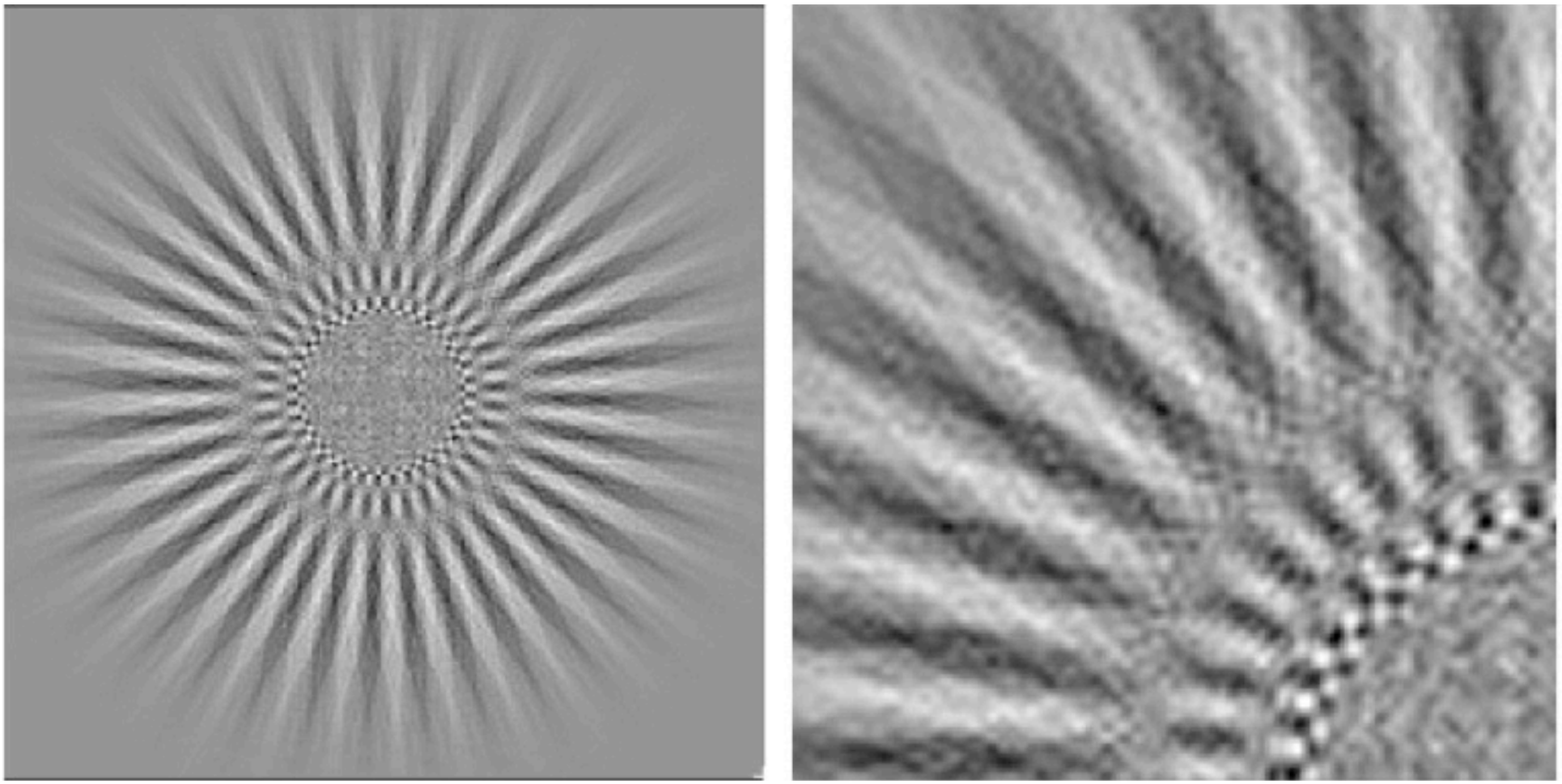
Δf = -5μm



Fernandez, 2012

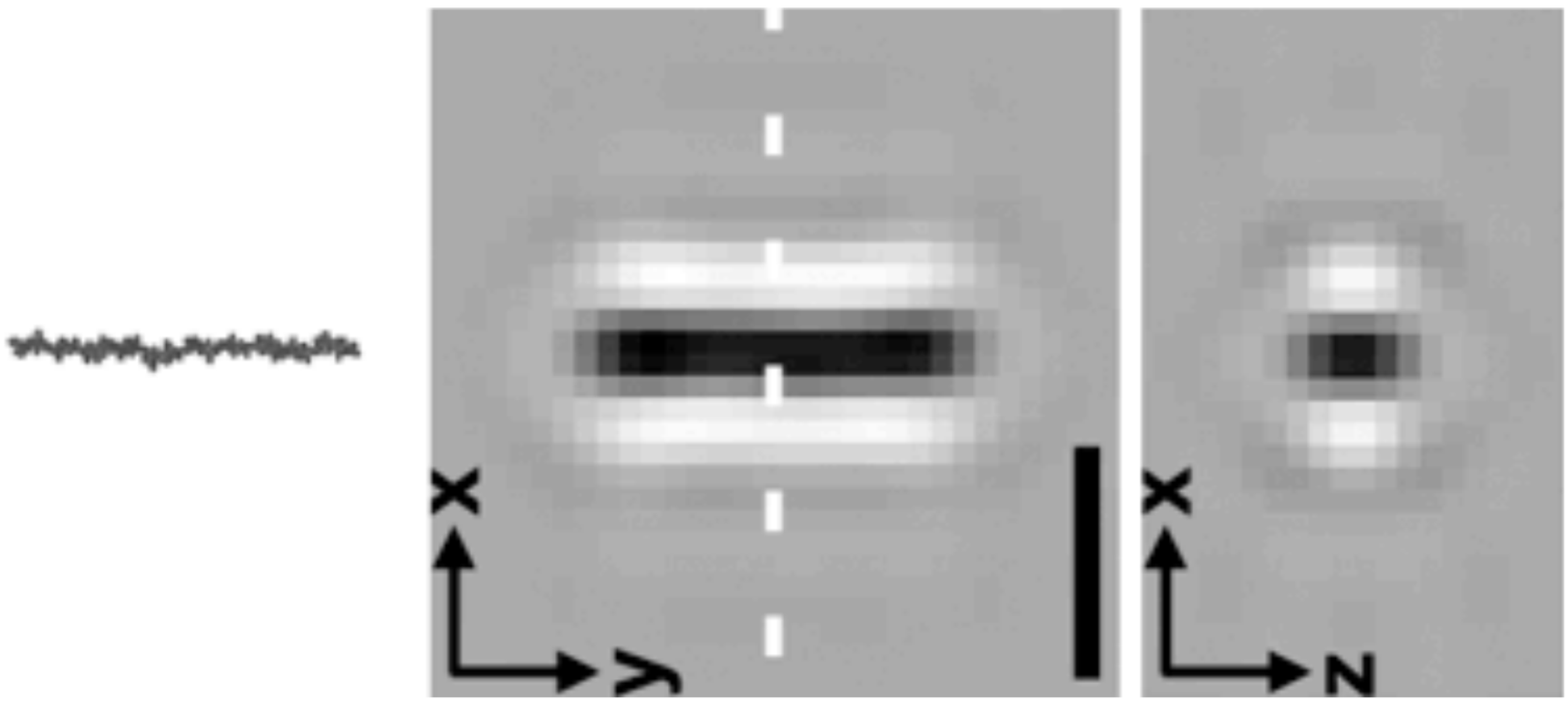
cryo-ET: large defocus

Δf = -8μm



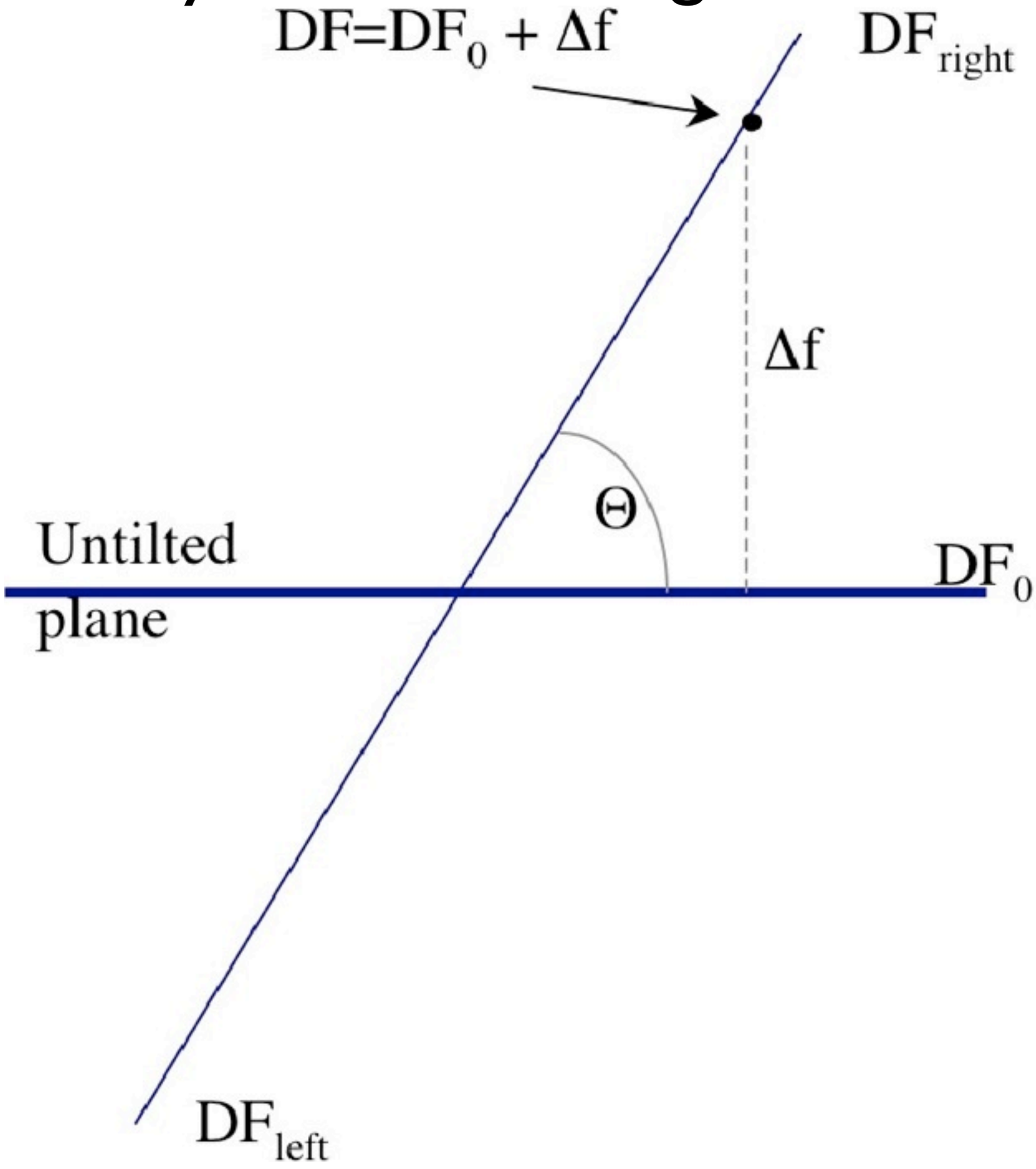
Fernandez, 2012

cryo-ET: large defocus



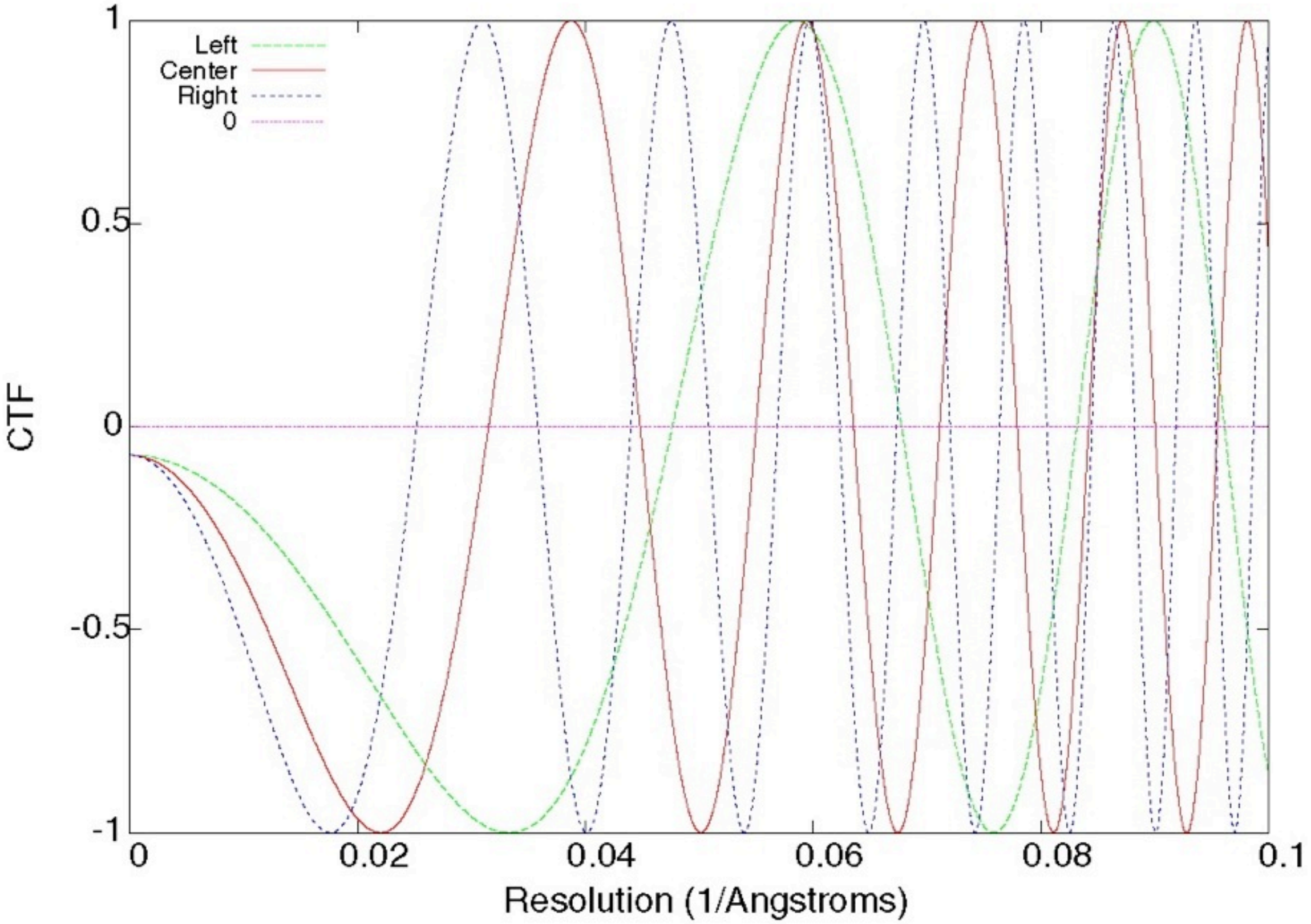
Gan, 2008

cryo-ET: defocus gradient



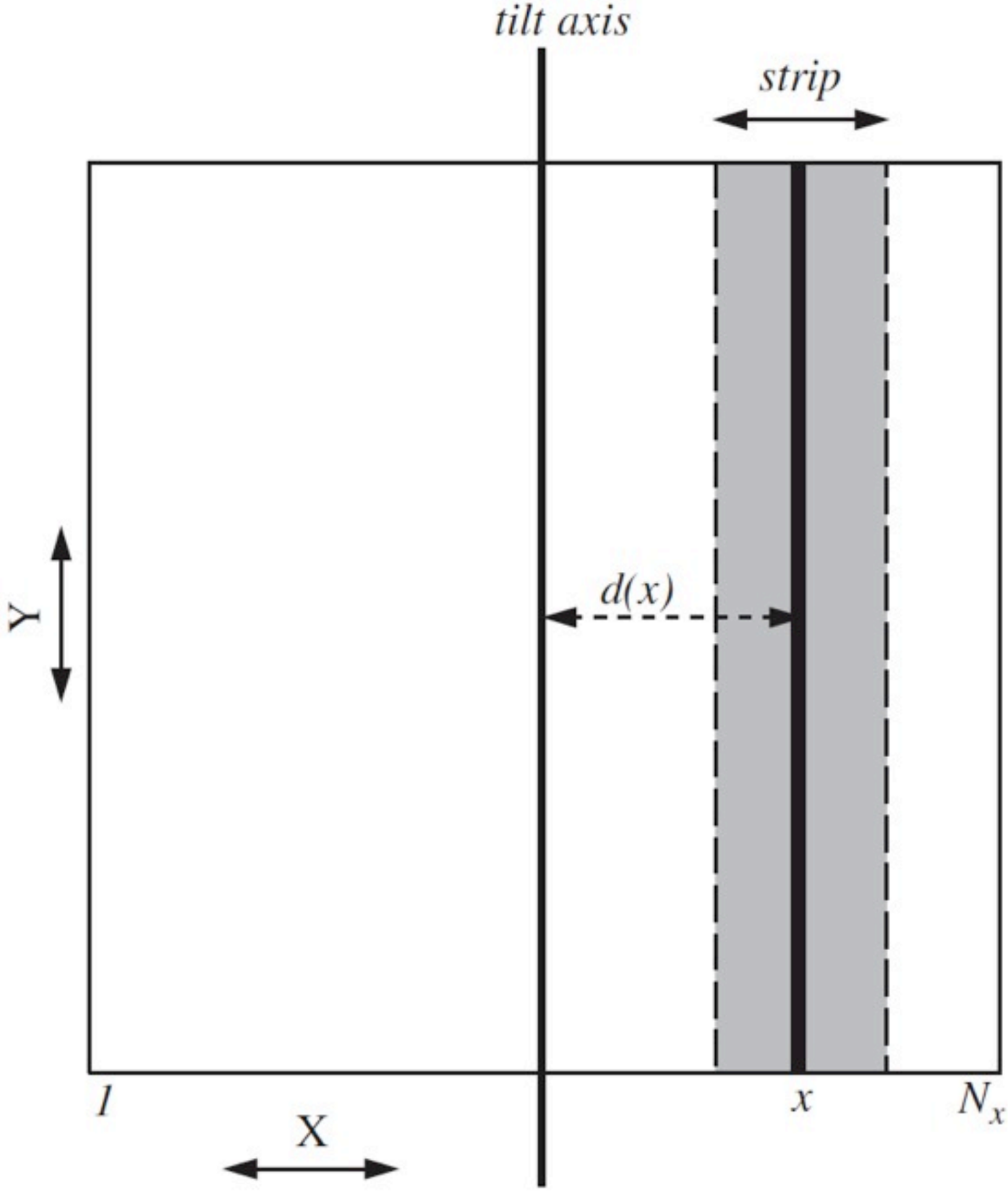
Fernandez, 2012

cryo-ET: defocus gradient



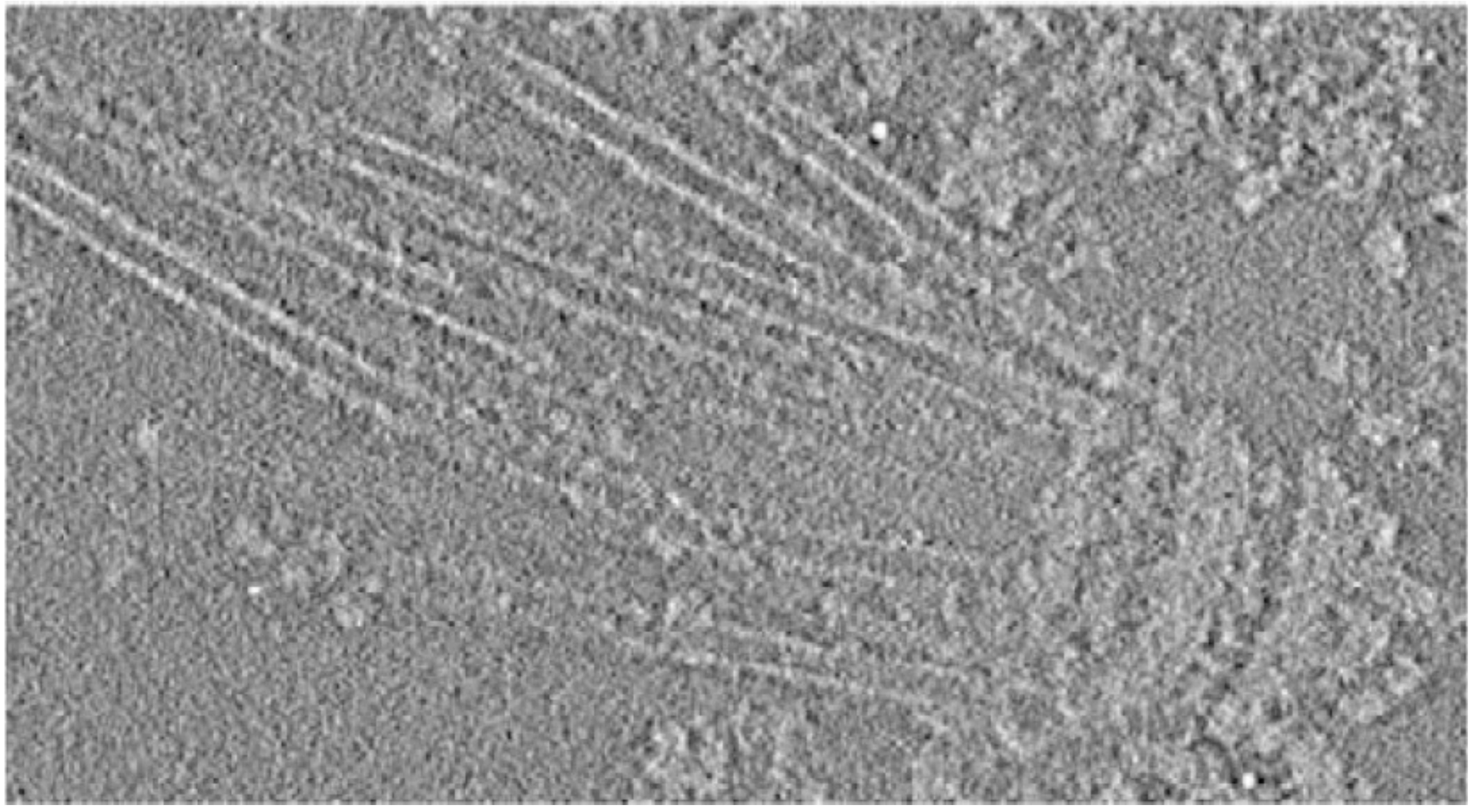
Fernandez, 2012

cryo-ET: defocus gradient



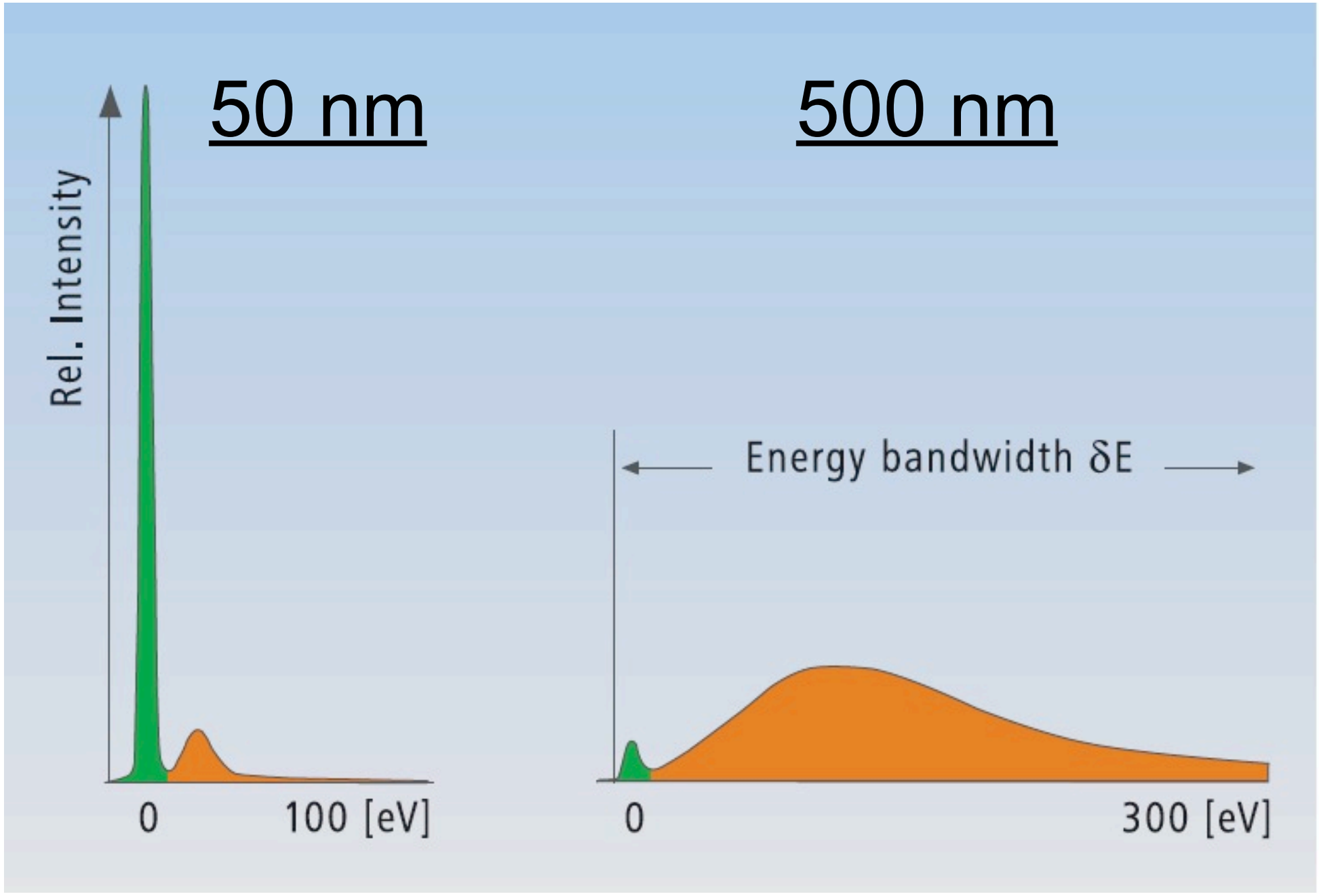
Fernandez, 2006

cryo-ET: defocus gradient

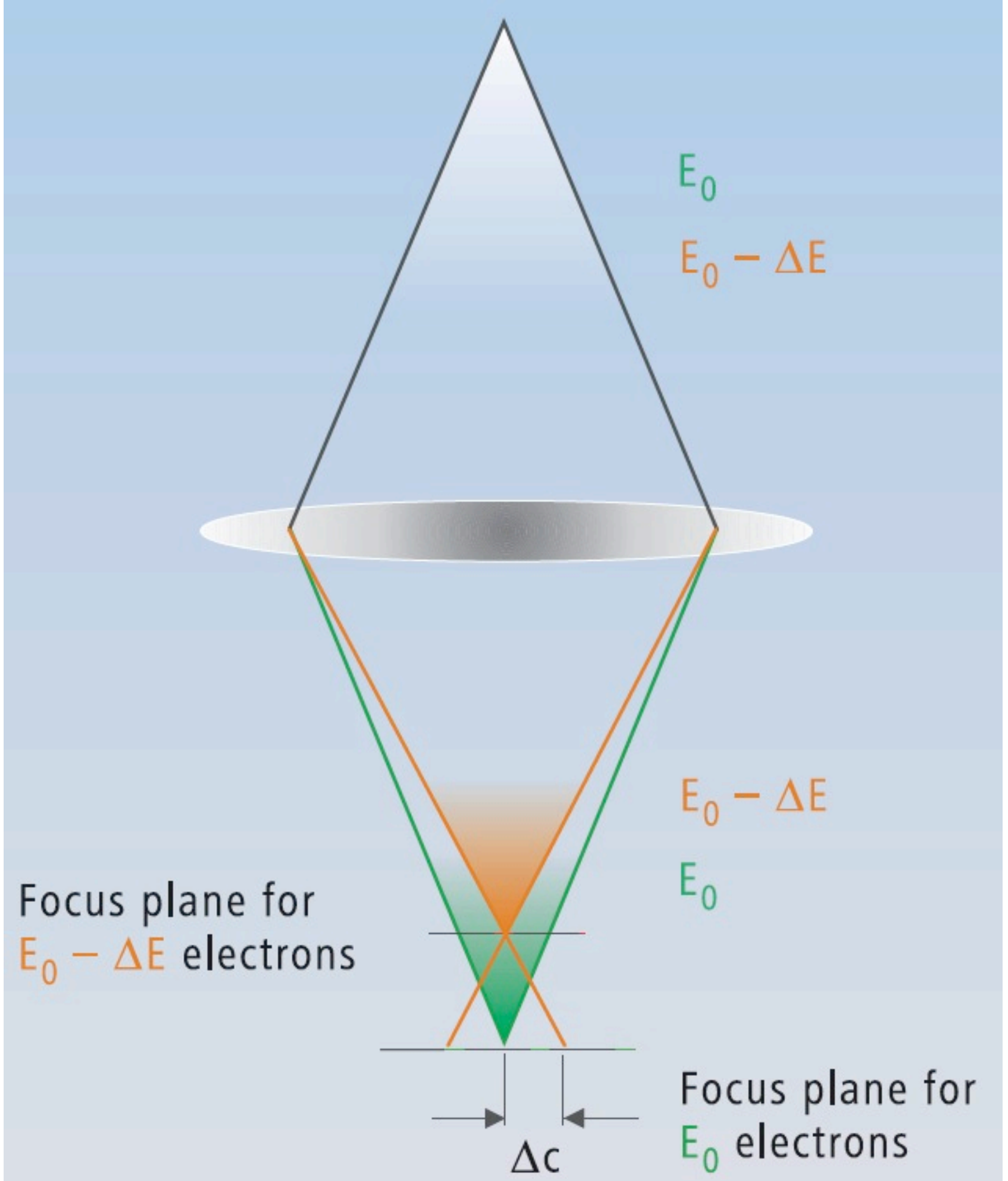


Fernandez, 2006

EELS Spectra, EPON sections



Zeiss NTS


E_0
$E_0 - \Delta E$
$E_0 - \Delta E$
E_0
Focus plane for
$E_0 - \Delta E$ electrons
Focus plane for
E_0 electrons
Δc

Zeiss NTS

How to deal with samples that are too thick

$$$$ Make them thinner

$$$$ Get rid of inelastically scattered electrons

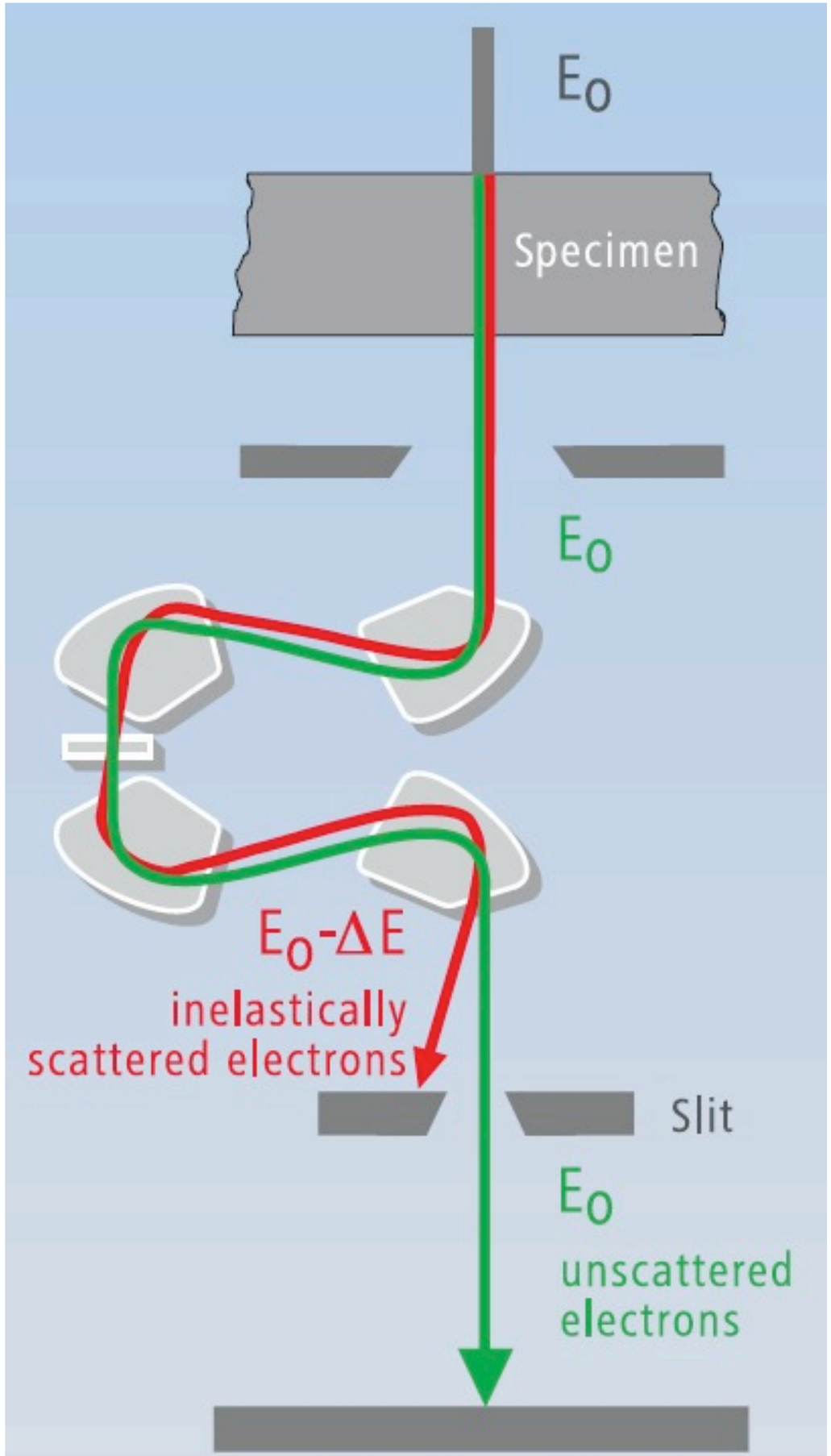
$$$$$$$$$$ Correct chromatic aberration

Energy filtered TEM (EF-TEM)

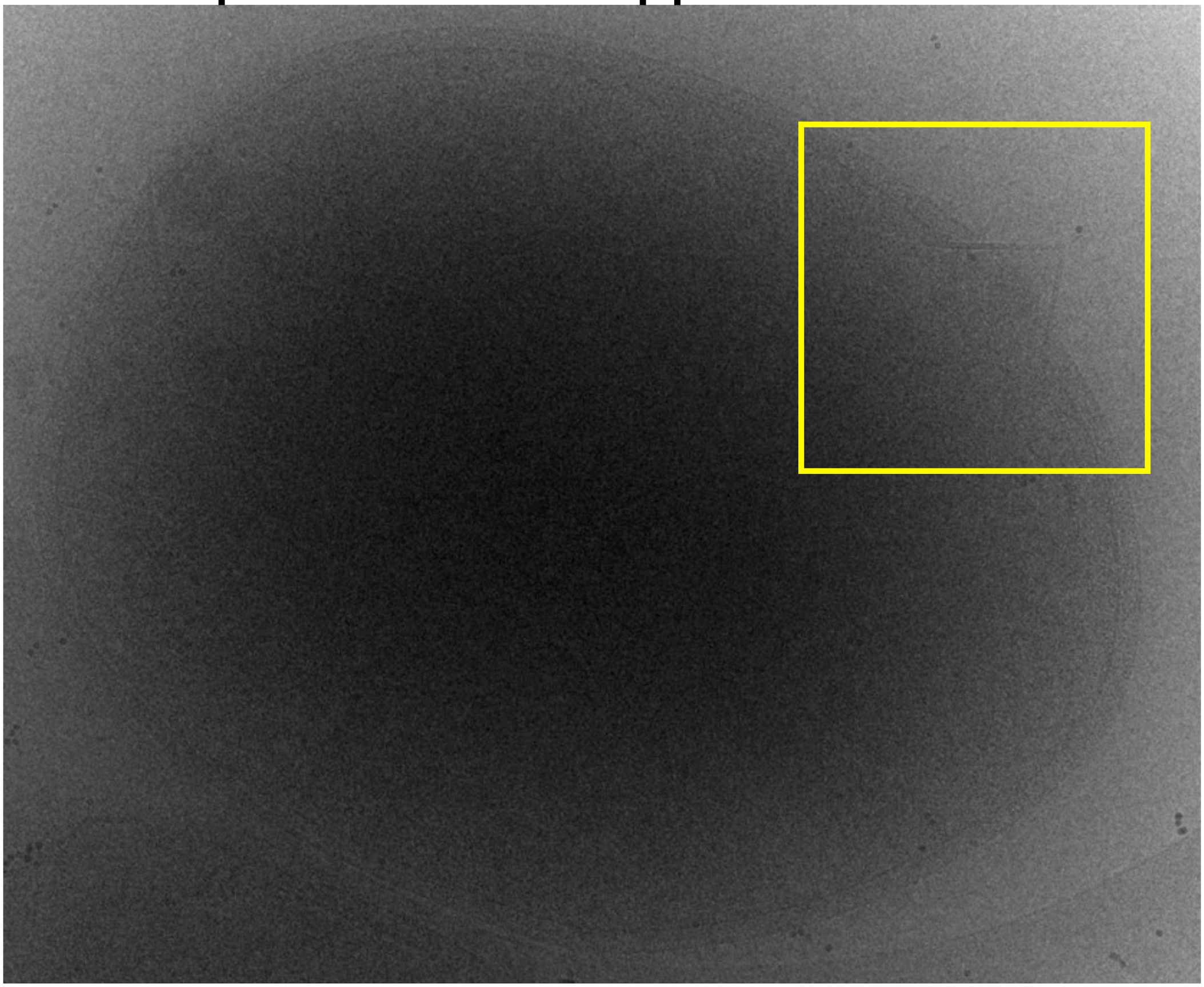
1. Disperse scattered electrons with an magnetic prism.

2. Remove inelastically scattered electrons with a selection slit.

When only the unscattered (E_0) electrons are selected, we call it "zero-loss" imaging mode.

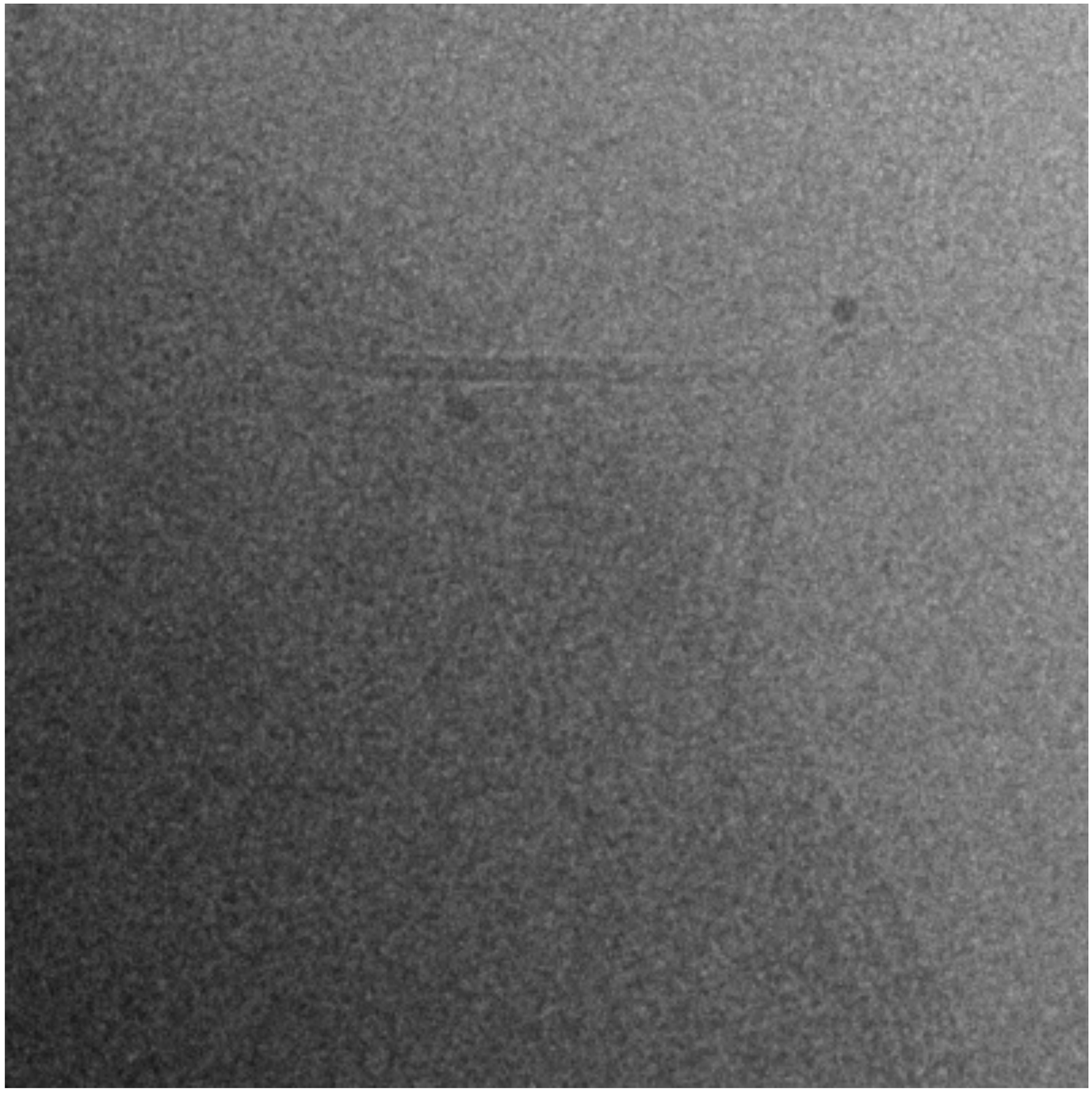


An example of EFTEM applied to archaeal cell



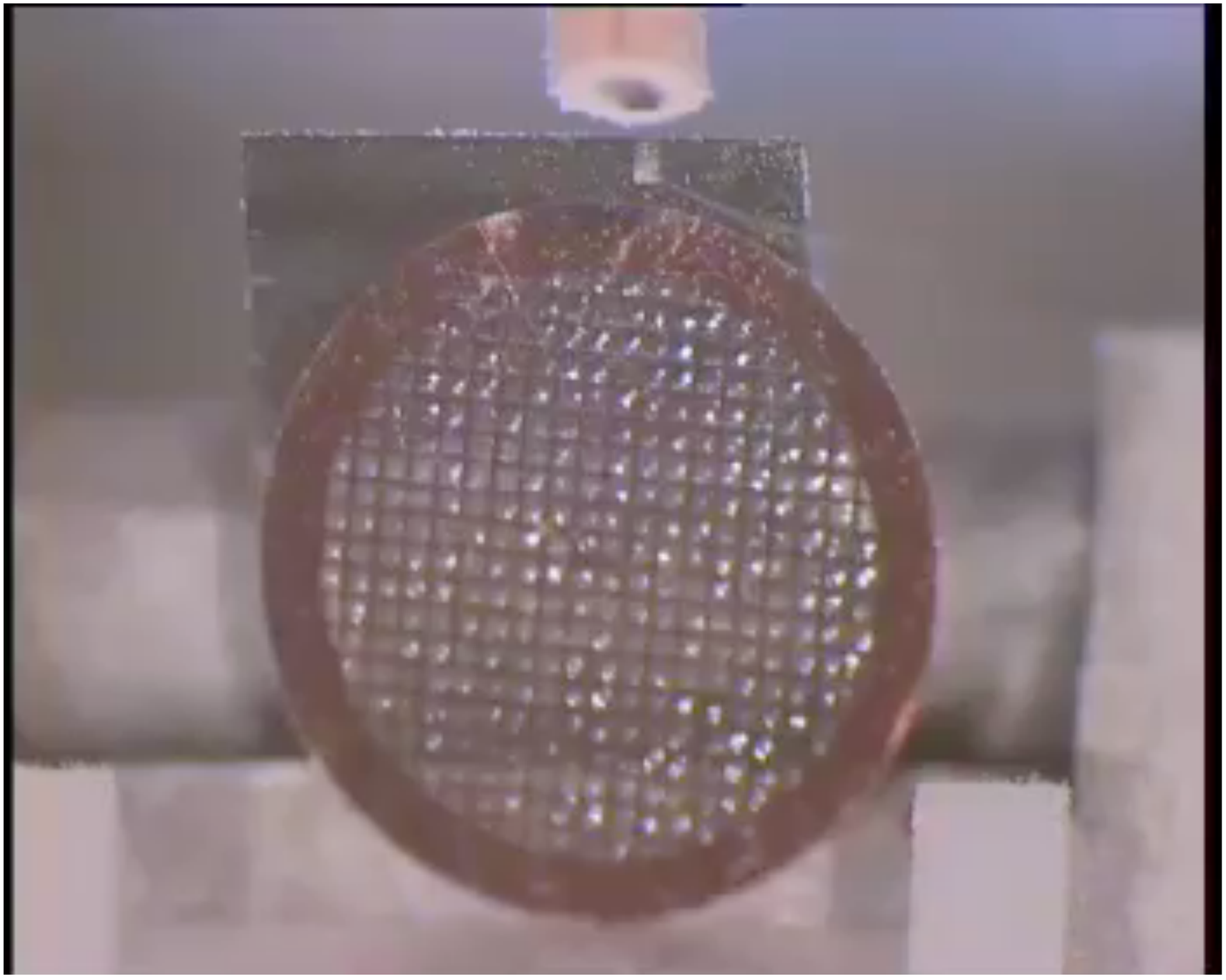
Fu, unpublished

An example of EFTEM applied to archaeal cell

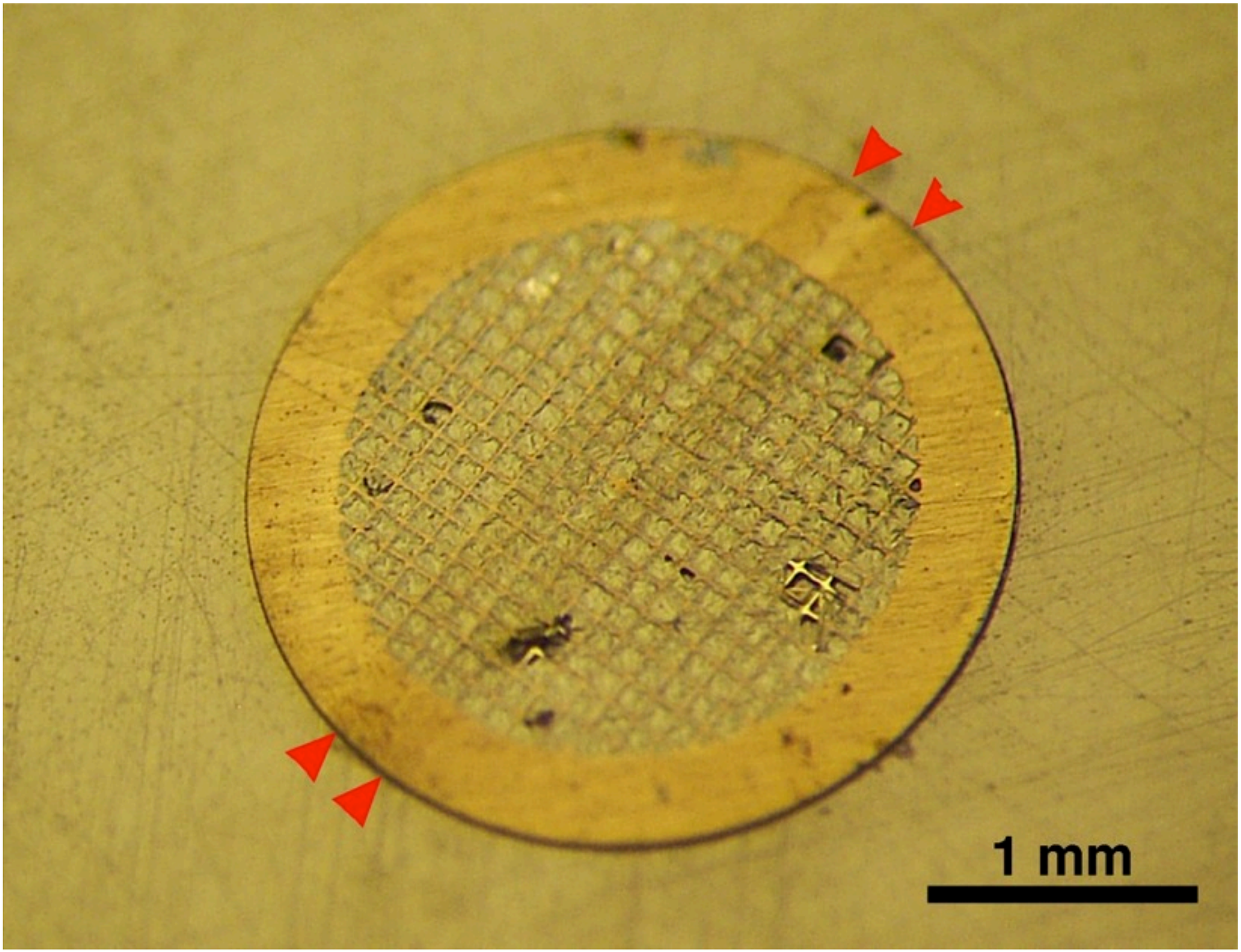


Fu, unpublished

microtomy: making ultrathin samples



Pierson, 2009



Ladinsky, 2006

You can't beat physics

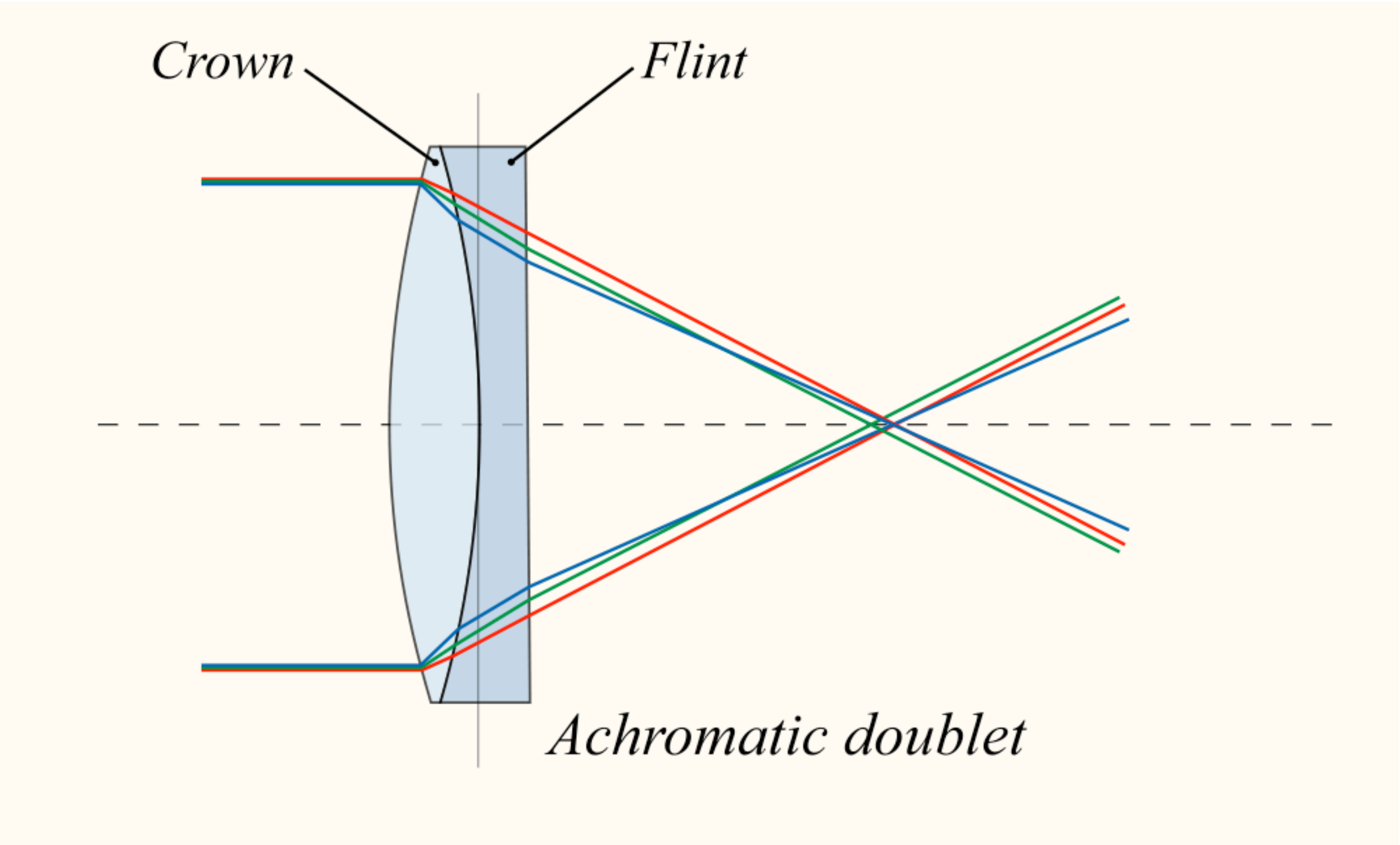
Table 3

Experimental and theoretical elastic and inelastic scattering cross-sections and mean free path lengths for electrons in amorphous ice layers. The experimental errors of the cross-sections and mean free path lengths are about 10–15%, resulting in an error of 20–30% for μ

	exp.	CD and HR [9]
σ_{in} $[10^{-4}\,nm^2]$	3.82	3.06
Λ_{in} [nm]	84.8	106
σ_{el} $[10^{-4}\,nm^2]$	1.15	0.86
Λ_{el} [nm]	283.4	379
$\mu = \sigma_{in} / \sigma_{el}$	3.34	3.15
120 keV, ρ_{ice} = 0.92 g/cm^3		

Angert, 1996

... or can you? Chromatic aberration correction



Wikipedia

C_s + C_c correction: the future of cryo-EM?

Haider, 2008

C_s + C_c correction: the future of cryo-EM?

CEOS

Tuesday, July 10, 12

1. What is tomography?

2. Sample preparation (what kind?)

3. Principles of reconstruction

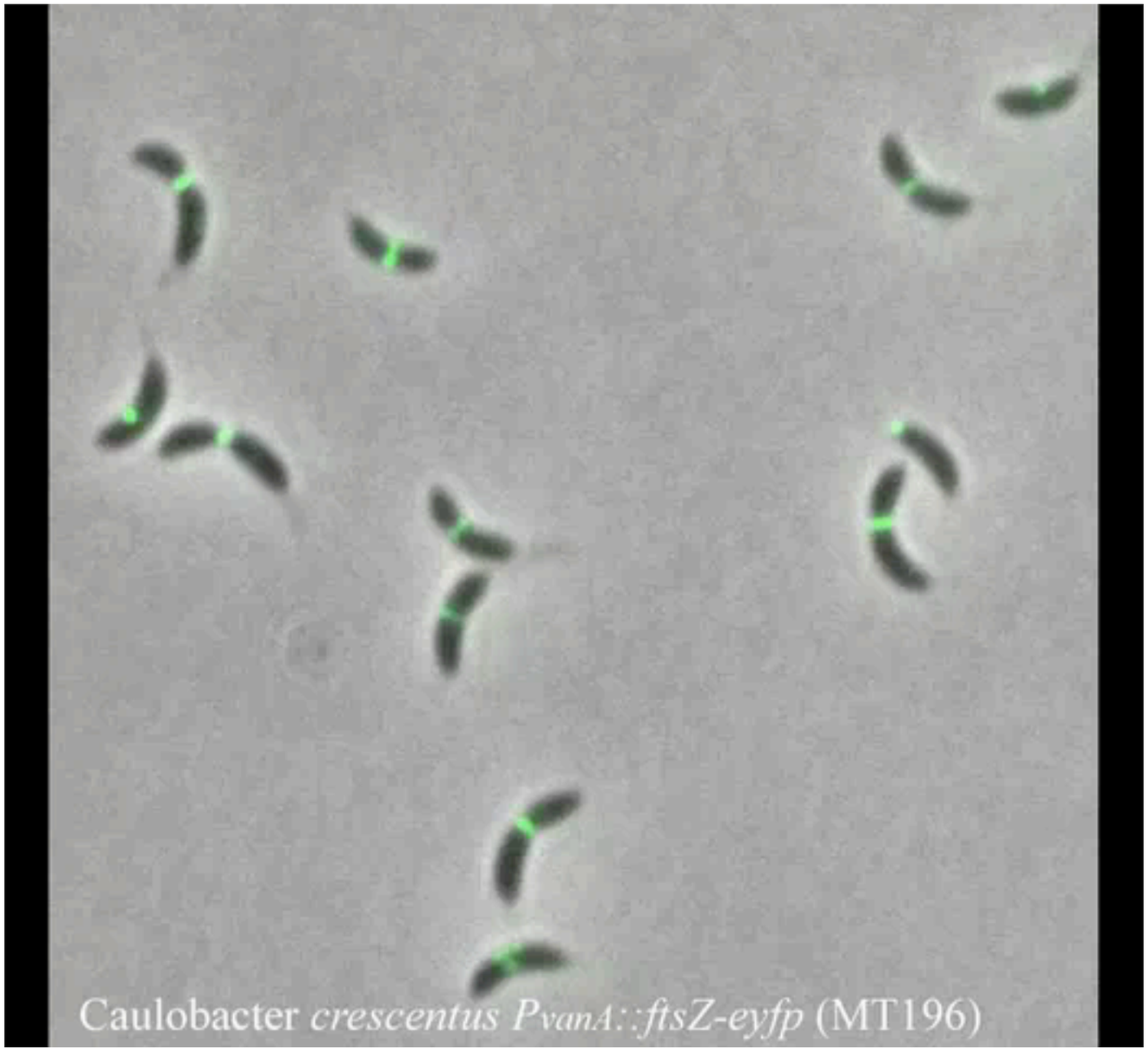
4. Beware of artifacts

5. Example studies

Examples

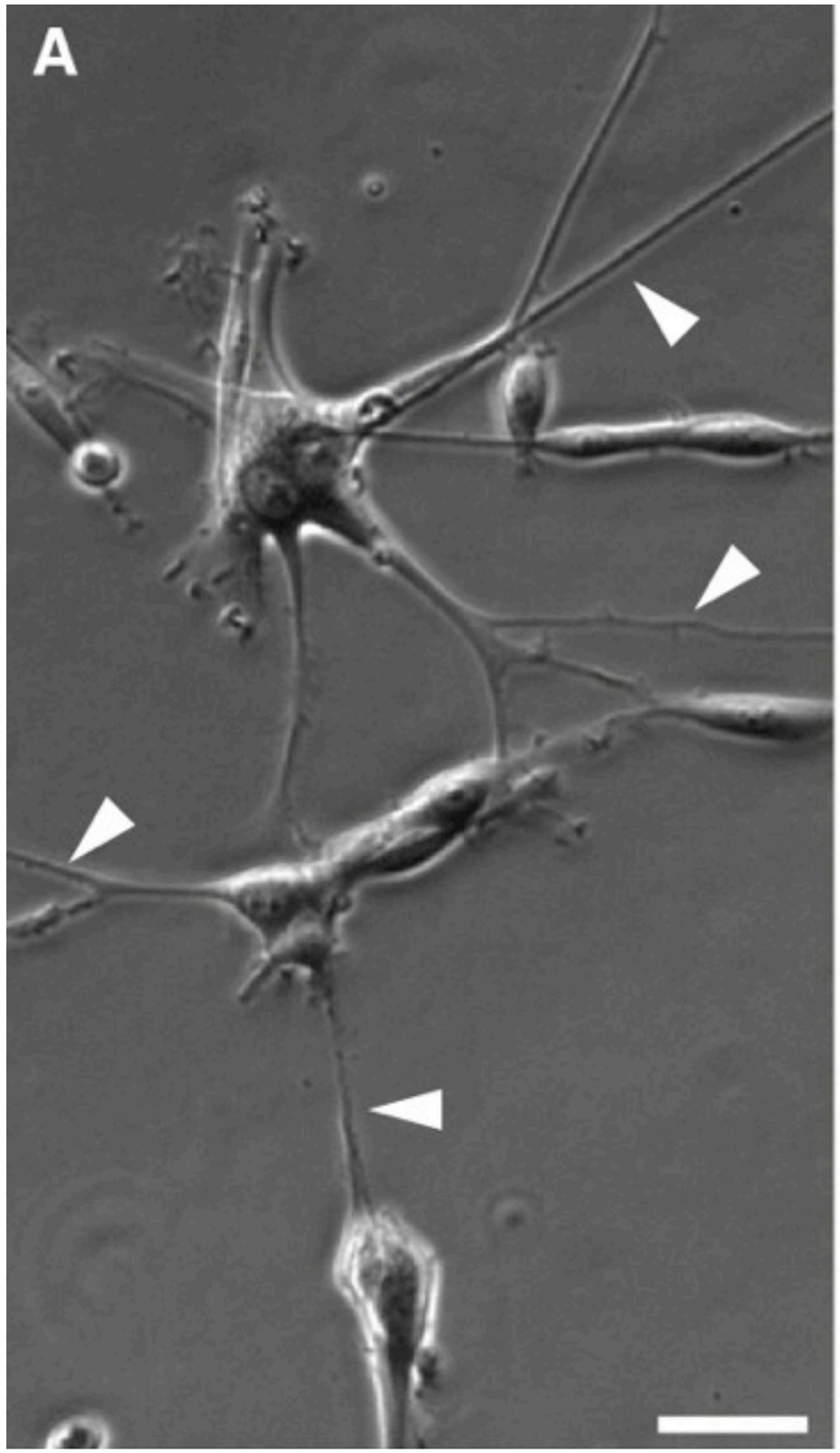
Li, 2007

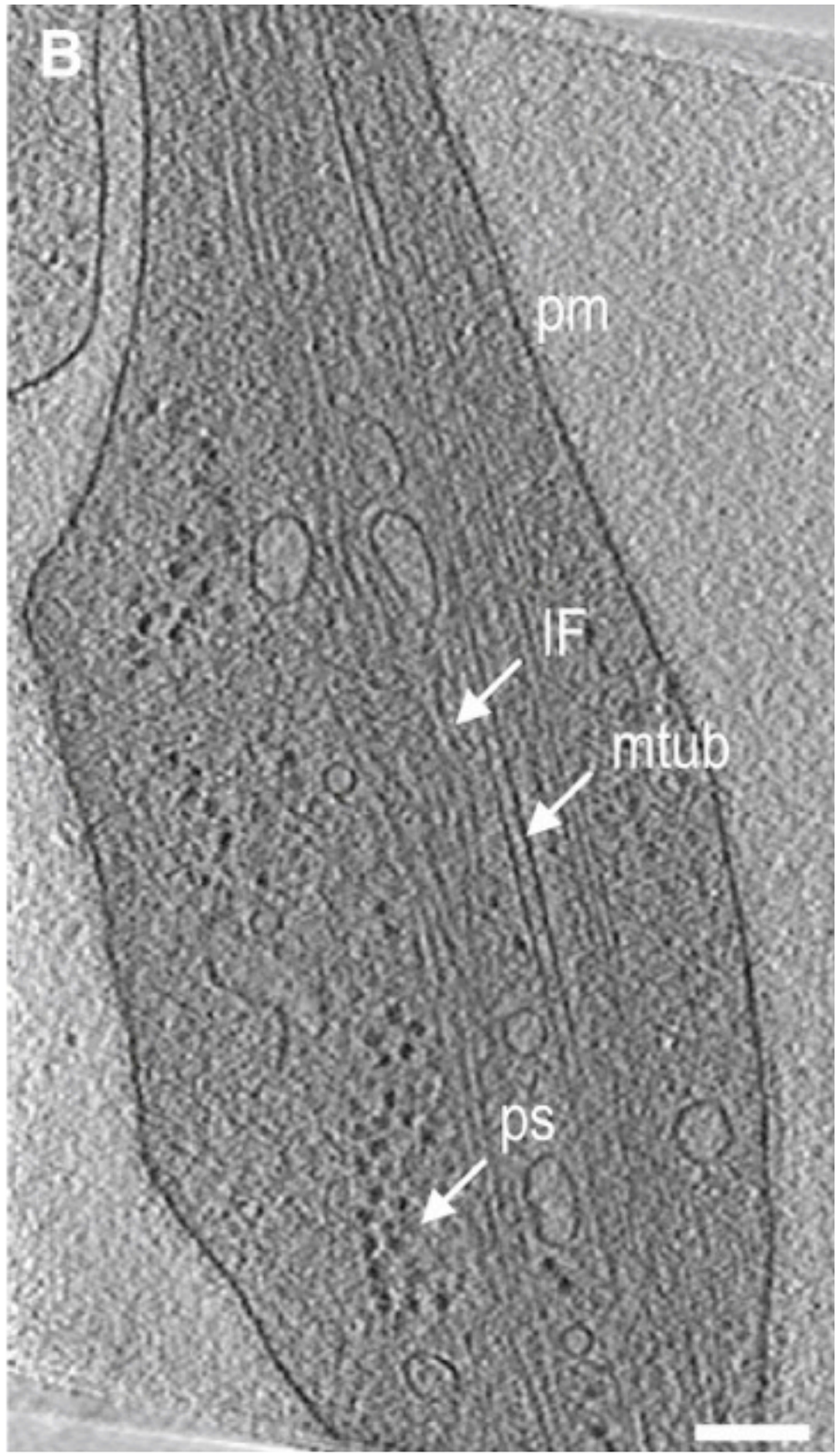
Examples



Li, 2007

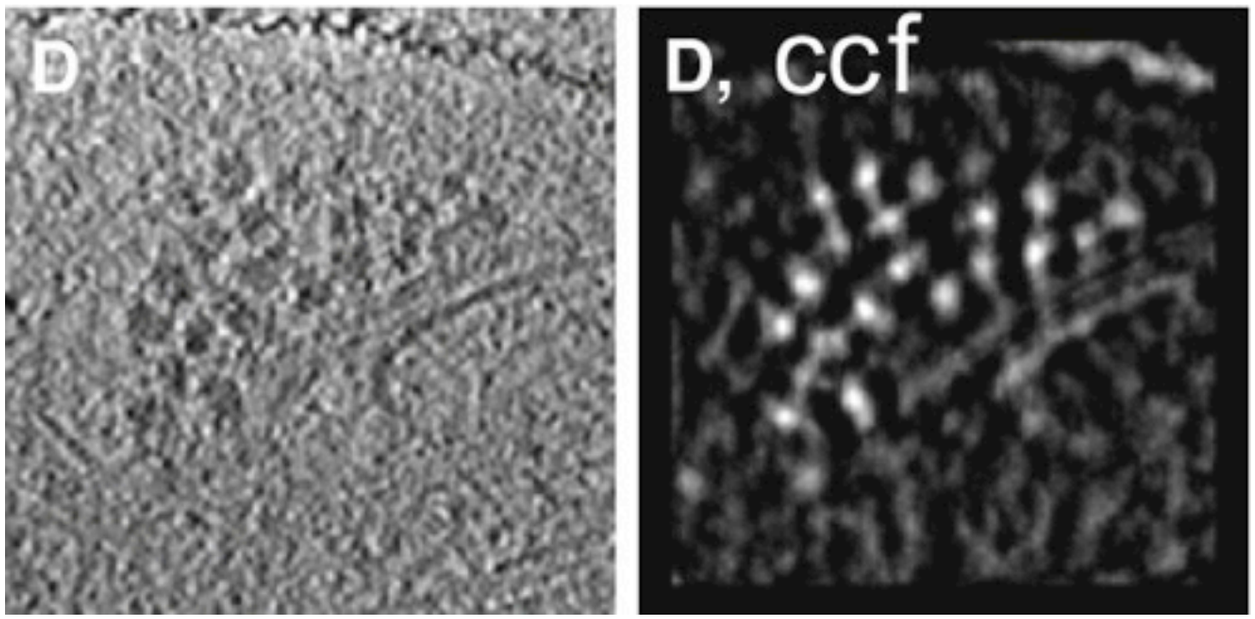
Examples





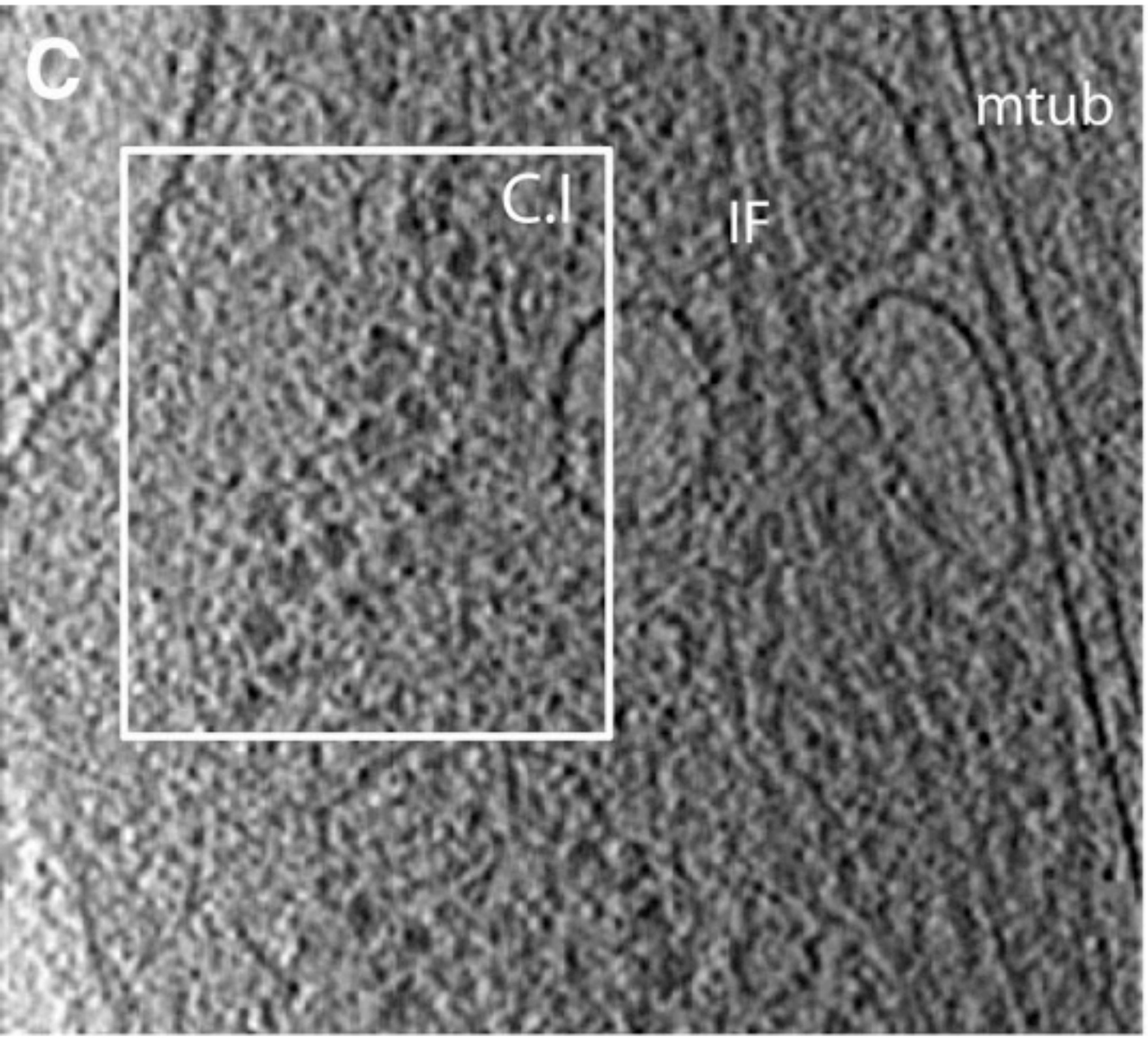
Brandt, 2010

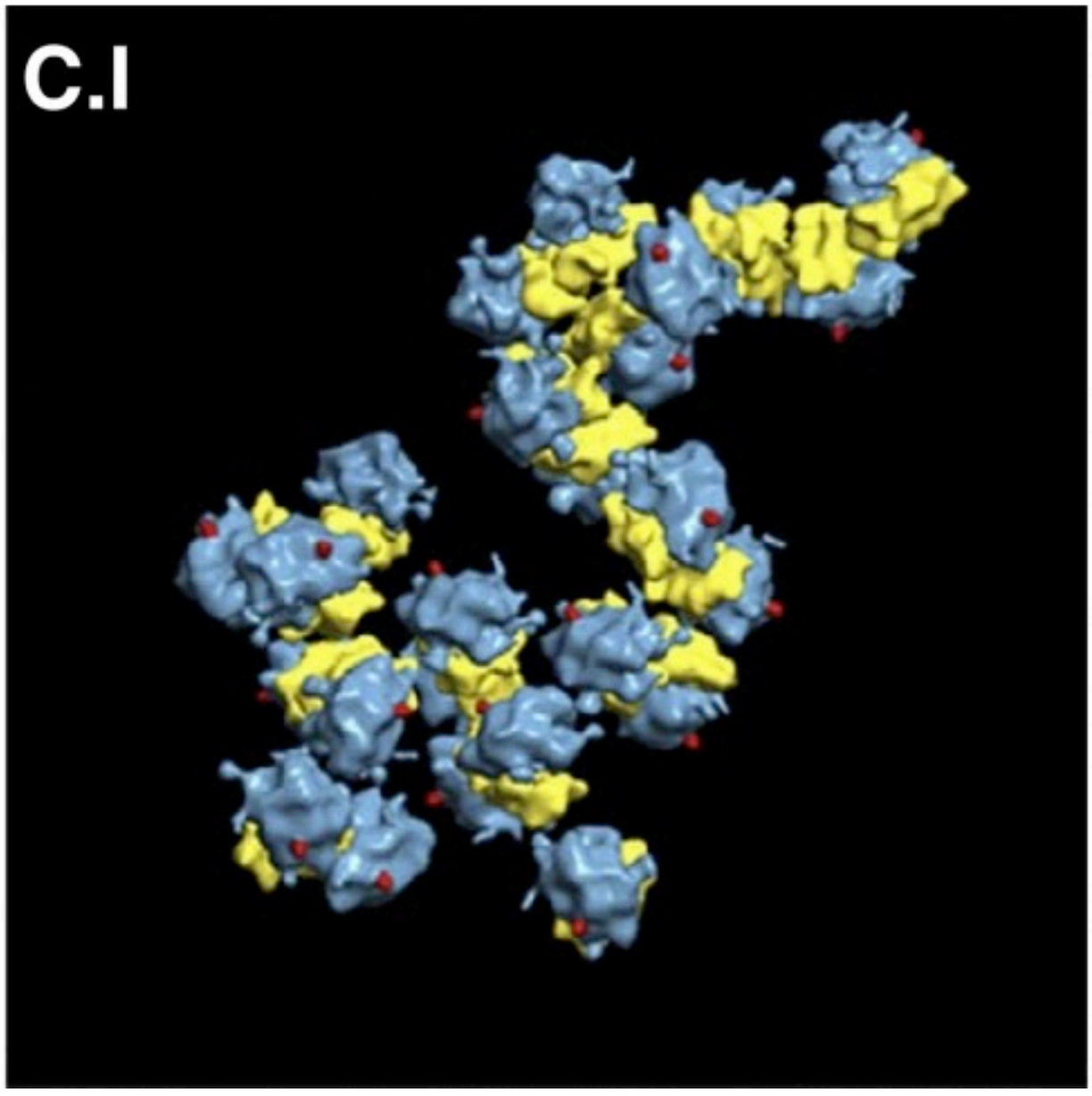
Examples



Brandt, 2010

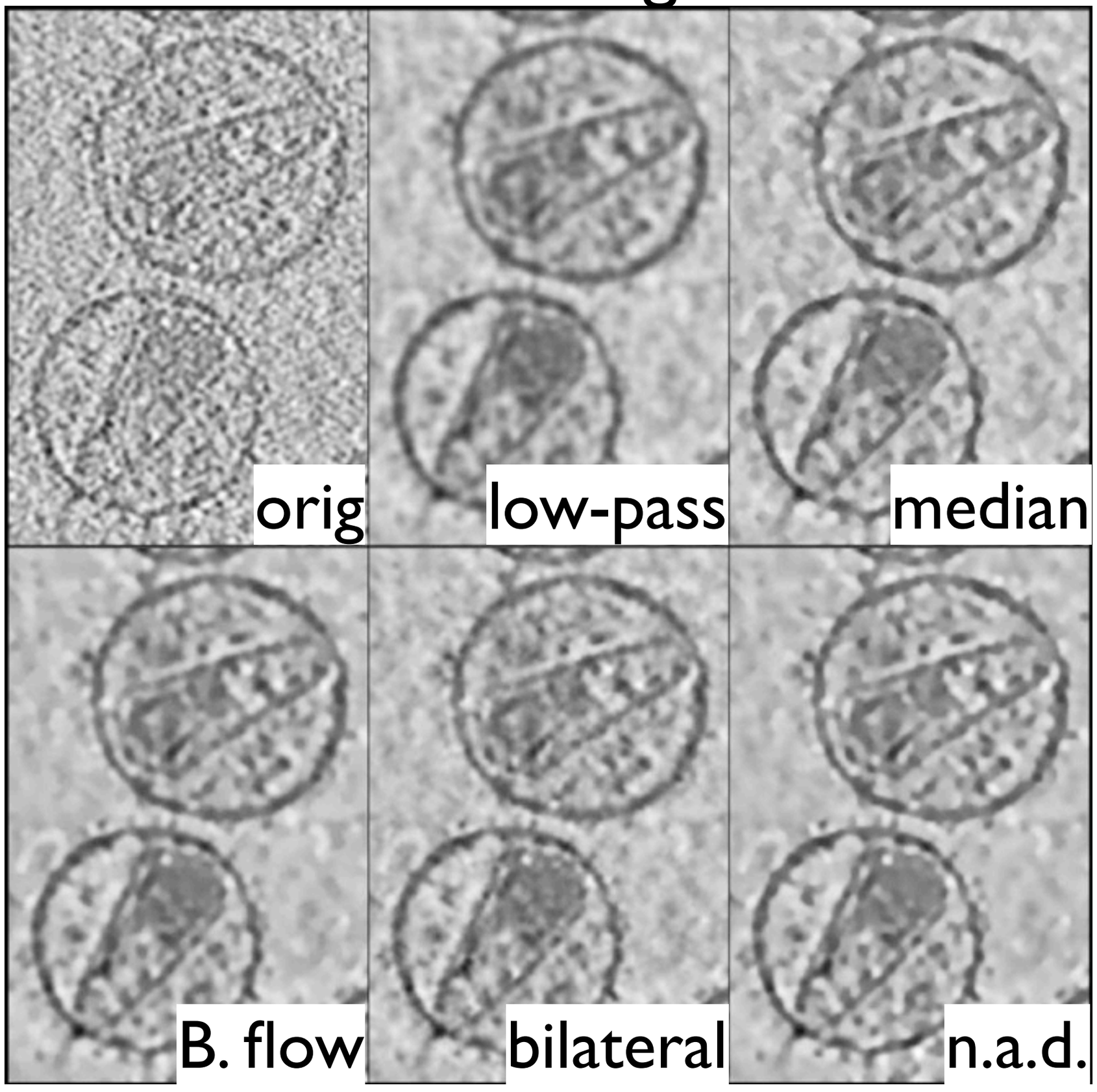
Examples





Brandt, 2010

Filtering

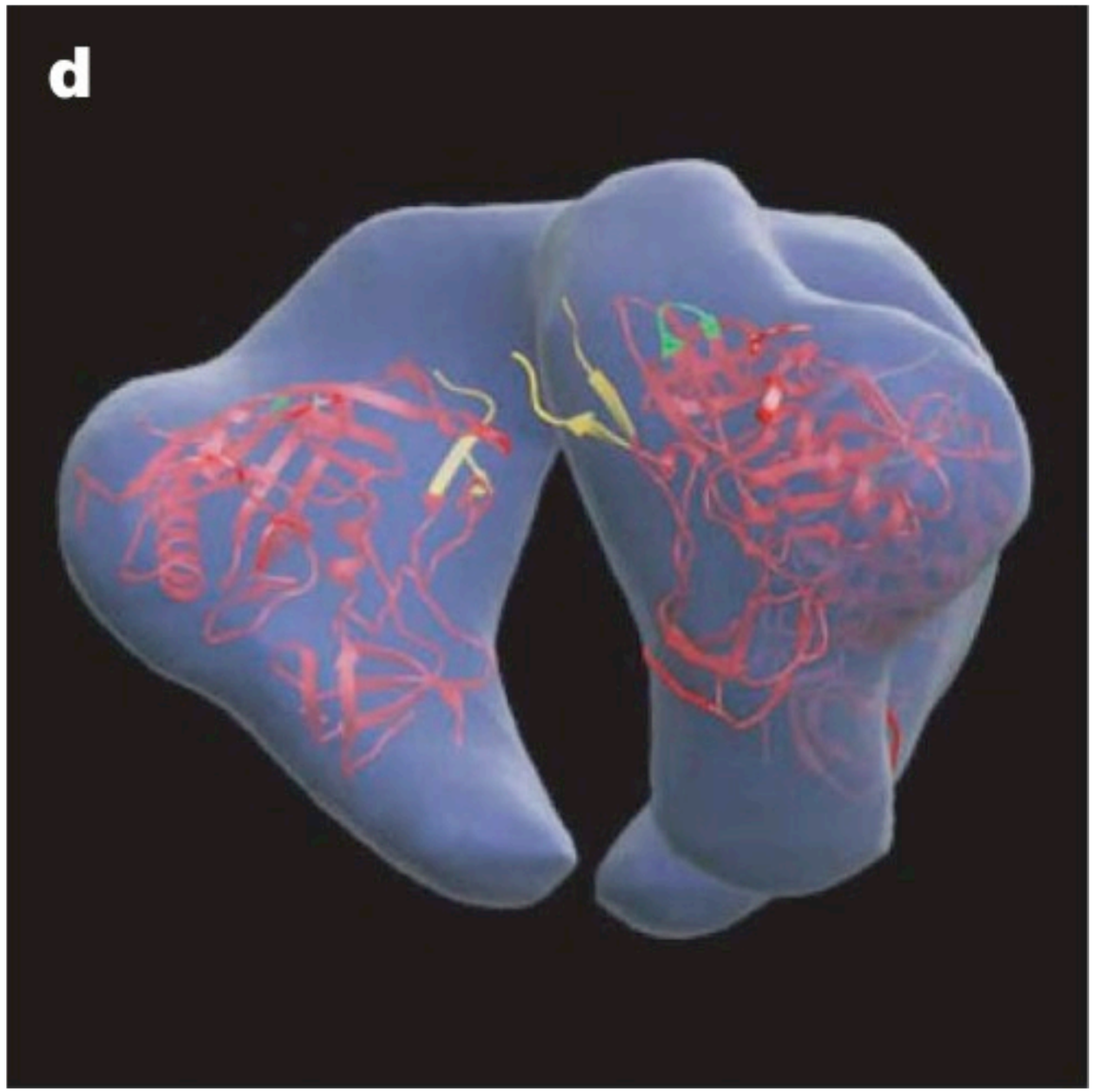


Fernandez, 2012

Filtering

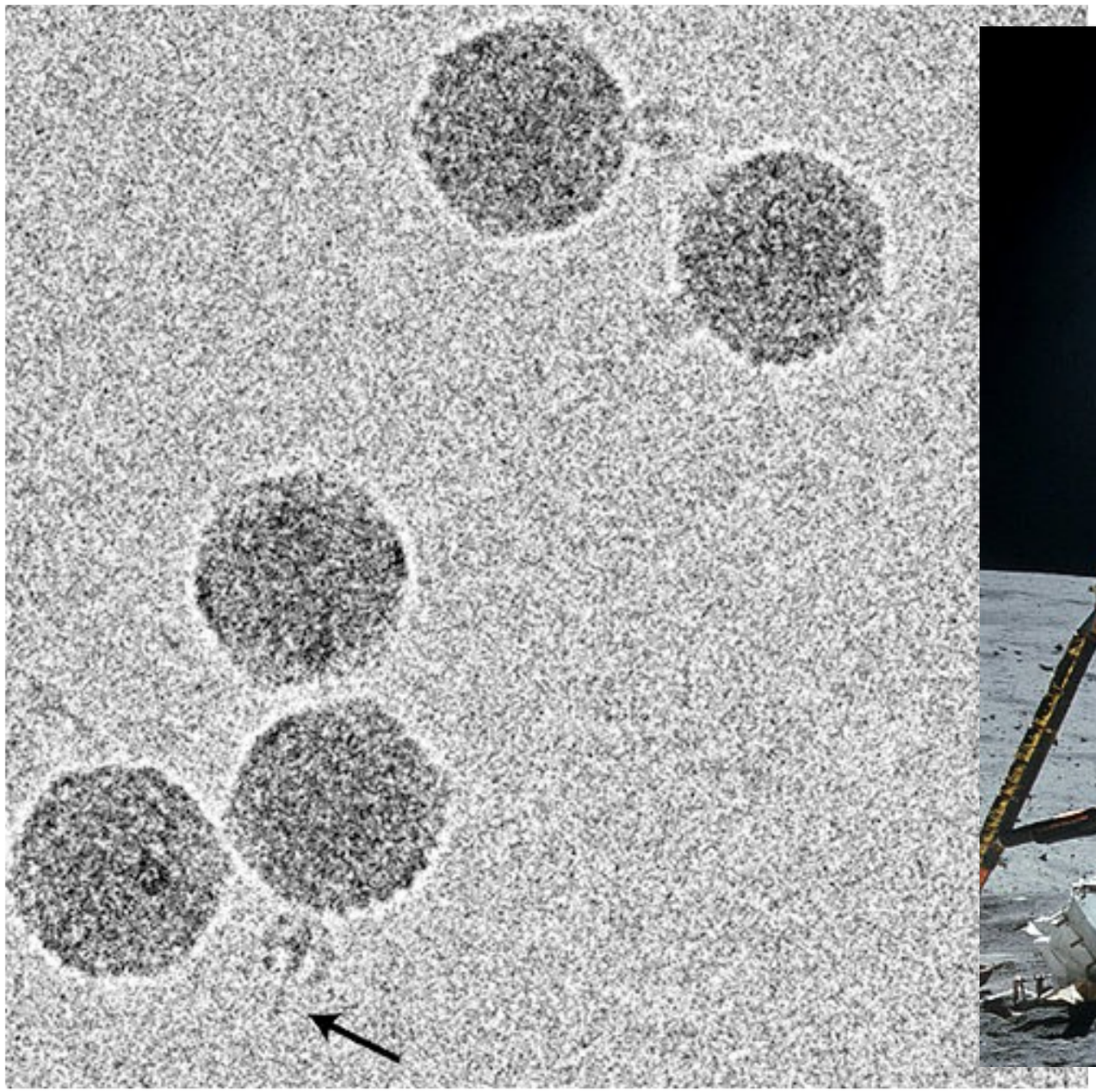
Fernandez, 2012

Subtomogram averaging (Mike Schmid)



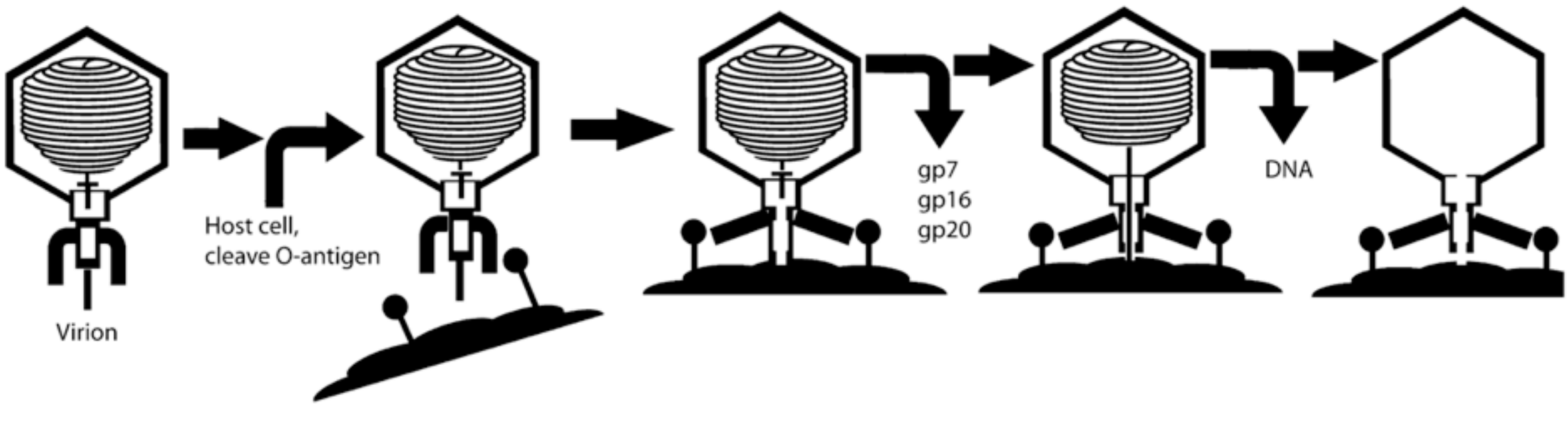
Liu, 2008

Viruses + cells



Chang, 2006

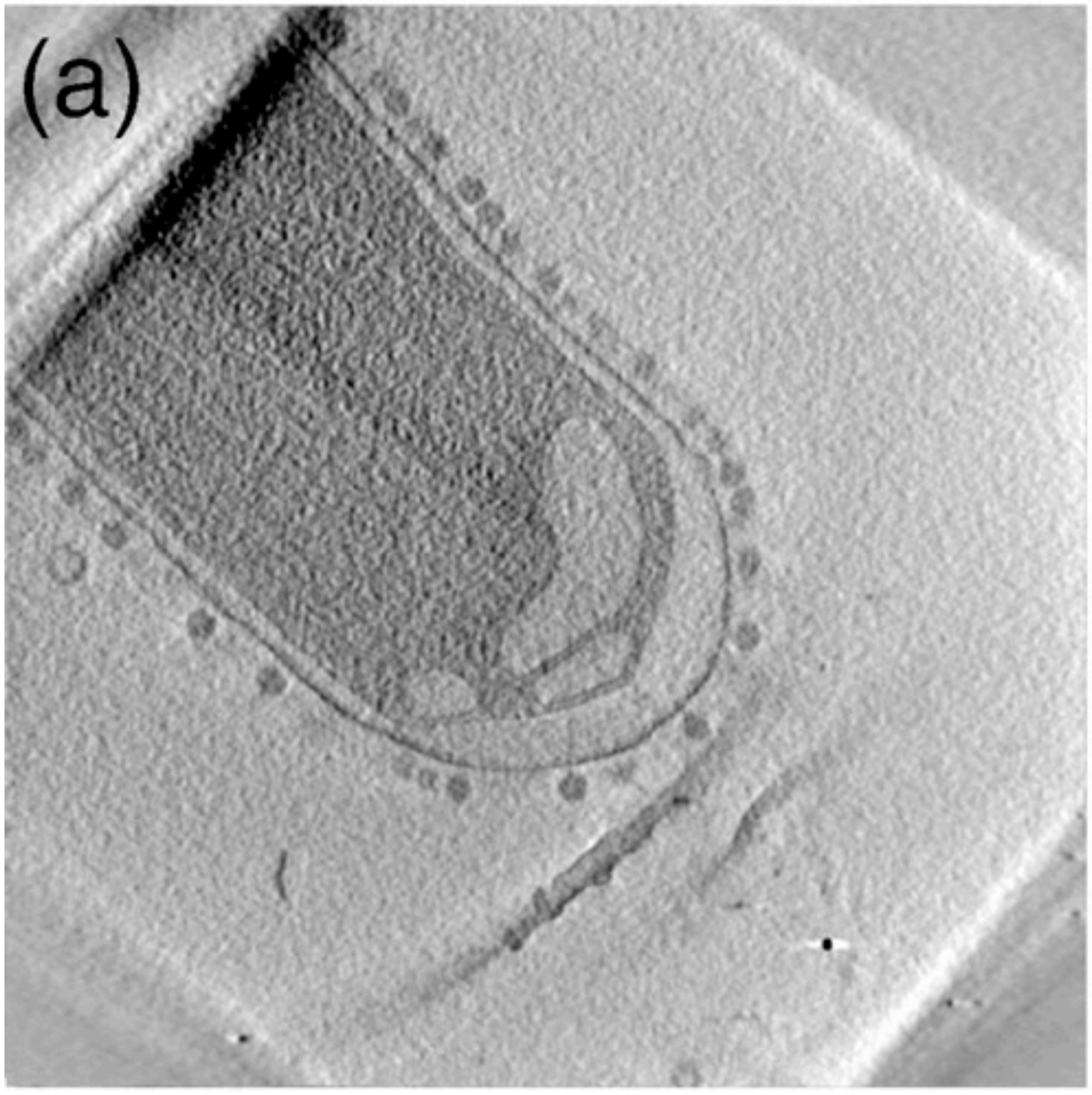
Viruses + cells



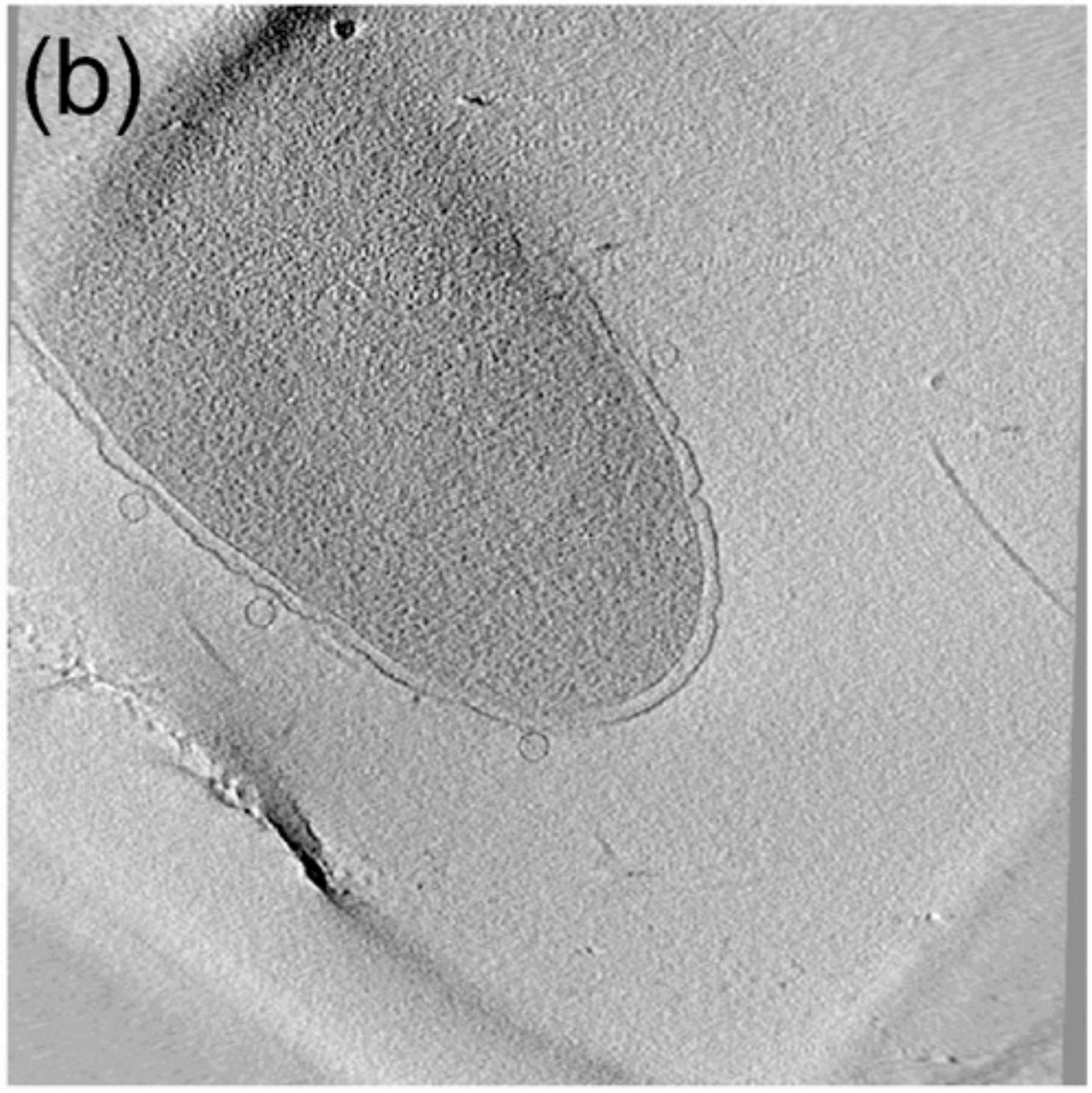
Chang, 2006

Viruses + cells

ε15 + Cell

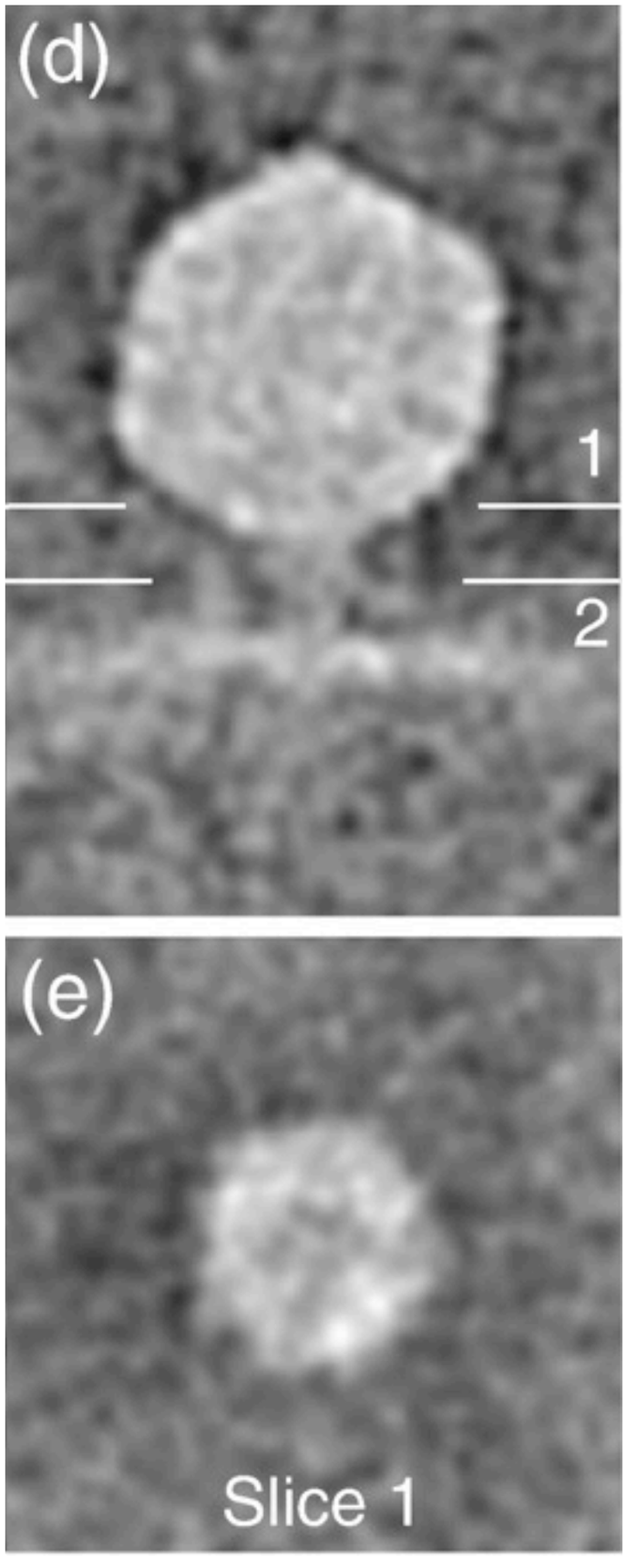


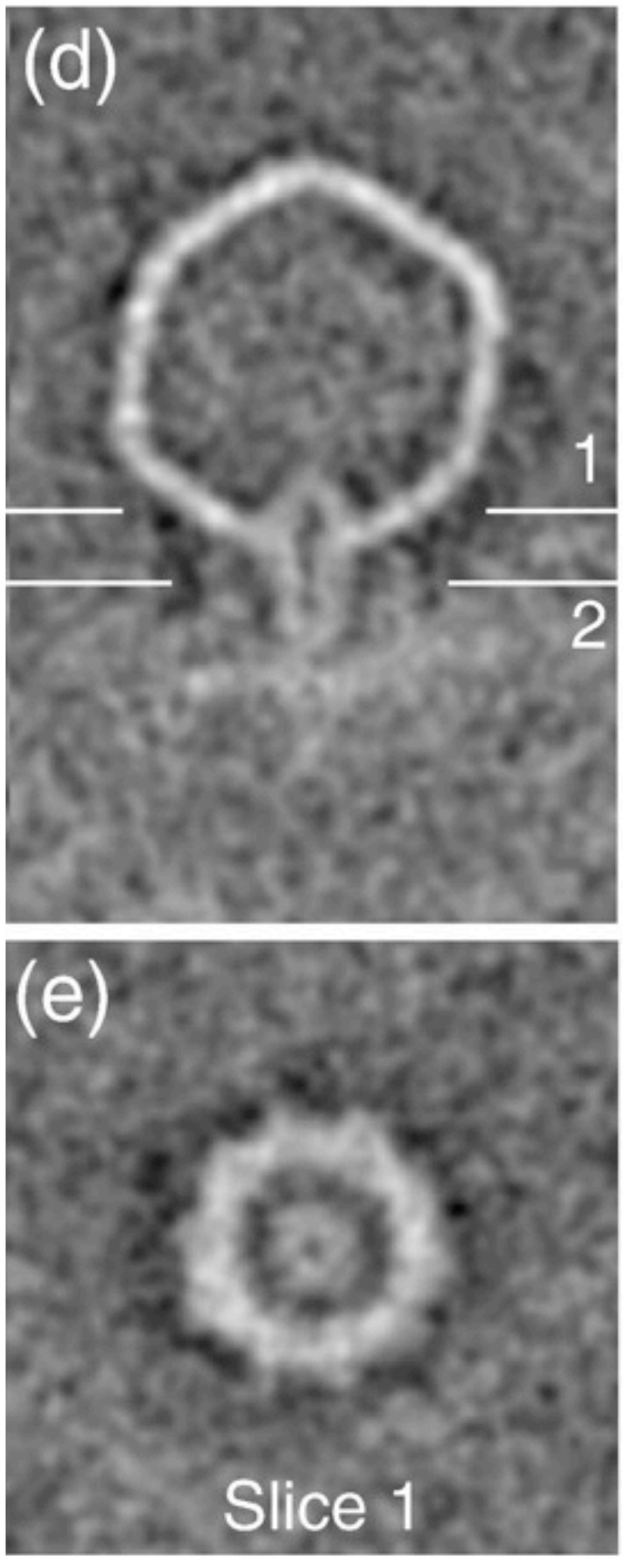
ε15 + Cell



Chang, 2010

Viruses + cells





Chang, 2010

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

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