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General Principles of EM Tomography

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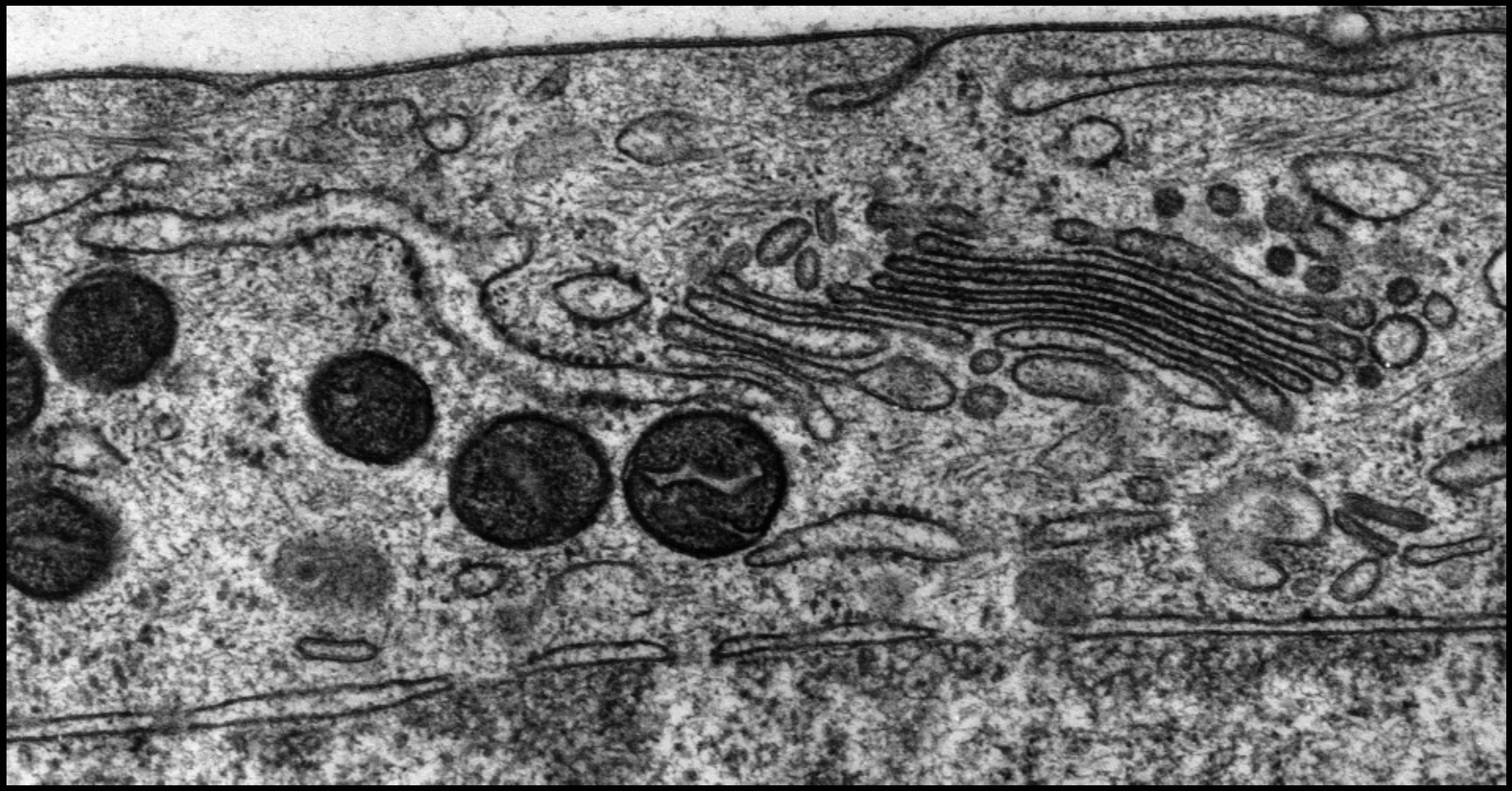
Why Tomography?



Tomography is needed to resolve features in the 1-20 nm size range

- To determine connectivity and relationships in 3-D
- To determine 3-D structure of organelles and associated macromolecules

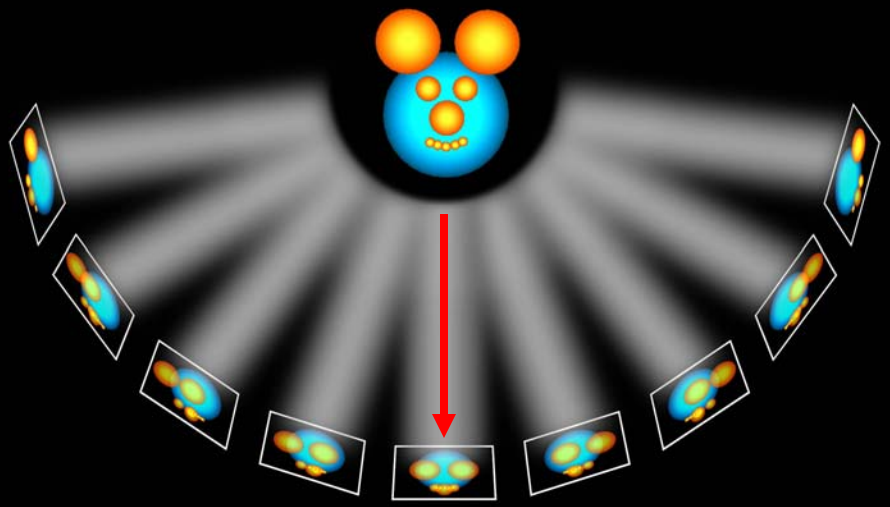
Electron tomography

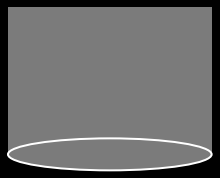
- method to obtain 3D information of an object by TEM
  ⇨ principle …

Principle of electron tomography

3D object

➲ set of 2D projections

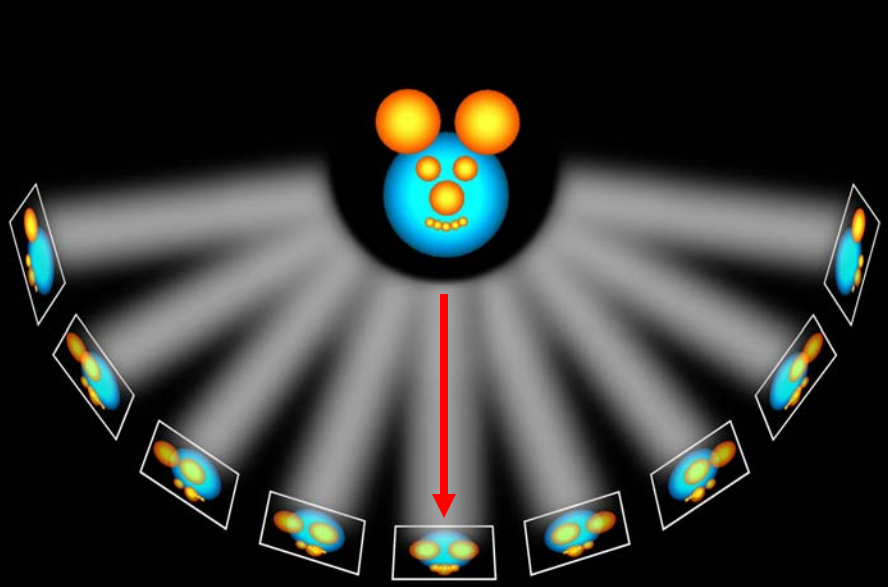


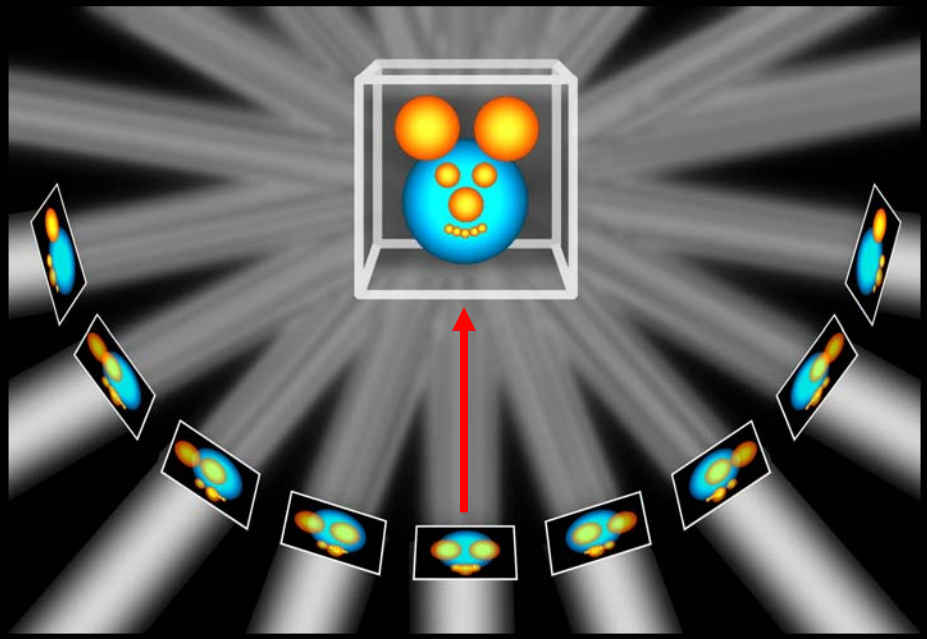


set of projections
(± 90°, 2° increment)

Principle of electron tomography

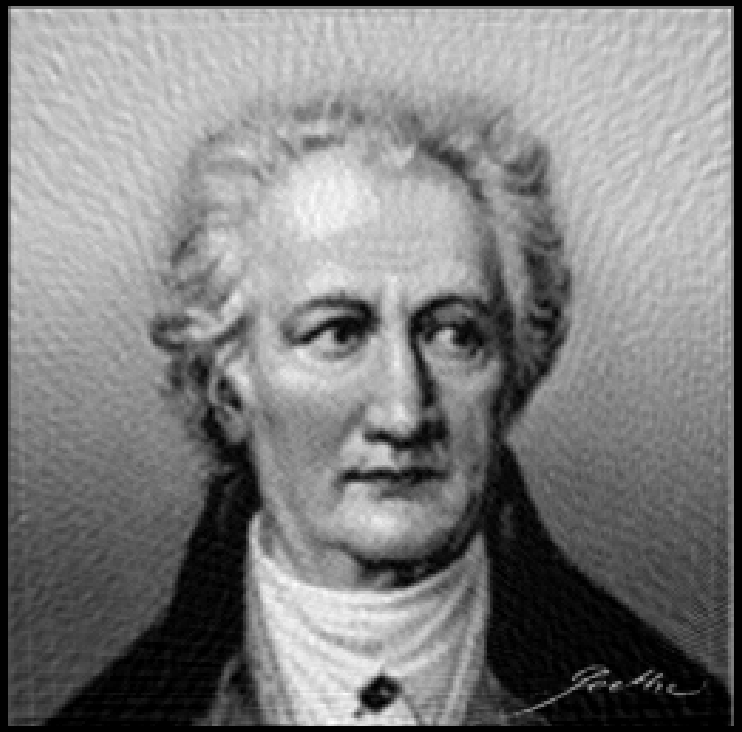
3D object ⇨ set of 2D projections ⇨ 3D reconstruction



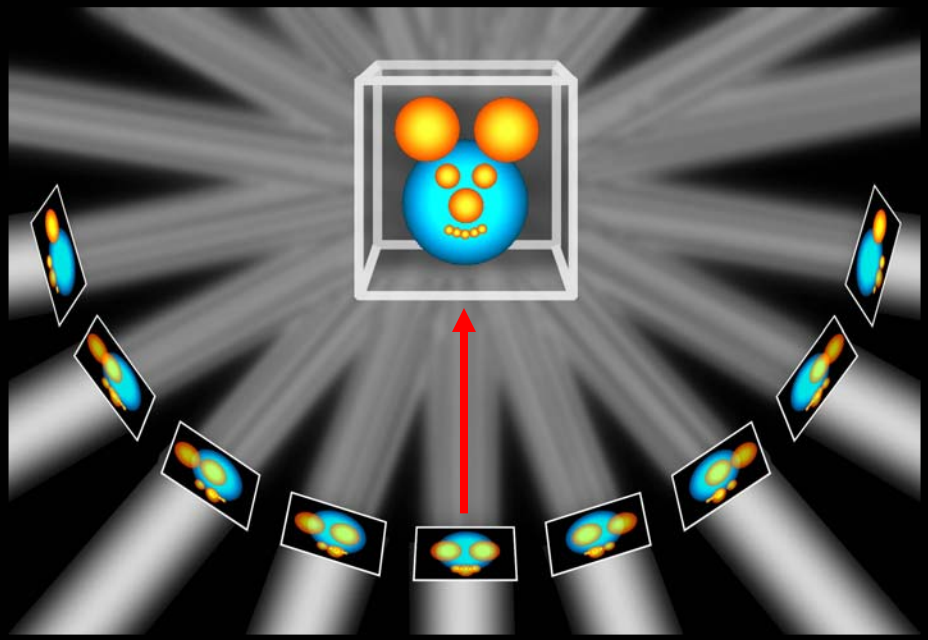


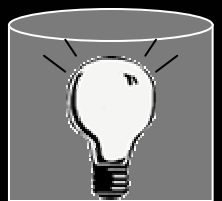
Principle of electron tomography

reconstruction



set of 2D projections
⇨ 3D reconstruction

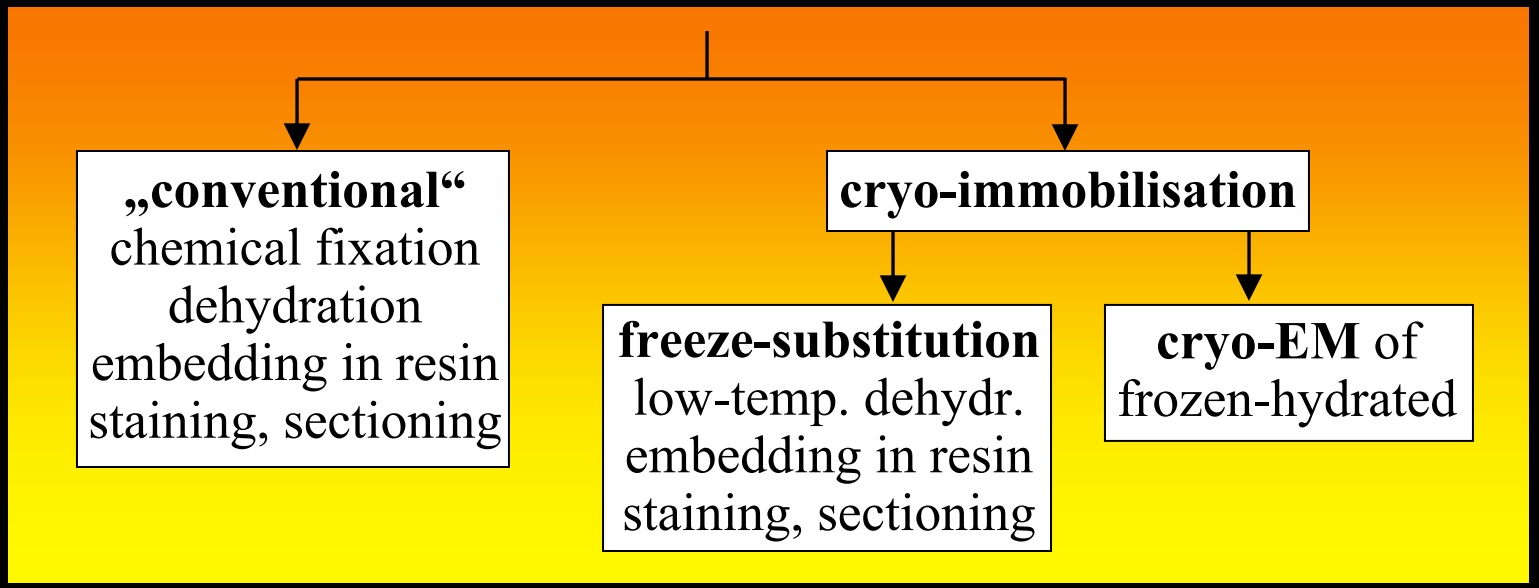




by weighted
backprojection

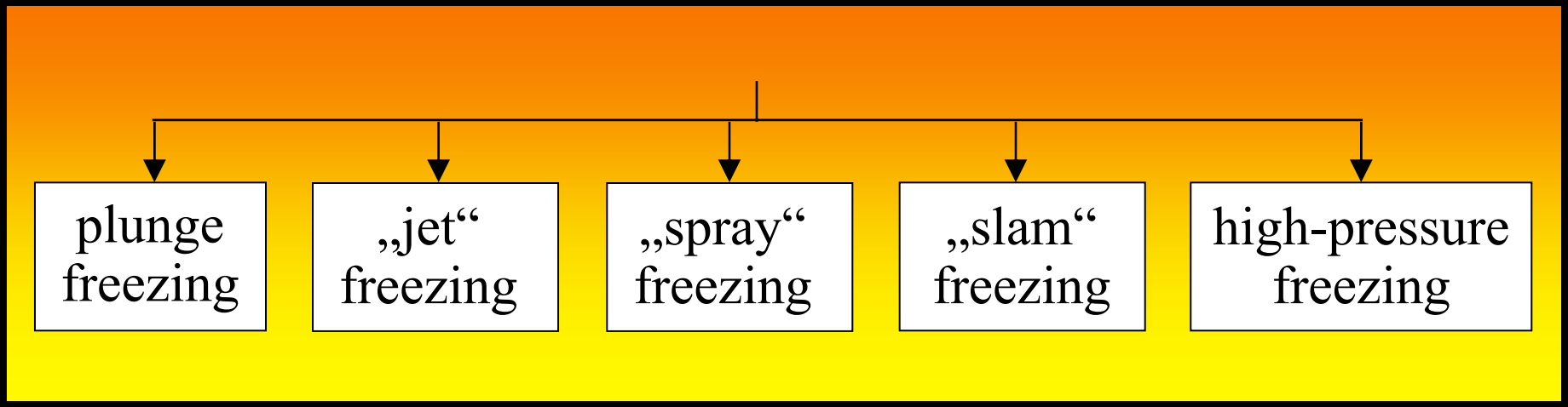
Electron tomography

- method to obtain 3D information of an object by TEM
  ⇨ principle
- preparation of the specimen for electron microscopy:
  goal: best possible structure preservation and resolution



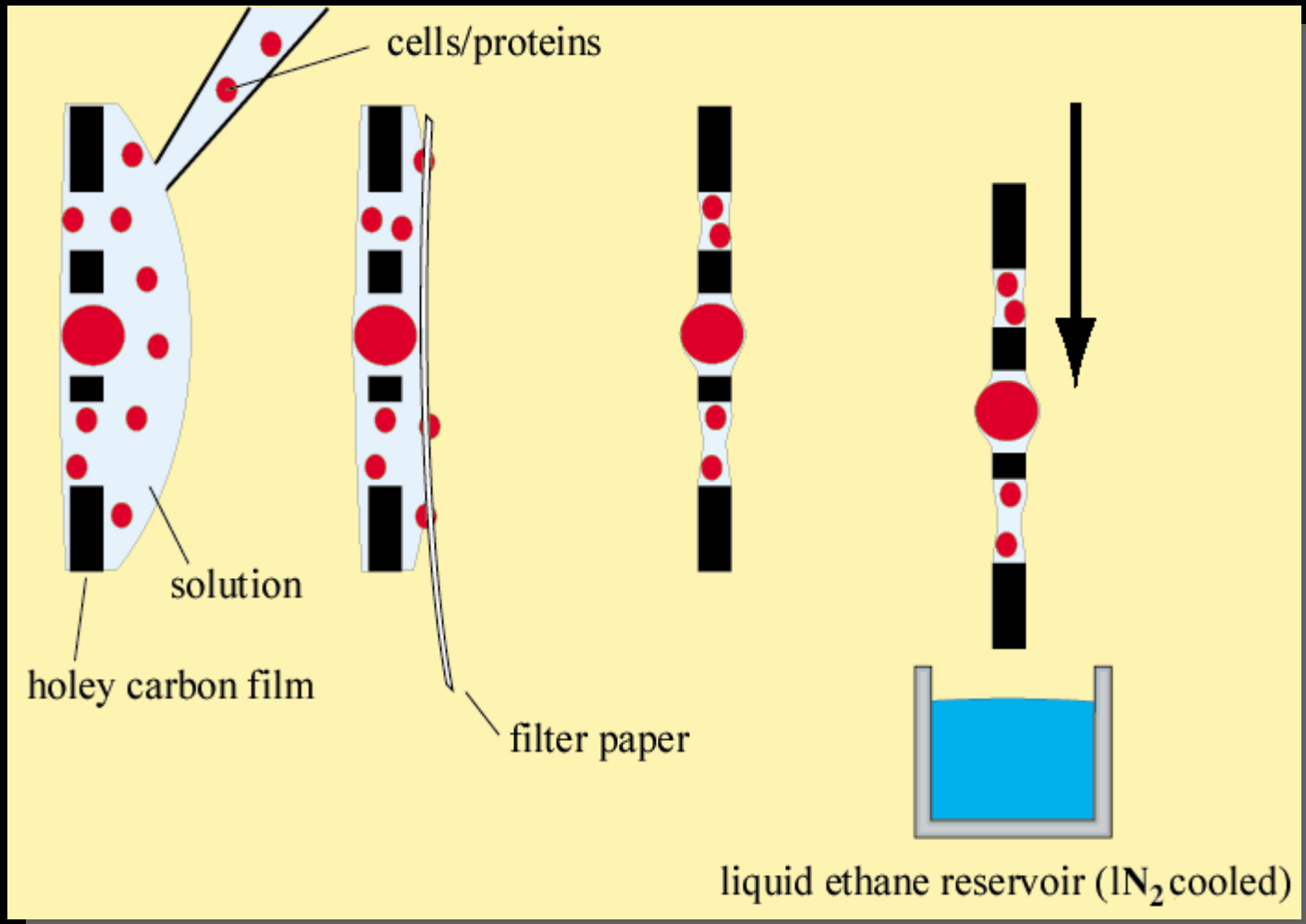
Cryo-immobilisation

- cooling rate approx. 10.000°C/sec.,
  water from liquid ➔ amorphous ice
- penetration thickness that is well frozen: ~ 10 µm,
  with high-pressure freezing: ~ 300 µm (x 2)
- advantages: structure preservation and permits time resolution of dynamic biological events (msec. range)



Specimen embedding in vitreous ice

- Plunging into liquid ethane:



Specimen embedding in vitreous ice

- High-pressure freezing:

Thicker samples (~300 µm per side of freezing hat) can be well frozen if the growth of ice crystals is slowed down by applying high pressure (~2,050 bar) immediately prior to freezing a sample (Dahl and Staehelin, 1989).

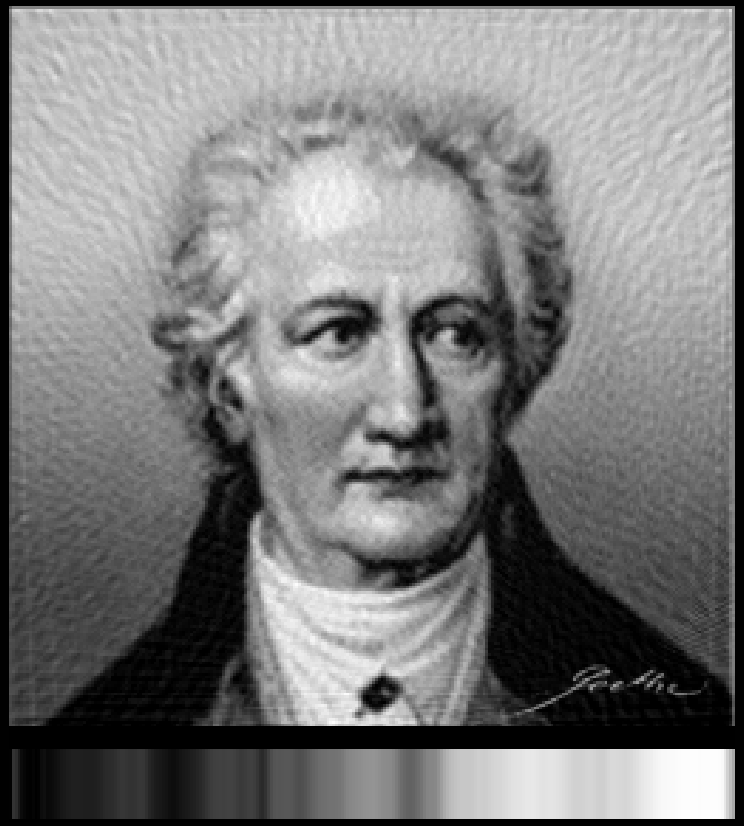
freezing hats

Electron tomography

- method to obtain 3D information of an object by TEM
  ⇨ principle
- preparation of the specimen for electron microscopy:
  goal: best possible structure preservation and resolution
- advantages ET:
  - 3-D information, non-invasive, resolution better than 10nm, applicable to many specimens
- limitation of electron tomography:
  - limited tilt angle range ⇨ “missing wedge”

Limitation of electron tomography

reconstruction of series with
± 90° tilt angle range



reconstruction of series with
± 60° tilt angle range

Dual-axis tilting and reconstruction

original image

reconstr. of first axis

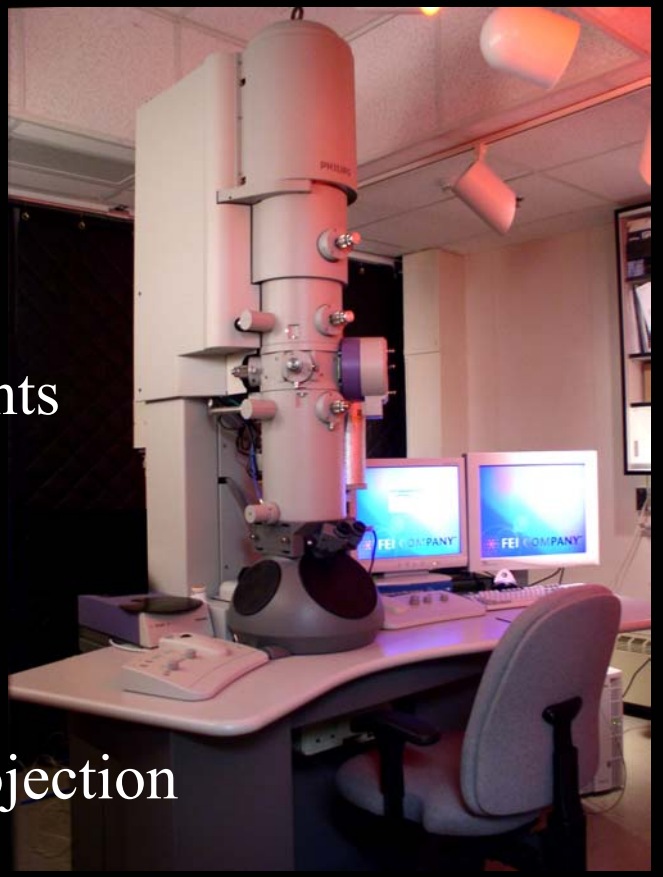
second axis (90º rotated)

combined tomogram

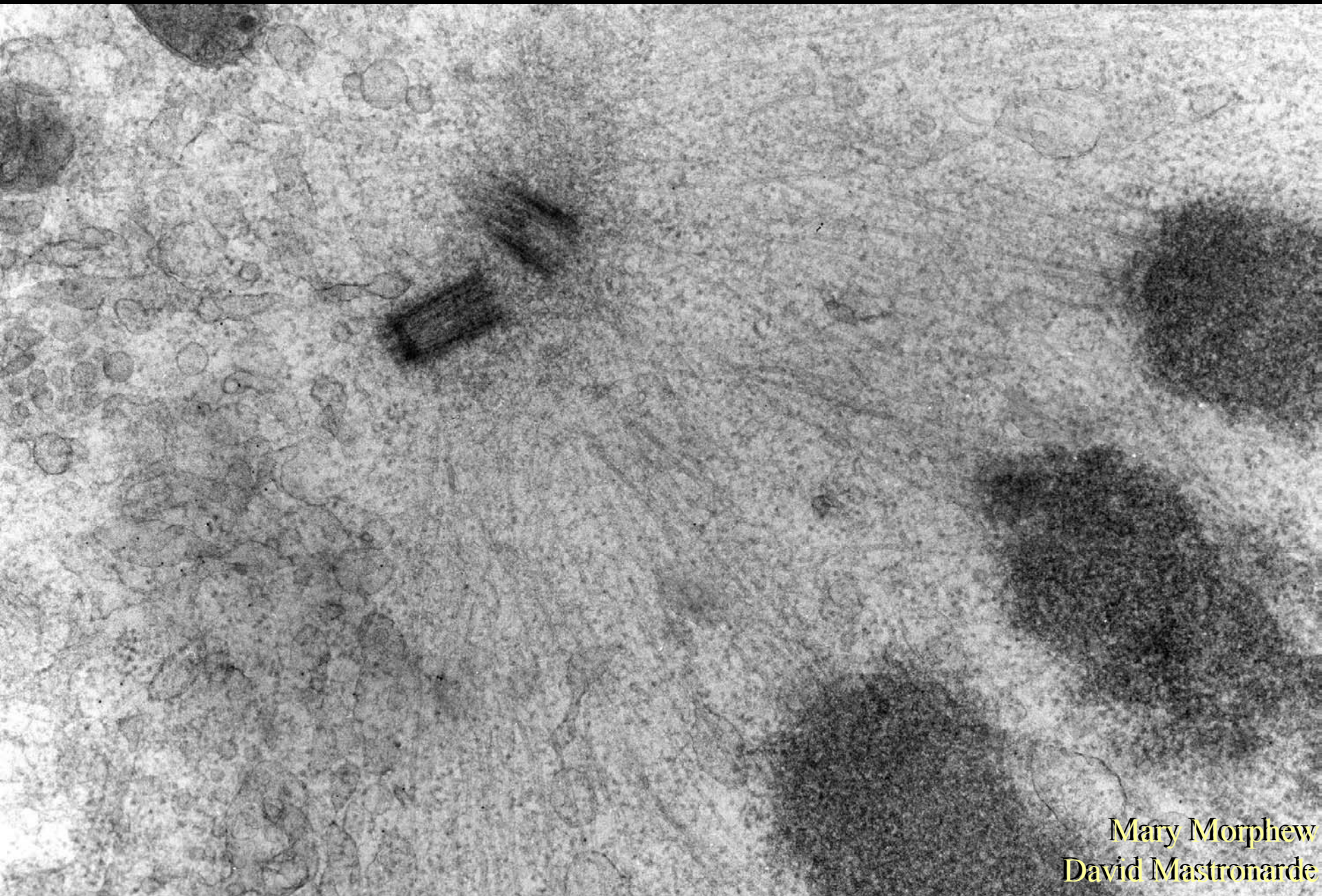
Tomography

- IVEM: Tecnai F30 operated at 300KV
- Automated tilt-series acquisition software, SerialEM written by David Mastronarde
- ±65° single axis tilt-series at 1-1.5° increments
- Projection alignment using colloidal gold as fiducials (or by cross-correlation methods) and

  Tomograms computed by weighted back-projection (using IMOD/Etomo software)

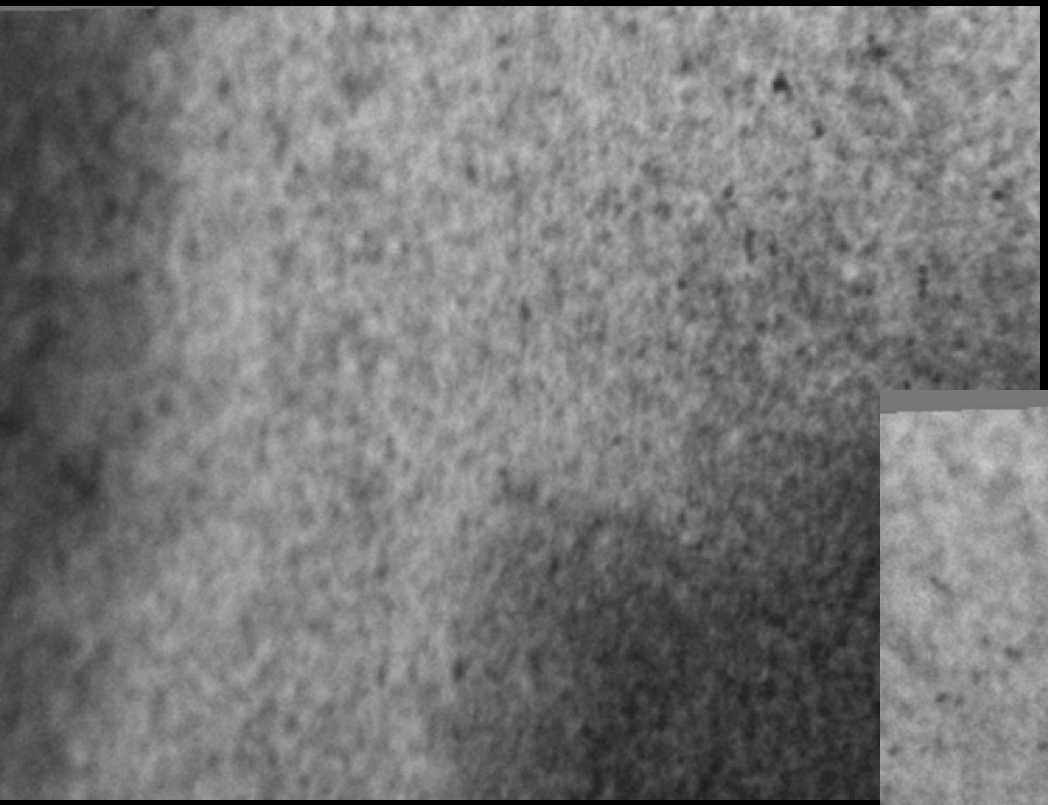


Mitotic spindle

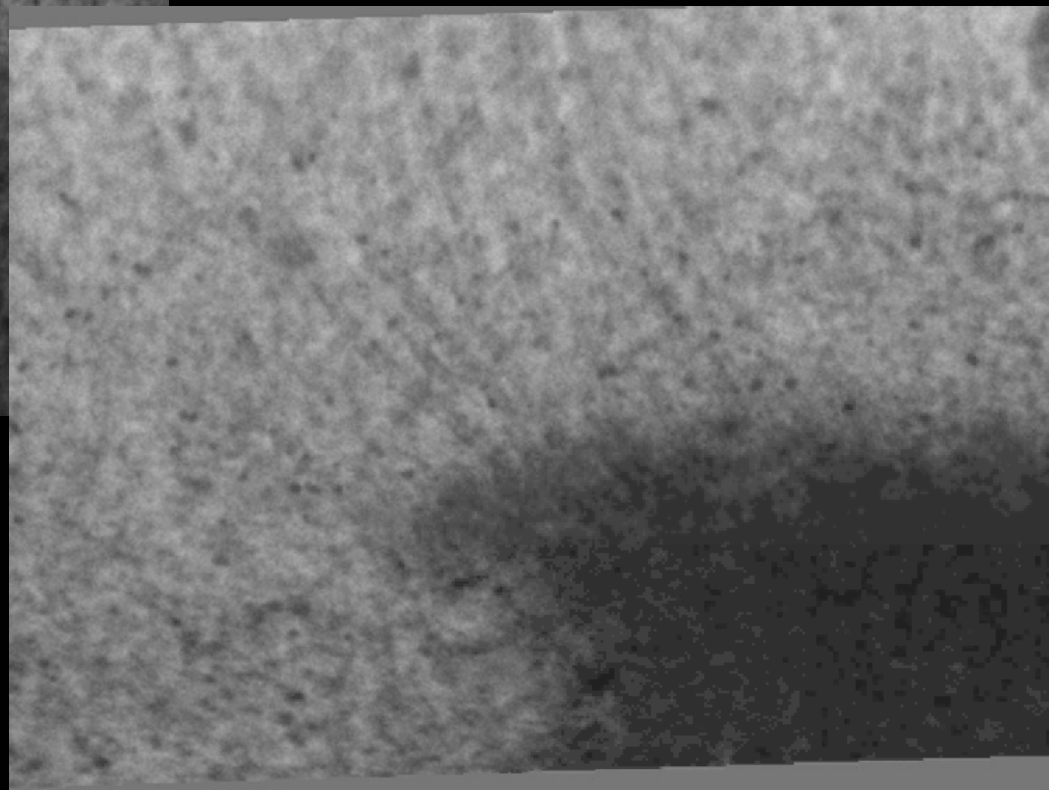


Dual-axis tomography of kinetochore in PtK cell

First axis: +/- 70° at 1.5° intervals

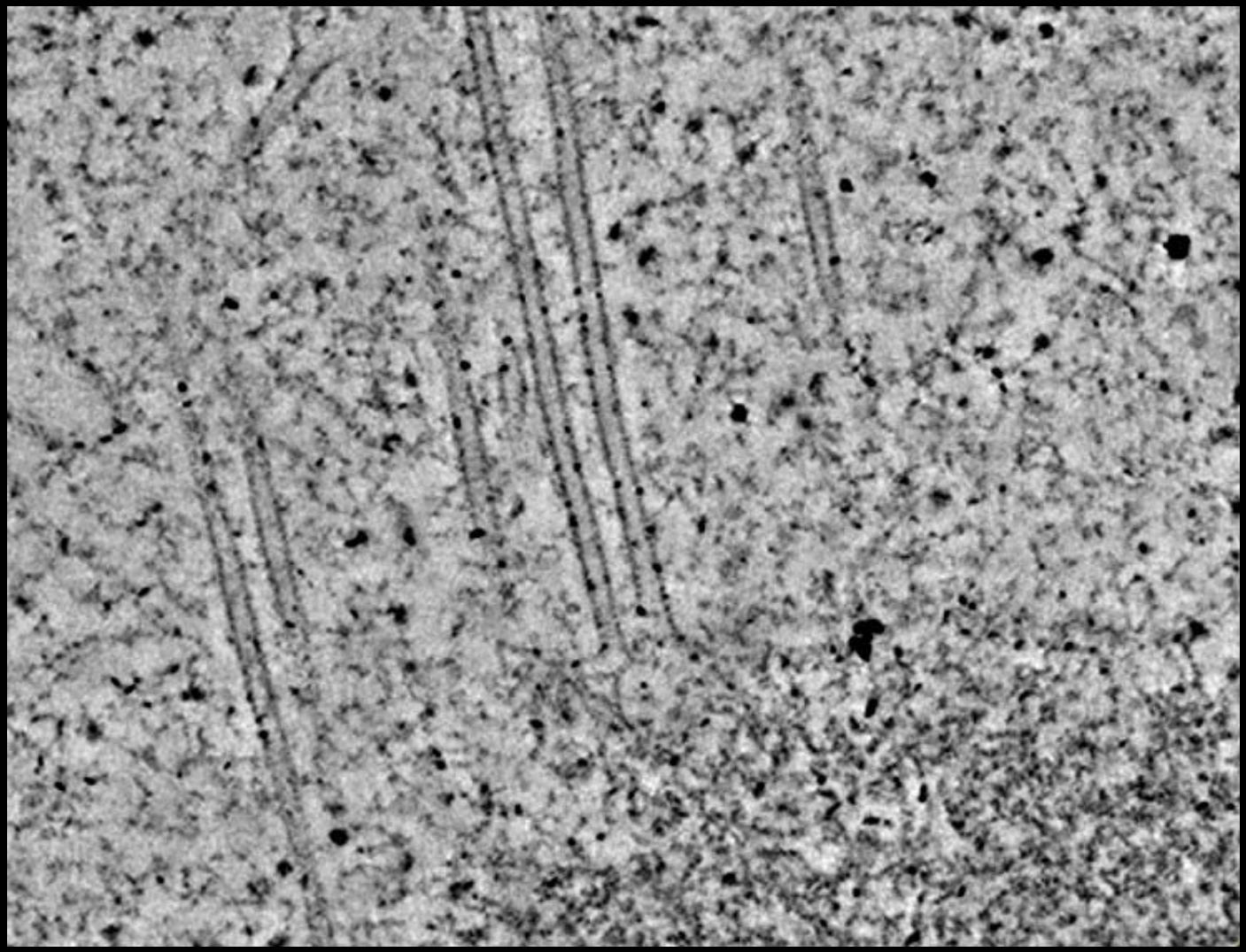


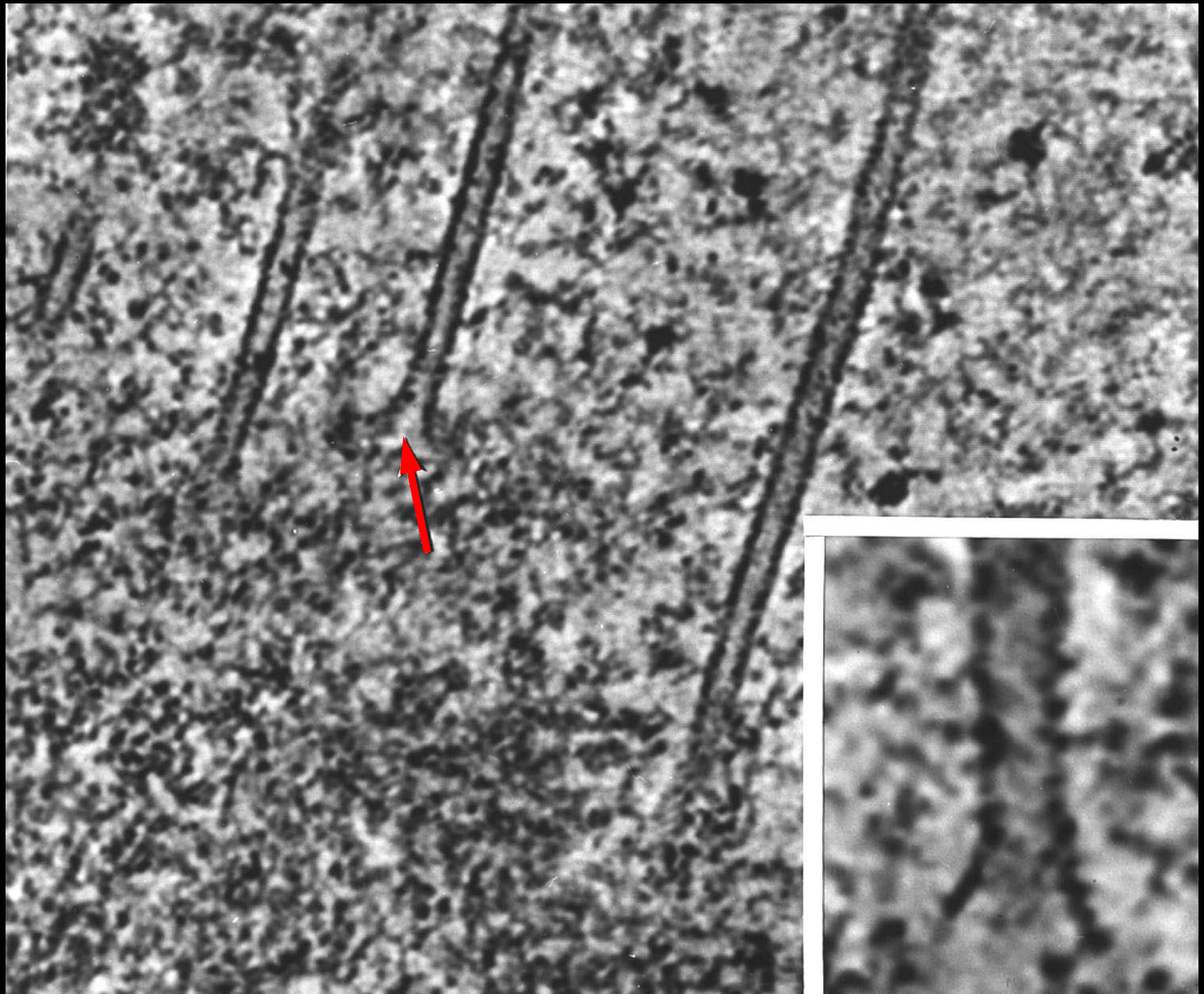
Second axis: +/- 70° at 1.5° intervals



Dual-axis tomography of kinetochore in PtK cell

Combined tomogram

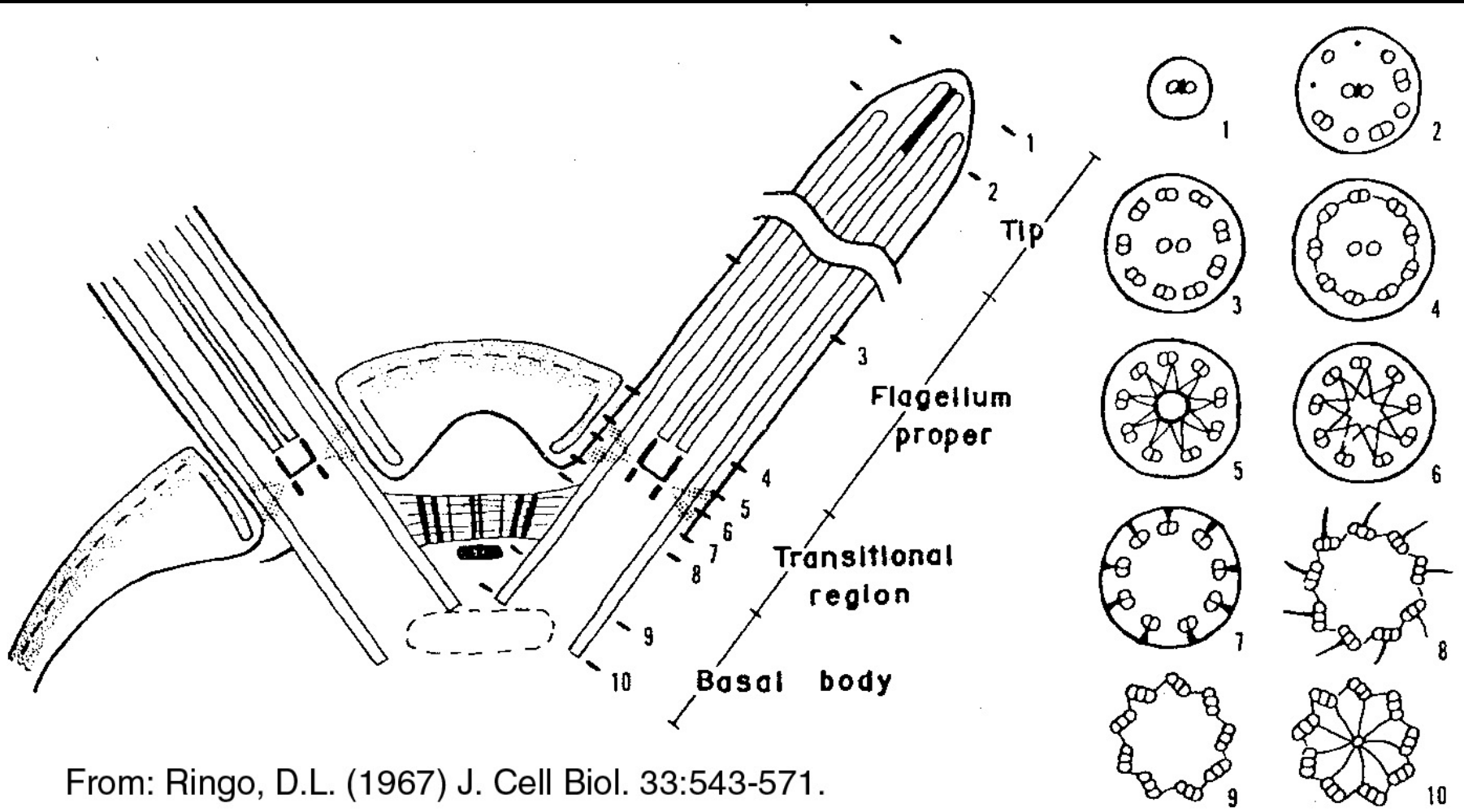




Studies of basal body/centriole assembly in *Chlamydomonas*

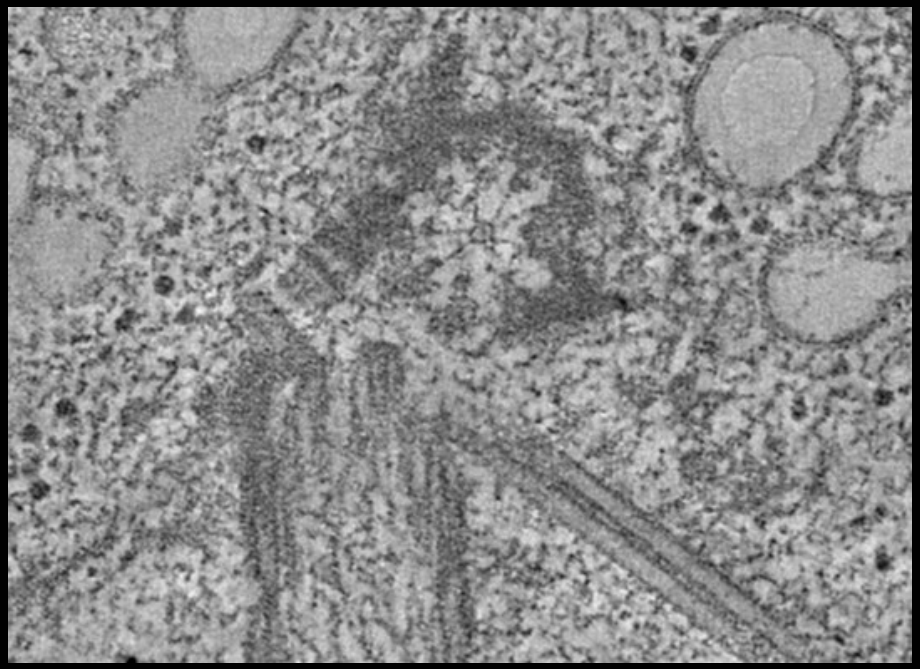
Eileen O'Toole
Susan Dutcher
Tom Giddings

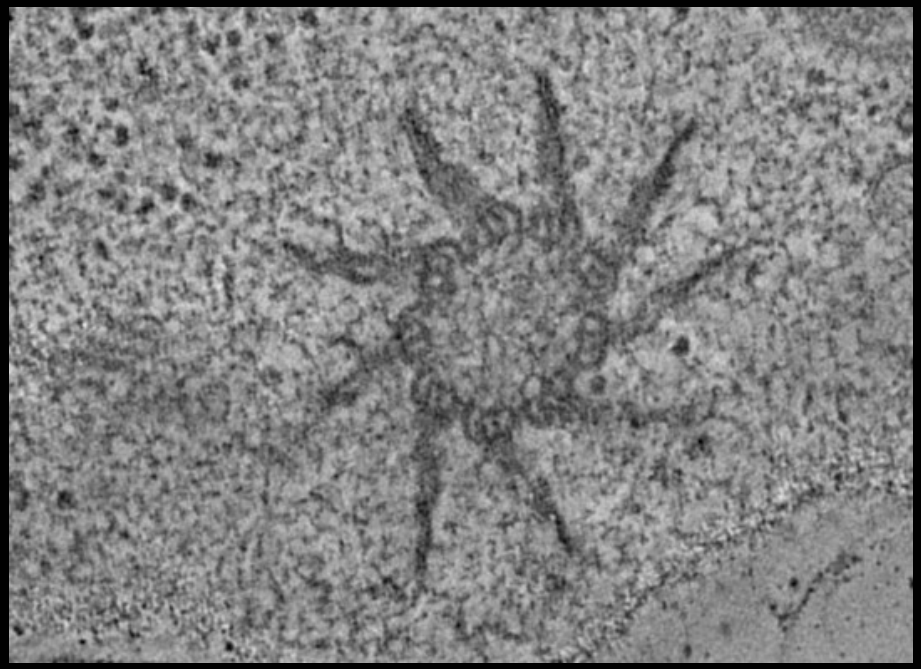
Serial thin sections reveal the organization of basal bodies and flagella



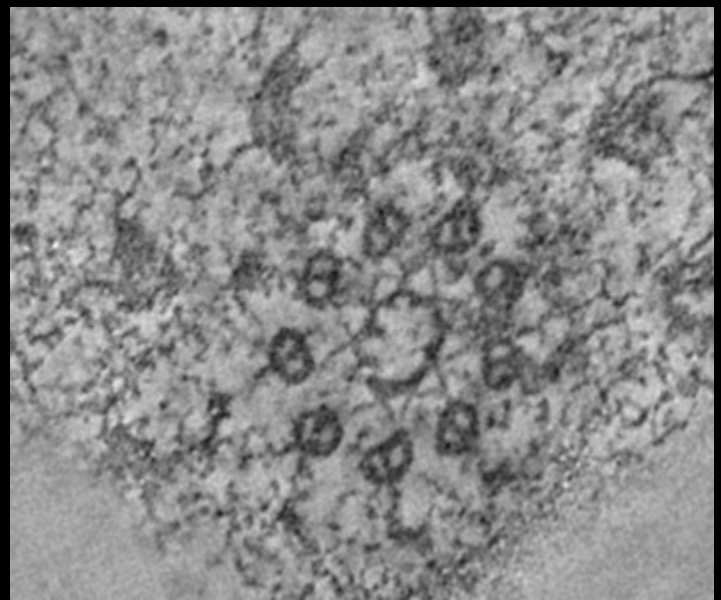
From: Ringo, D.L. (1967) J. Cell Biol. 33:543-571.

Serial section tomography (~700nm)



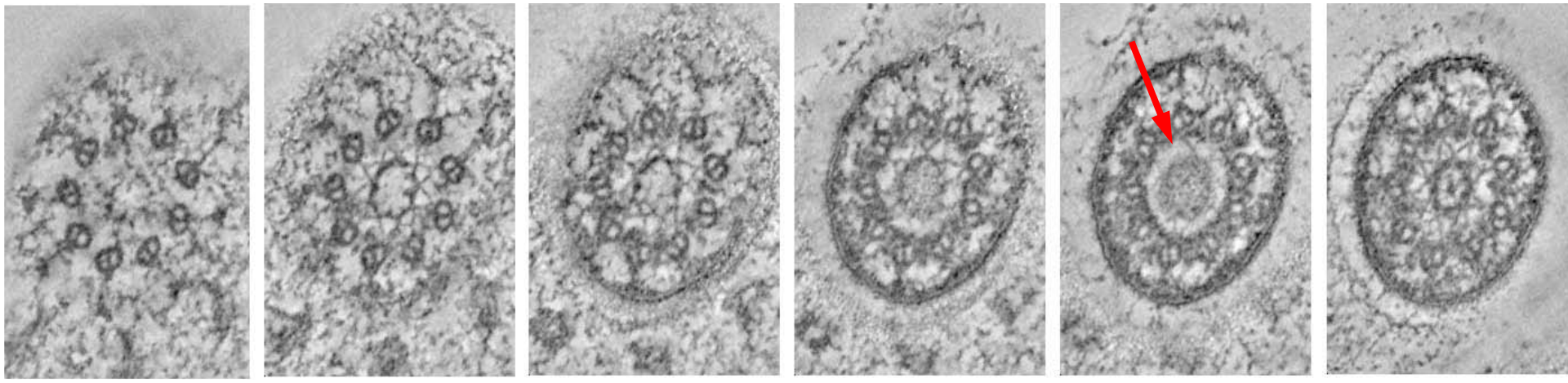


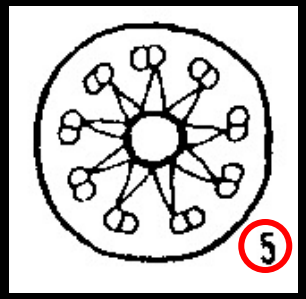
proximal

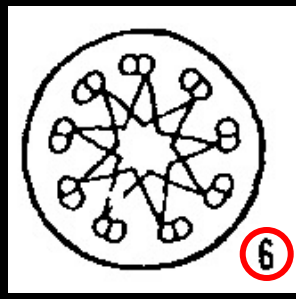


distal

Electron tomography reveals new structures in the wildtype transition zone





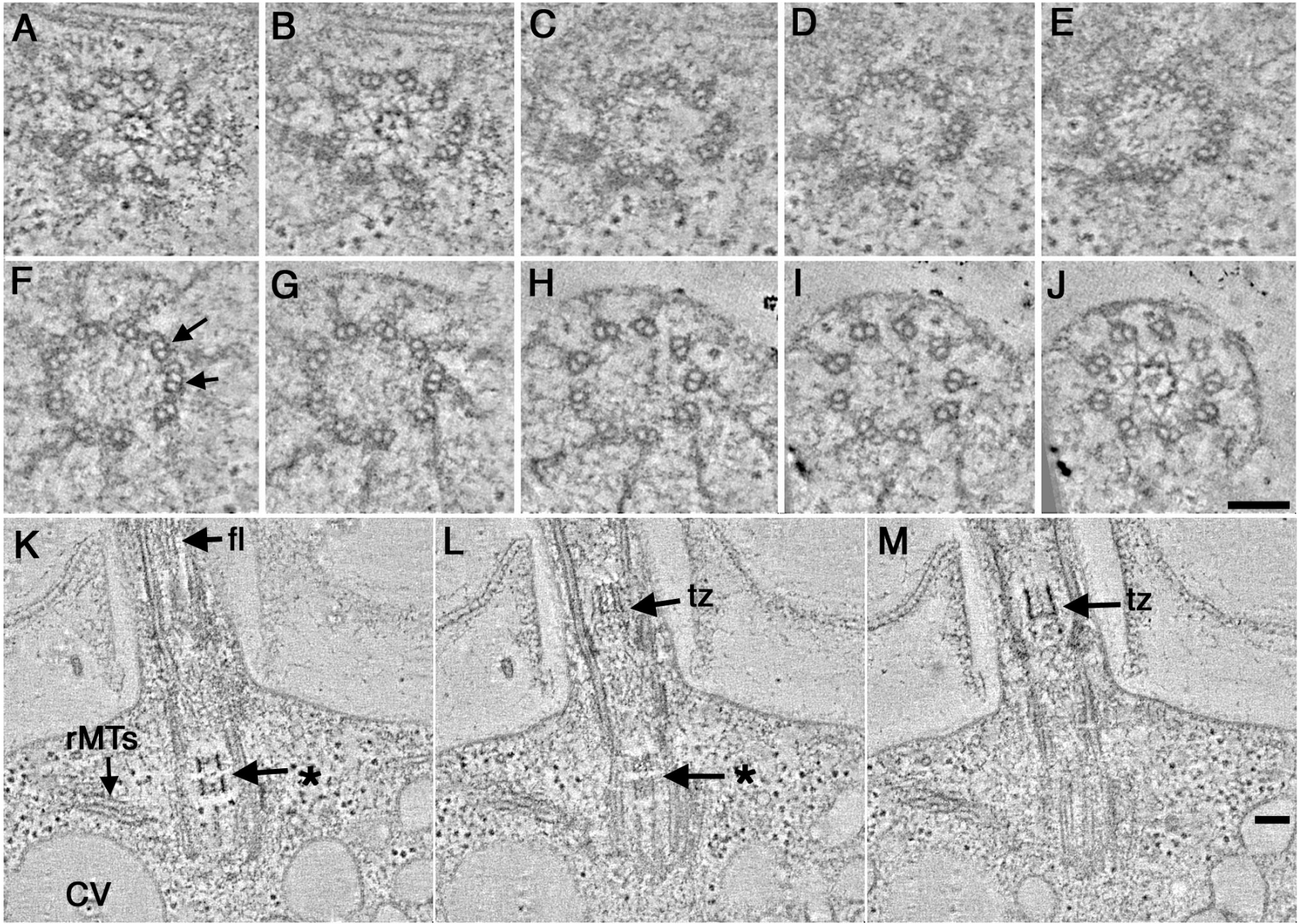


From: Ringo, D.L. (1967) J.Cell Biol. 33:543-571

The *UNI3* gene is required for basal body assembly

- *UNI3* gene encodes d-tubulin (tubulin superfamily)
  (Dutcher and Trabuco, Mol. Biol. Cell 9:1293-1308, 1998).
- Mutant *uni3-1* cells assemble 0 , 1 or 2 flagella.
- Electron microscopy shows doublet rather than triplet microtubules in the basal body

uni3-1: ectopic assembly of transition zone structures in the bb

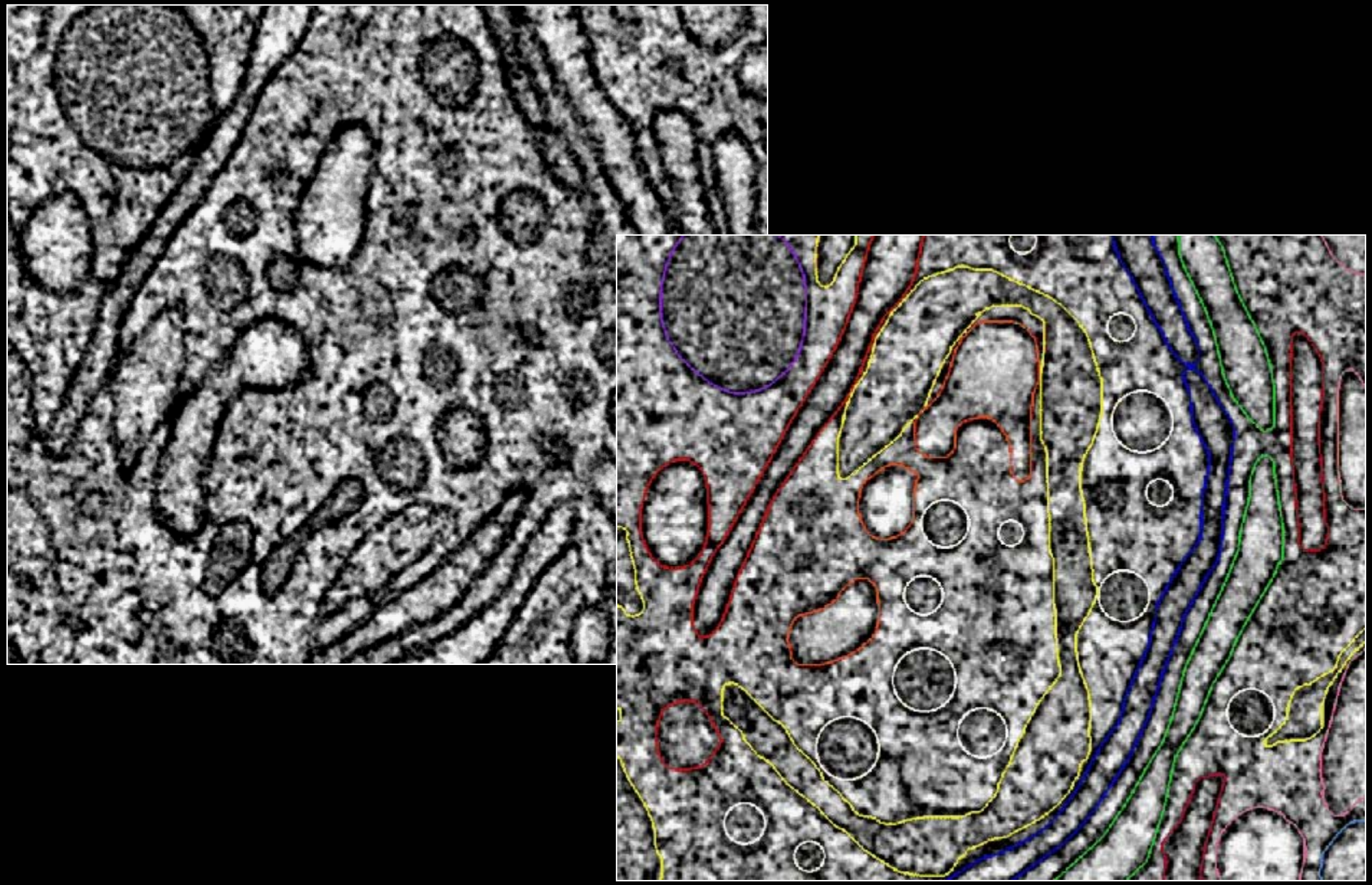


Studies of insulin granule formation in pancreatic Beta cells

Serial Section Tomogram of Golgi in HIT Cell

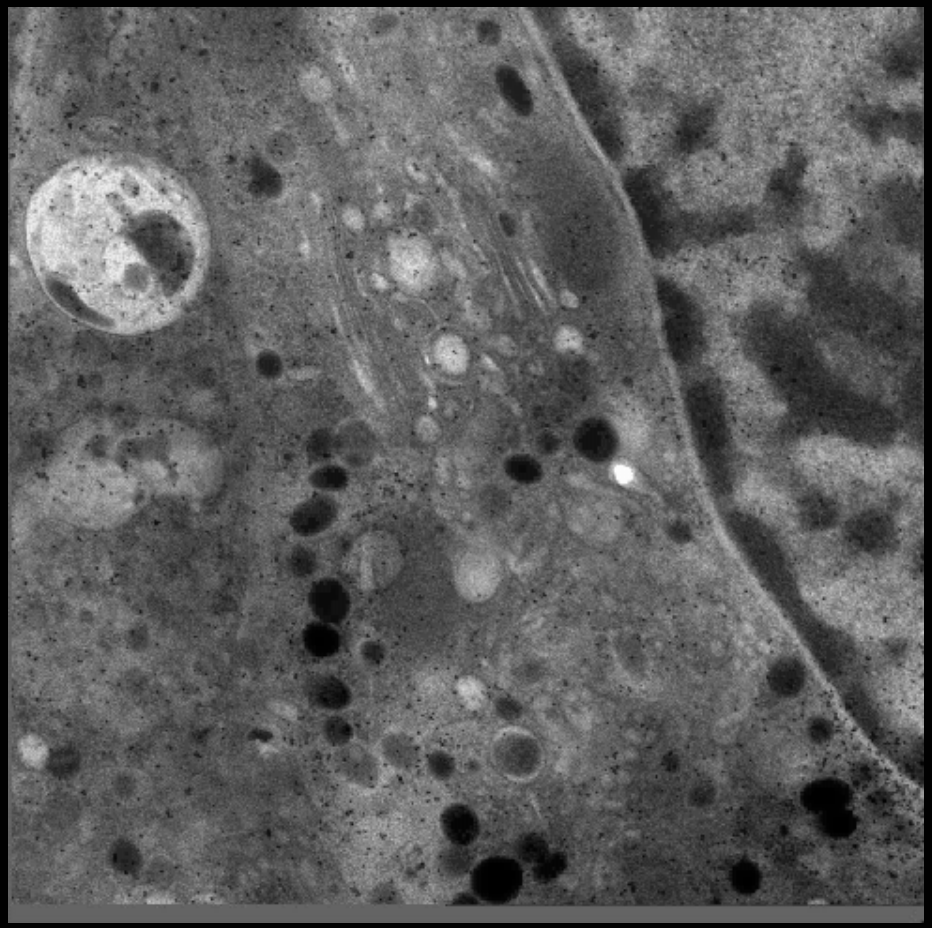
Brad Marsh
Kathryn Howell

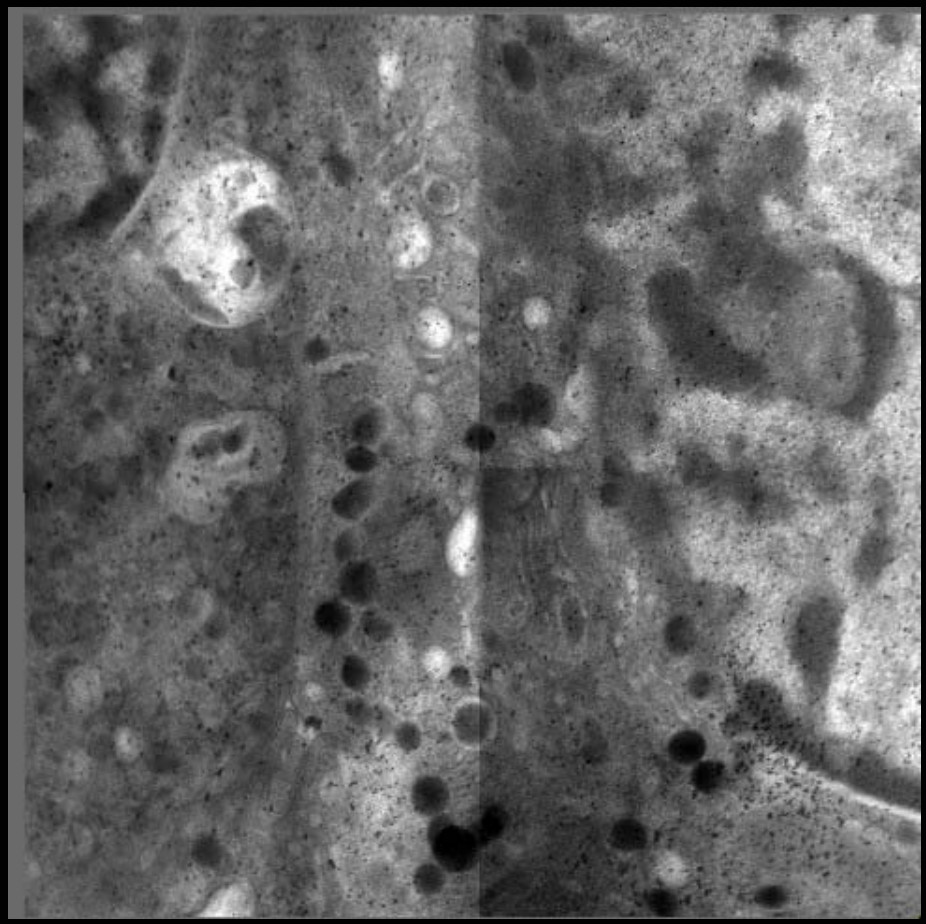
Modeling in the tomogram (IMOD software)



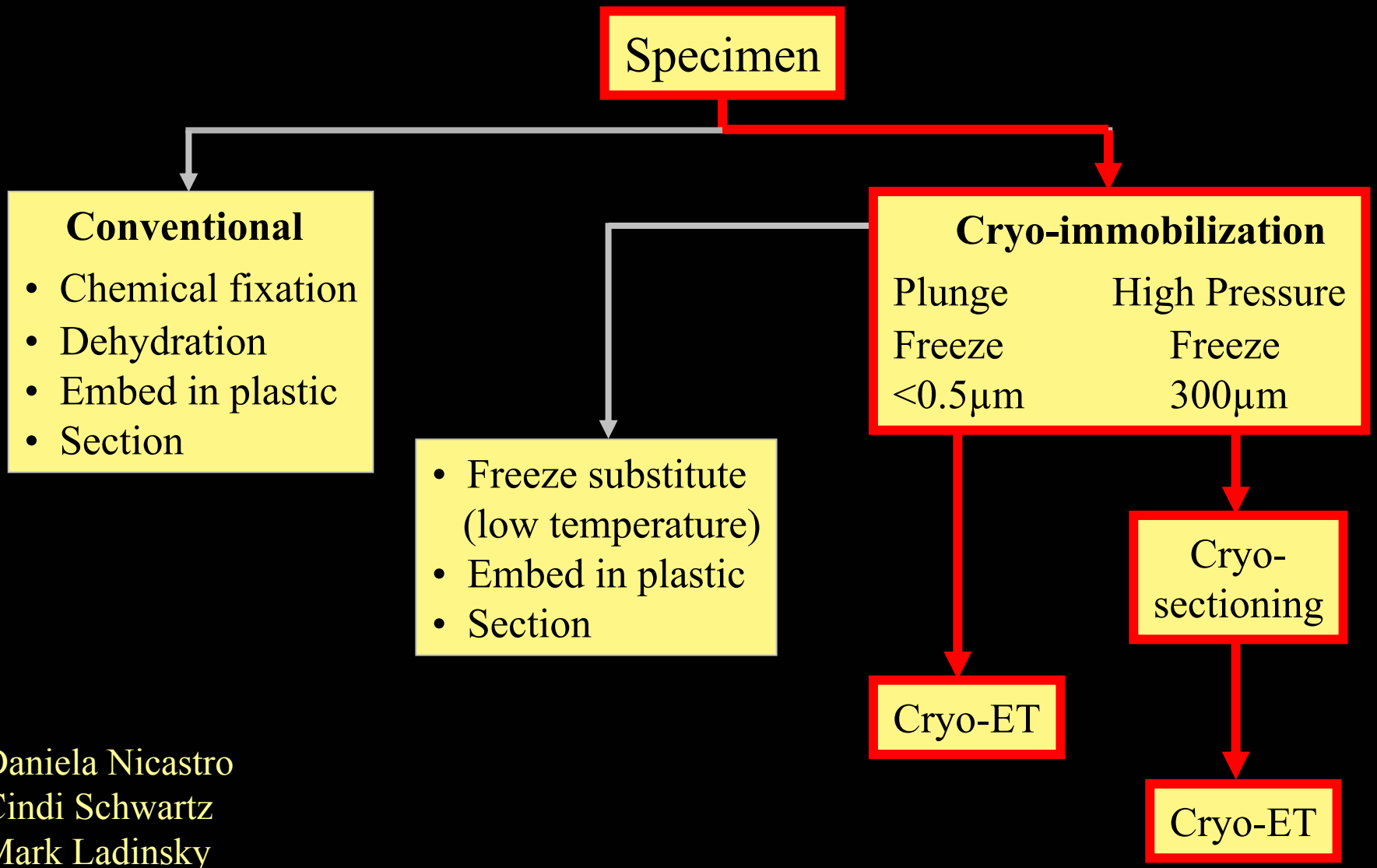
Complete model:

2 by 2 Montage acquired on 300 kV Tecnai





Cryo-Electron Tomography

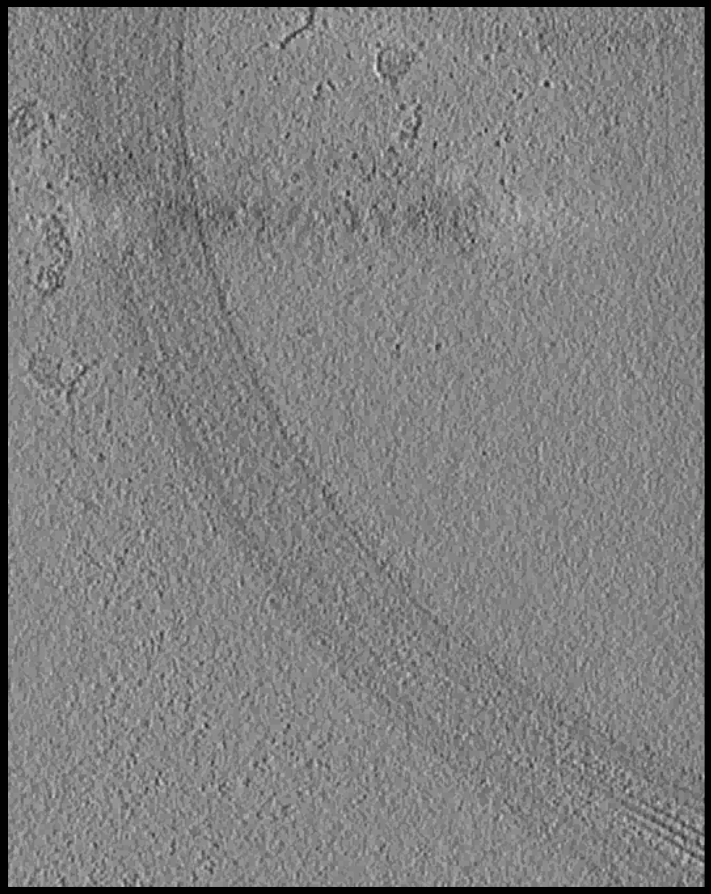


Daniela Nicastro
Cindi Schwartz
Mark Ladinsky
Mary Morphew
David Mastronarde

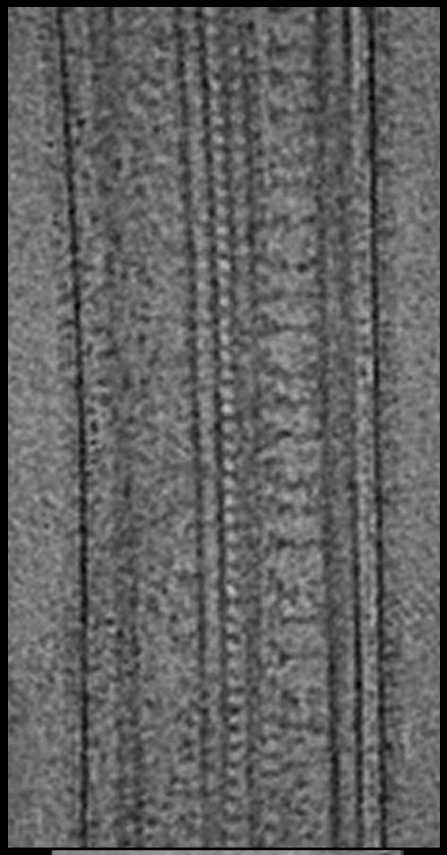
Original EM-image and 3-D reconstruction of sea urchin sperm flagellum

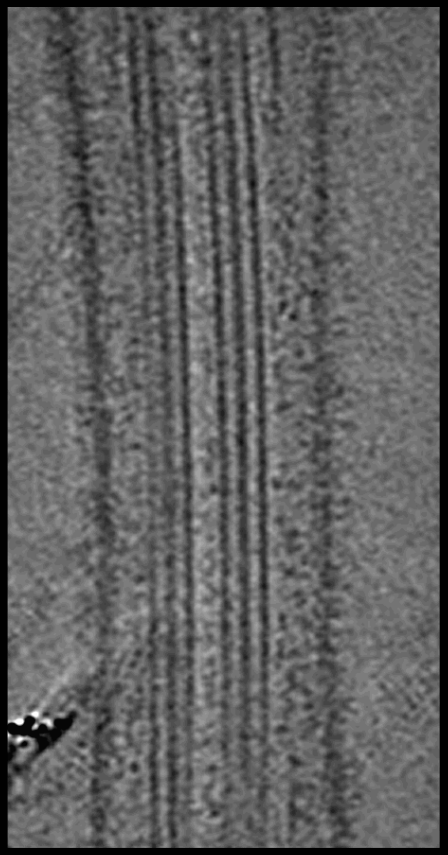
0°-Projections of single-axis tilt series +/- 63°, 1.5° increment

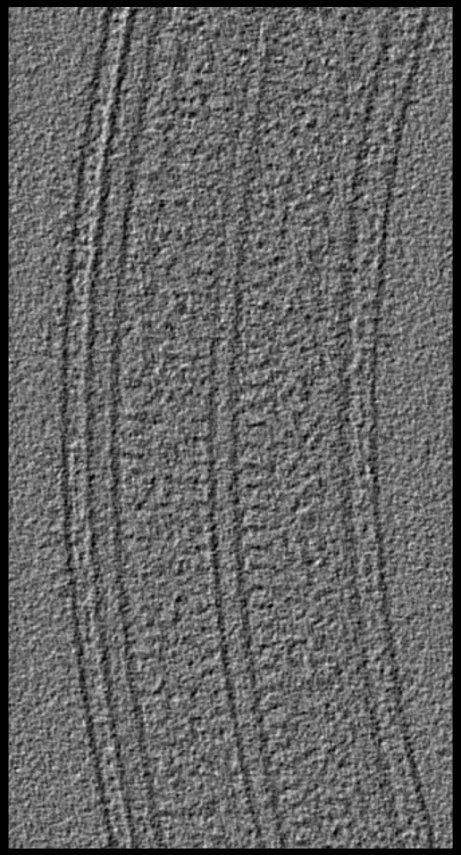
3-D reconstruction movie along z-axis

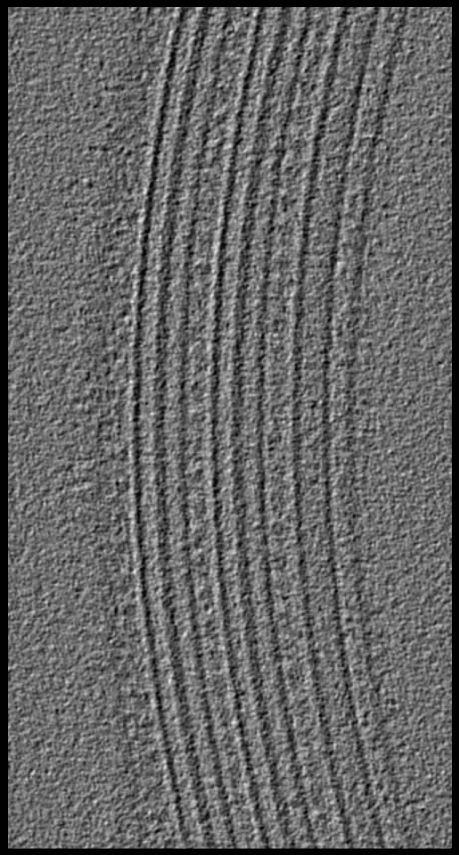


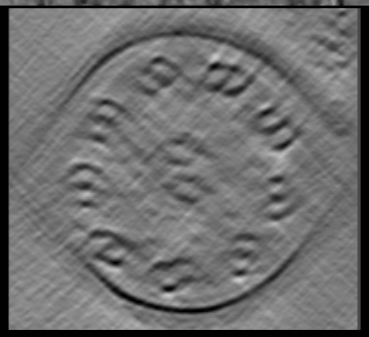
3-D reconstructions - selected detail



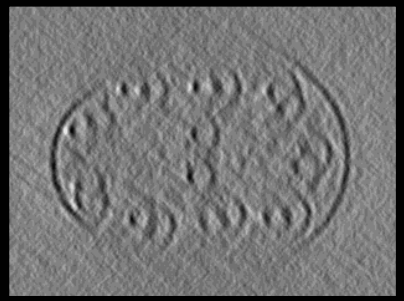






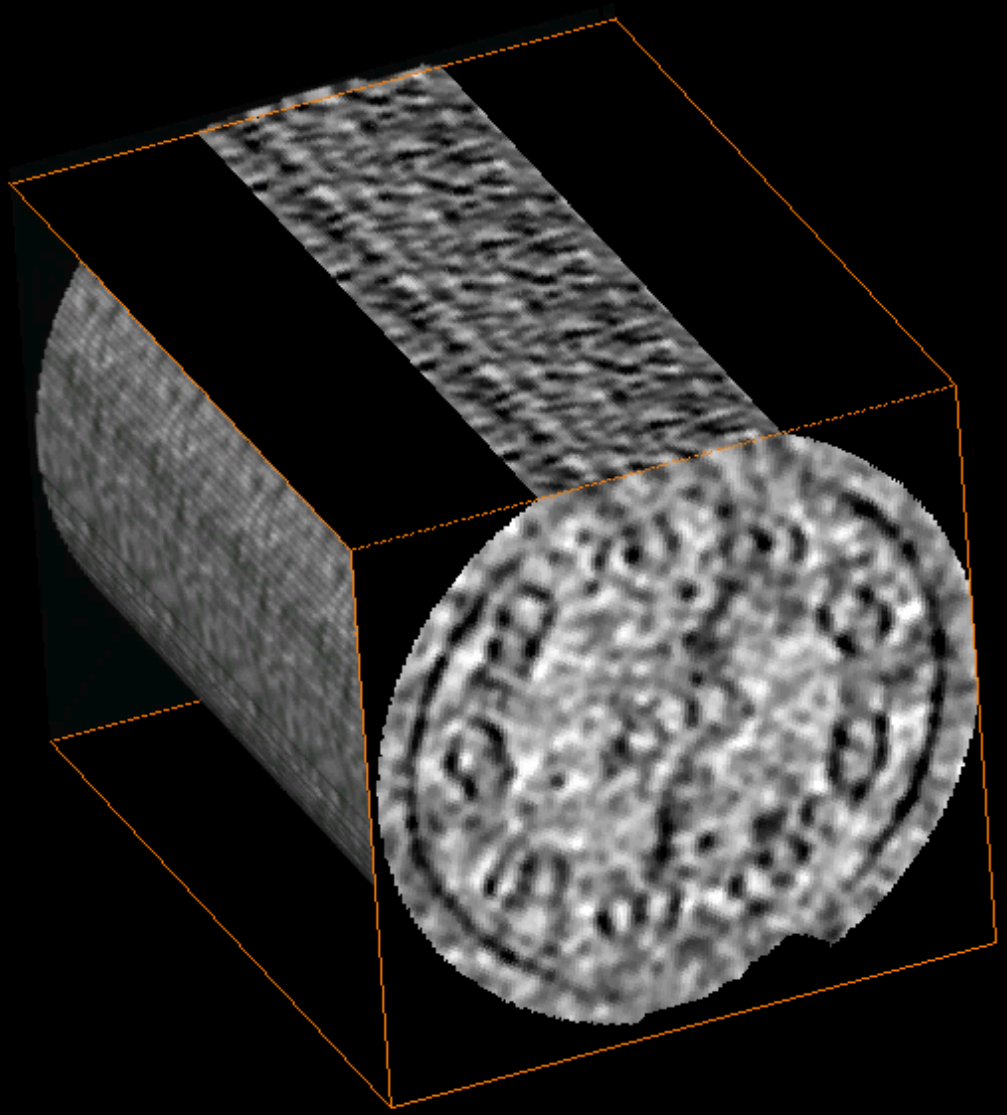


quiescent state

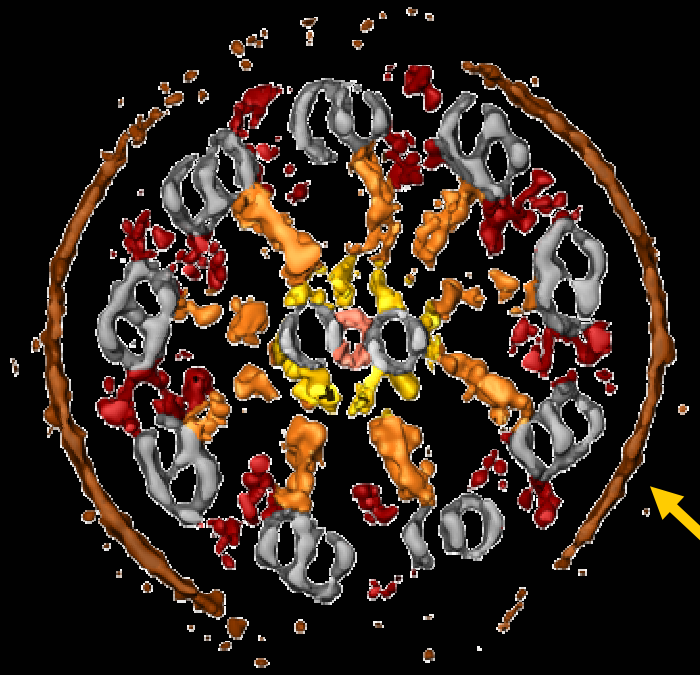


active state

Visualization of a sea urchin sperm flagellum

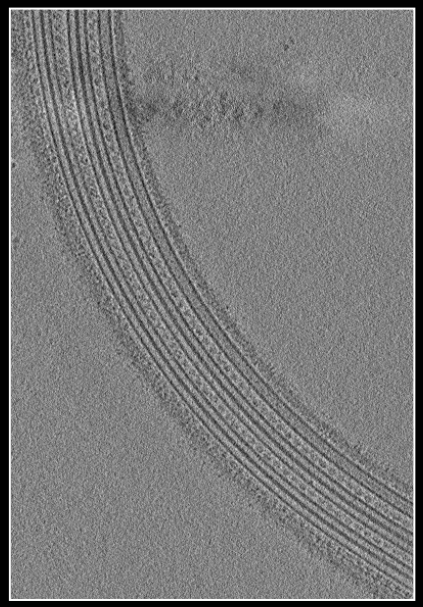


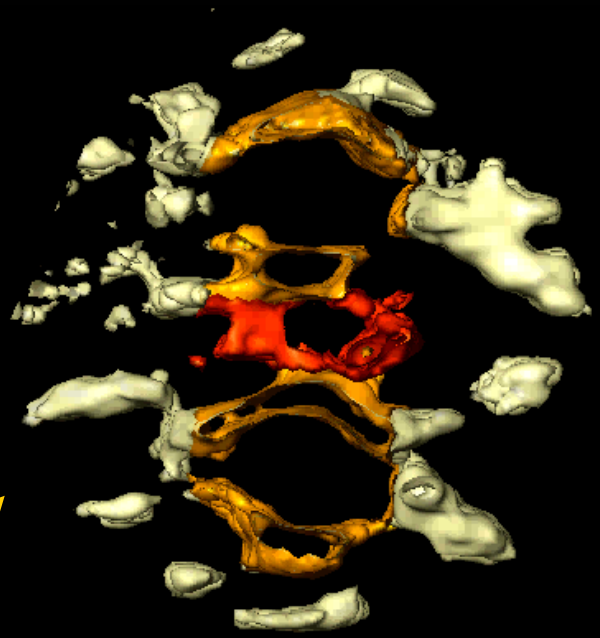
Analysis



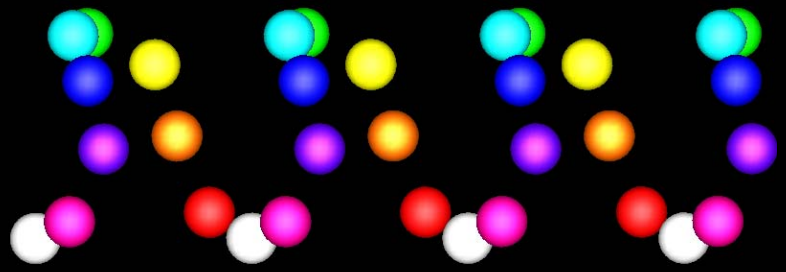
3-D Visualization of the flagellum

reconstruction

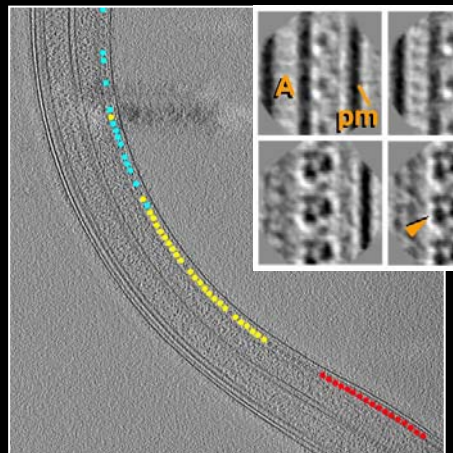


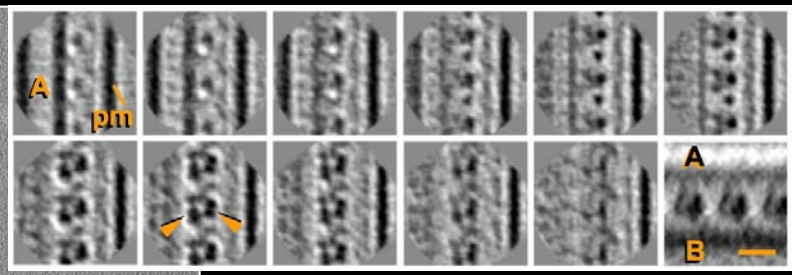


Central pair complex



3-D arrangement radial spokes

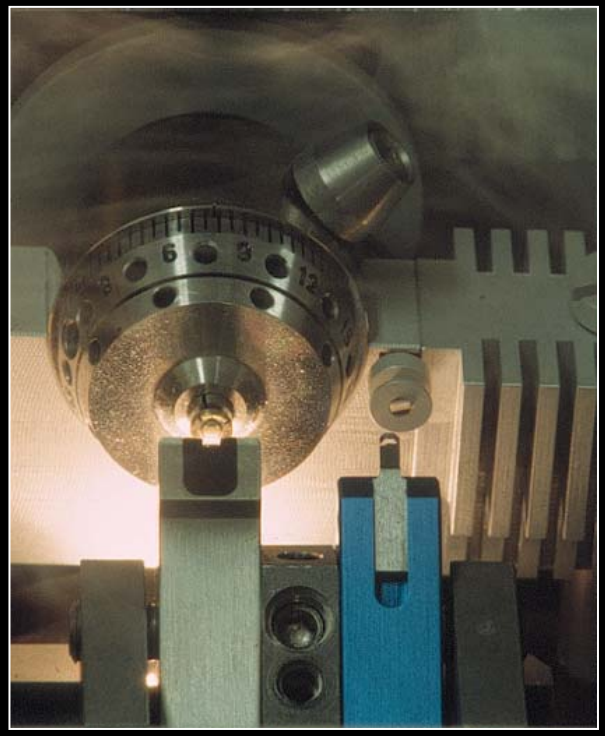


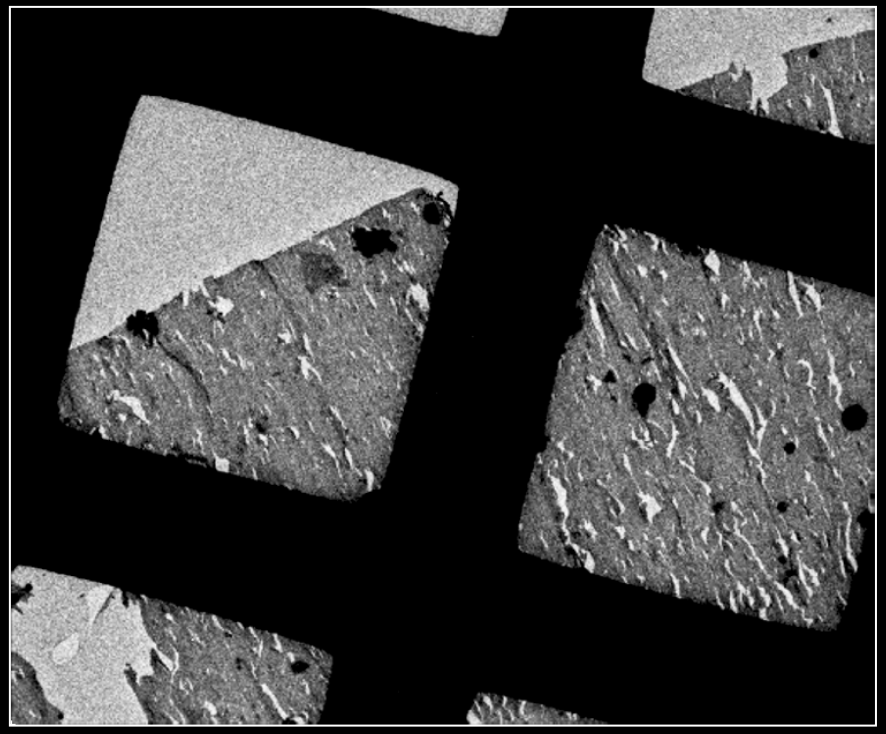


Outer dynein arms

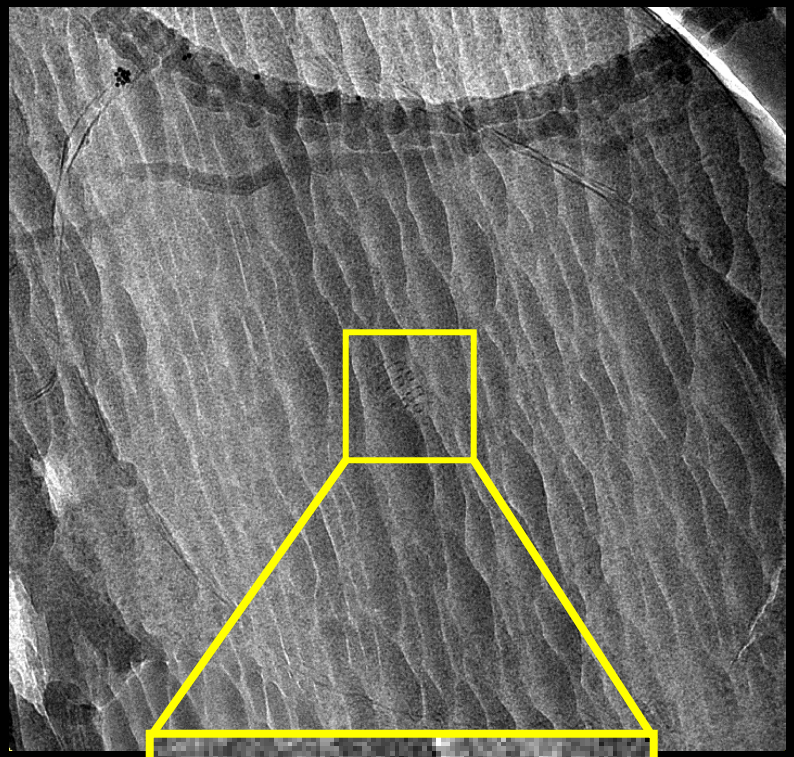
Cryo-Microtomy

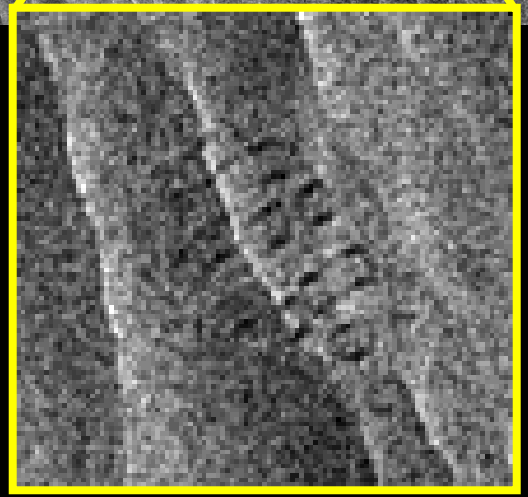
- High Pressure Freezing hats were treated with soy lecithin (“dome”)
- Leica Ultracut UCT with EMFCS cryobox, and static ionizer
- 35° cryo-diamond knife, chamber temp -160°C
- 100-200 nm thick sections with very small and rectangular block face



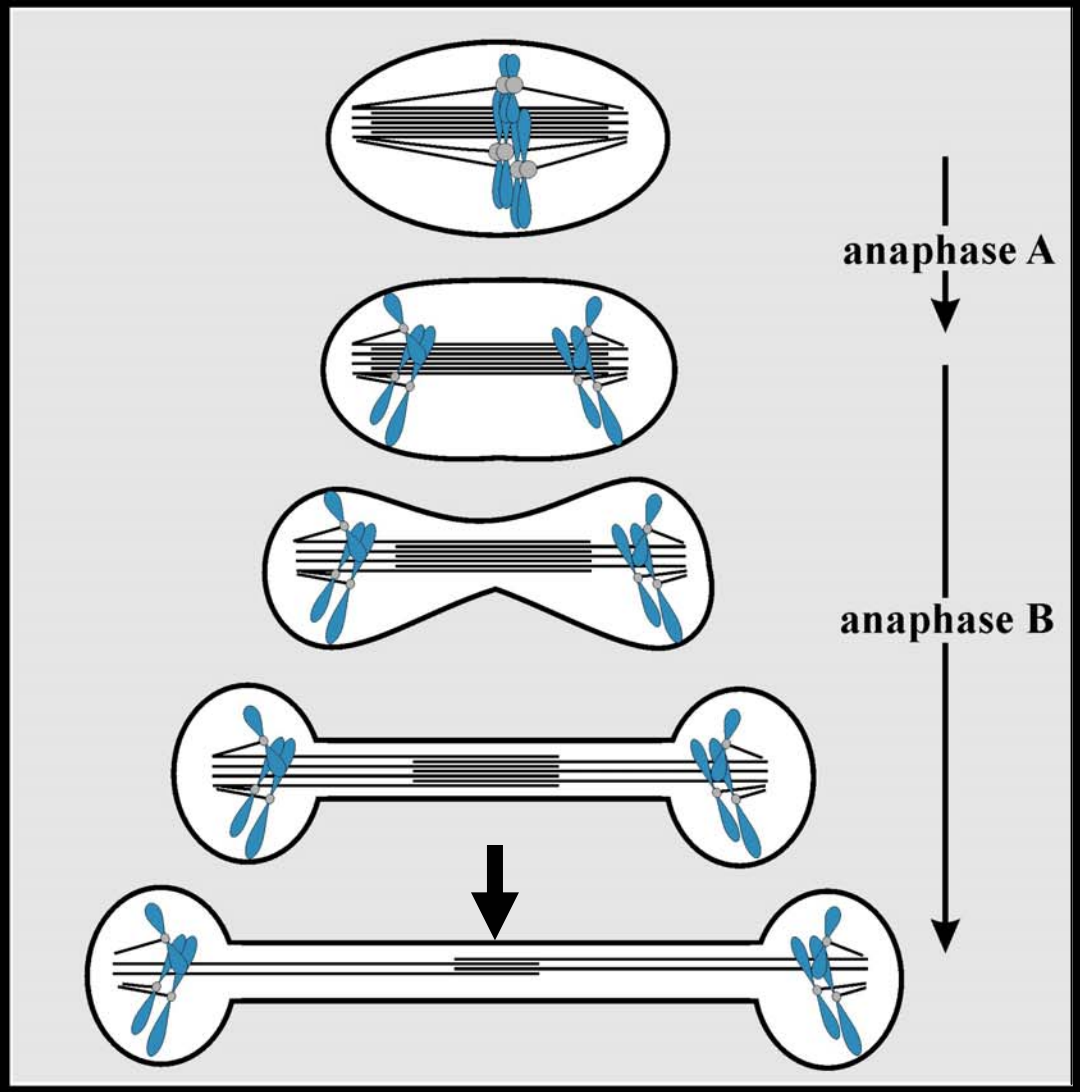


Cryo-sections (fission yeast)



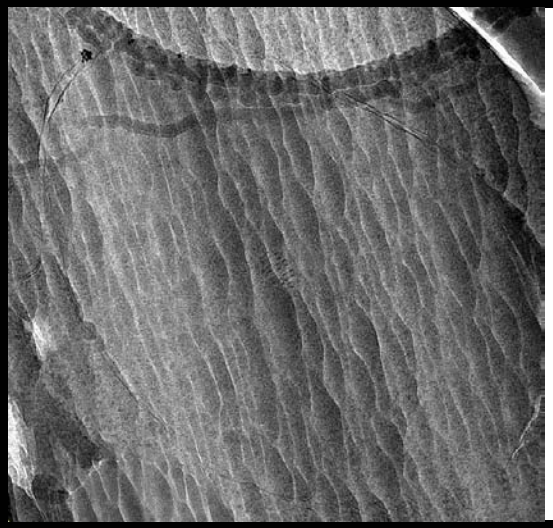


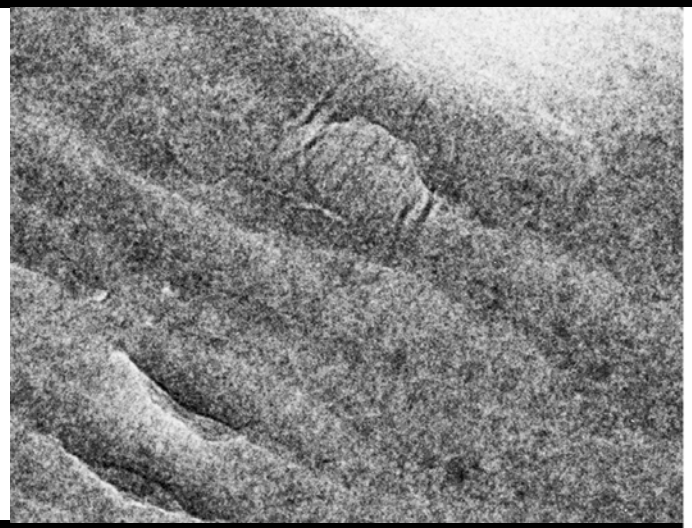
Mitotic Spindle Dynamics in *S. pombe*

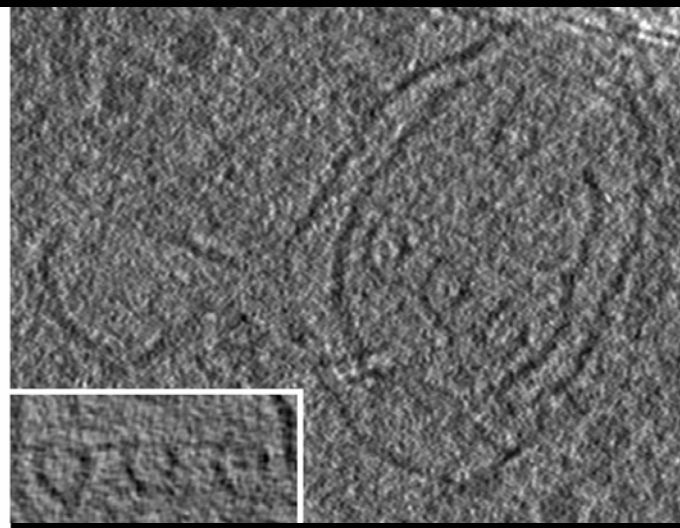


- Chromosomes line up at metaphase plate
- Chromosomes pulled to the spindle poles
- Spindle elongates
- Nuclear envelope never breaks down
- The next data set will show spindle midzone MTs at late anaphase B

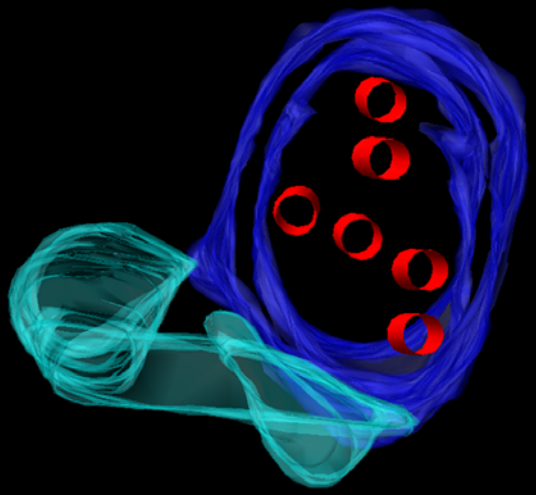
Electron tomography of high-pressure frozen/ cryo-sectioned eukaryotic cells

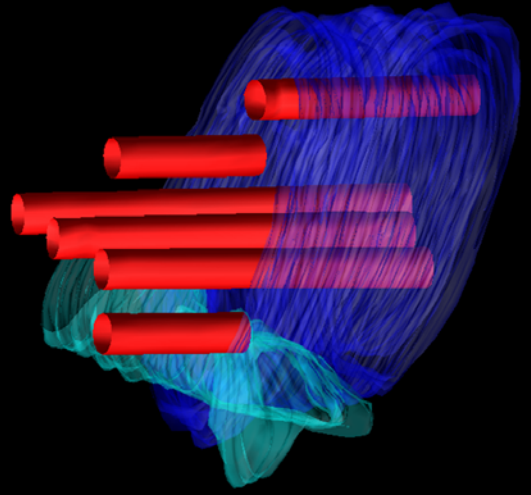




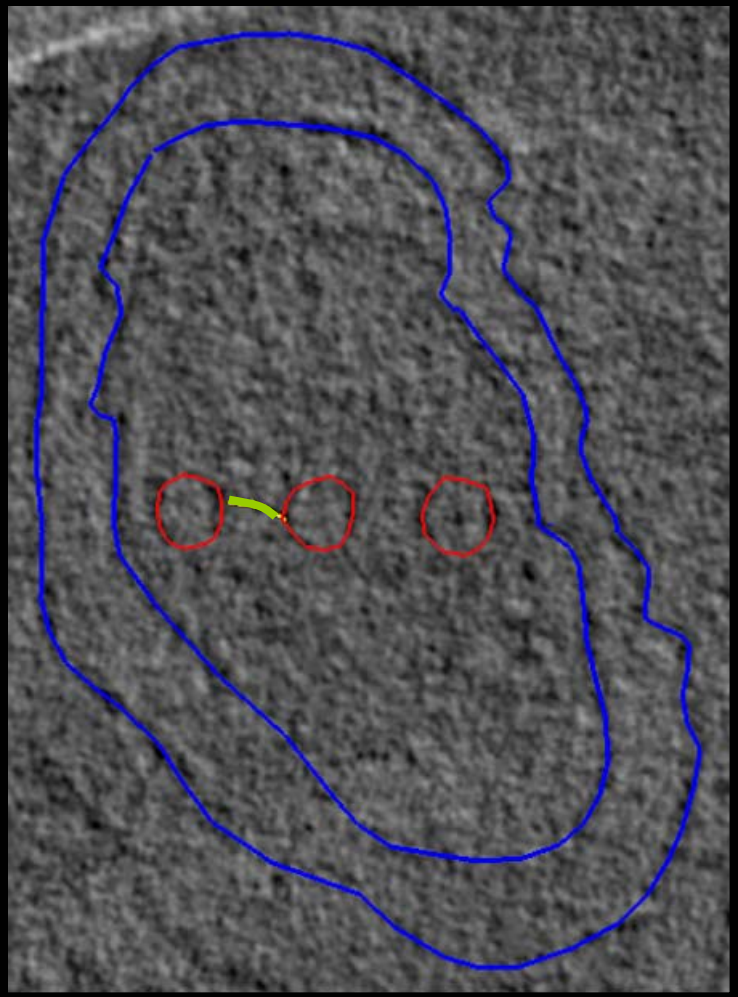


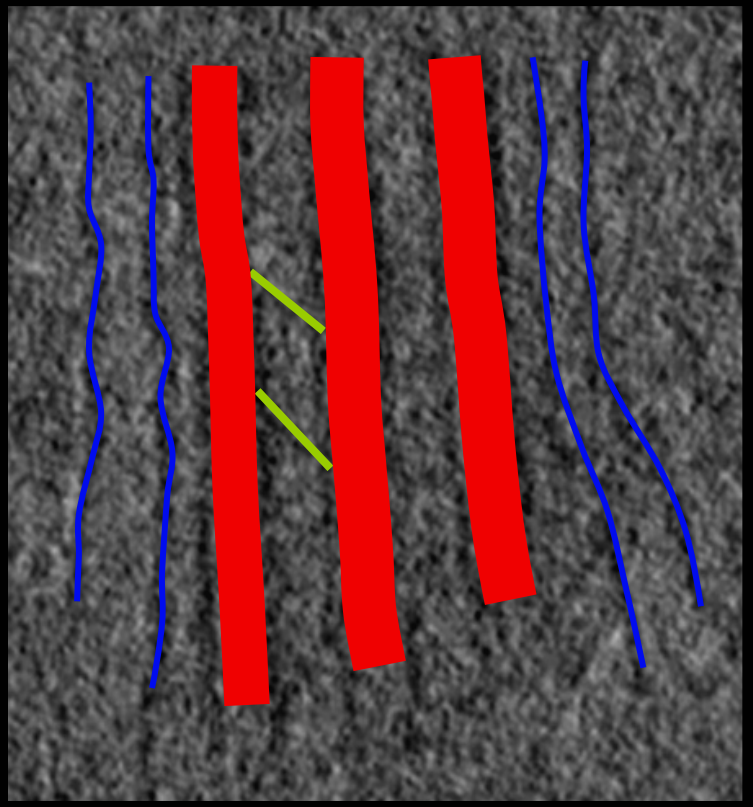
Spindle mid-zone
in fission yeast
(anaphase B)





Details





Nuclear Envelope

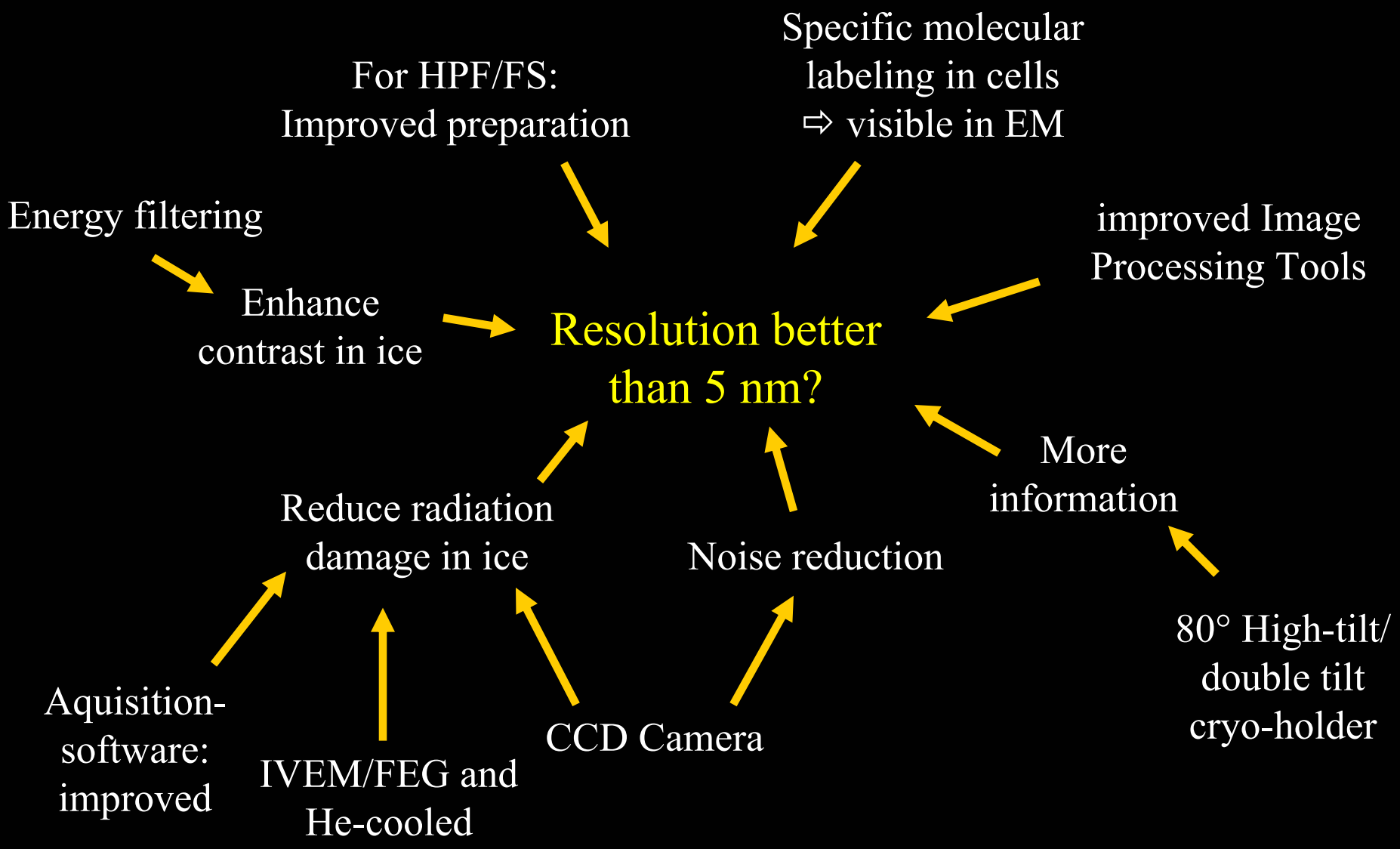
Spindle MTs

Bridges between MTs

	Cryo	Plastic *
MT diameter	24.8nm +/- 1.5	23.8nm +/- 1.5
Center-to-center distance	40.8nm +/- 1.2	48.2nm +/- 2.7

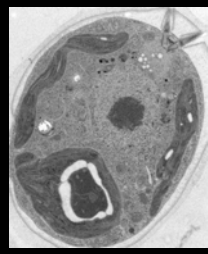
* Ding R., et al. (1993) *J. Cell Bio* **120:**141-152

Future directions in electron tomography of cells



Acknowledgements

Kinetochore in PtK cells: Mary Morphew, David Mastronarde



Basal body/centriole assembly in *Chlamydomonas*:
Eileen O'Toole, in collaboration with Susan Dutcher (Washington University) and Tom Giddings (University of Colorado)

Studies of Insulin Granule Formation in Pancreatic Beta Cells
Brad Marsh, in collaboration with Kathryn Howell (University of Colorado, Health Sciences Center)

Cryo-Electron Tomography: Daniela Nicastro, Cindi Schwartz, Mark Ladinsky, Mary Morphew, David Mastronarde

Boulder Lab for 3D EM of Cells: J. Richard McIntosh (PI)
http://bio3d.colorado.edu

National Center for Research Resources

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