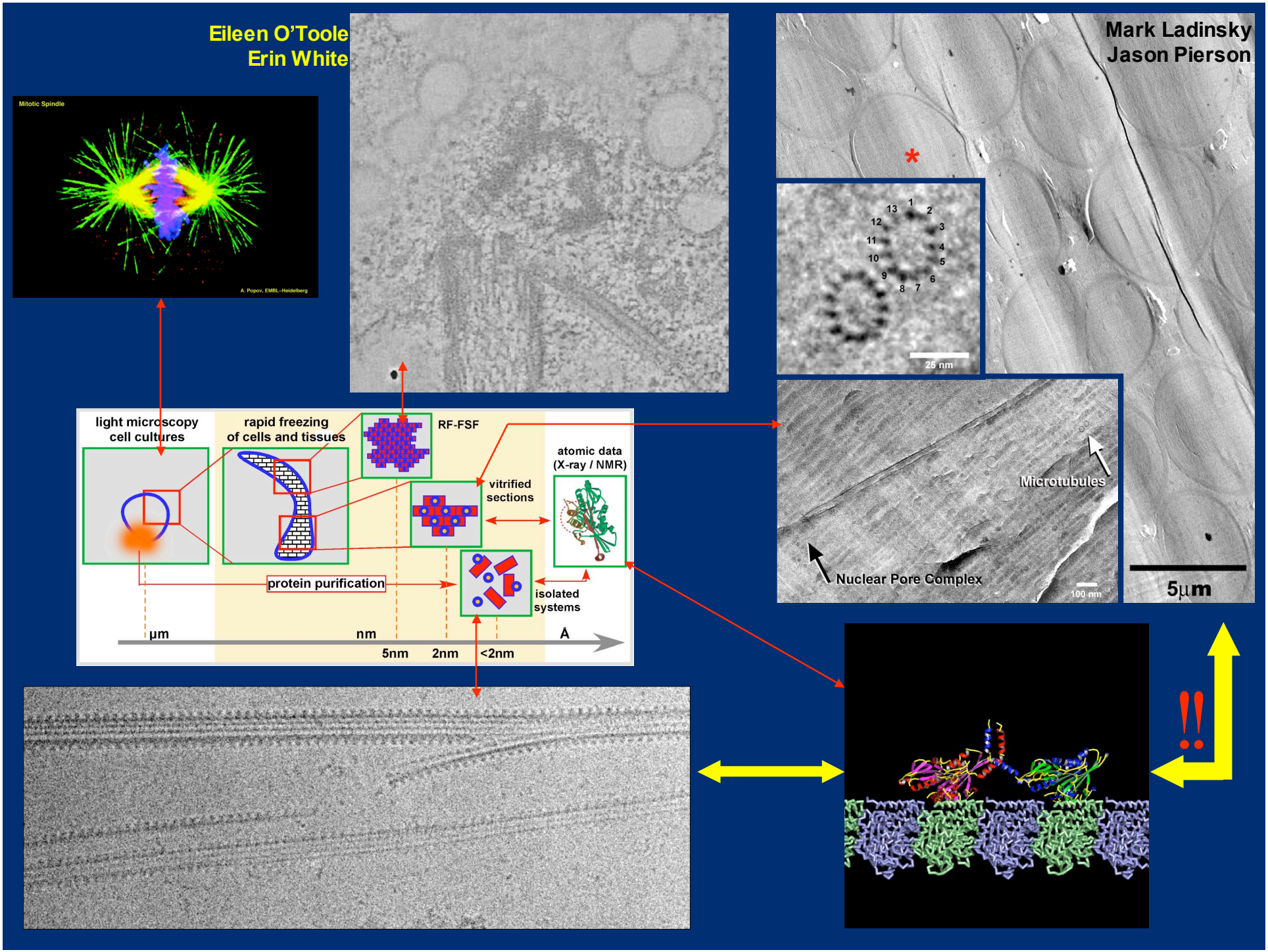
Cryo-Electron Microscopy and Tomography


Eileen O'Toole
Erin White
Mitotic Spindle
A. Popov, EMBL–Heidelberg
Mark Ladinsky
Jason Pierson
13
1
2
12
3
11
4
10
5
9
6
8
7
25 nm
Microtubules
Nuclear Pore Complex
100 nm
5µm
light microscopy
cell cultures
rapid freezing
of cells and tissues
RF-FSF
atomic data
(X-ray / NMR)
vitrified
sections
protein purification
isolated
systems
µm
nm
Å
5nm
2nm
<2nm
!!

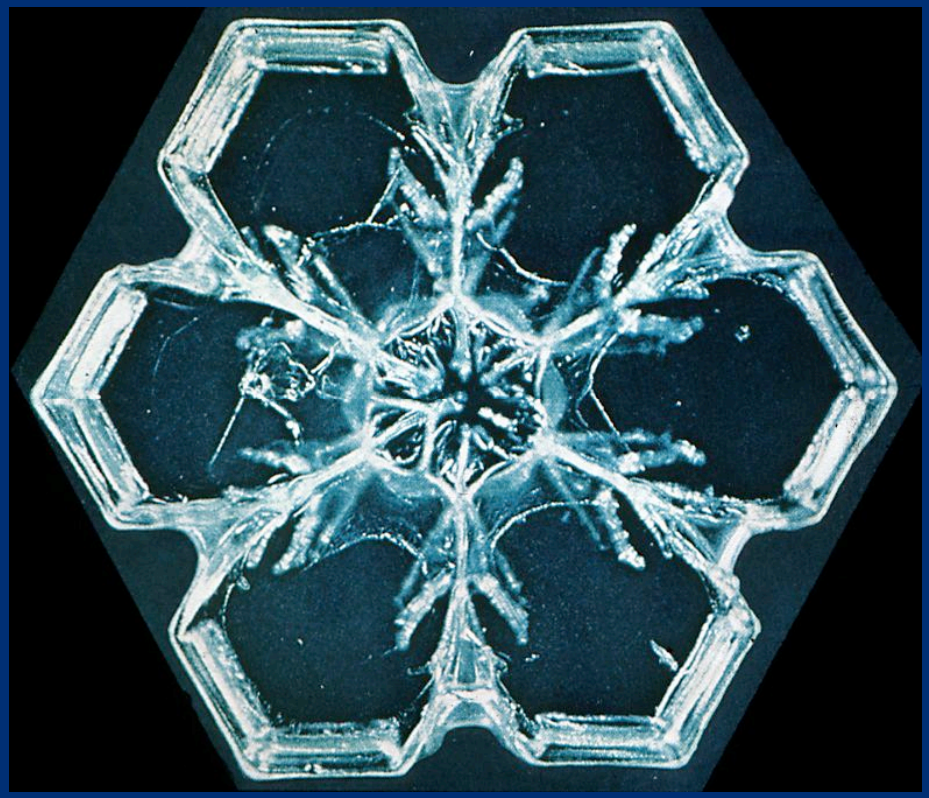
Embedding in vitreous ice:

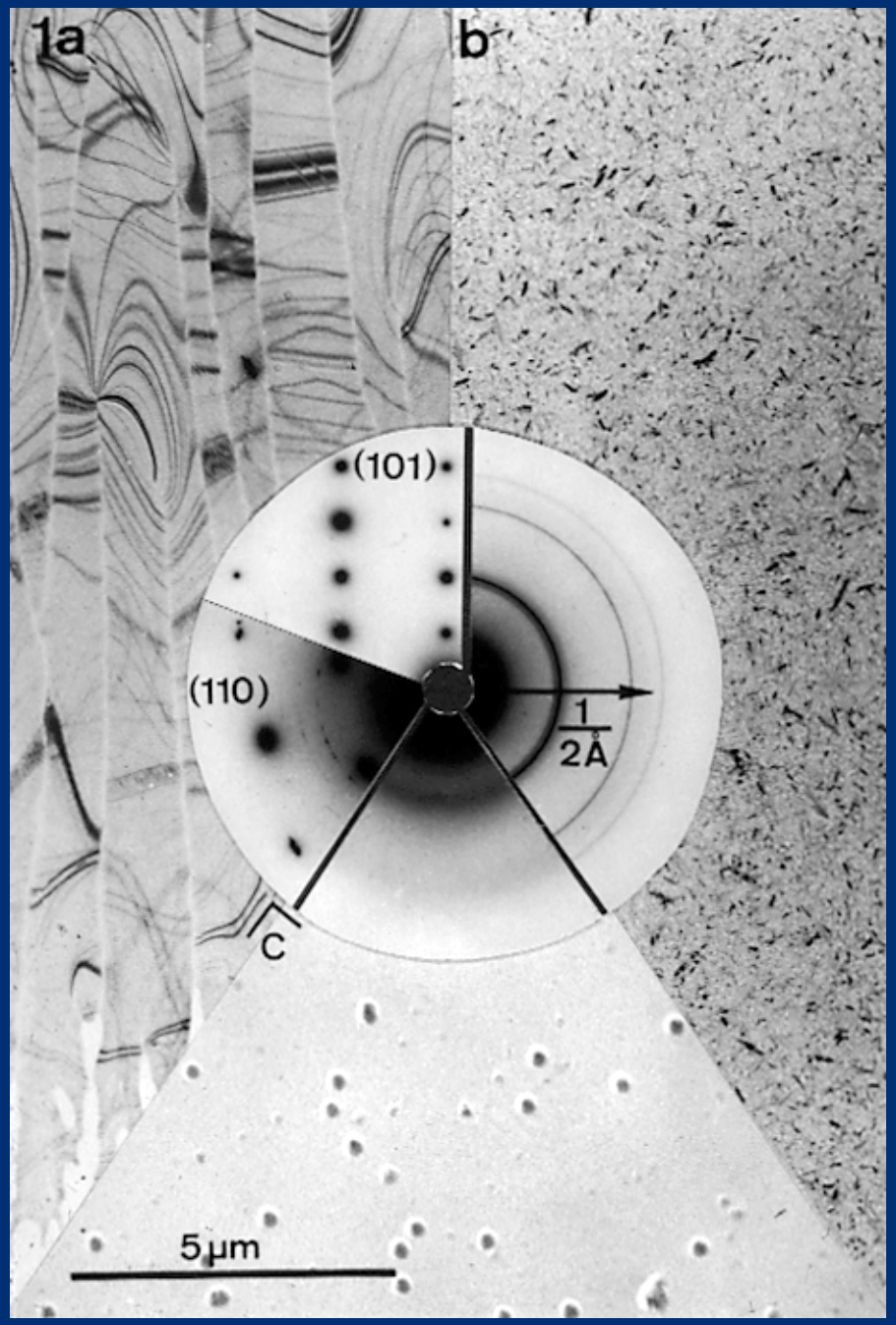
- Specimens remain fully hydrated and in physiological buffer. They are quick-frozen in liquid ethane or propane
- Contrast is generated solely by the density difference between protein and ice

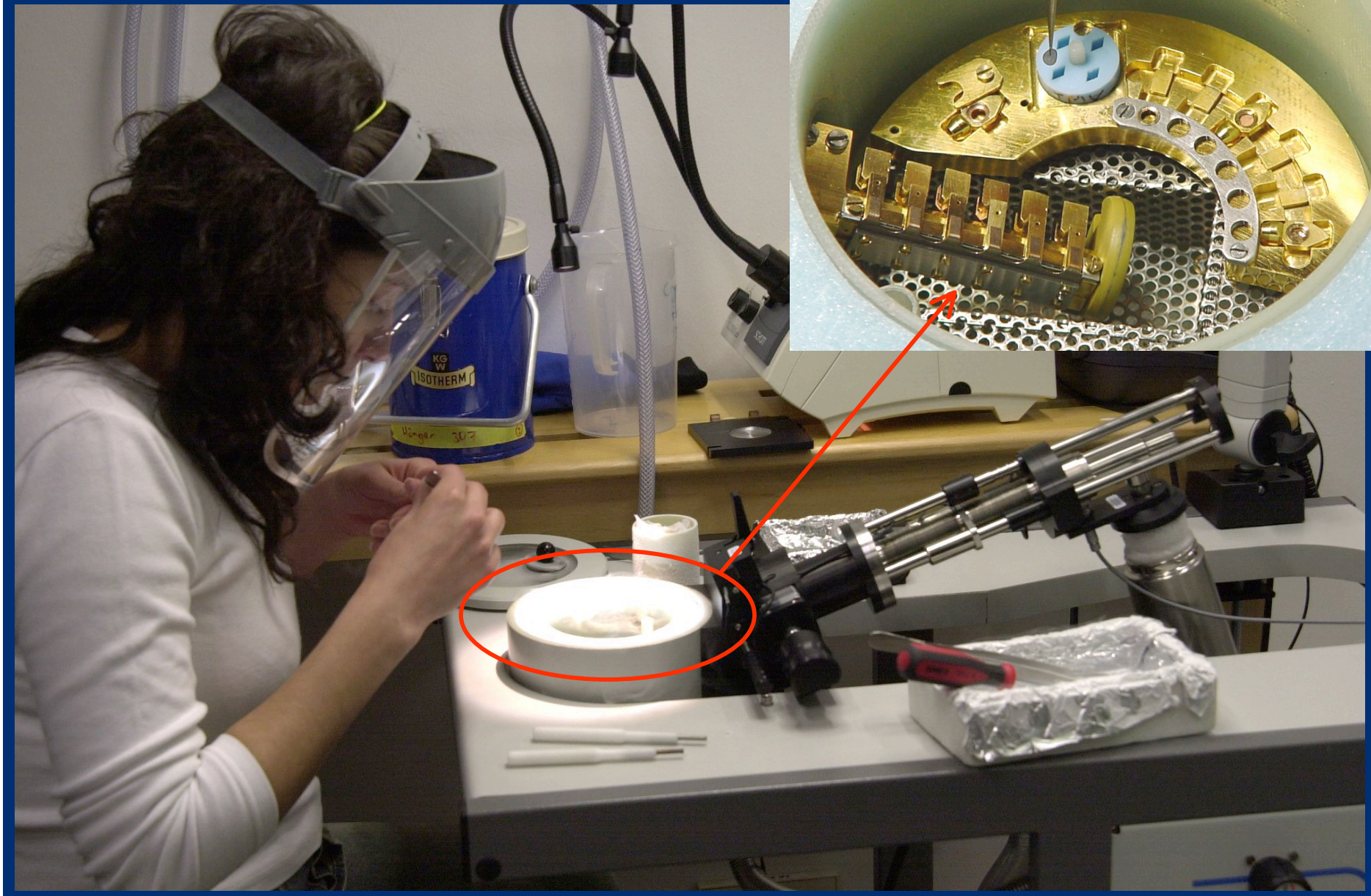
- 2-D and 3-D information to (at least in theory ...) atomic resolution
- Specimen preservation at most physiological conditions and with very few distortions (e.g. no flattening)
- Quick-freezing allows to capture dynamic states
- Reproduces the actual shape of a molecule or complex and allows to view internal structures

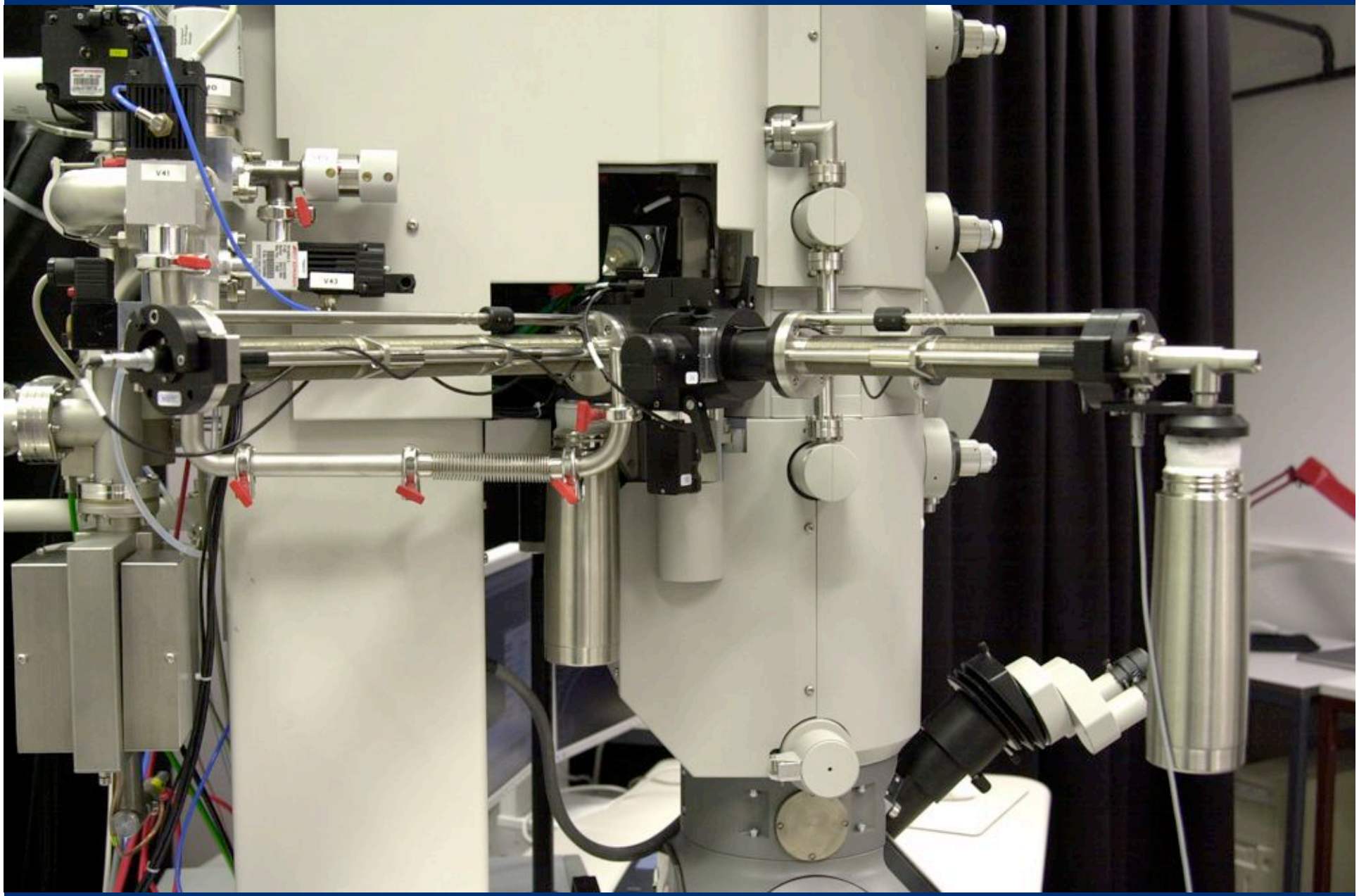
- Low contrast
- Requires expensive equipment

solid states of ice:
hexagonal
cubic
amorphous

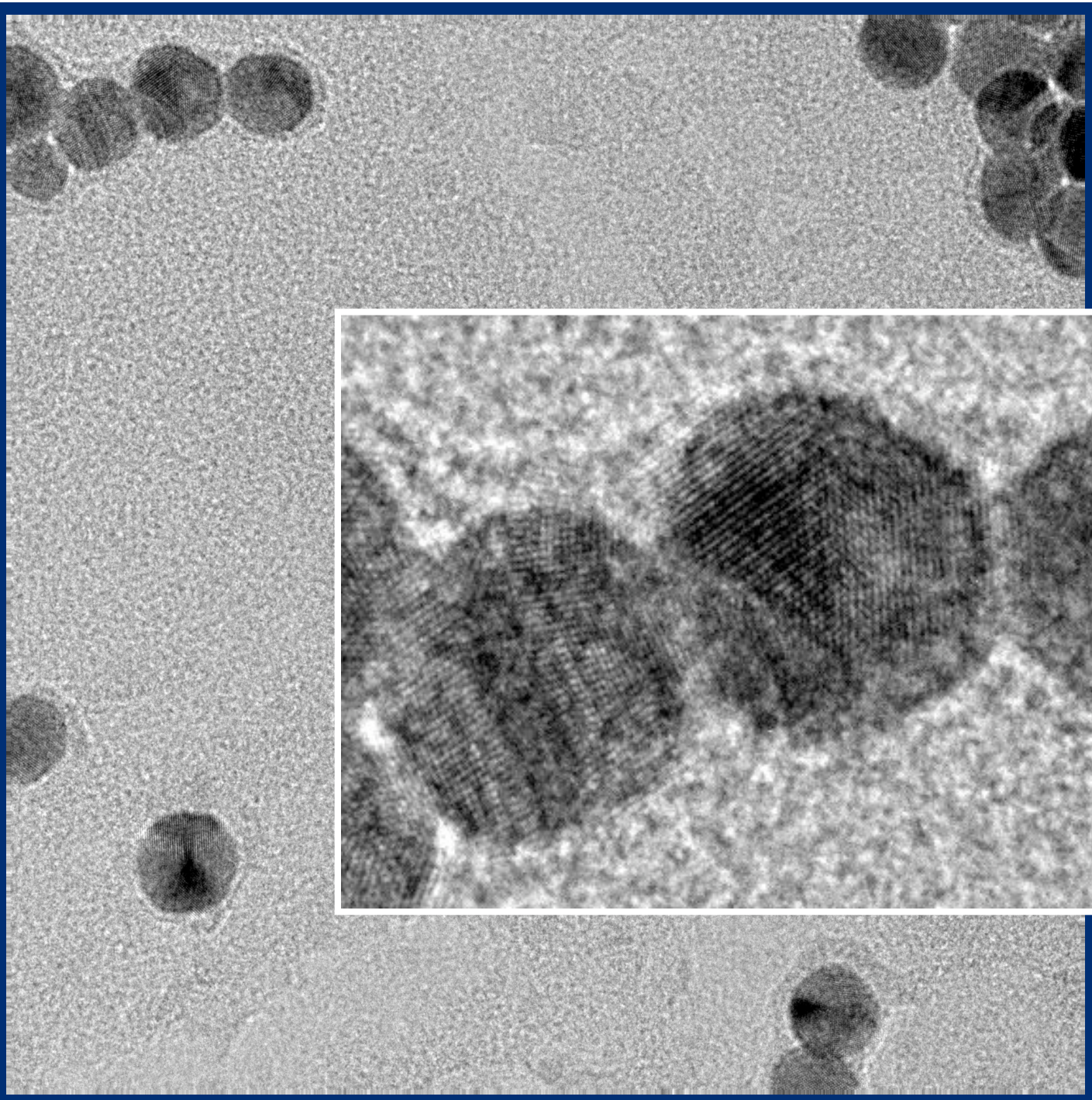




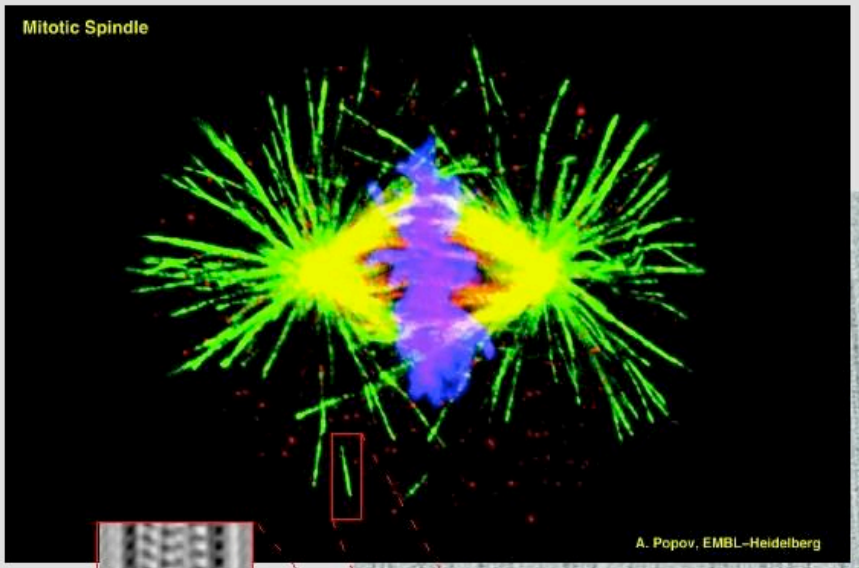

ISOTHERM

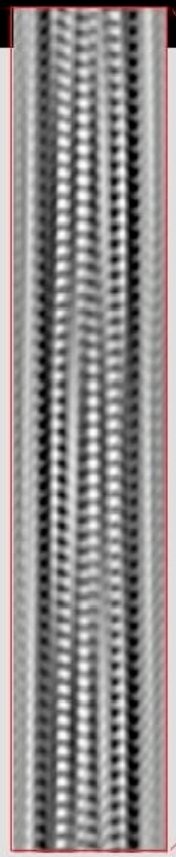


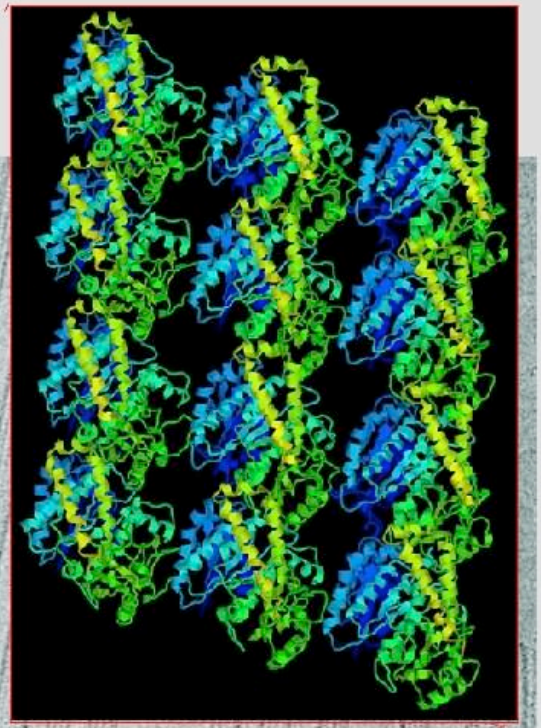
Anorganic Specimens



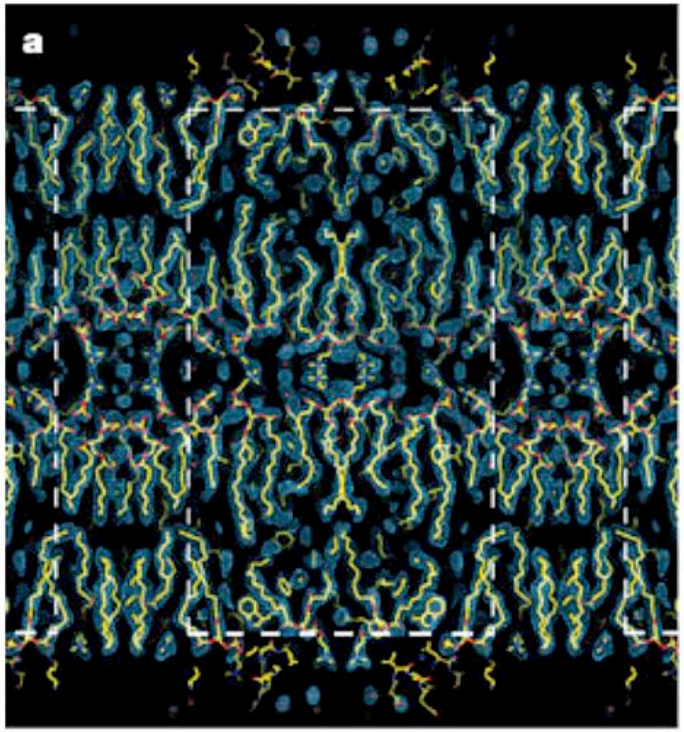
Resolving power of cryo-EM techniques

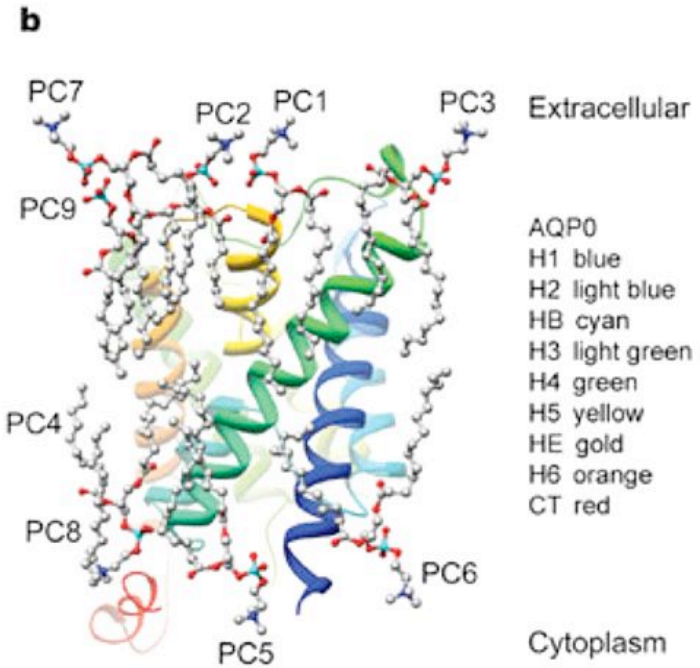


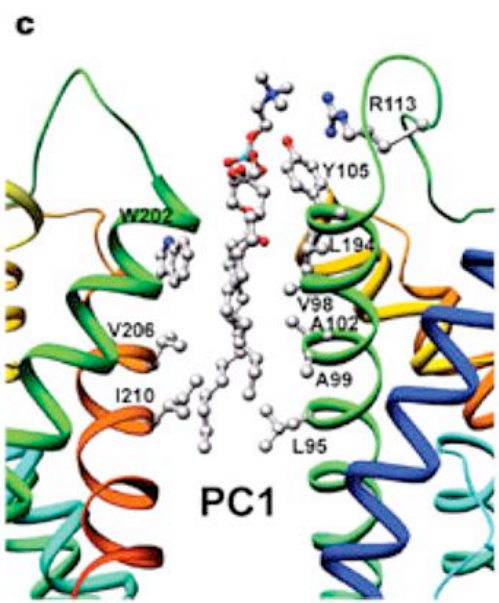


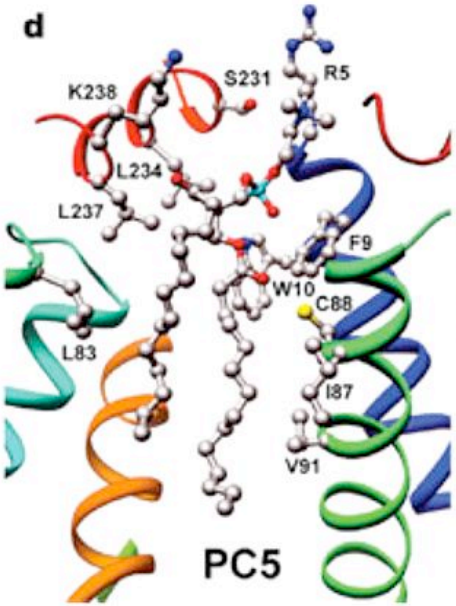


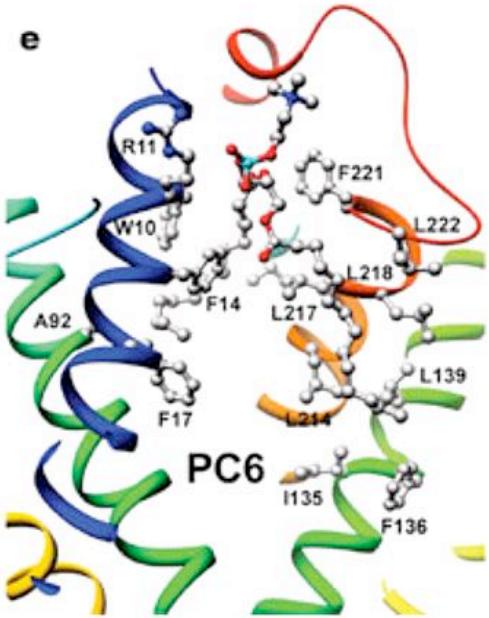
Gonen et al., 2005
Nature 441:248

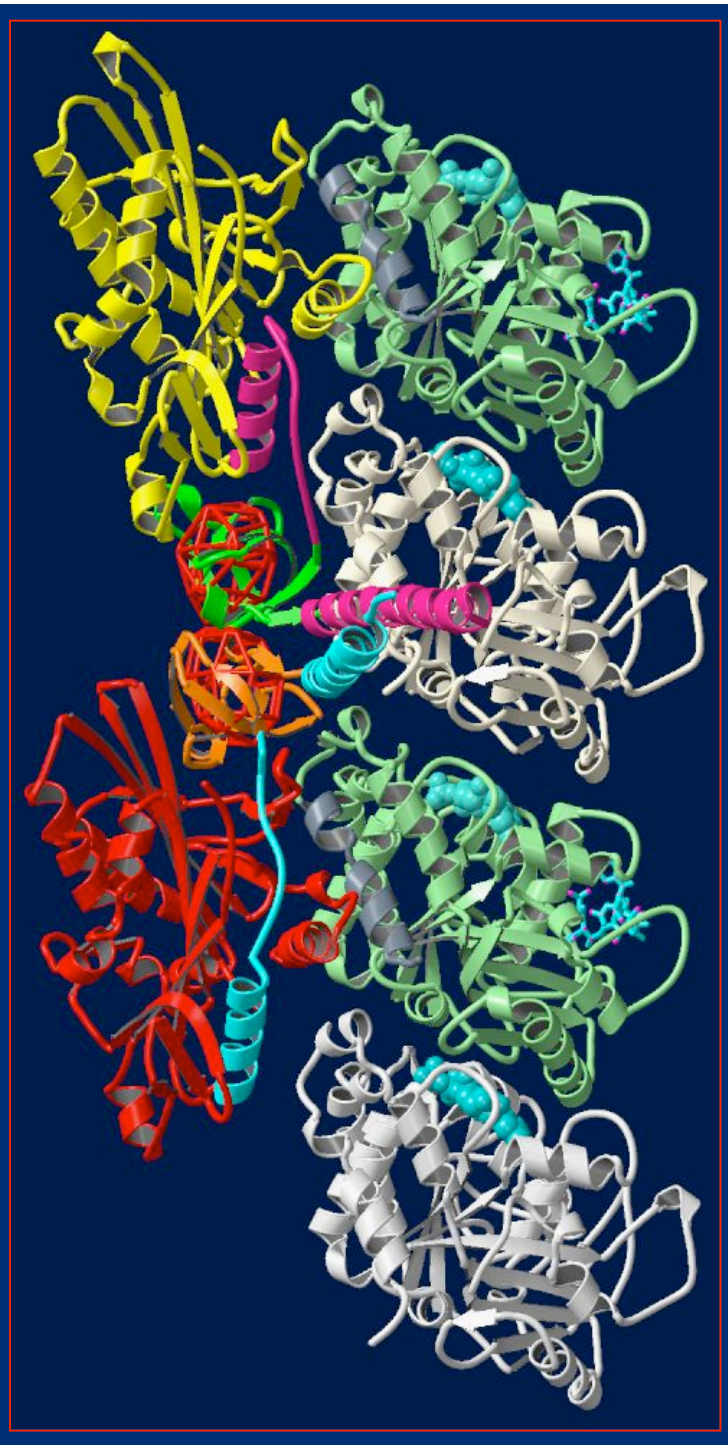


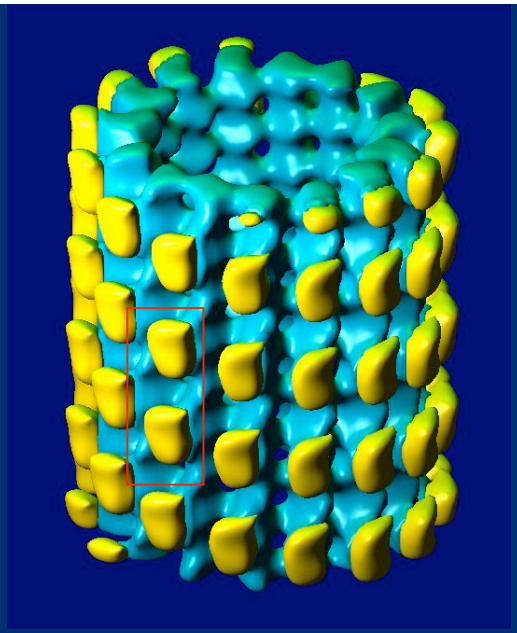


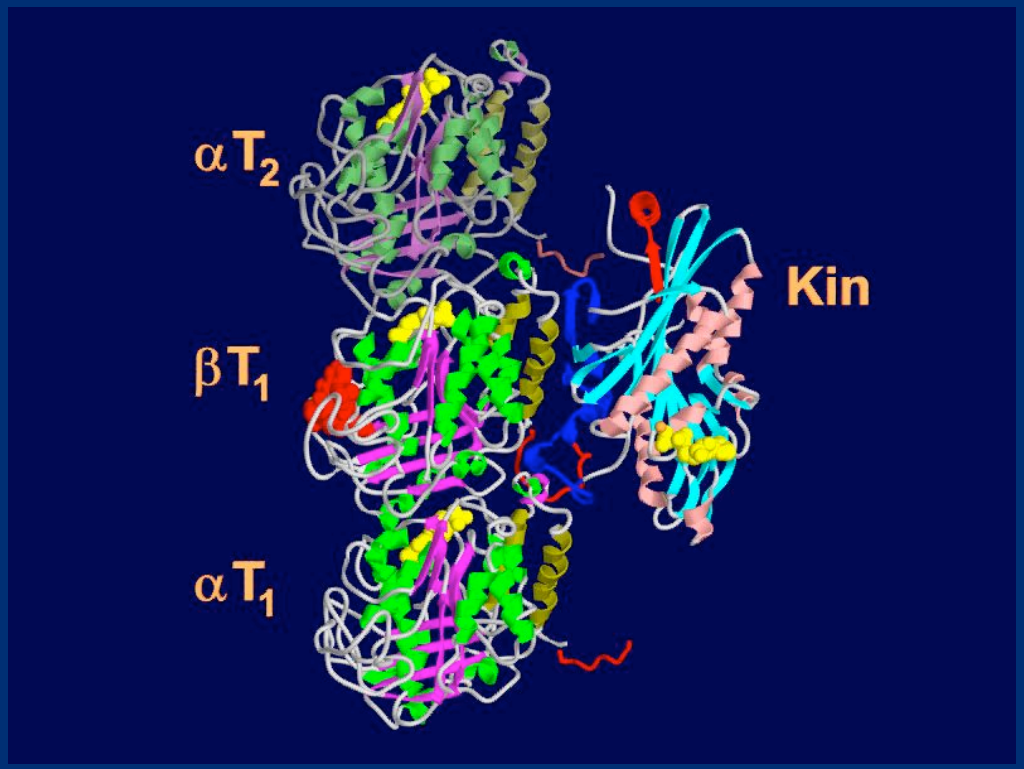




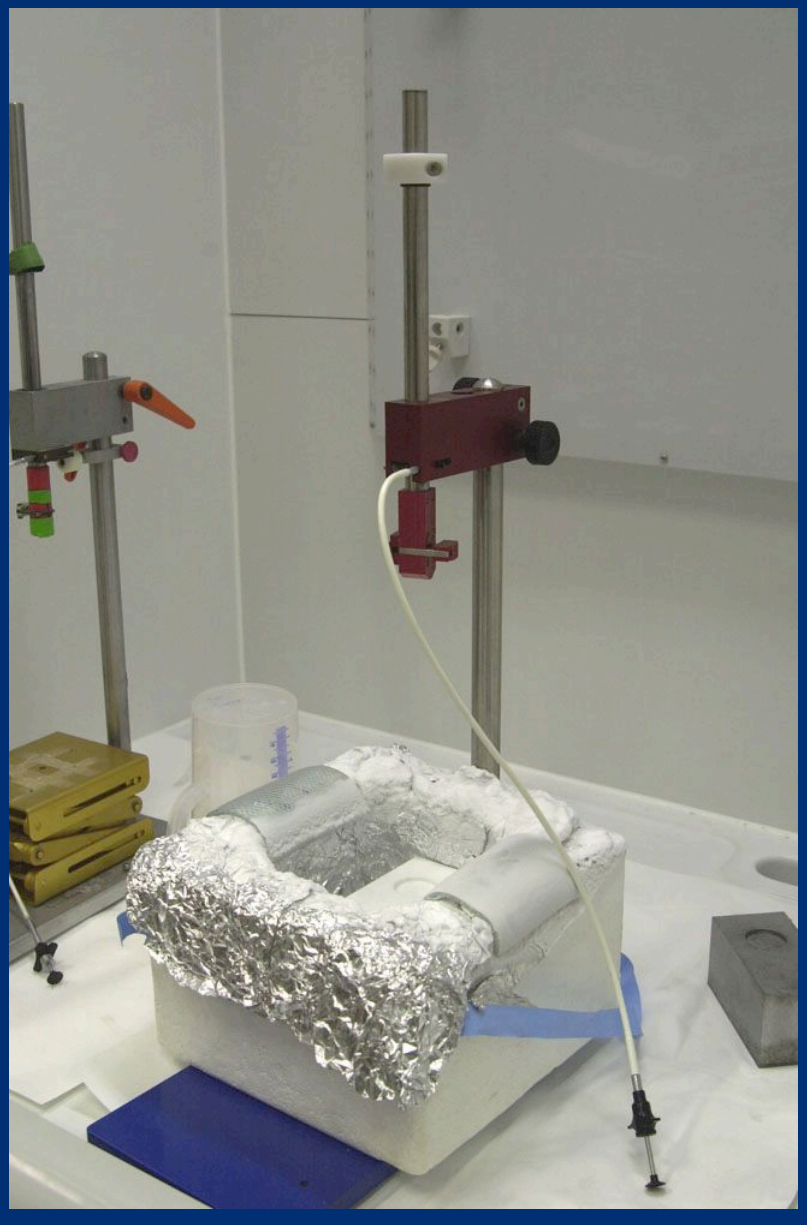


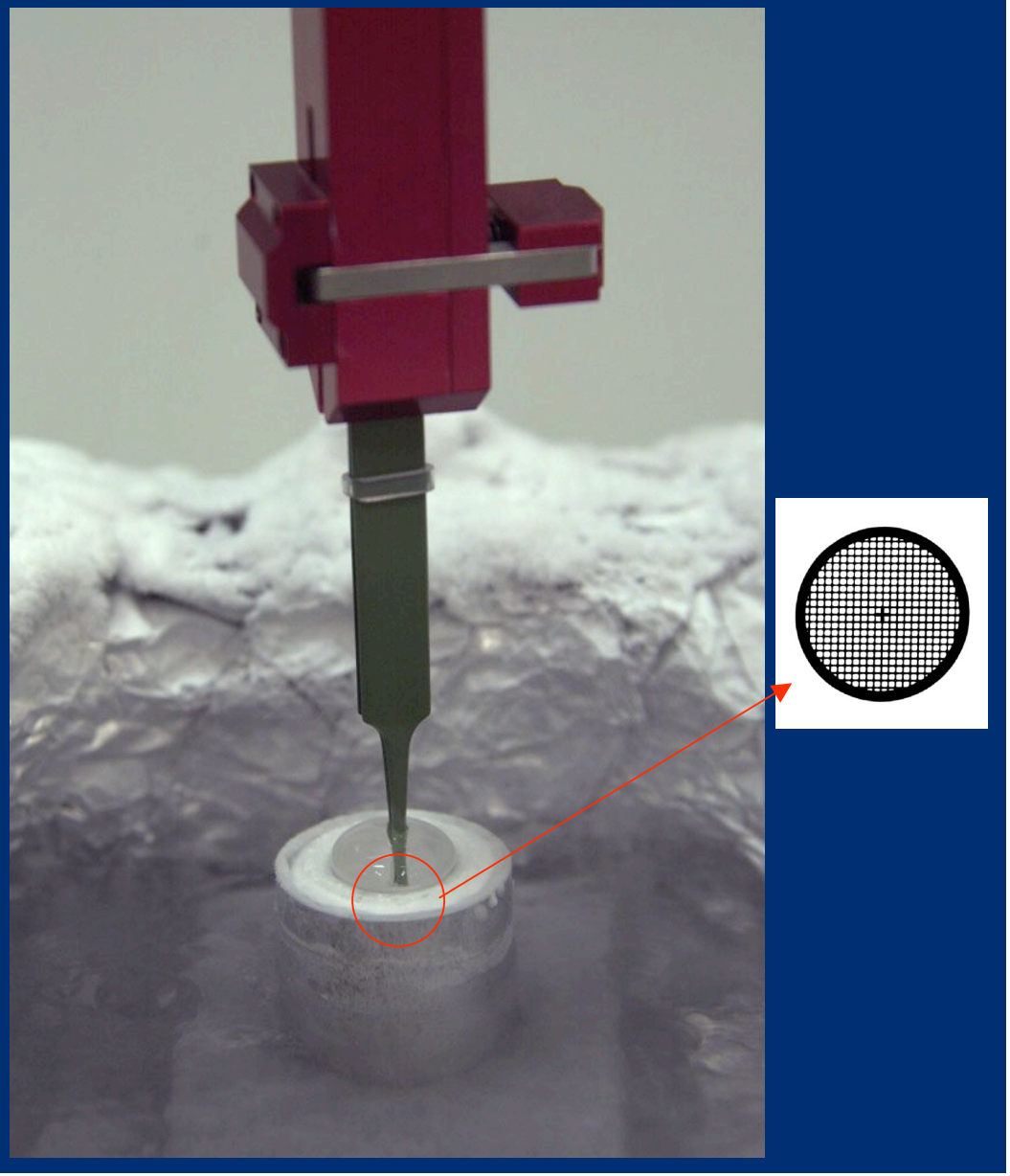


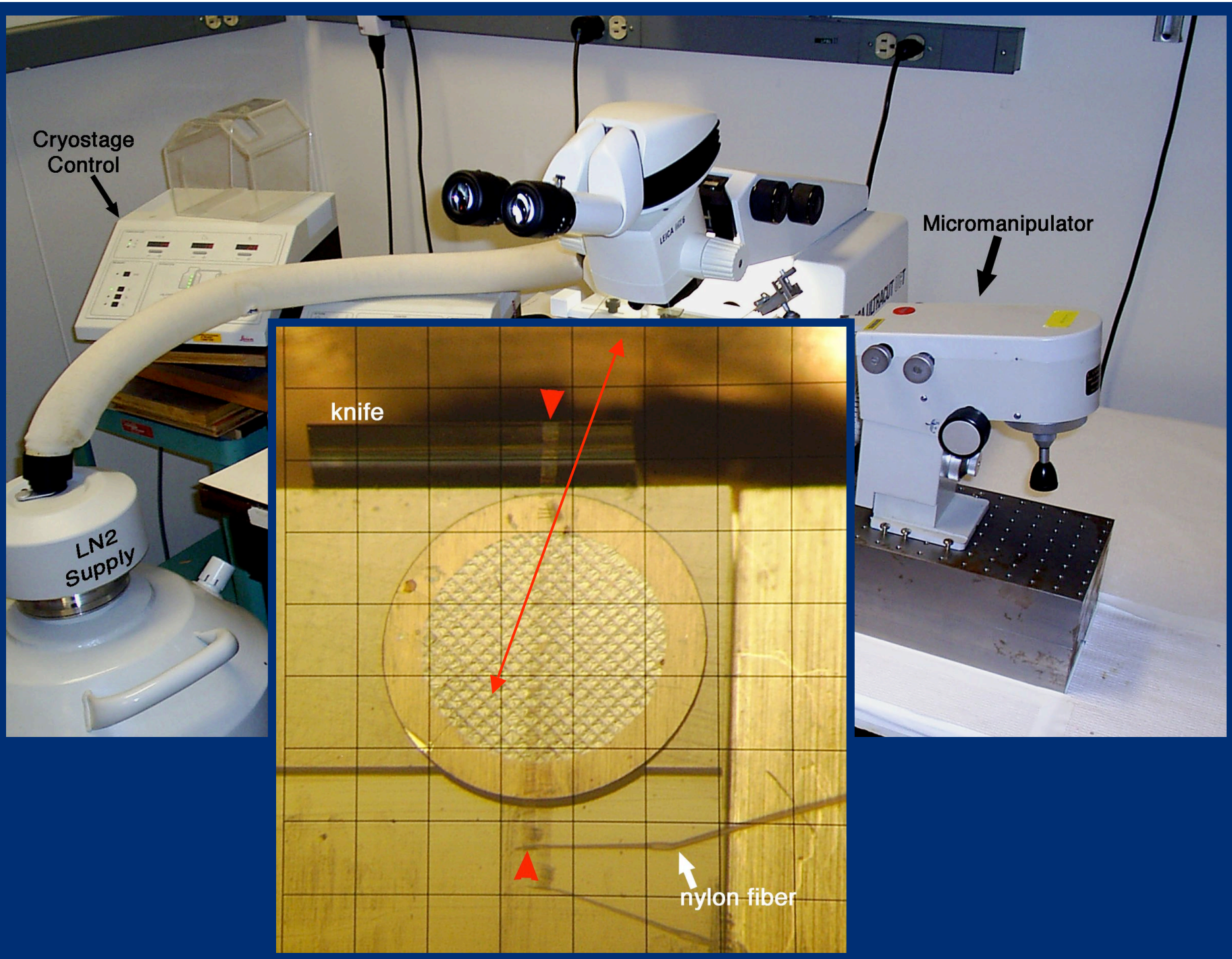



αT2
βT1
αT1
Kin

Freezing Grids for Cryo-Electron Microscopy



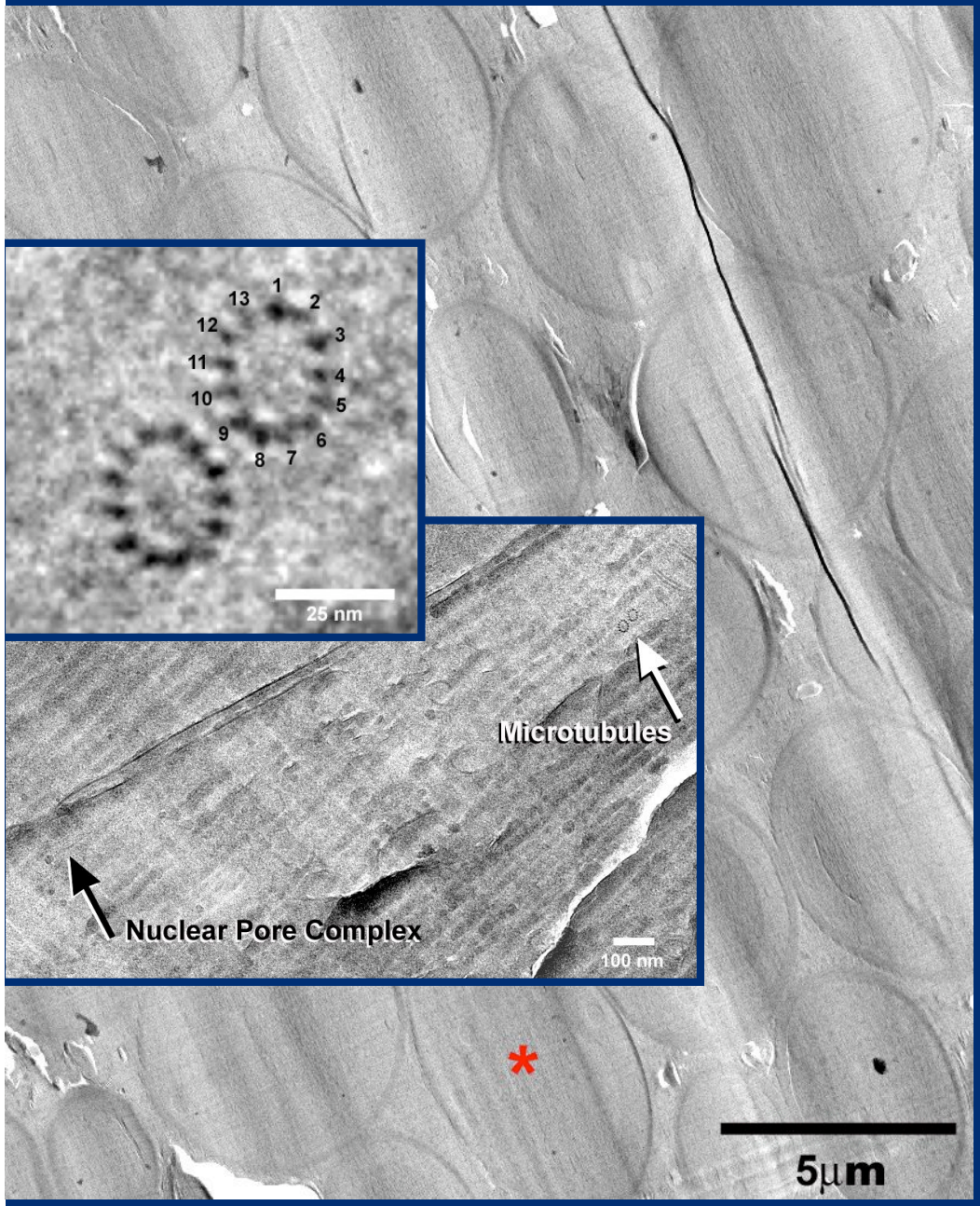


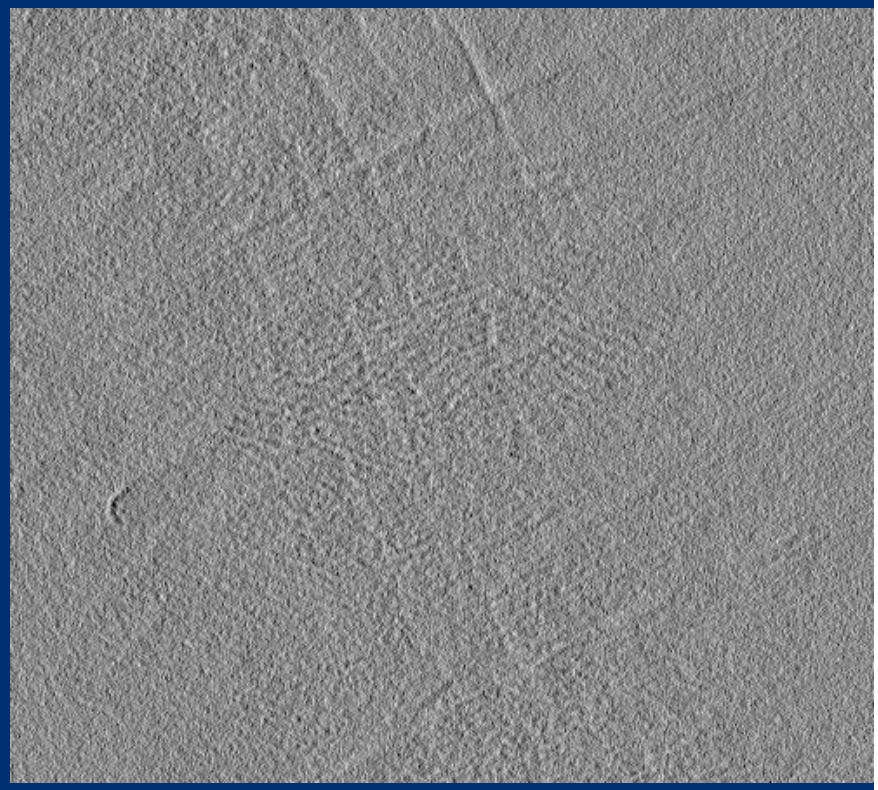

Cryostage
Control
Micromanipulator
LN2
Supply
knife
nylon fiber

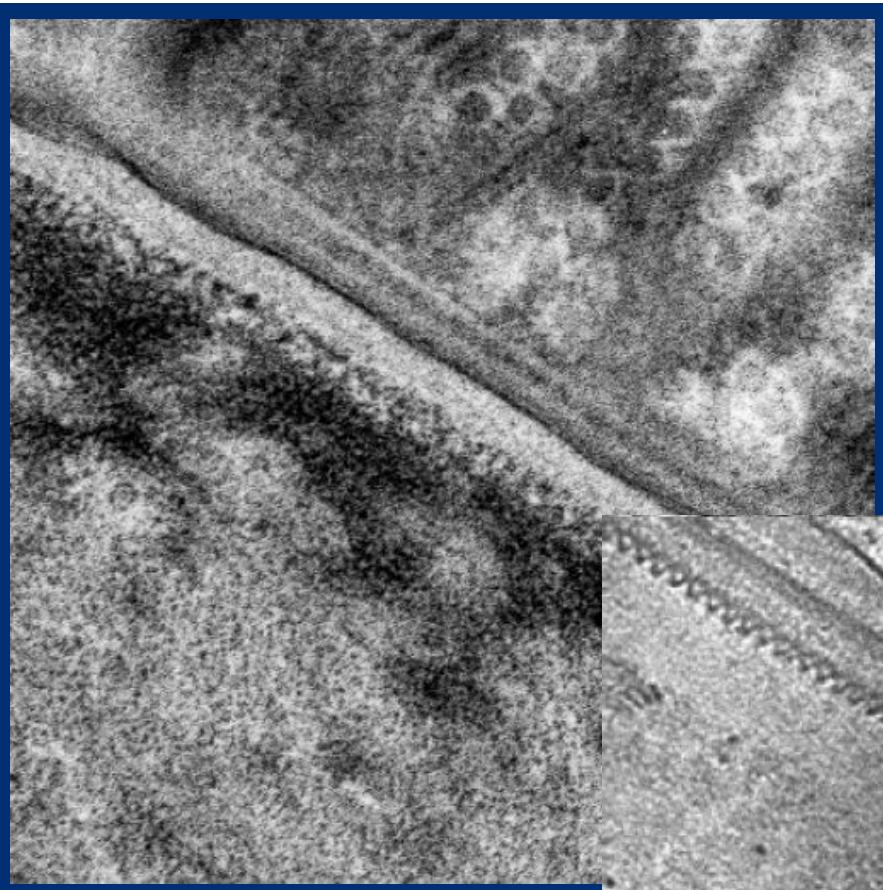
Vitrified Sections:

Link between cellular and molecular structures

Mark Ladinsky
Jason Pierson

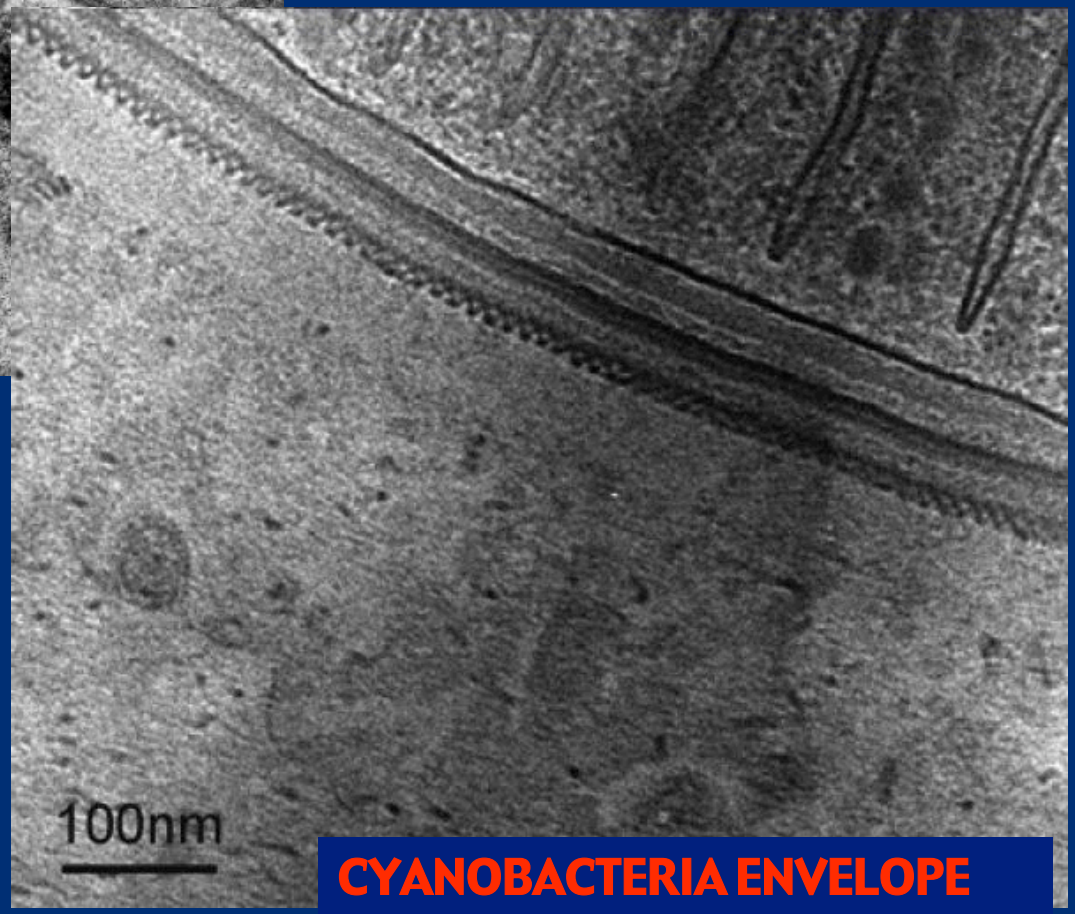






Cryo-Substitution / Fixation

(D. Studer, Berne CH)

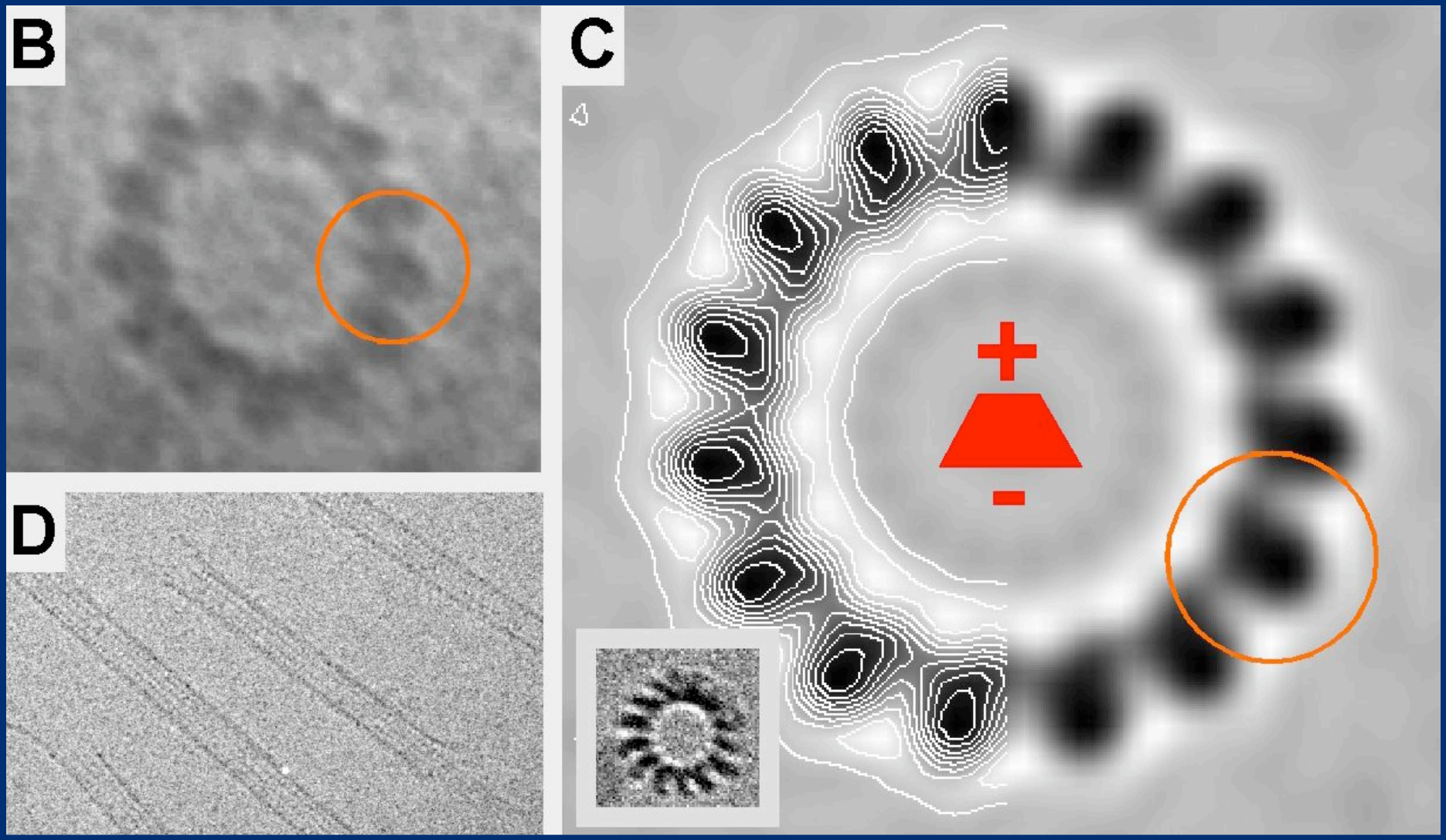


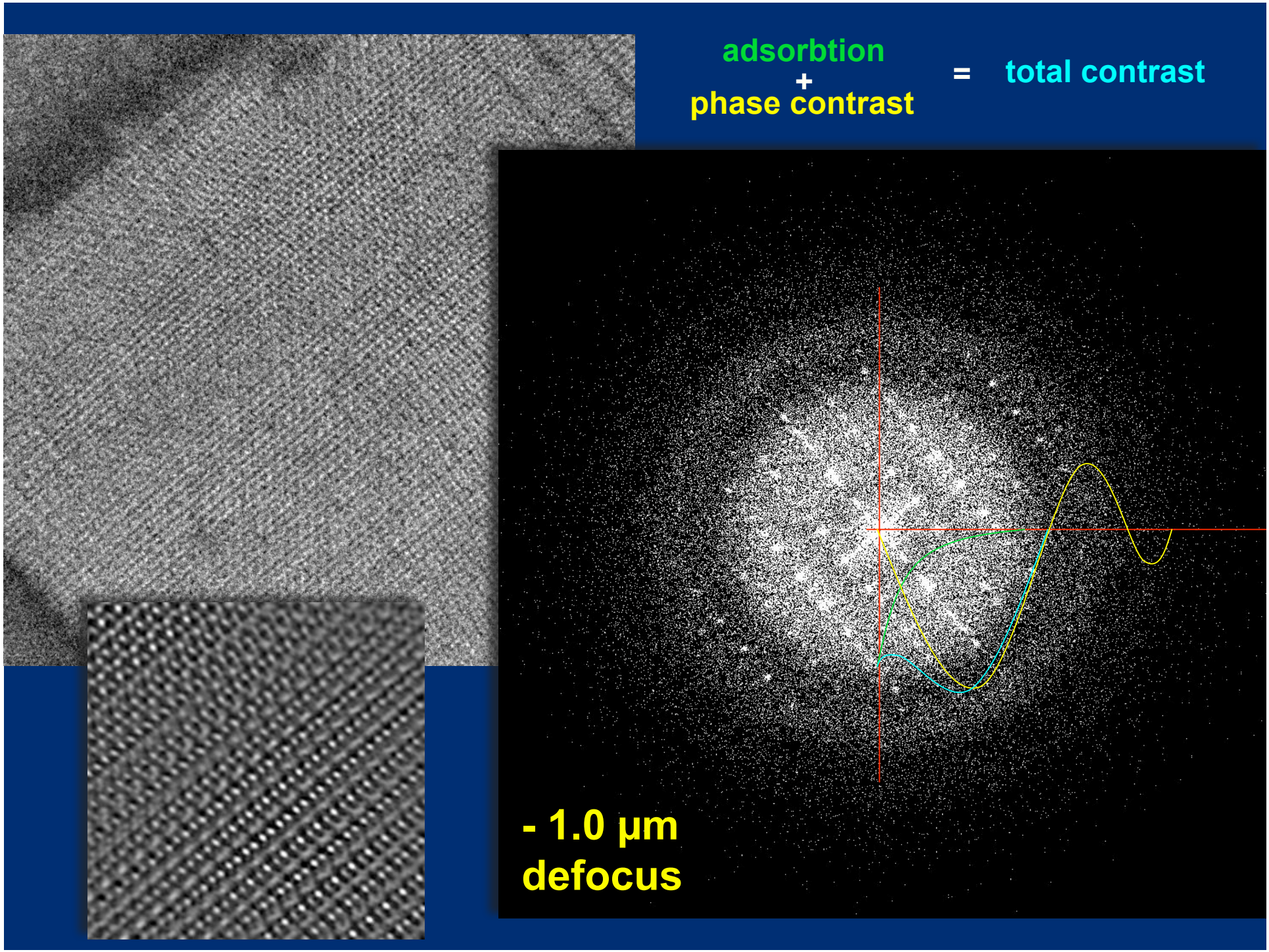
CYANOBACTERIA ENVELOPE

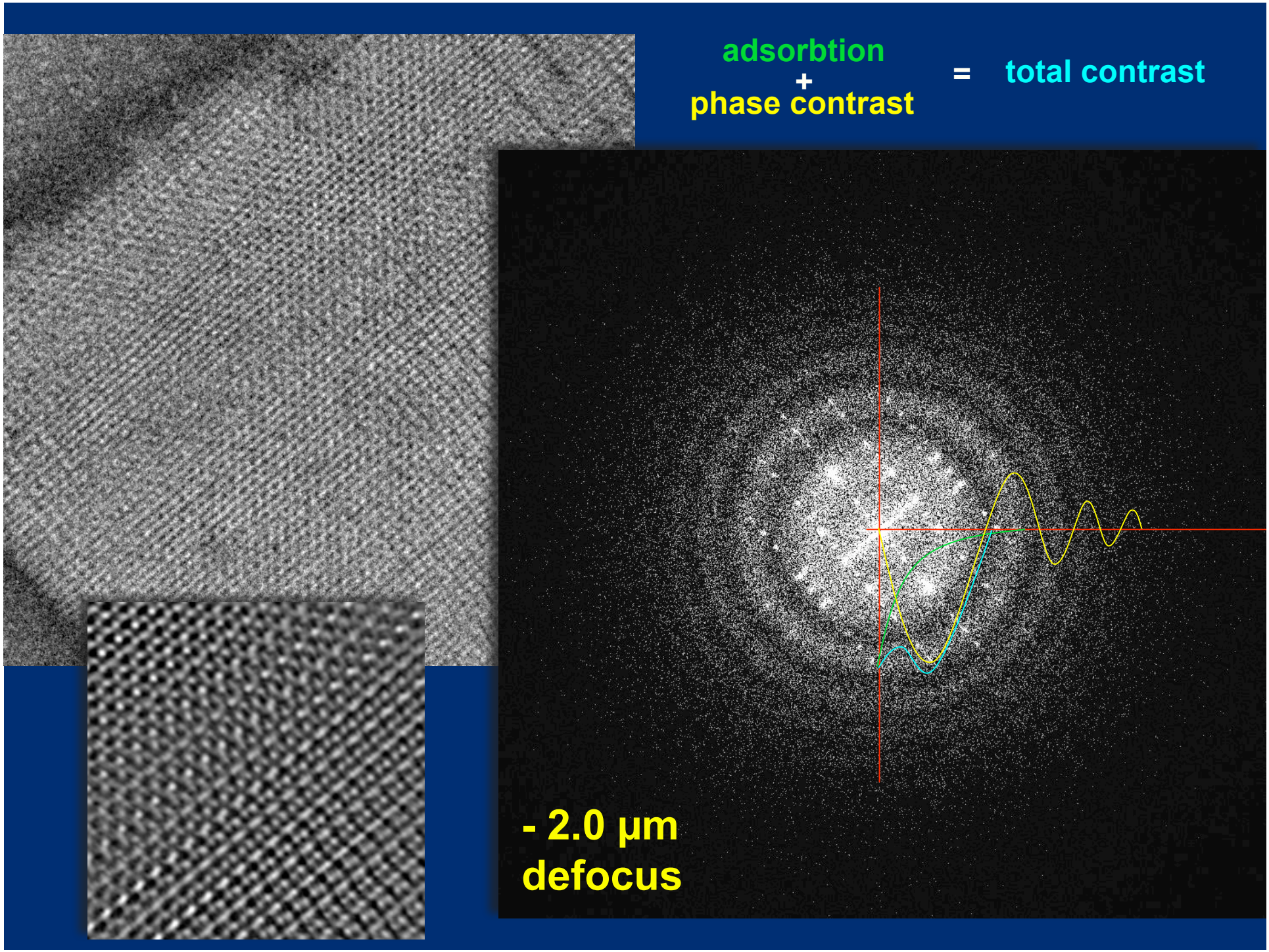
CEMOVIS

Ashraf Al Amoudi
Dubochet Lab, Lausanne CH

Biological Specimens



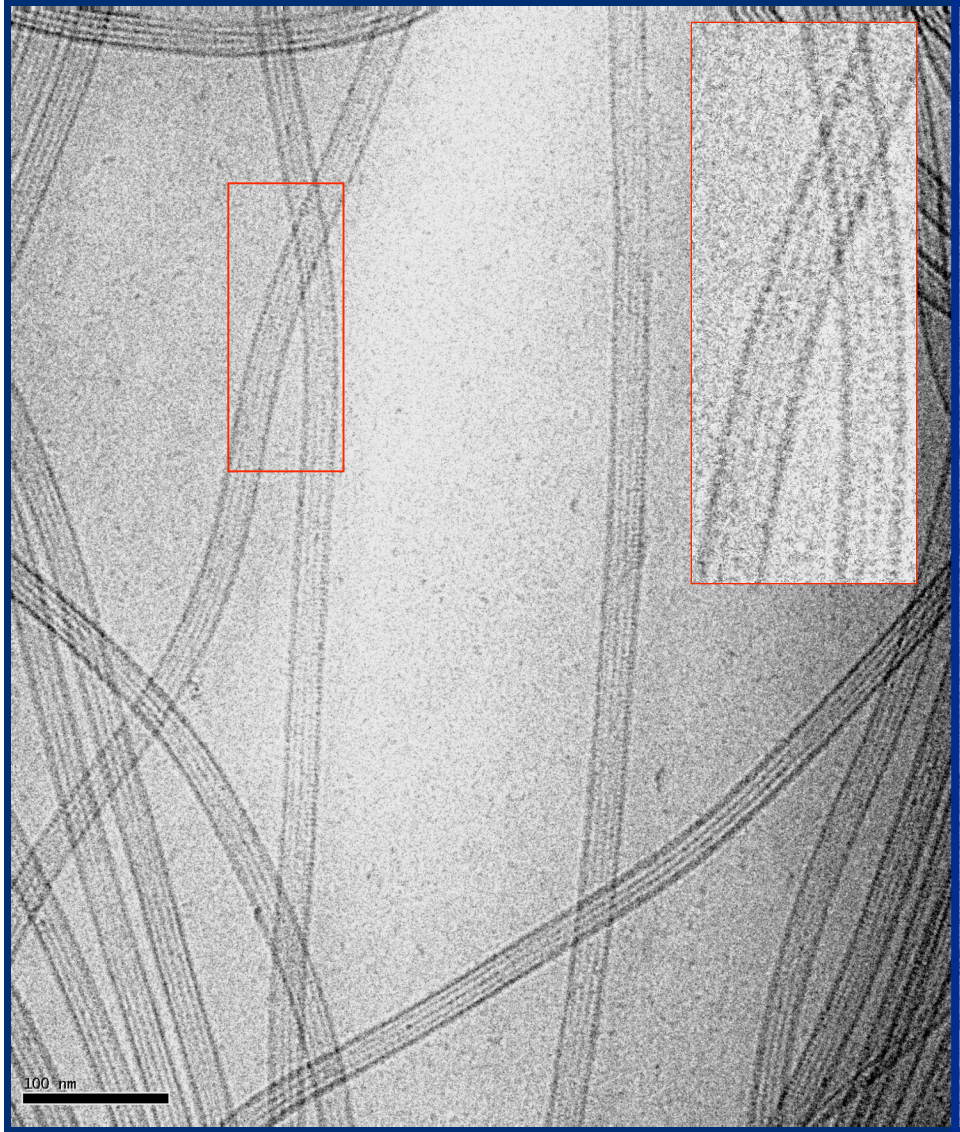

adsorbtion
+
phase contrast
=
total contrast
- 1.0 µm
defocus


adsorbtion
+
phase contrast
=
total contrast
- 2.0 µm
defocus

Energy Filtering

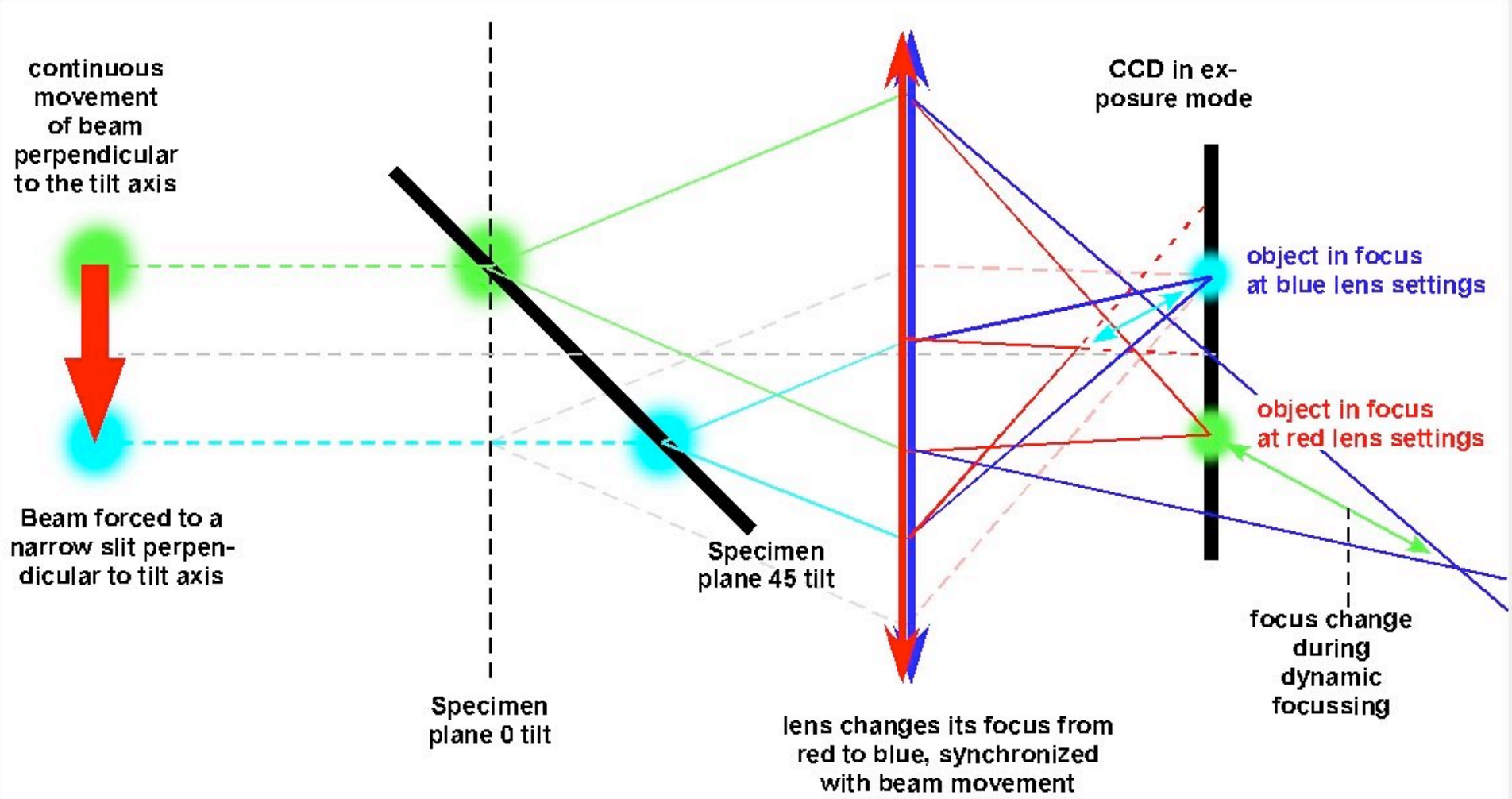
Microtubules recorded at 70 degree tilt

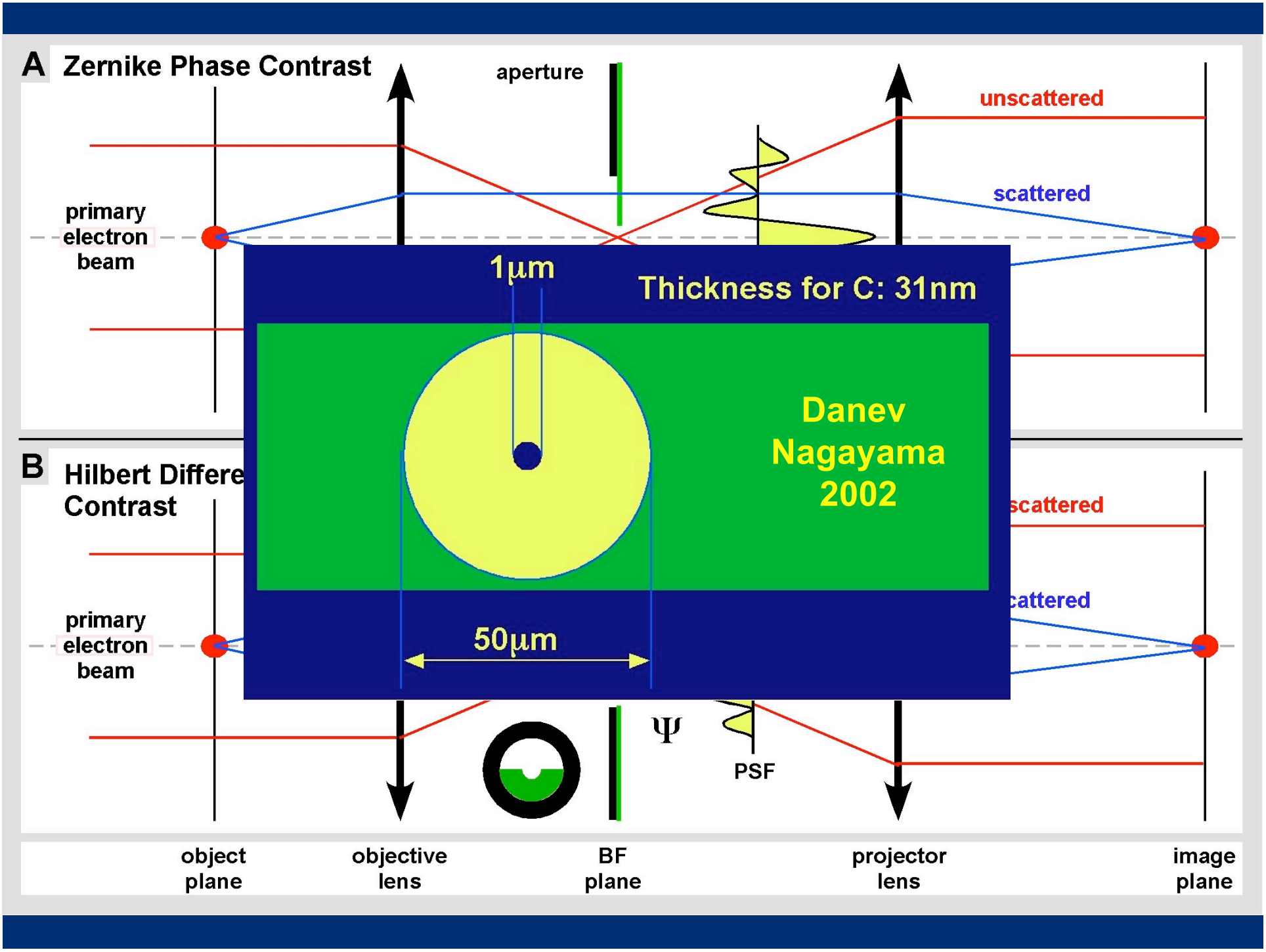
Zero-loss filtering



No filtering

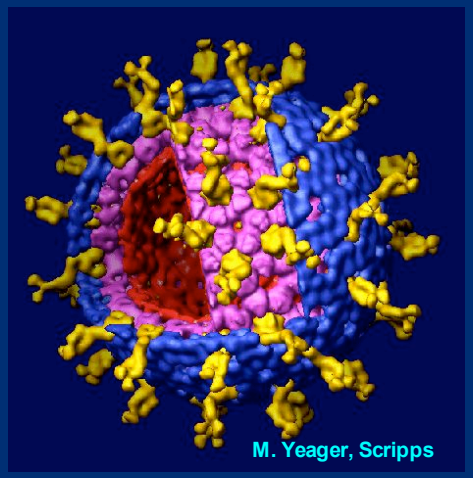
Dynamic Focusing

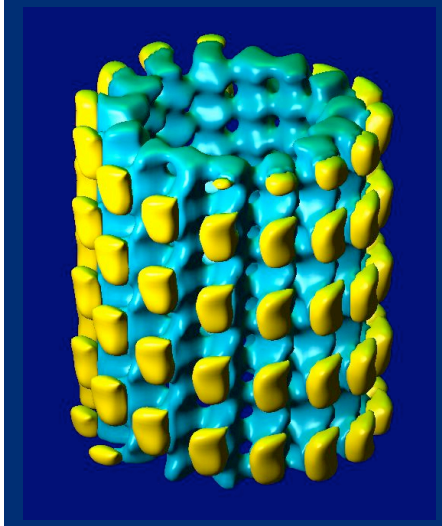



A Zernike Phase Contrast
aperture
unscattered
scattered
primary electron beam
1μm
Thickness for C: 31nm
Danev
Nagayama
2002
B Hilbert Differe
Contrast
scattered
cattered
primary electron beam
50μm
Ψ
PSF
object plane
objective lens
BF plane
projector lens
image plane

Averaging and 3D Image Reconstruction

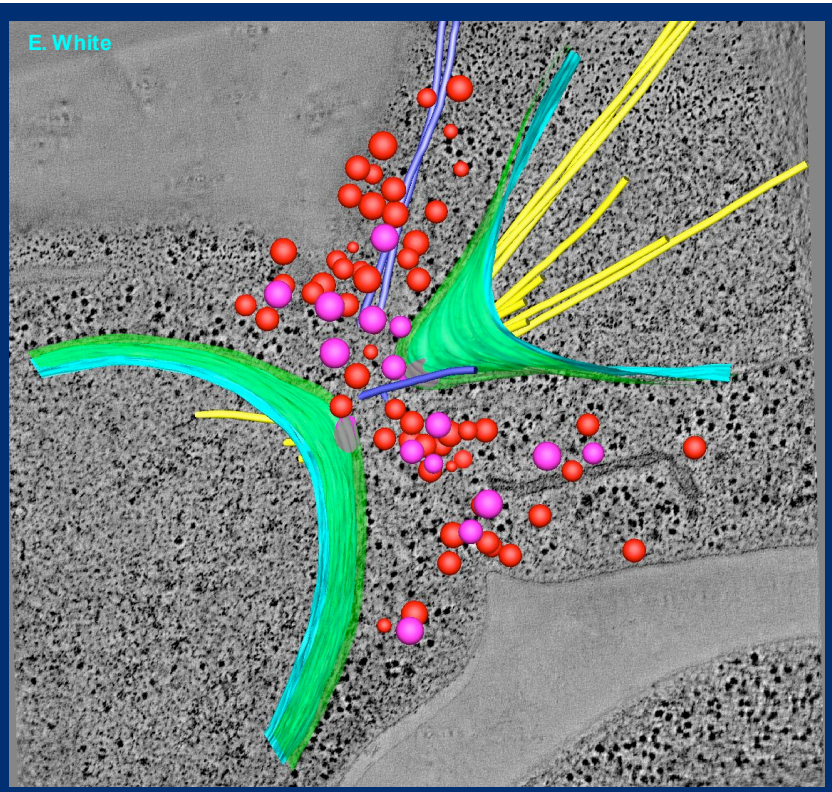
Why Tomography ?

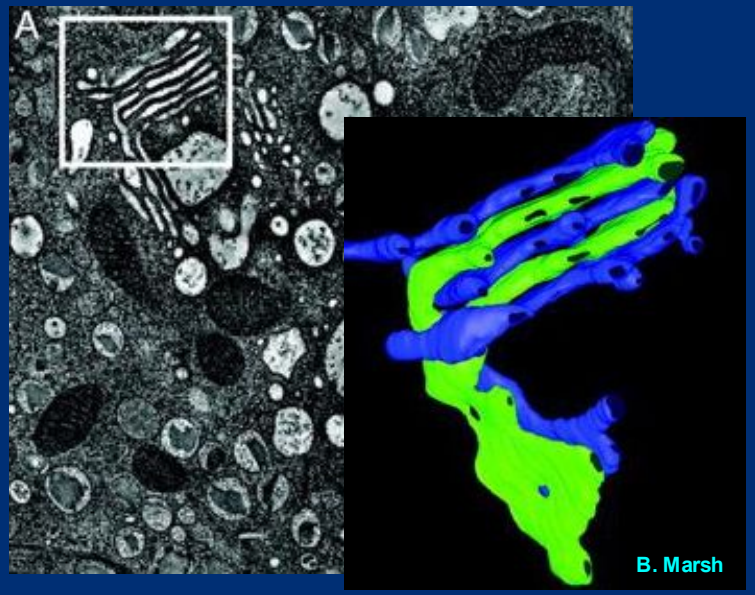




Reproducibly shaped structures

Large complex assemblies with flexible morphology





image

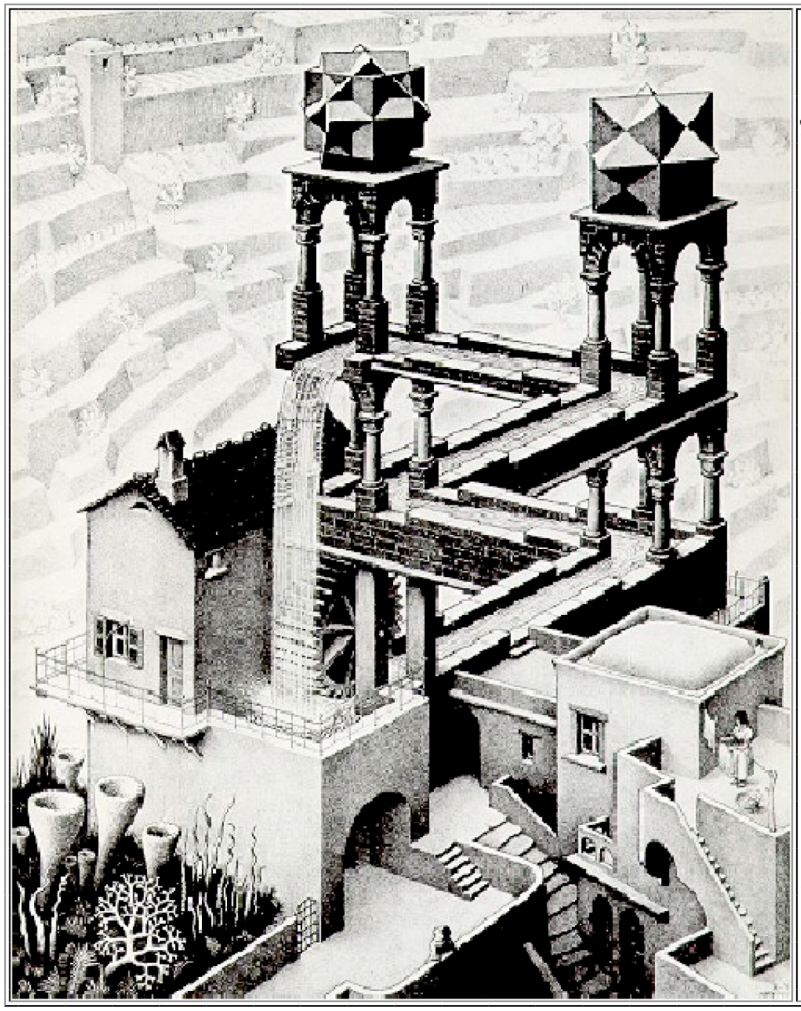
0°

projection

backprojection

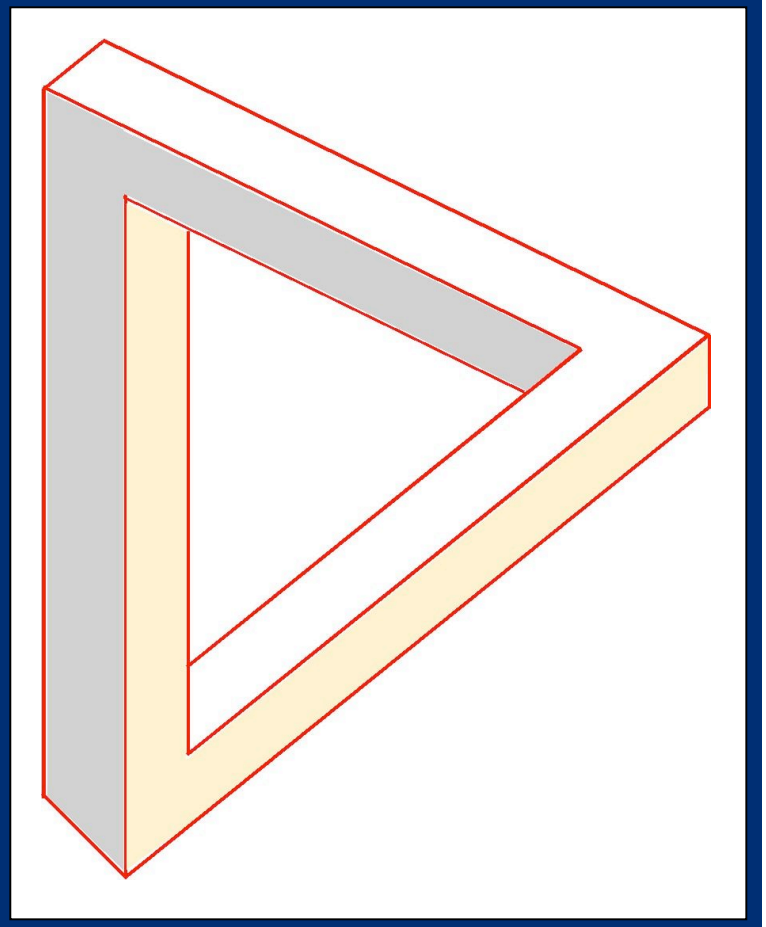
0 proj. used

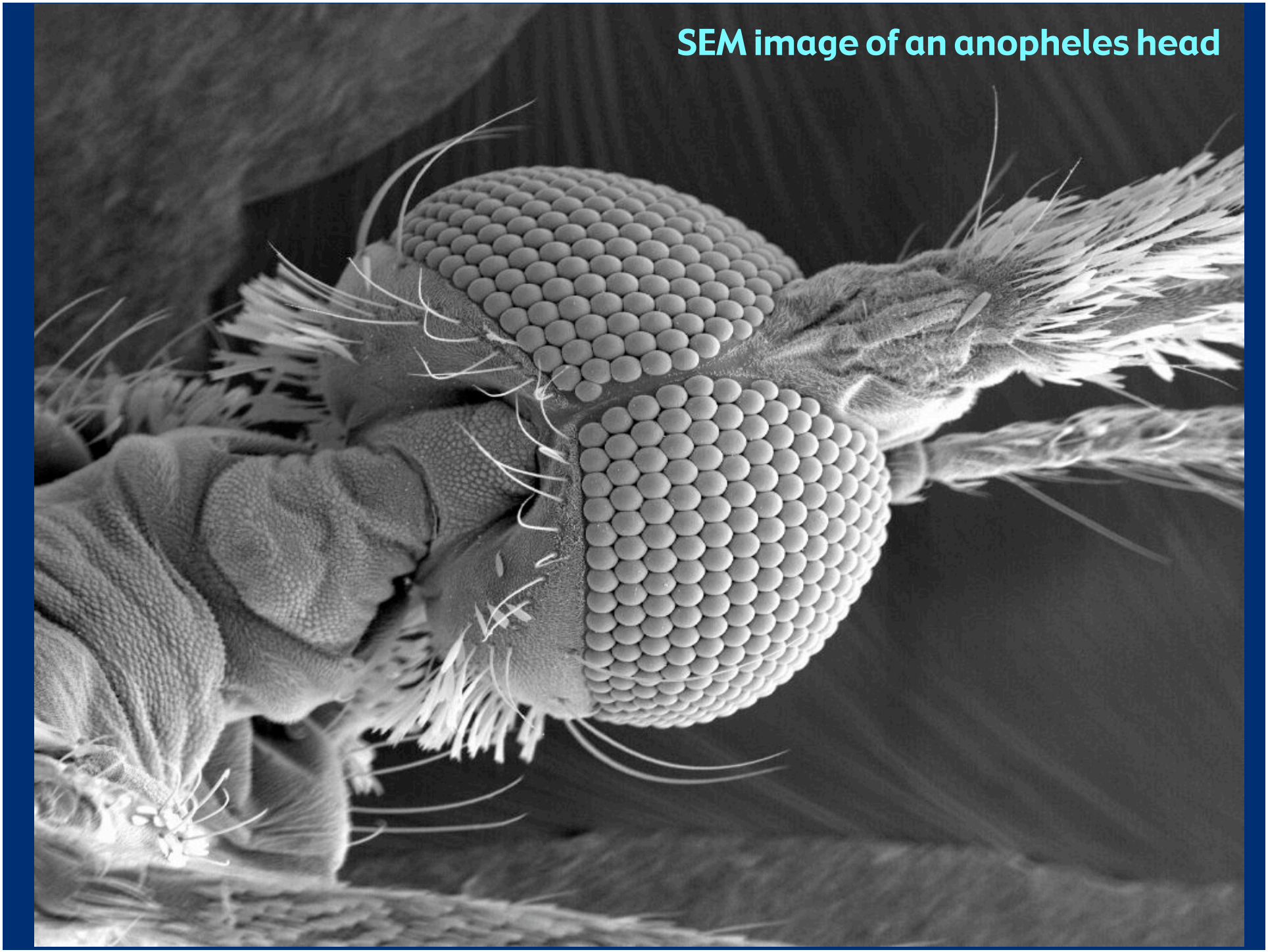
Wasserfall
M. C. Escher



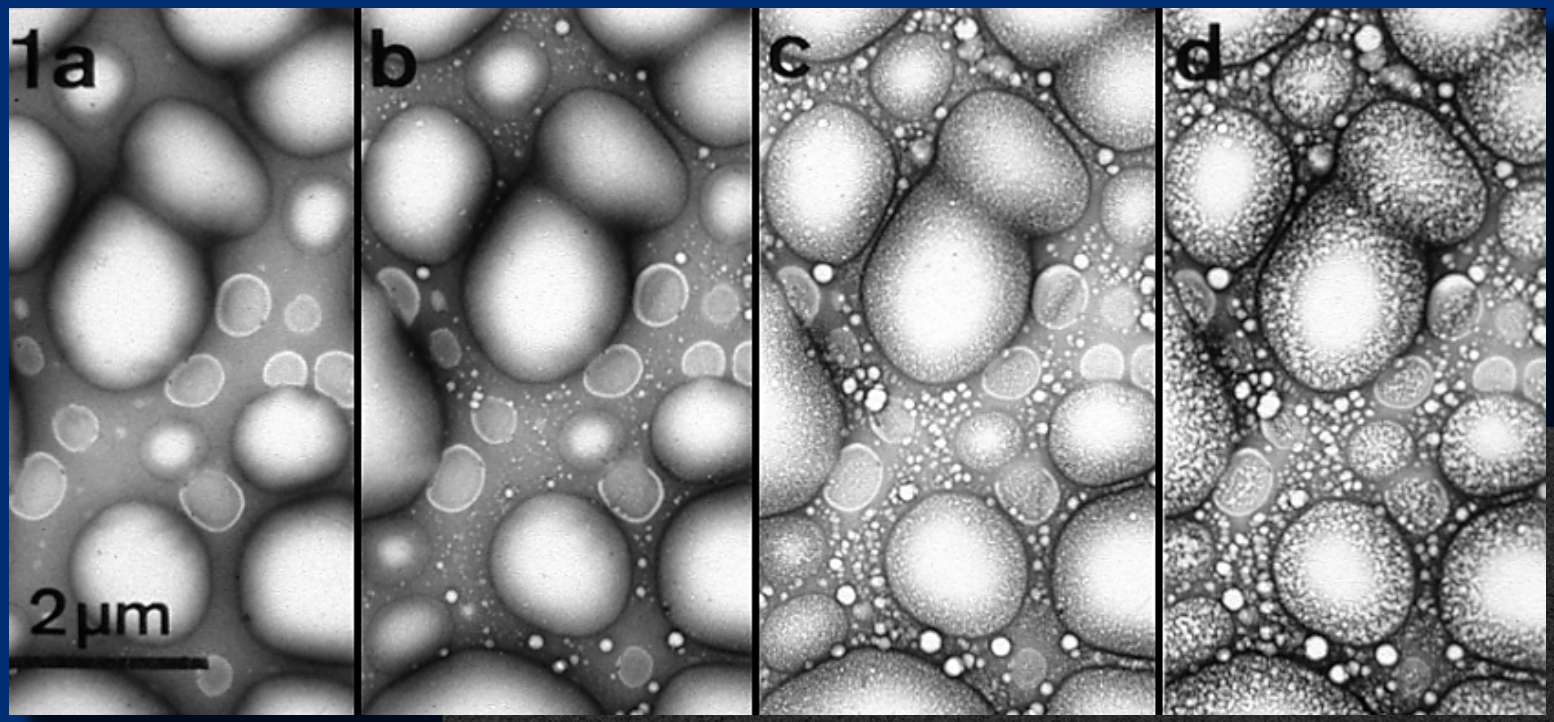
Titel
Wasserfall
Jahr
1961
Größe
28.0 x 28.0
Technik
Lithographie
Farbe
schwarz/weiß
Motiv
Haus mit unmöglichem Wasserfall

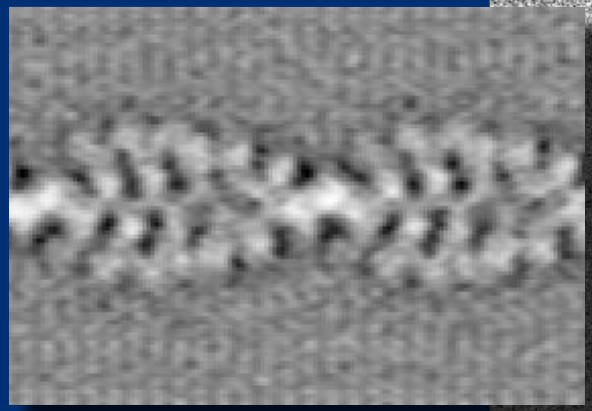
Bemerkungen
Der Wasserfall ist Eschers Weiterentwicklung des Tribars. An der Spitze der Säulen finden sich ein Drillingsoktaeder und ein Drillingshexaeder.

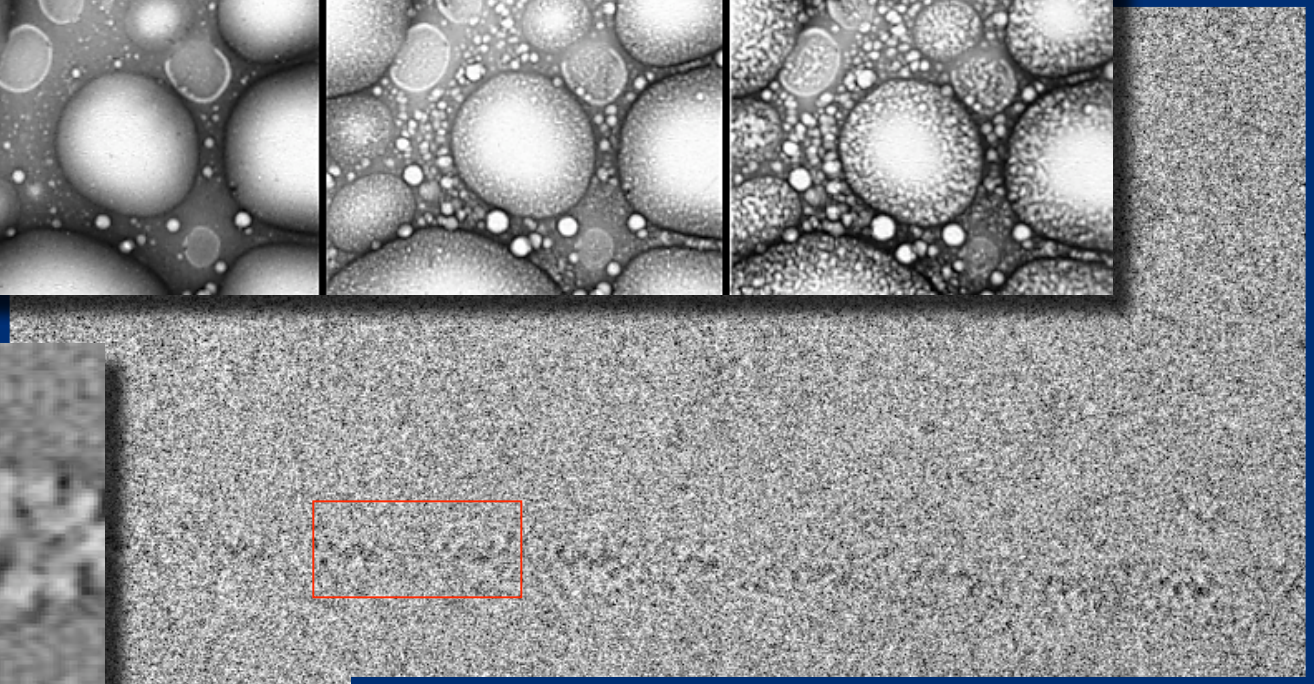



SEM image of an anopheles head

Beam damage / low dose imaging

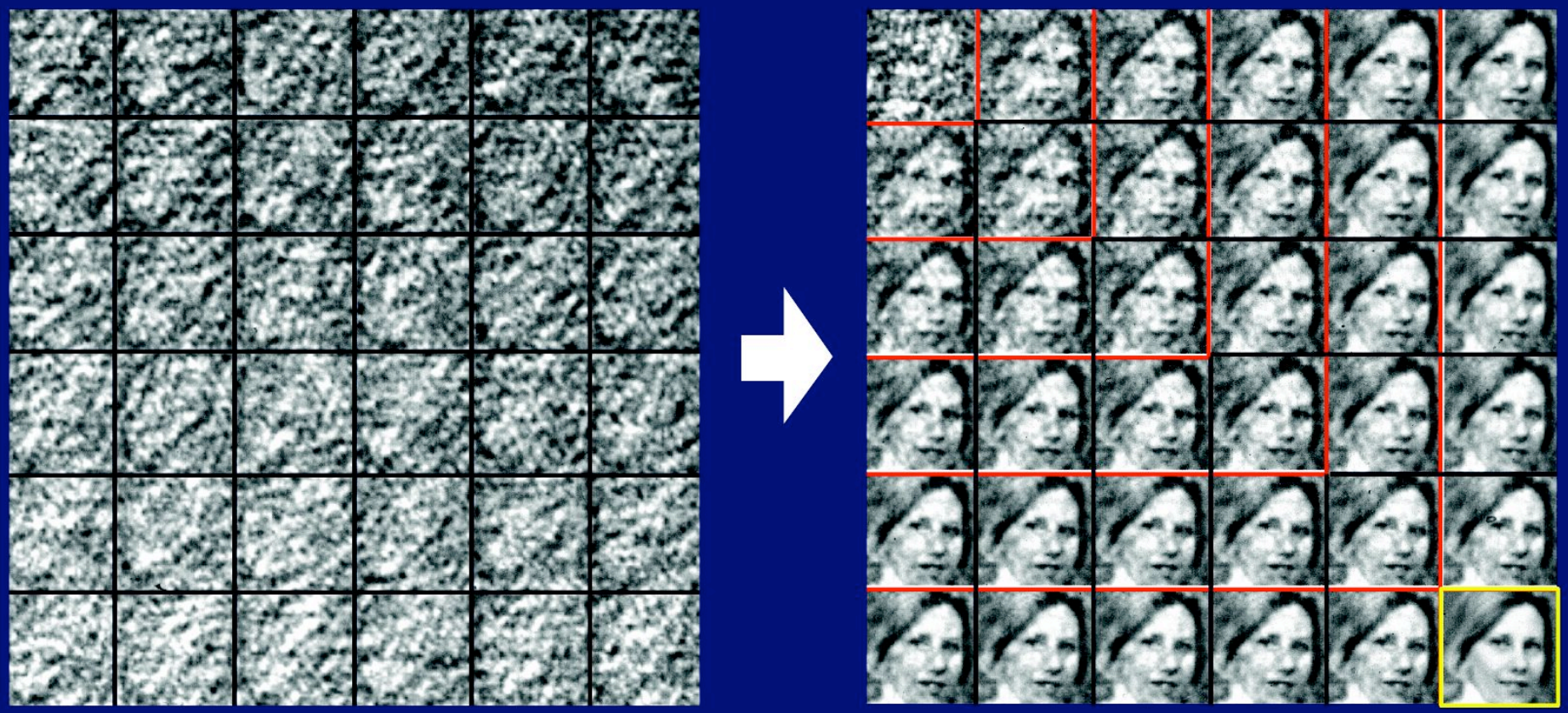


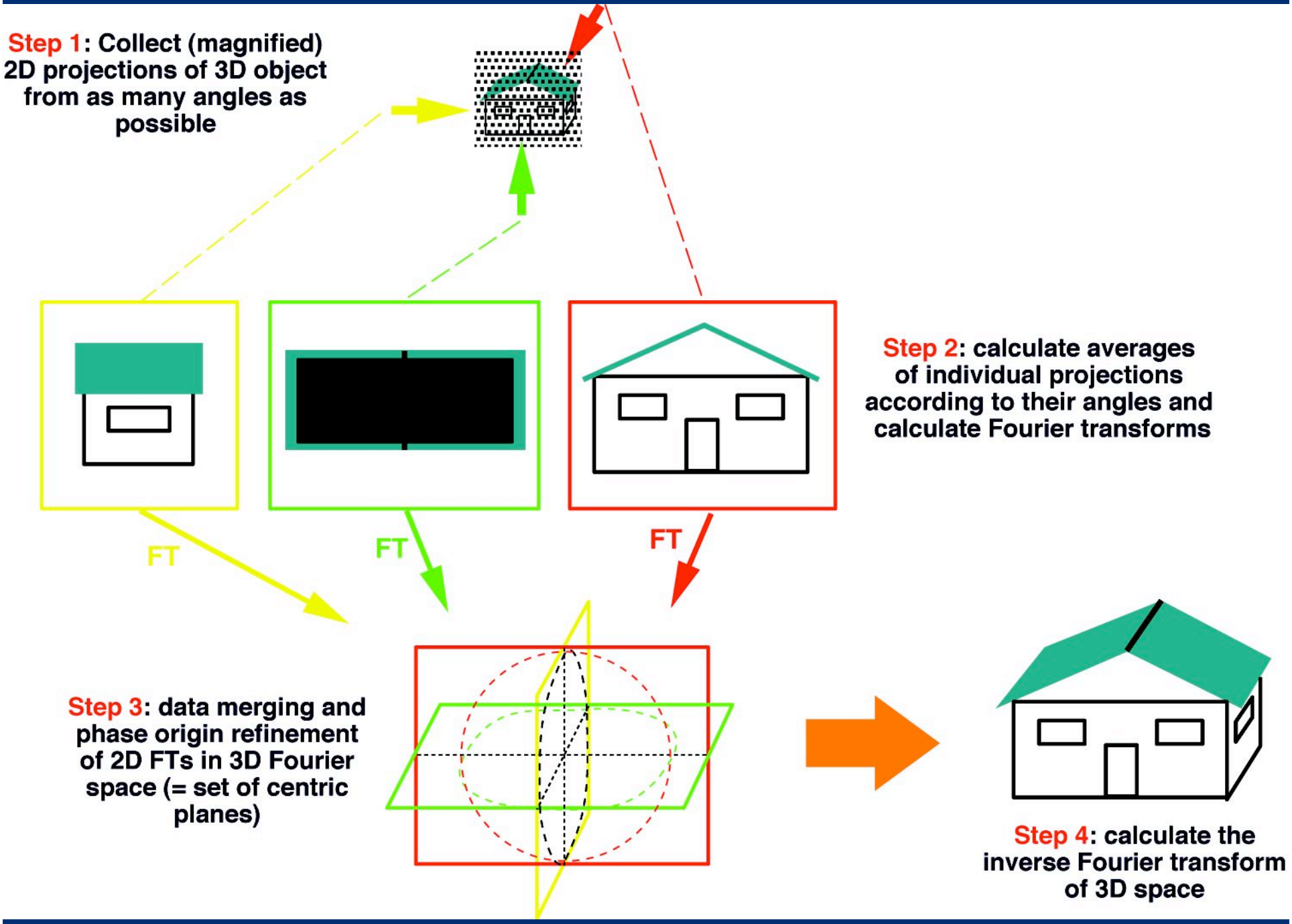




Actin filament decorated with Myosin Heads

averaging of noisy identical image elements




Step 1: Collect (magnified) 2D projections of 3D object from as many angles as possible
Step 2: calculate averages of individual projections according to their angles and calculate Fourier transforms
FT
FT
FT
Step 3: data merging and phase origin refinement of 2D FTs in 3D Fourier space (= set of centric planes)
Step 4: calculate the inverse Fourier transform of 3D space

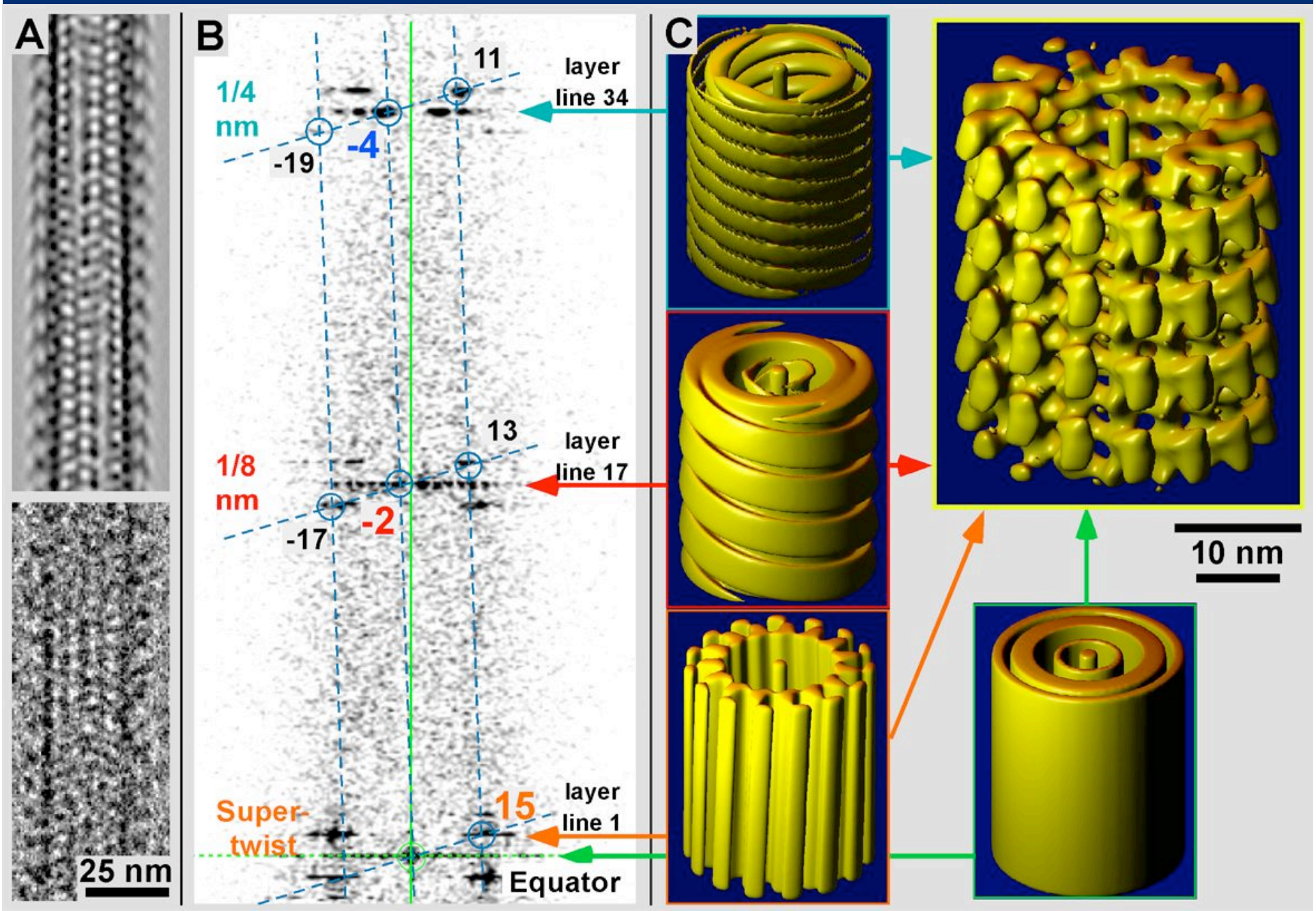
3-D Reconstruction Methods for EM:

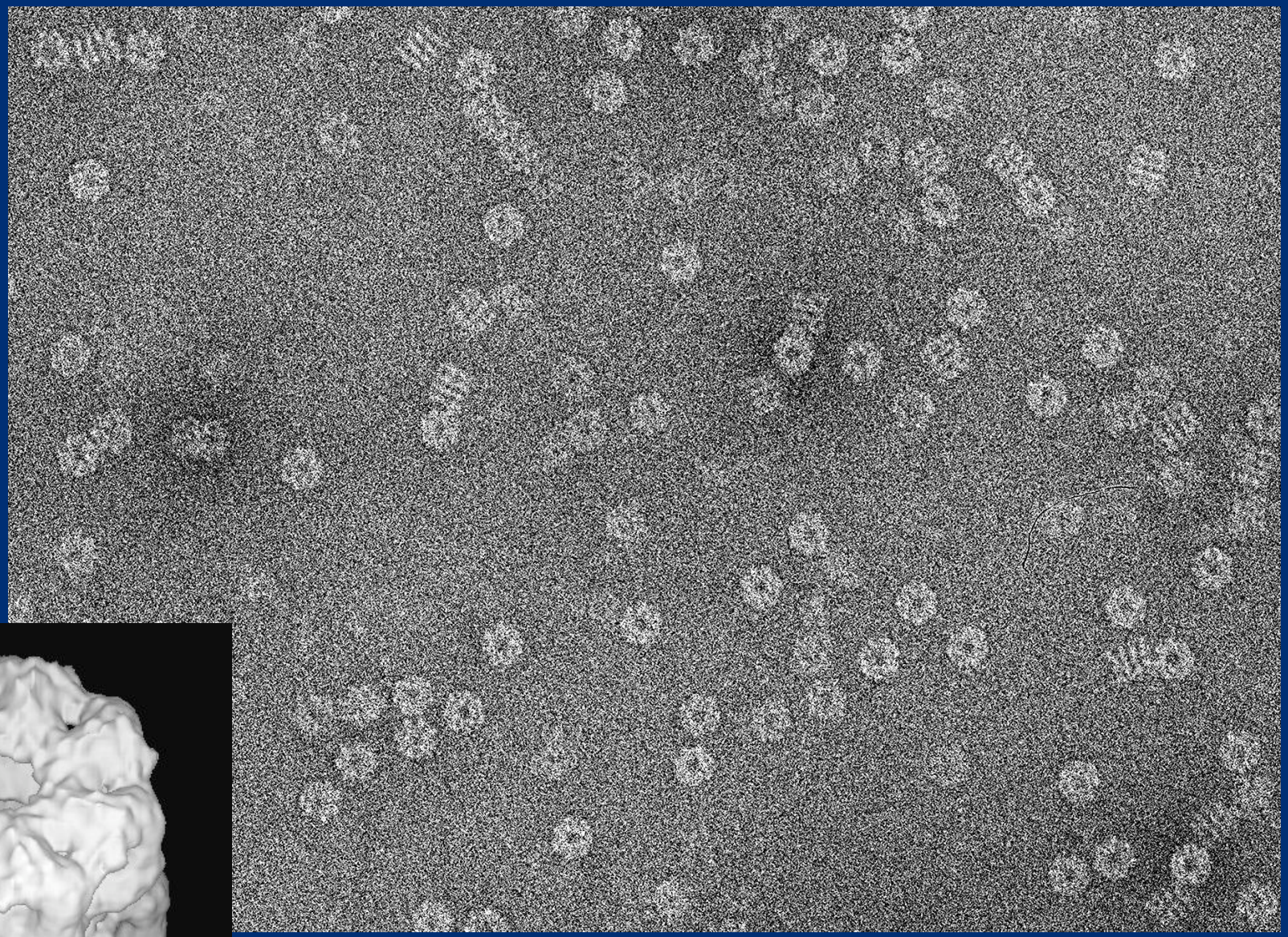
Tilt-series reconstruction from 2-D crystalline arrays

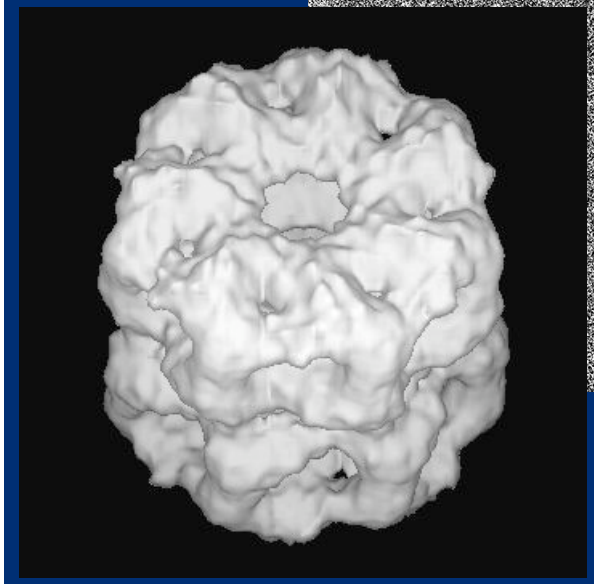
Single particle averaging / icosahedral particles

3-D reconstruction of helical assemblies

Tomographic reconstruction of irregular objects


A
25 nm
B
1/4
nm
11
-19
-4
layer
line 34
1/8
nm
13
-17
-2
layer
line 17
Super-
twist
15
layer
line 1
Equator
C
10 nm

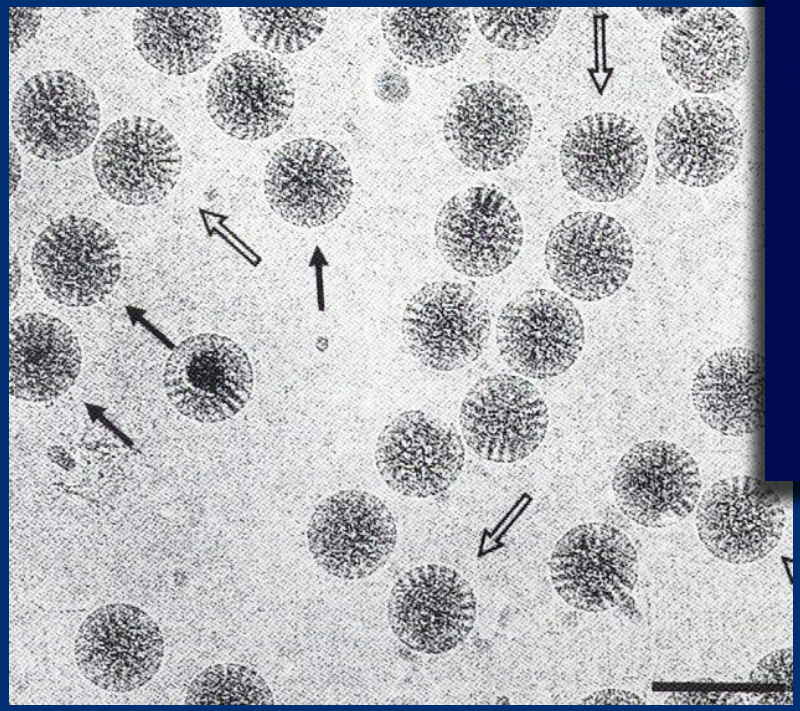


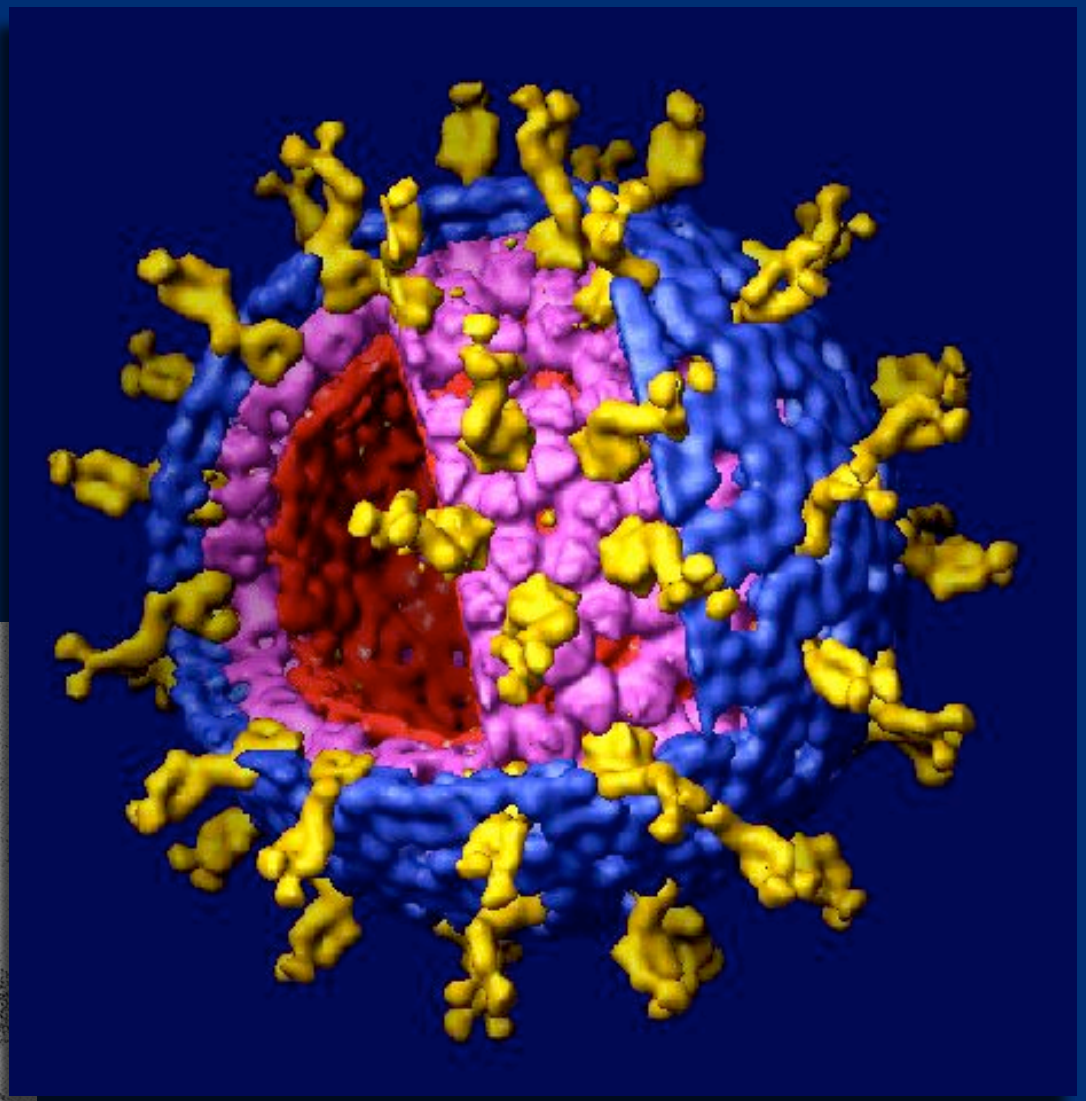


GroEL: Cryo-negative Staining (De Carlo et al.)

Rotavirus

(Yeager Lab
Scripps, San Diego)





image

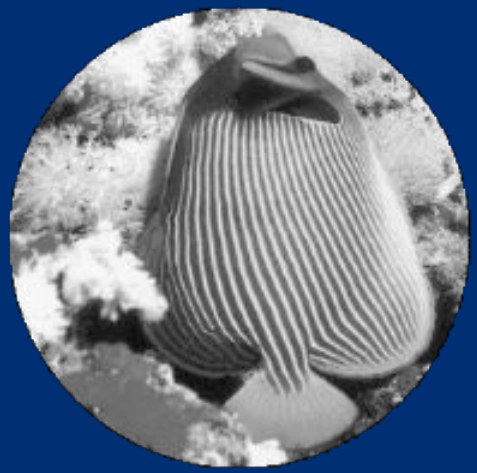
0°

projection

backprojection

0 proj. used

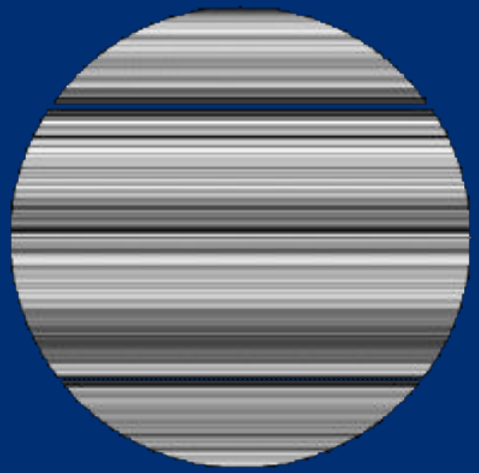
image



0°

projection

backprojection



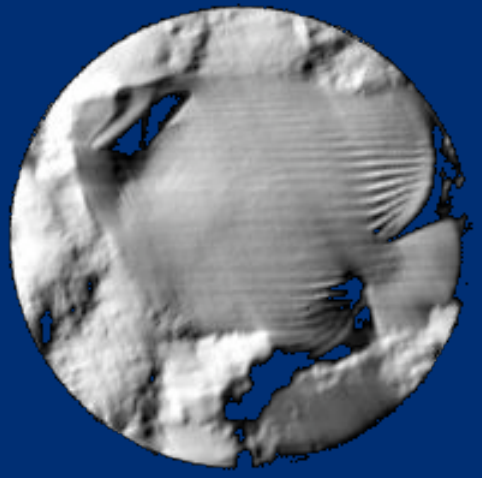
1 proj. used

image

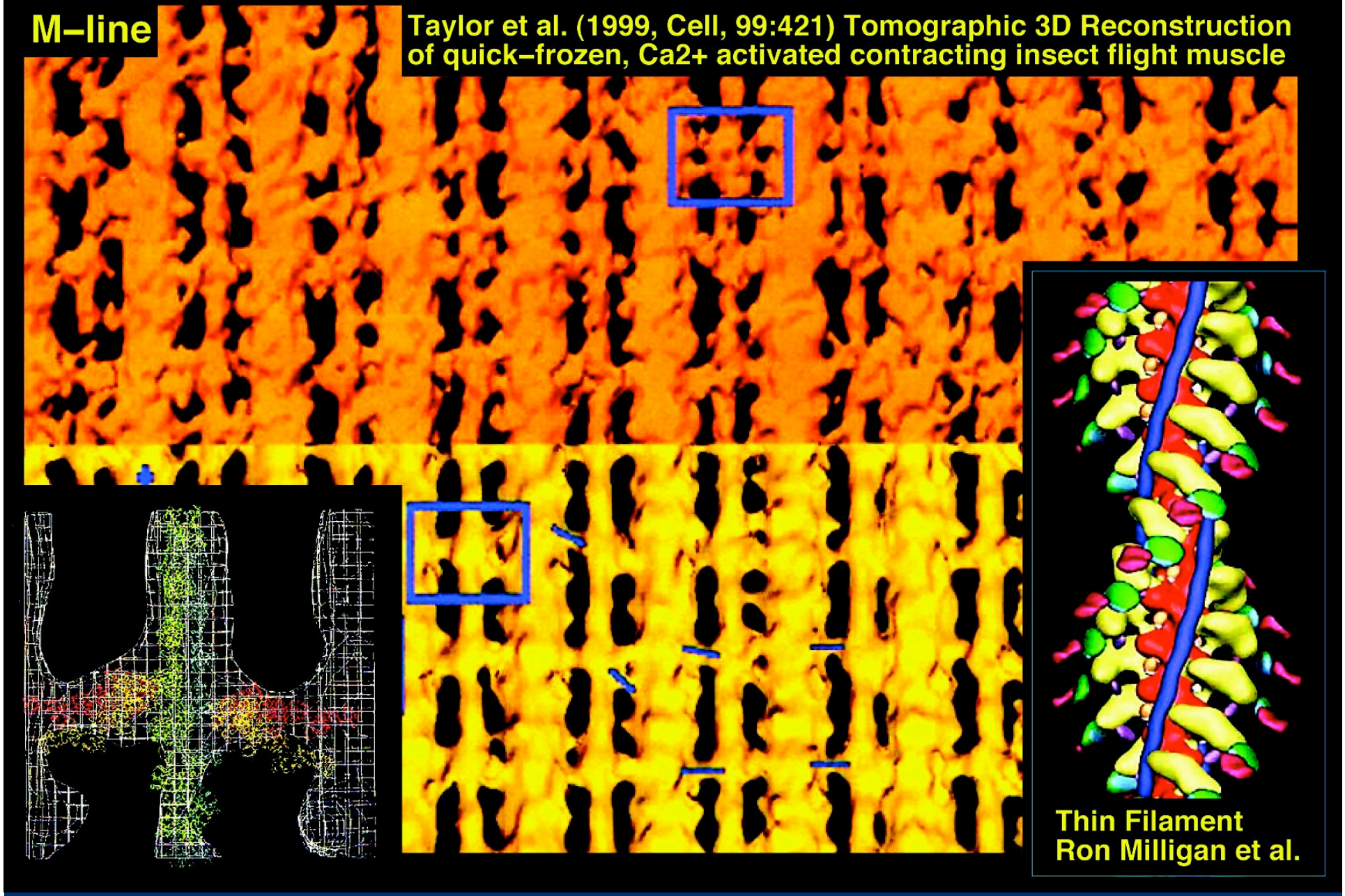
60°

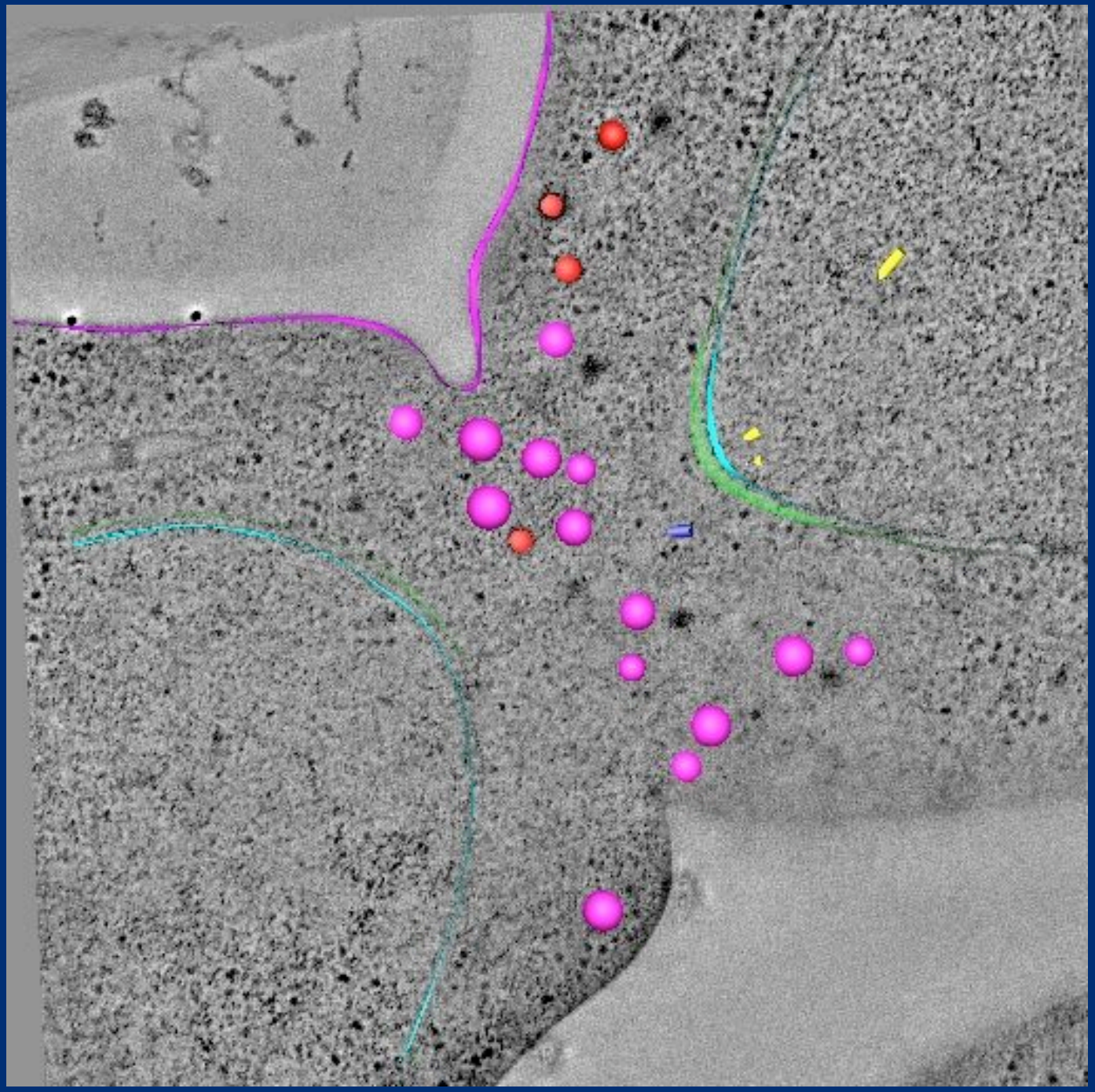
projection

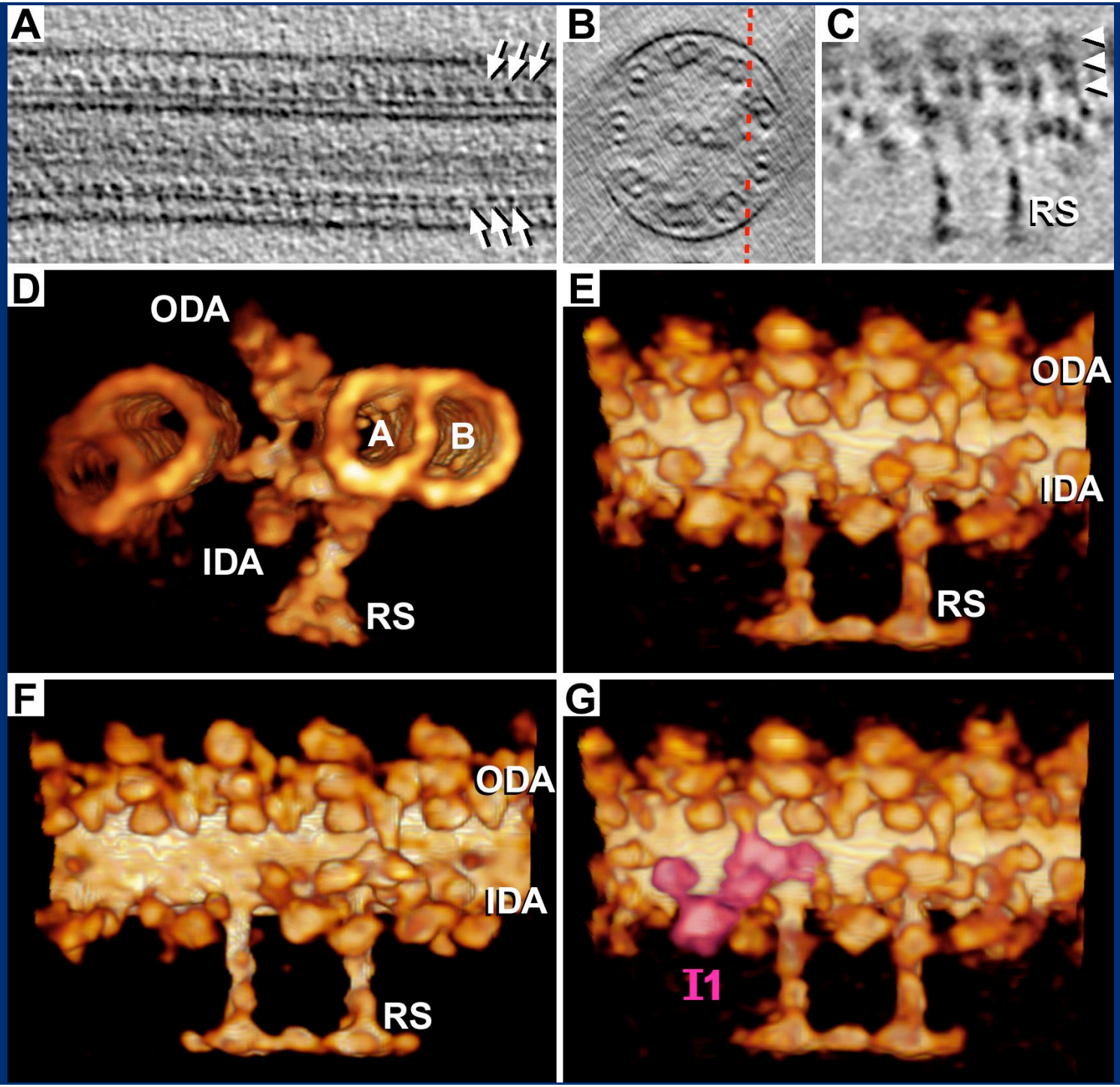
backprojection

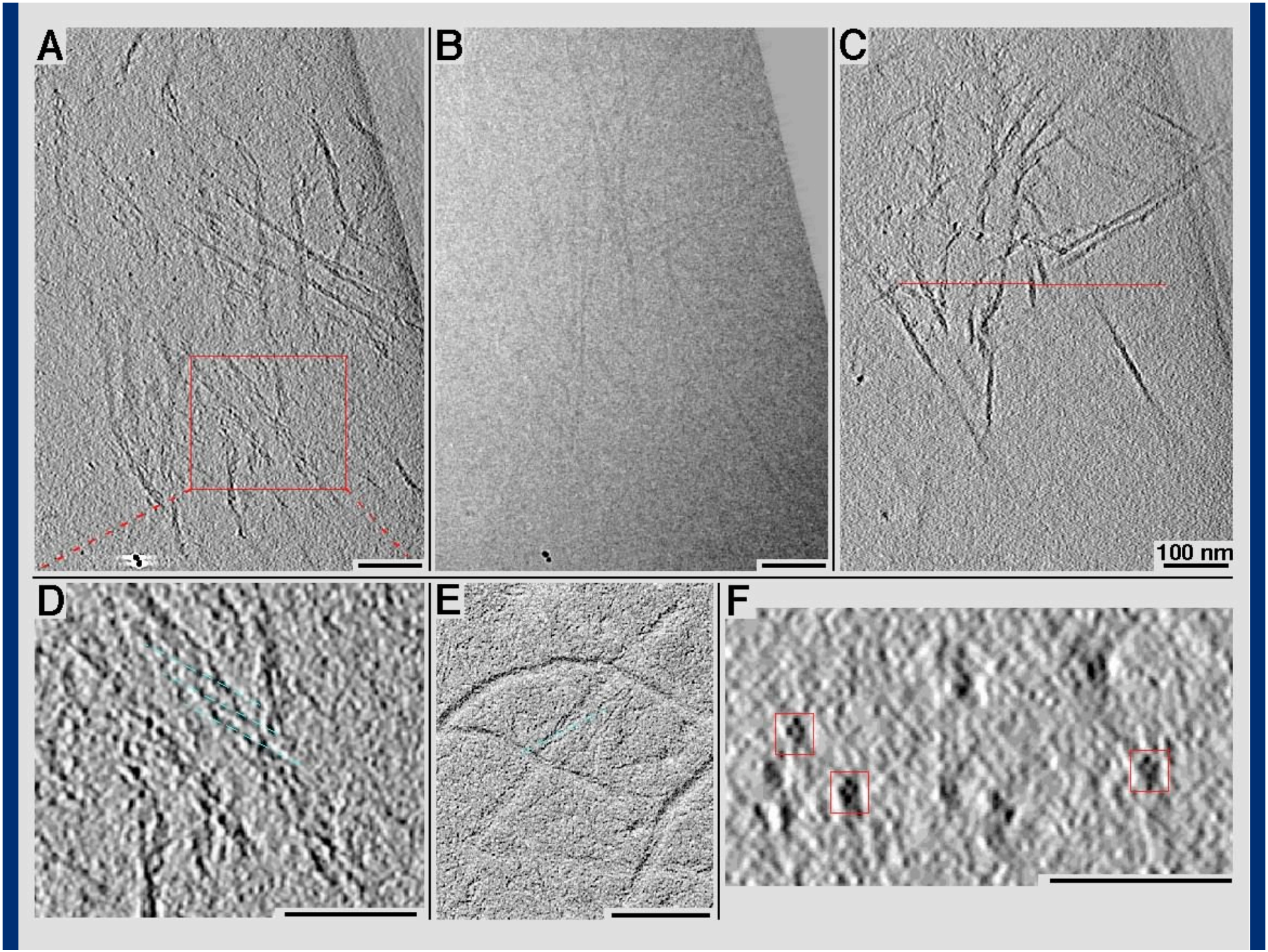


240 proj. used

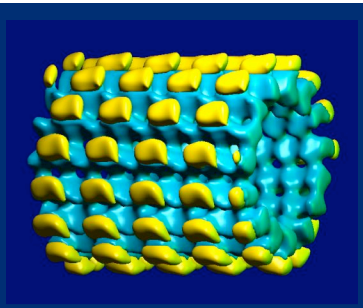

M–line
Taylor et al. (1999, Cell, 99:421) Tomographic 3D Reconstruction of quick–frozen, Ca2+ activated contracting insect flight muscle
Thin Filament
Ron Milligan et al.

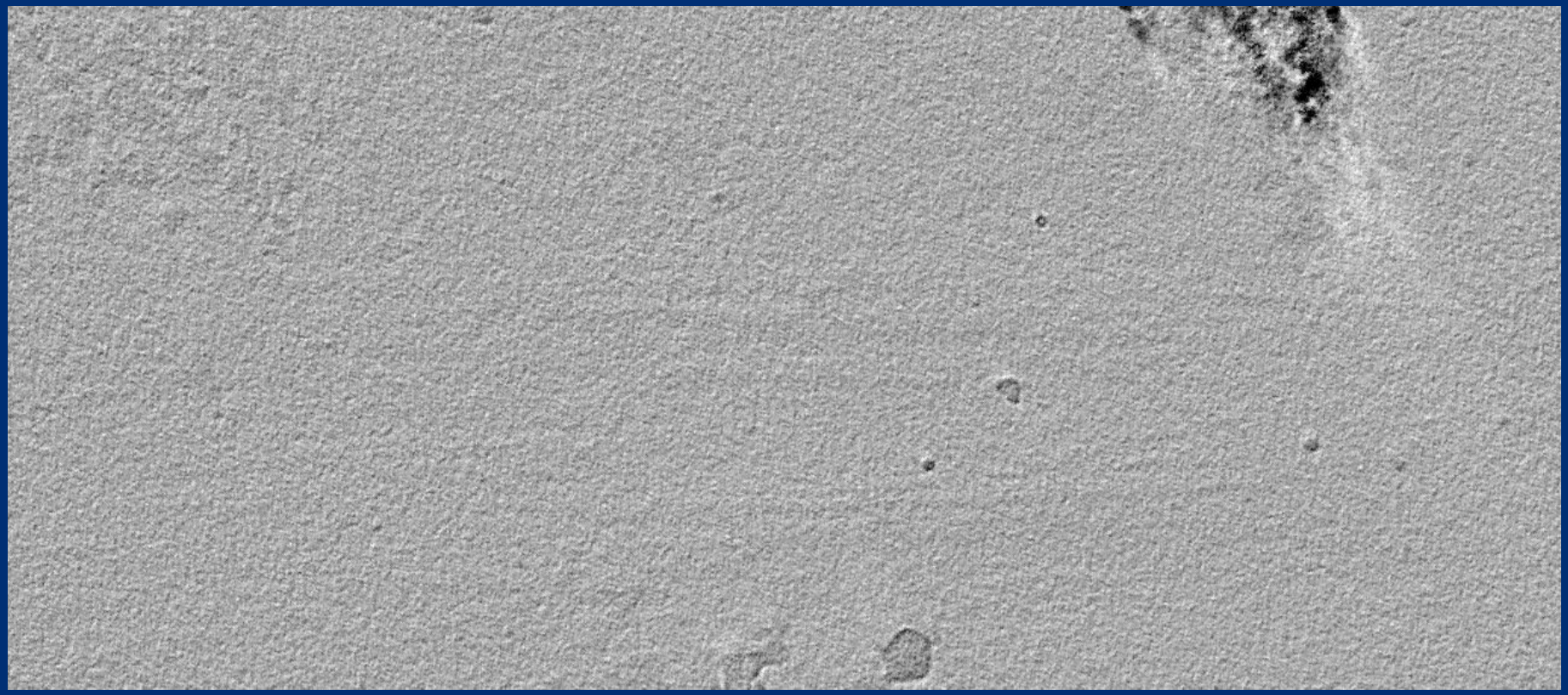



A
B
C
RS
D
ODA
A
B
IDA
RS
E
ODA
IDA
RS
F
ODA
IDA
RS
G
I1


A
B
C
100 nm
D
E
F

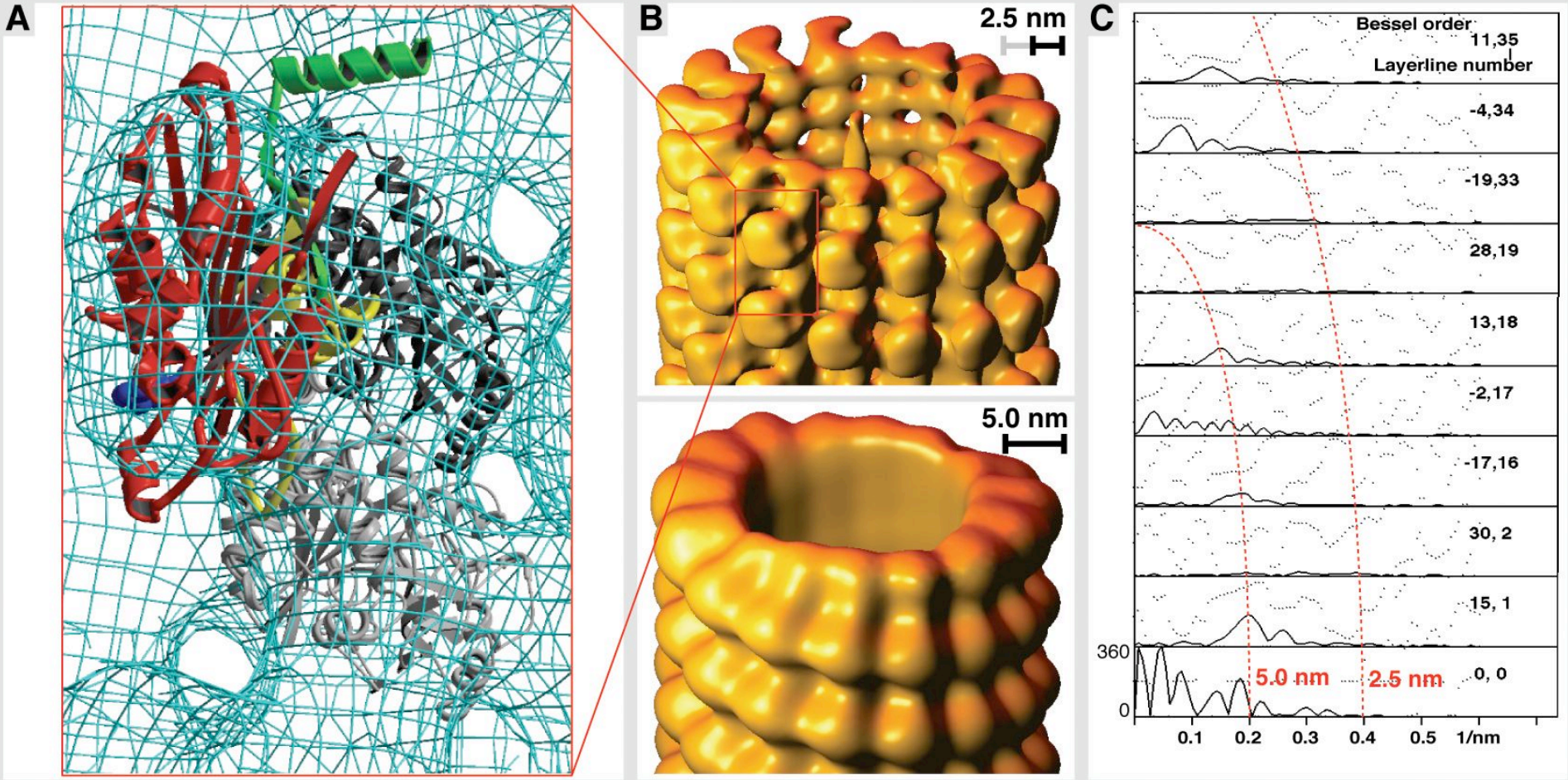
High-Resolution
Cryo-electron Tomography





Microtubules Decorated with Eg5 Motor Domains

(Mary Morphew, IVEM-Lab, Boulder, CO / Tecnai F20)


A
B
2.5 nm
5.0 nm
C
Bessel order
11,35
Layerline number
-4,34
-19,33
28,19
13,18
-2,17
-17,16
30, 2
15, 1
0, 0
360
0
5.0 nm
2.5 nm
0.1
0.2
0.3
0.4
0.5
1/nm