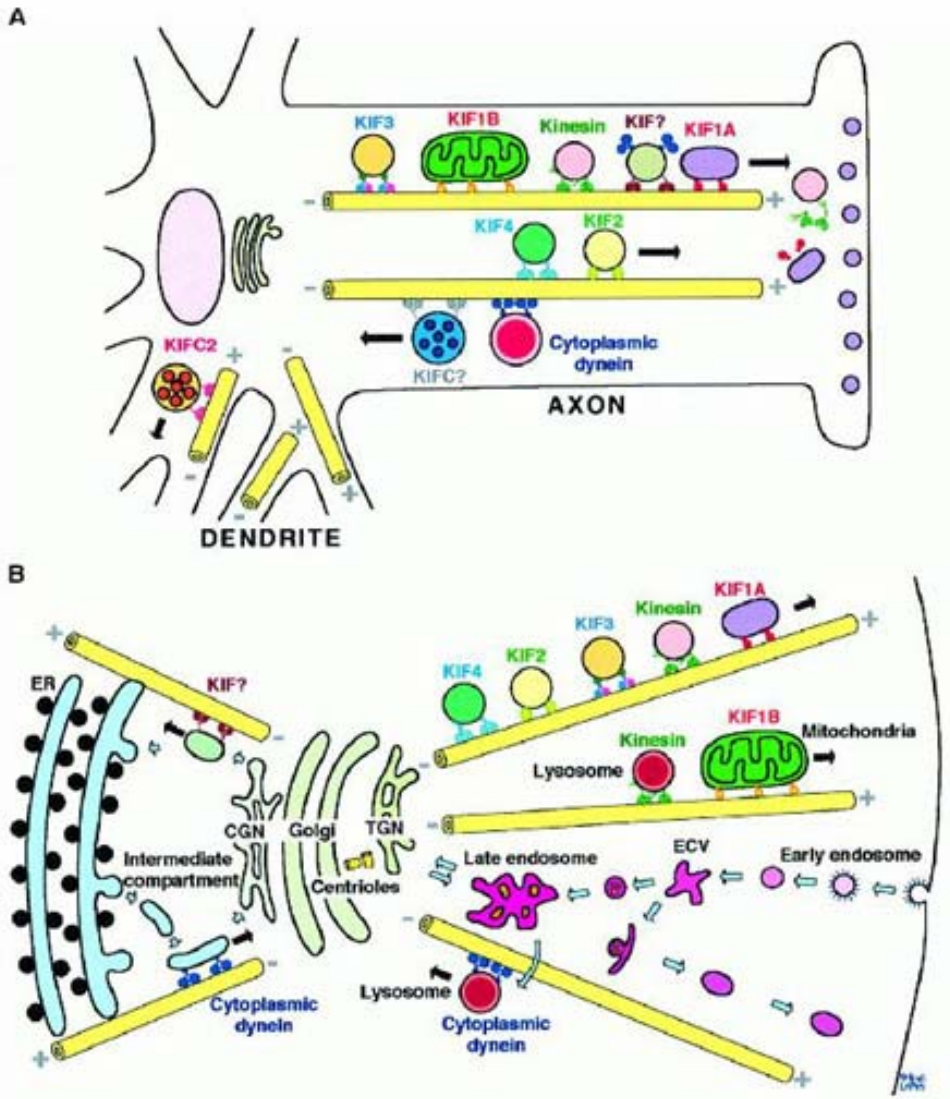
Study on the structural aspect of the dynein-microtubule interaction

Naoko Mizuno

University of Texas Southwestern Medical Center

Dynein

KLFs and ctoplasmic dynein in nerve axons

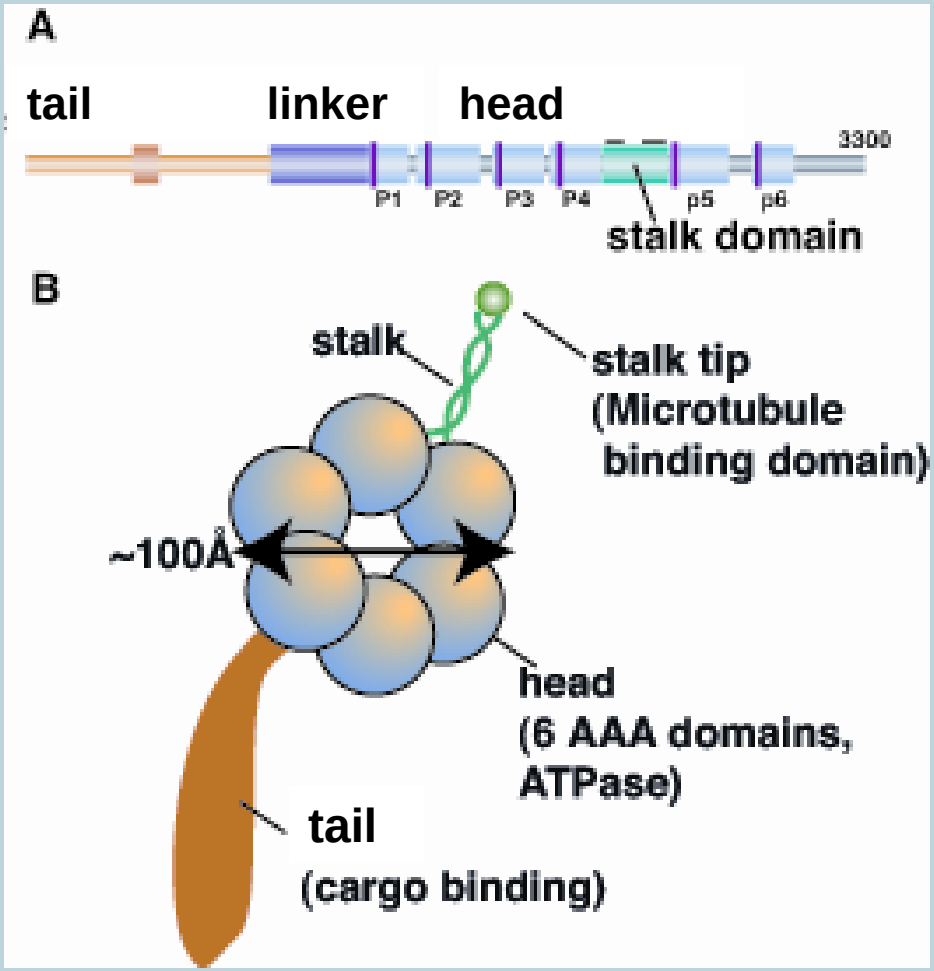


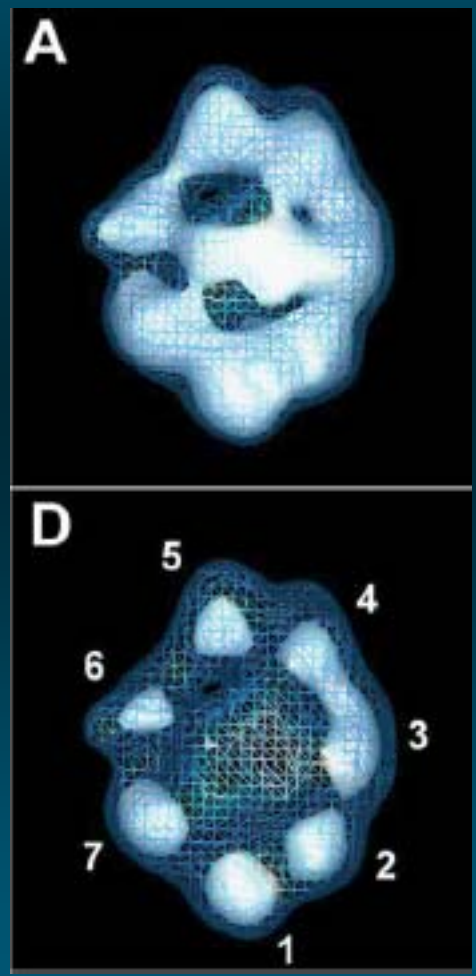
Hirokawa, N. *Science* 1998 279:519-526.

Contents

- Cytoplasmic dynein introduction
- CryoEM
- Imaging technique

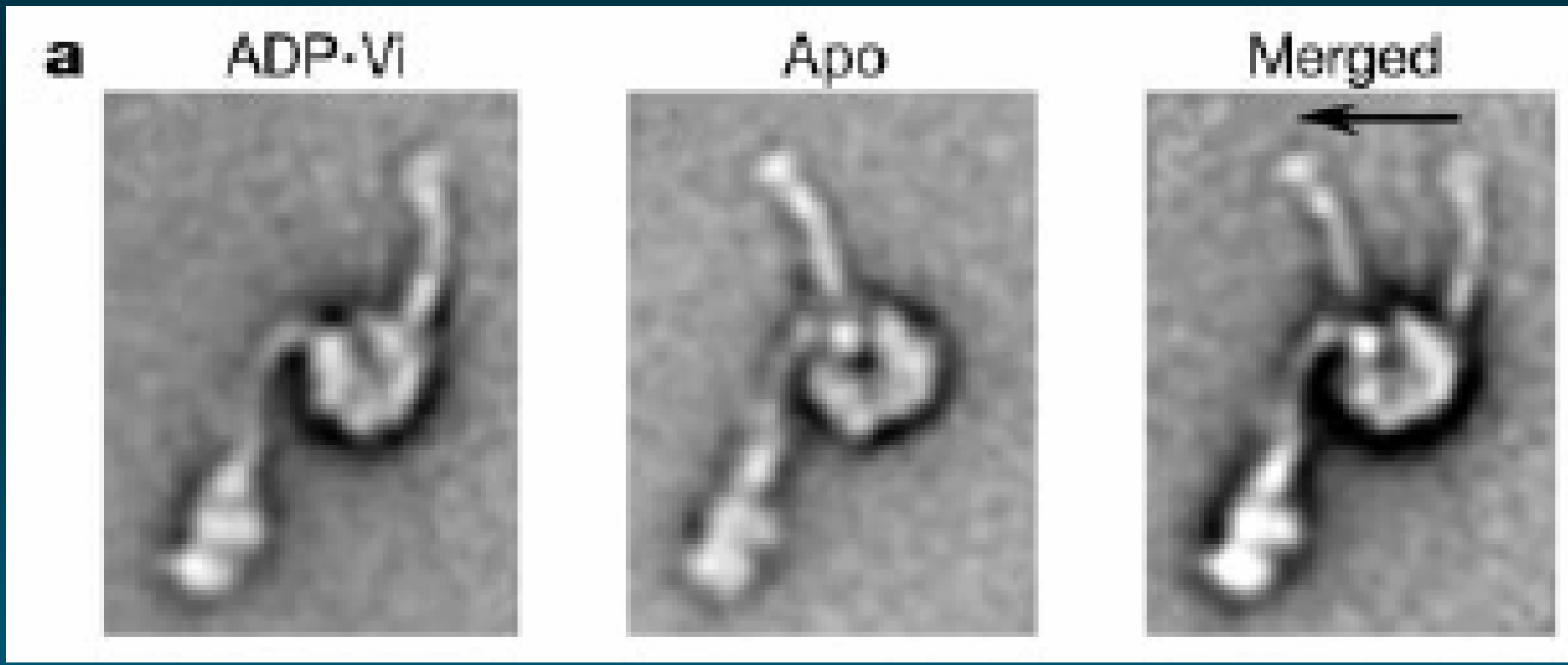
Dynein - AAA+ protein





Samso and Koonce, 2004

Dynein's nucleotide-dependent structure change



Burgess et al. *Nature* 2003

Aim of the research

- 3D reconstruction of dynein –microtuble complex

  380kDa Minimal functional domain
- Detection of the tail region

CryoEM Procedure Results

CryoEM advantage

- Representation of a native condition
  - Buffer condition, pH, Concentrations
  - No staining
- Macromolecule observation

Cryo-EM @ UT Southwestern

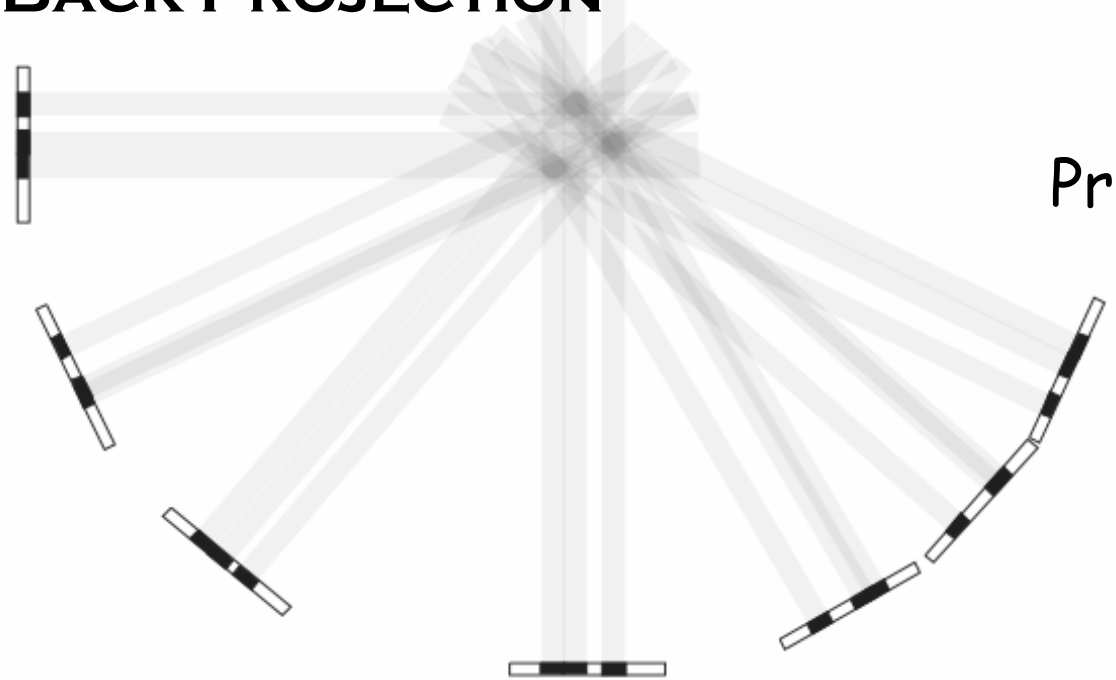
- 200kV
- Electron gun:

Field emission

How do we build a 3D object from 2D projections? The principle of “Back Projection”

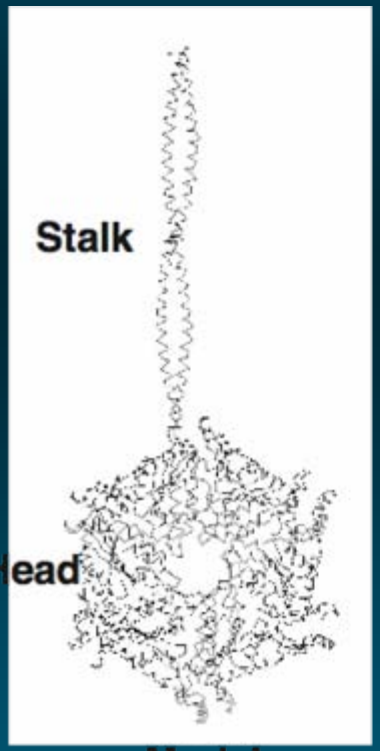
(1) Microscopy
(2) Back-Projection

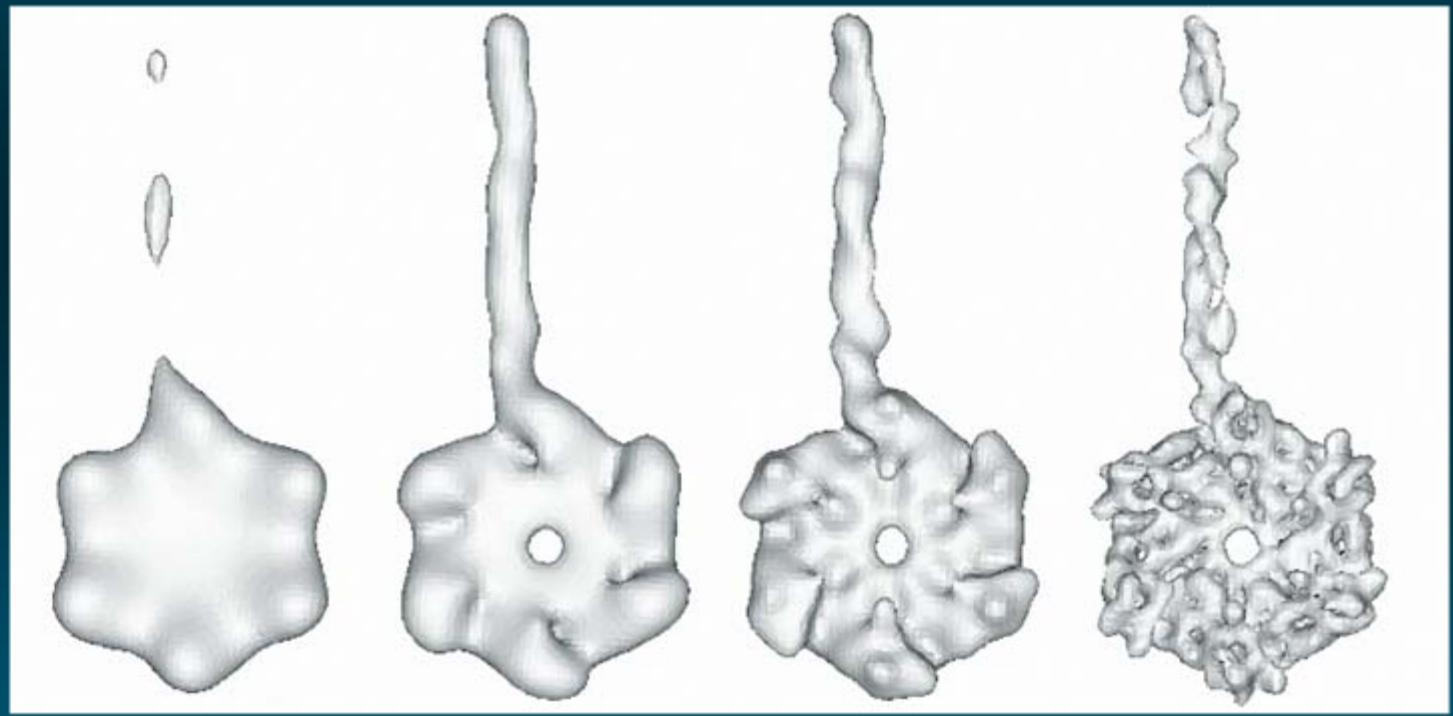
“Molecule”



Projection

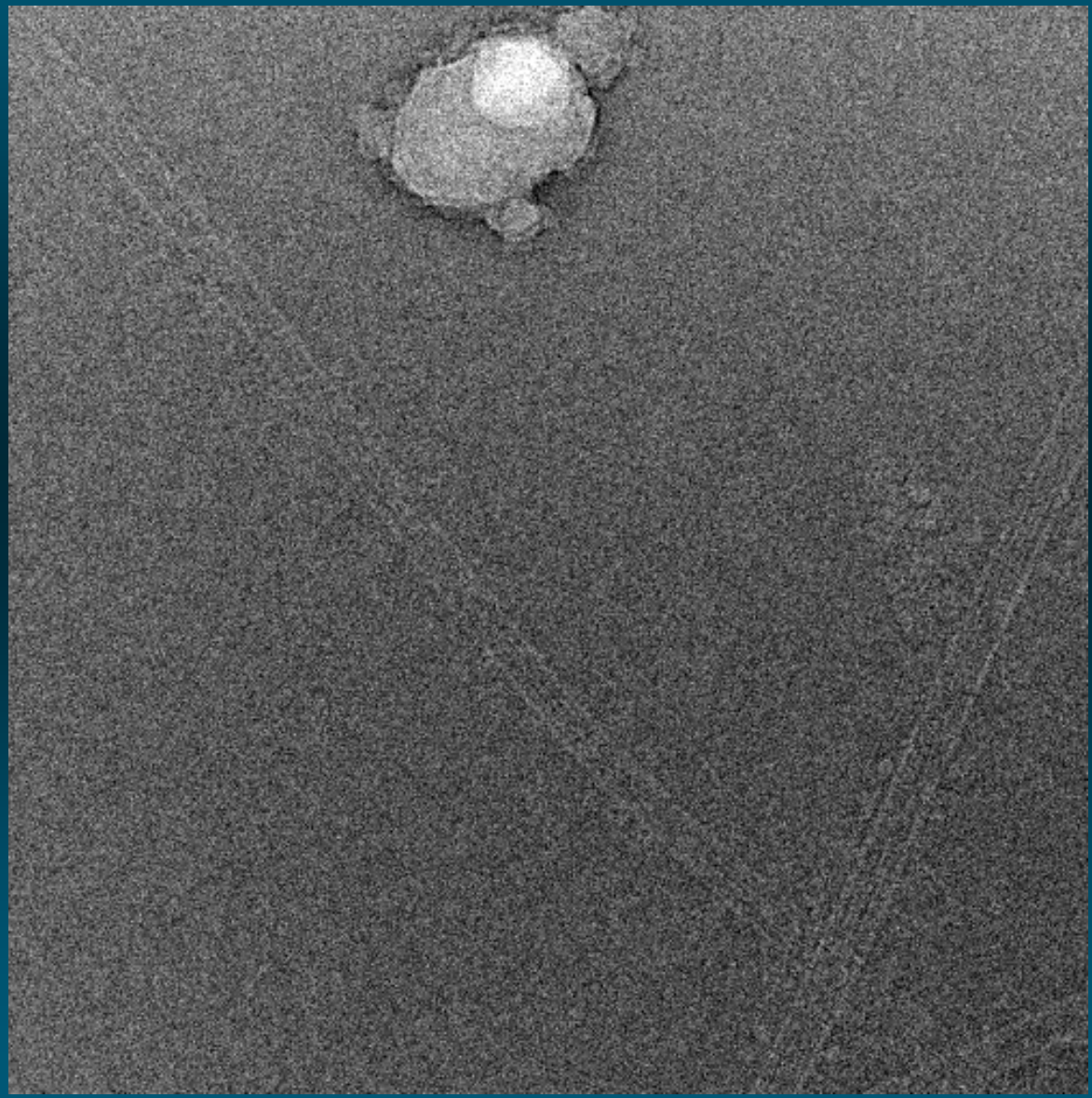
The power of resolution





Actual Image Analysis

CryoEM Raw Image



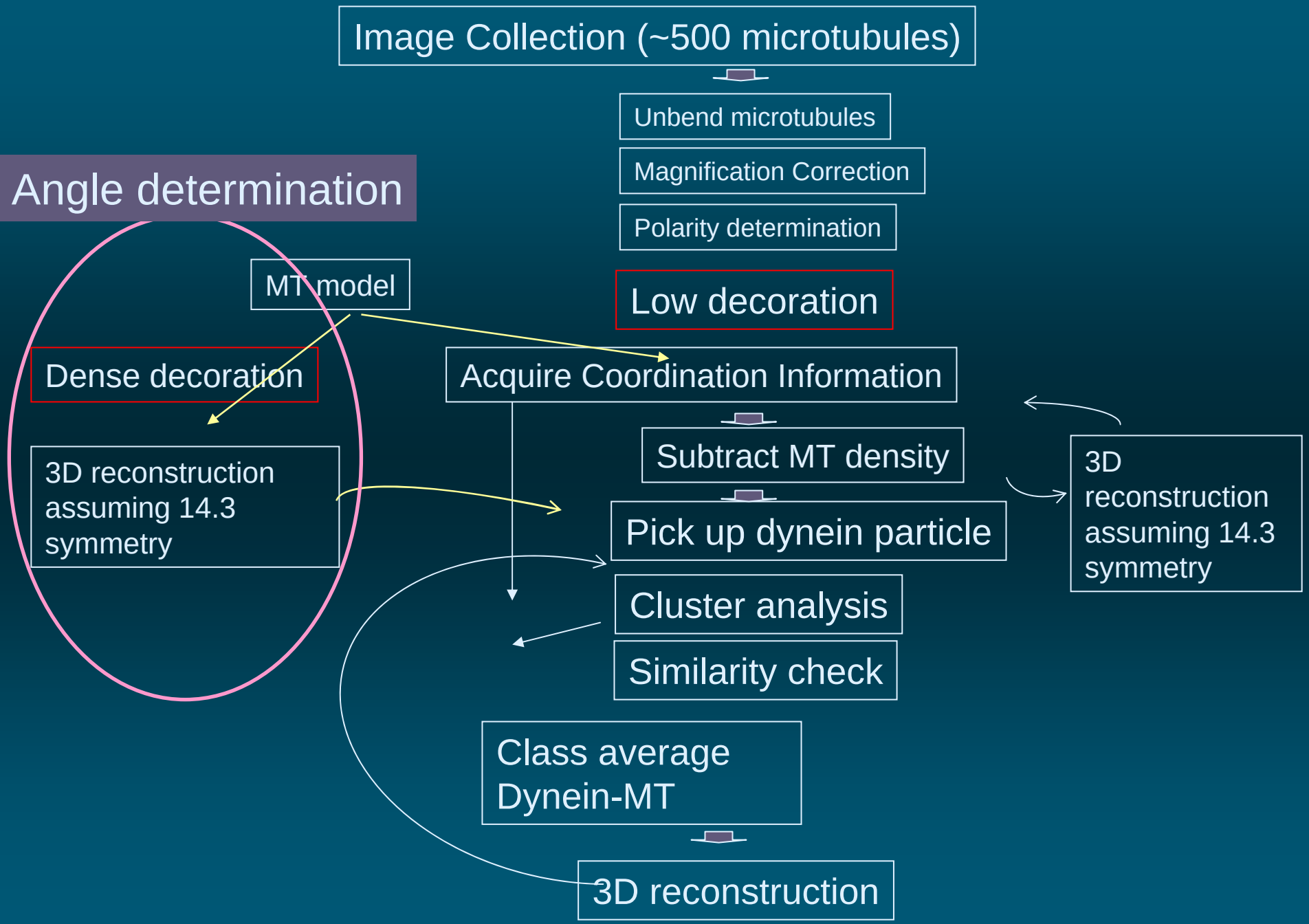
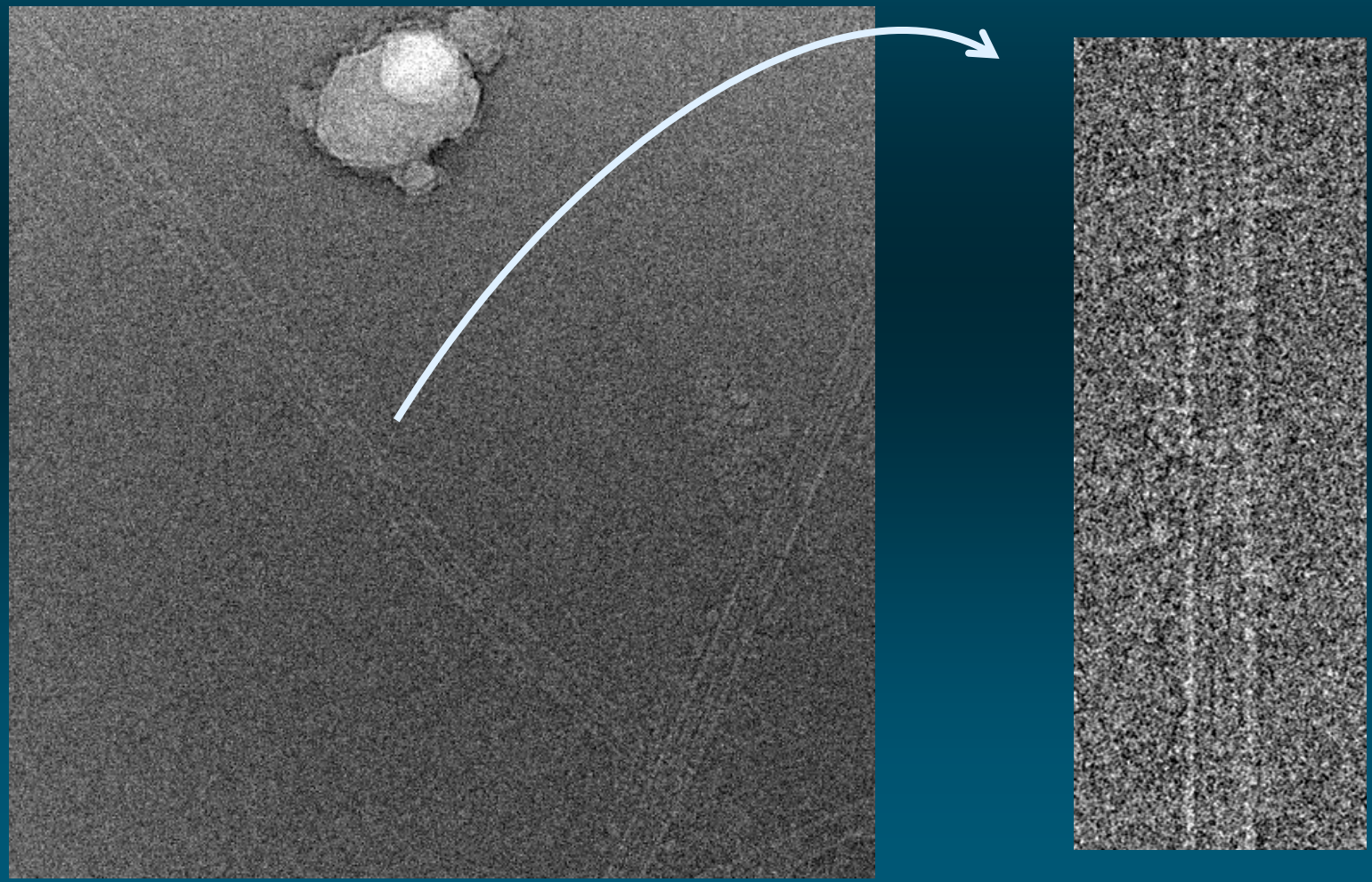
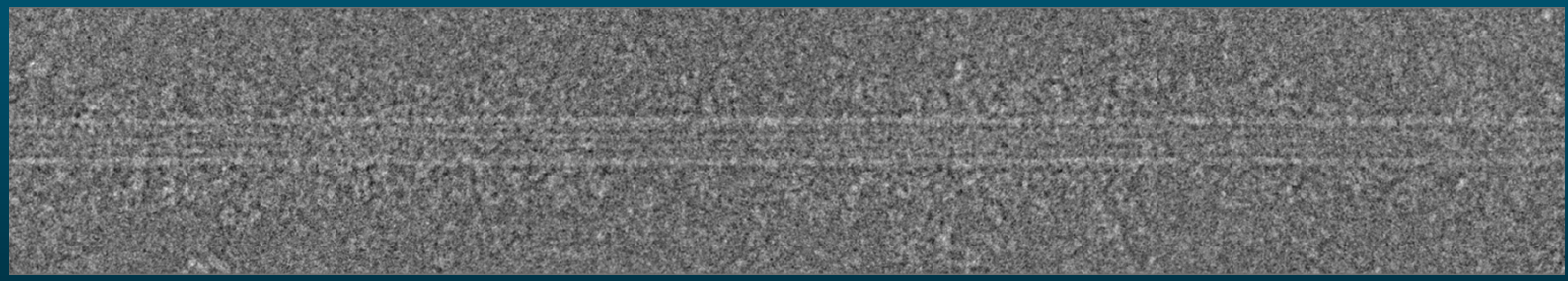


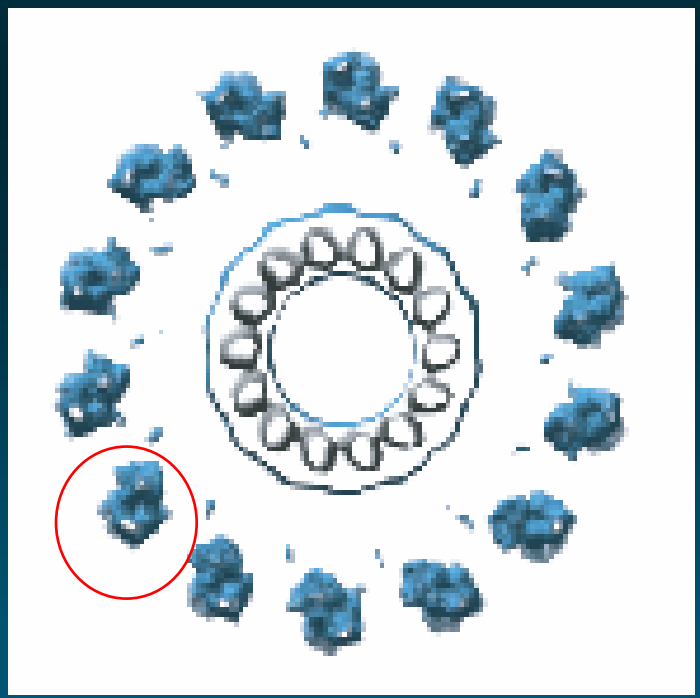
Image Collection (~500 microtubules)
Unbend microtubules
Magnification Correction
Polarity determination
Angle determination
MT model
Low decoration
Dense decoration
Acquire Coordination Information
3D reconstruction assuming 14.3 symmetry
Subtract MT density
3D reconstruction assuming 14.3 symmetry
Pick up dynein particle
Cluster analysis
Similarity check
Class average Dynein-MT
3D reconstruction

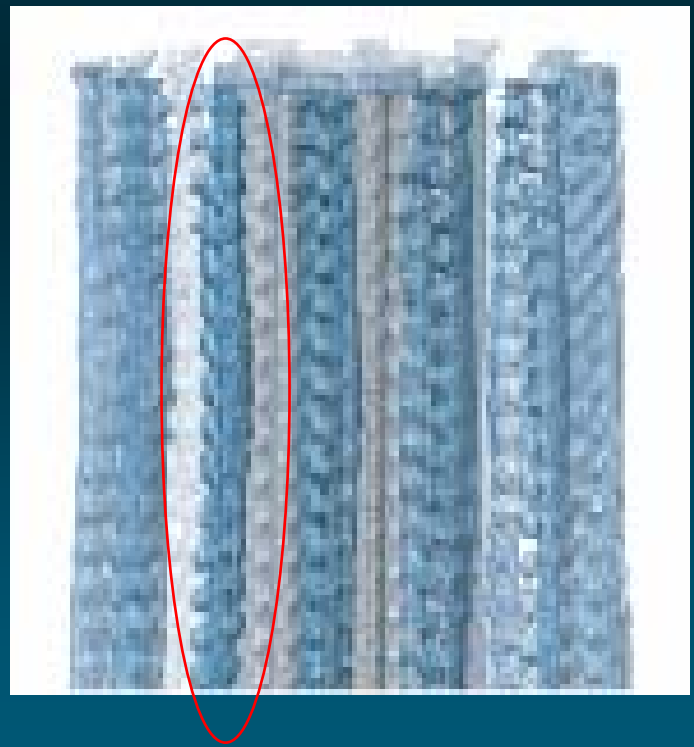
Image acquire and process

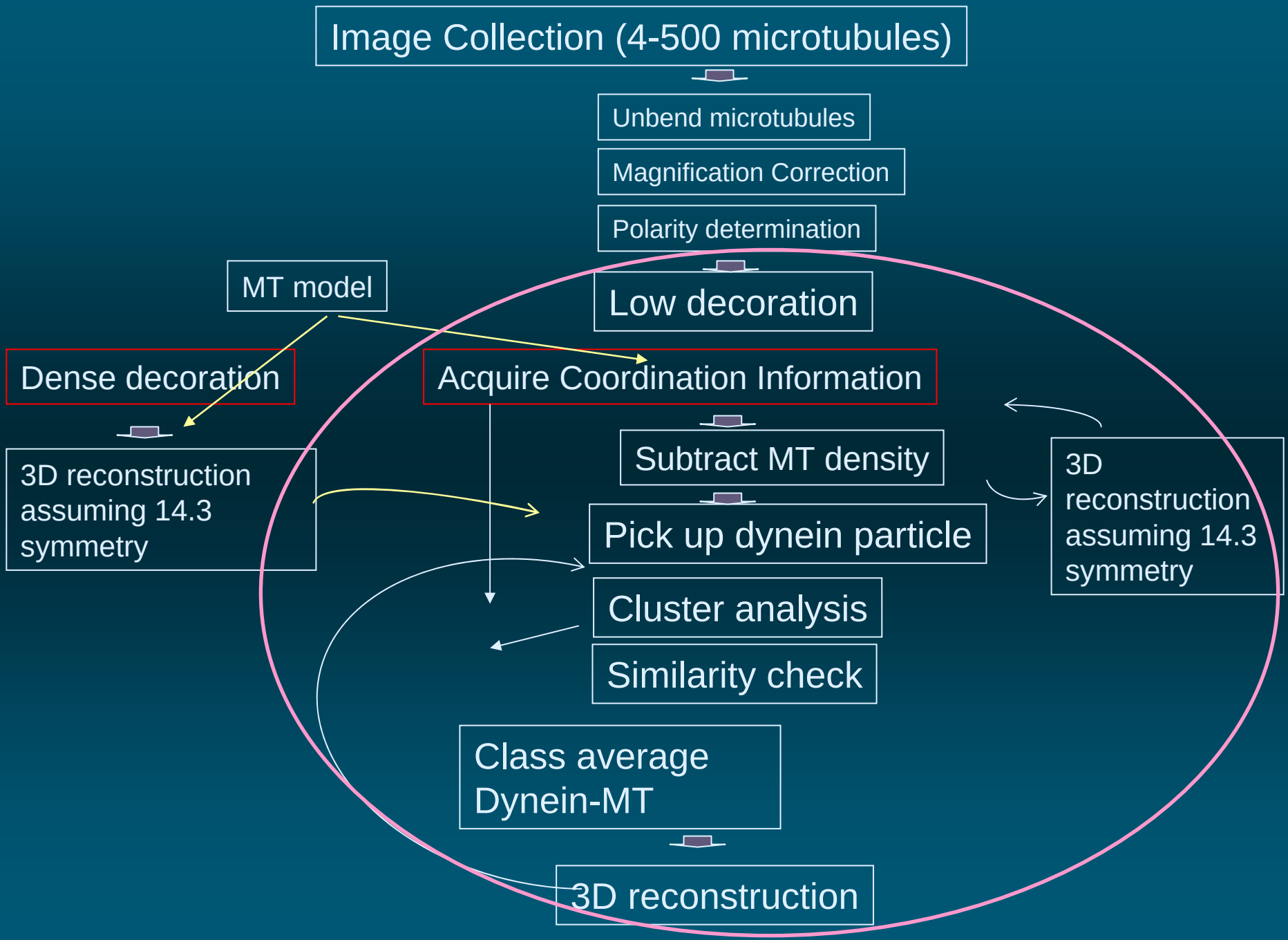


Dense decoration reference





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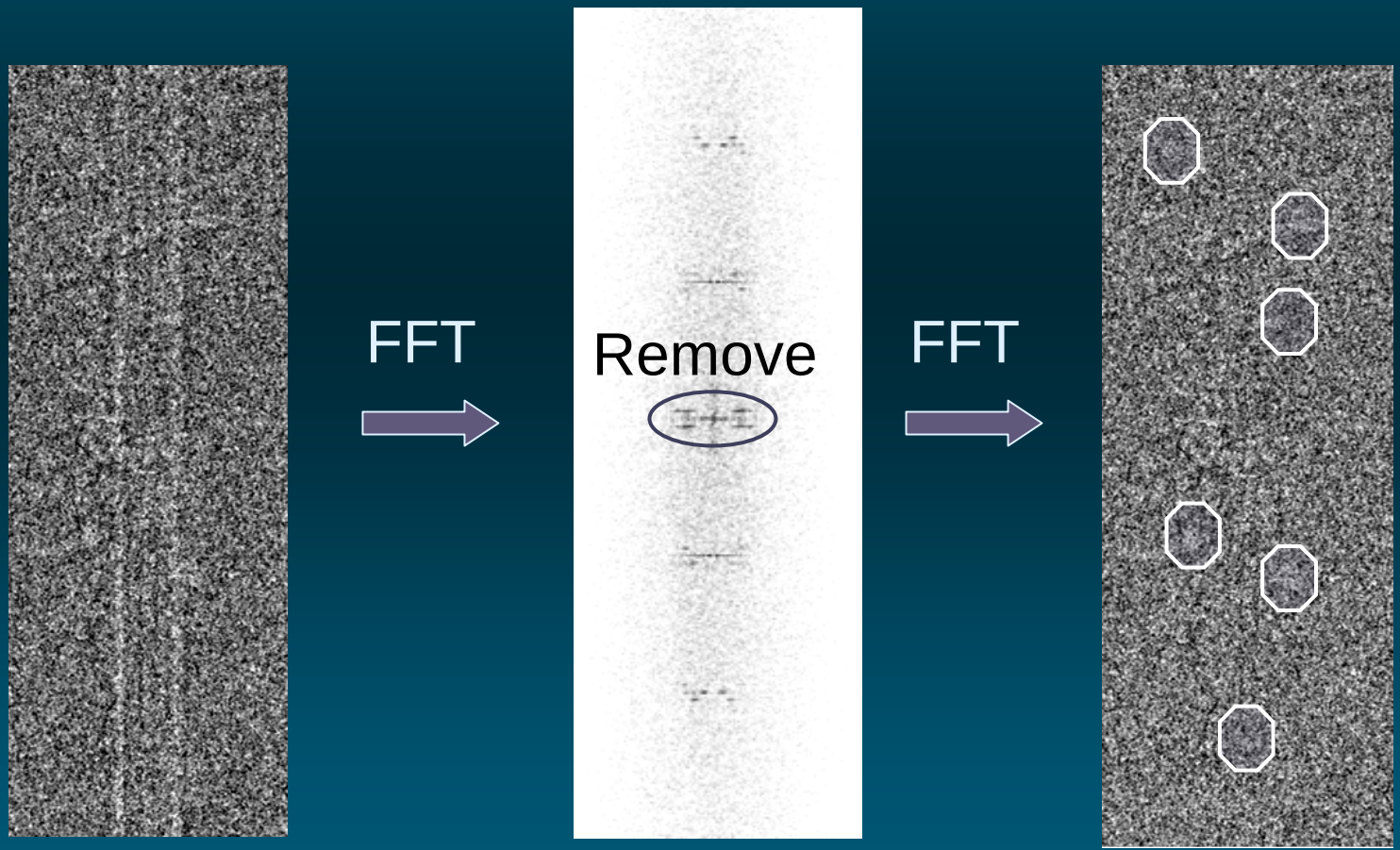
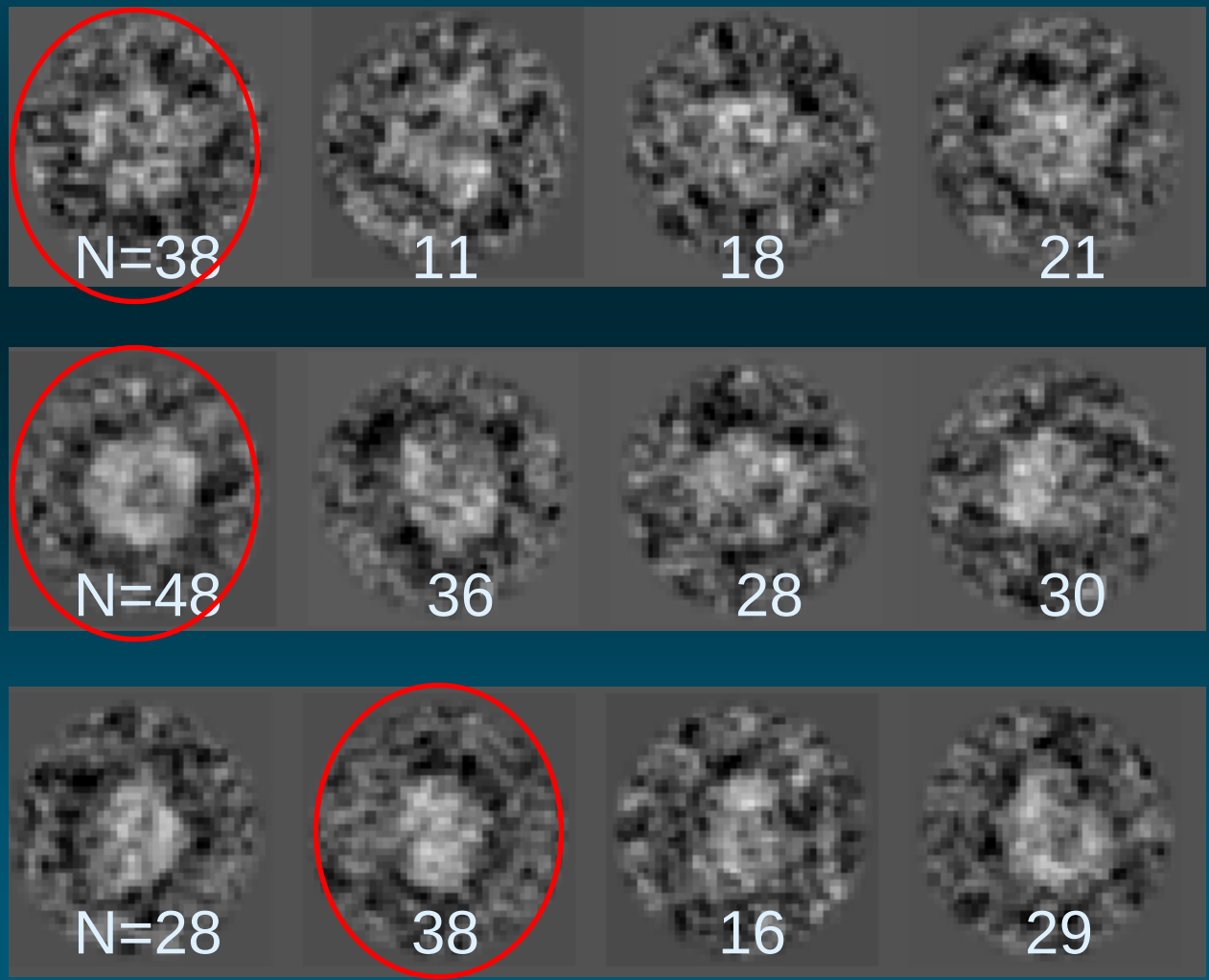
Similarity check
Class average Dynein-MT
3D reconstruction

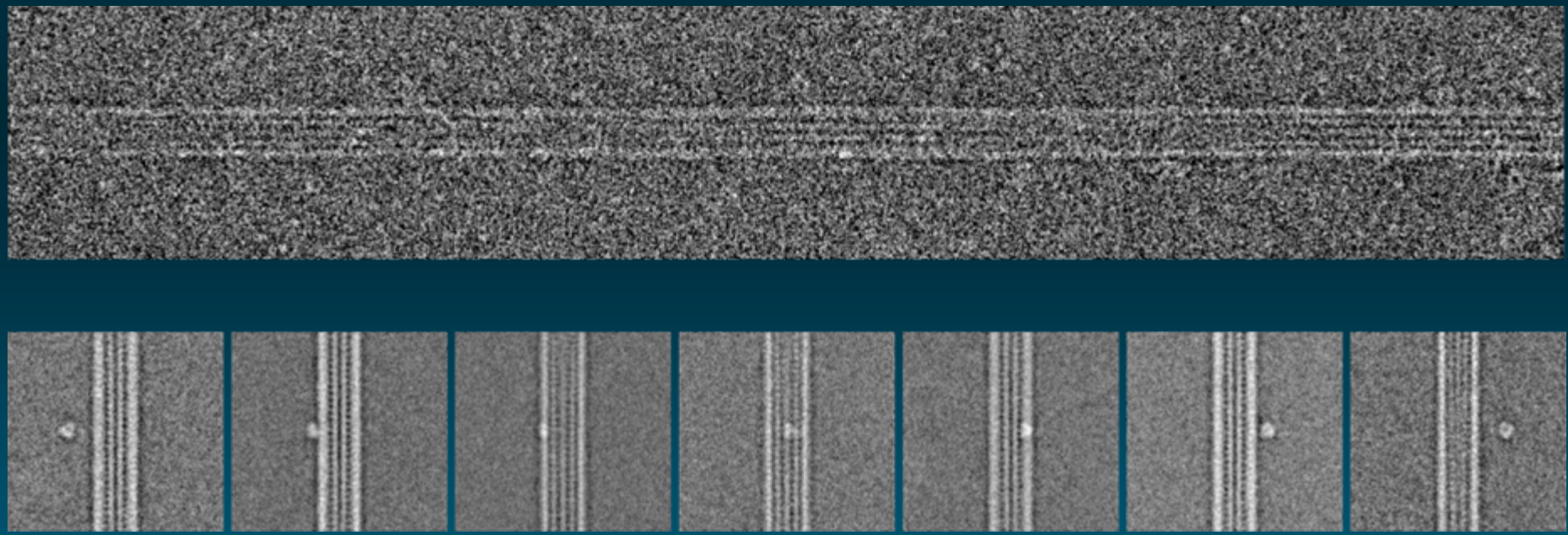
Microtubule Subtraction - Pick up particles



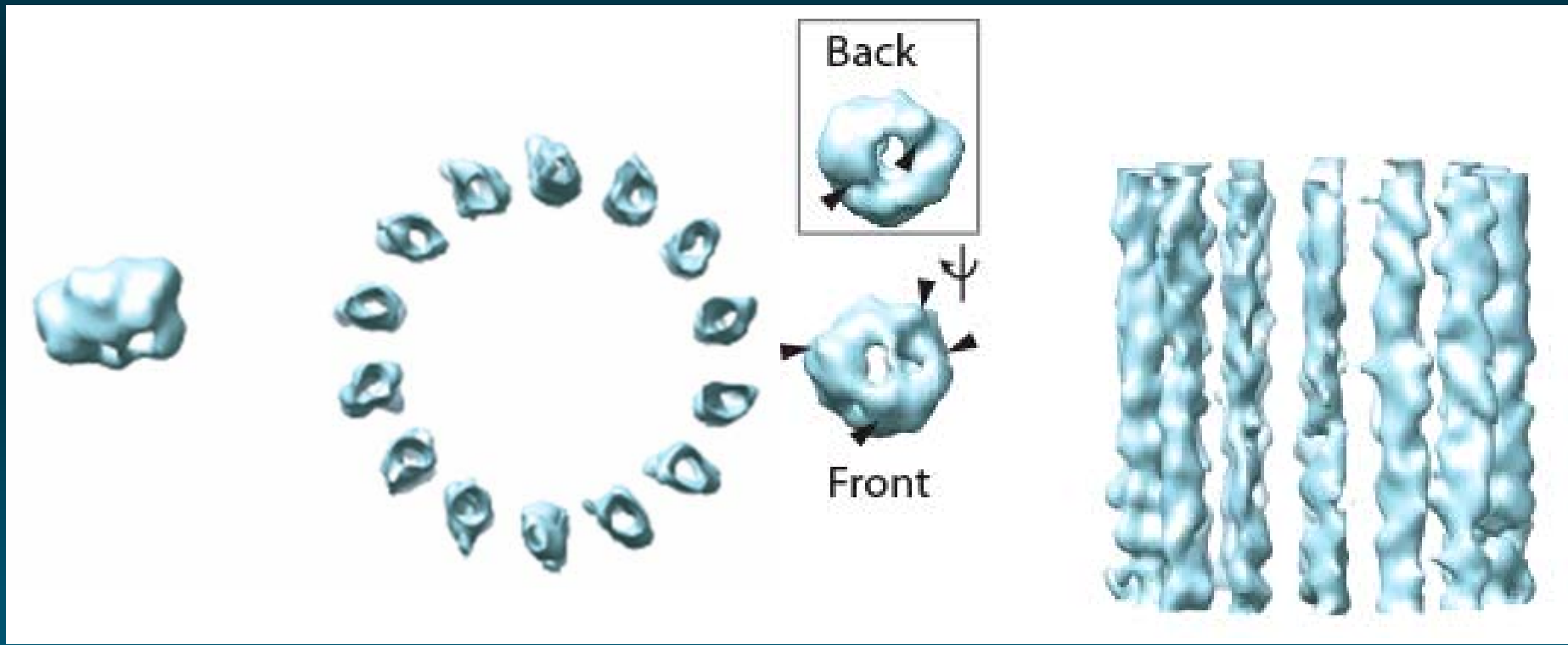
Cluster analysis to make a reference model

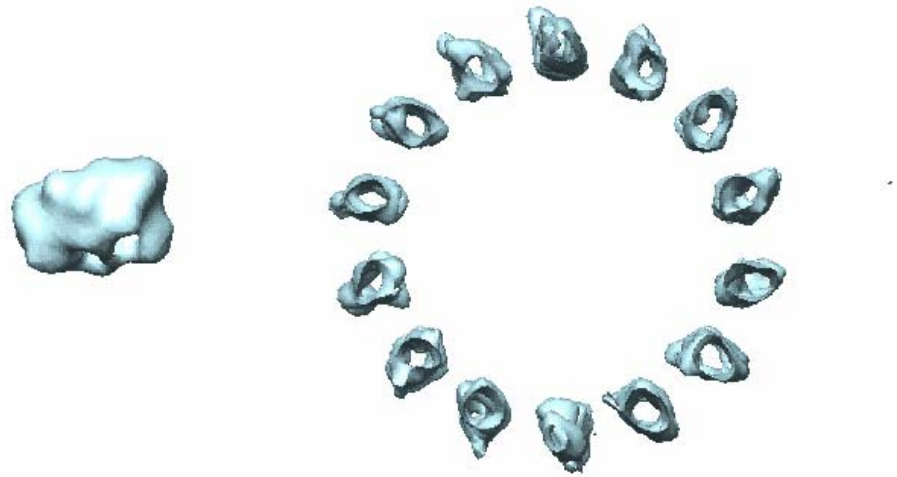


Class averages

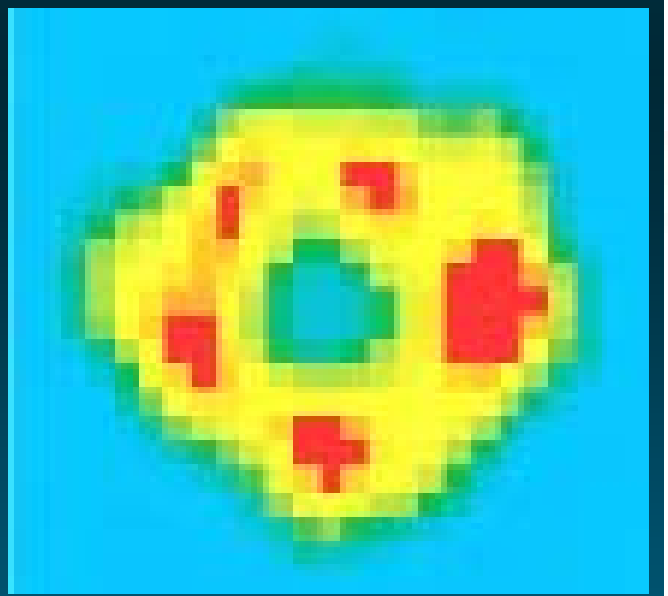


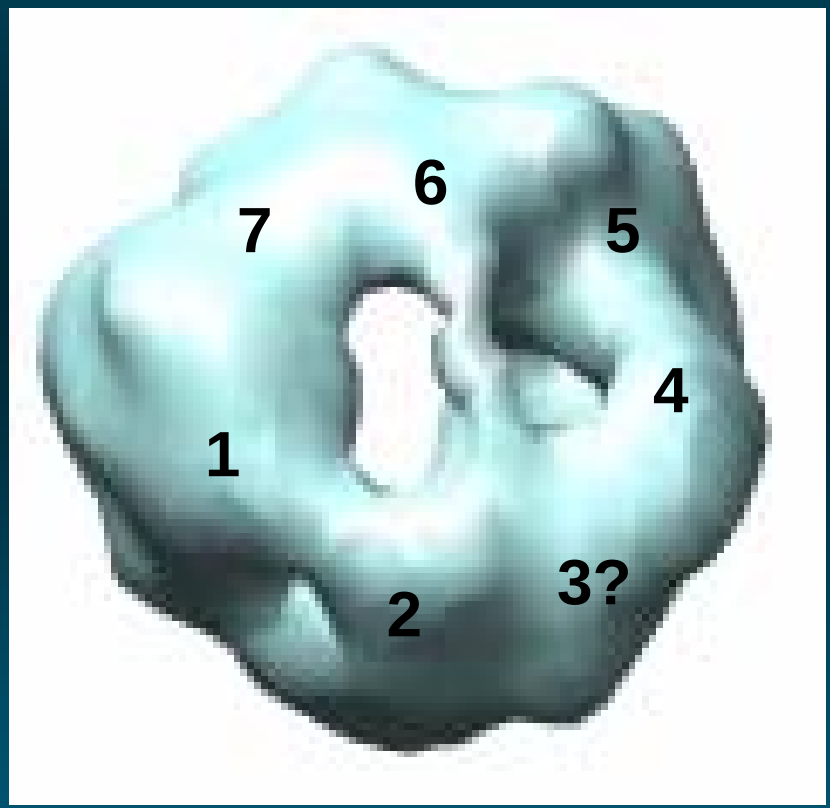
3D Reconstruction



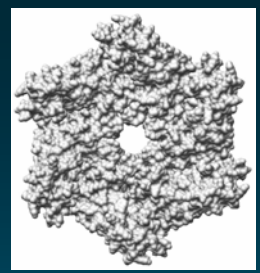


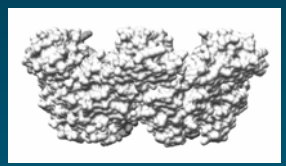
Sub-domain determination





Ref



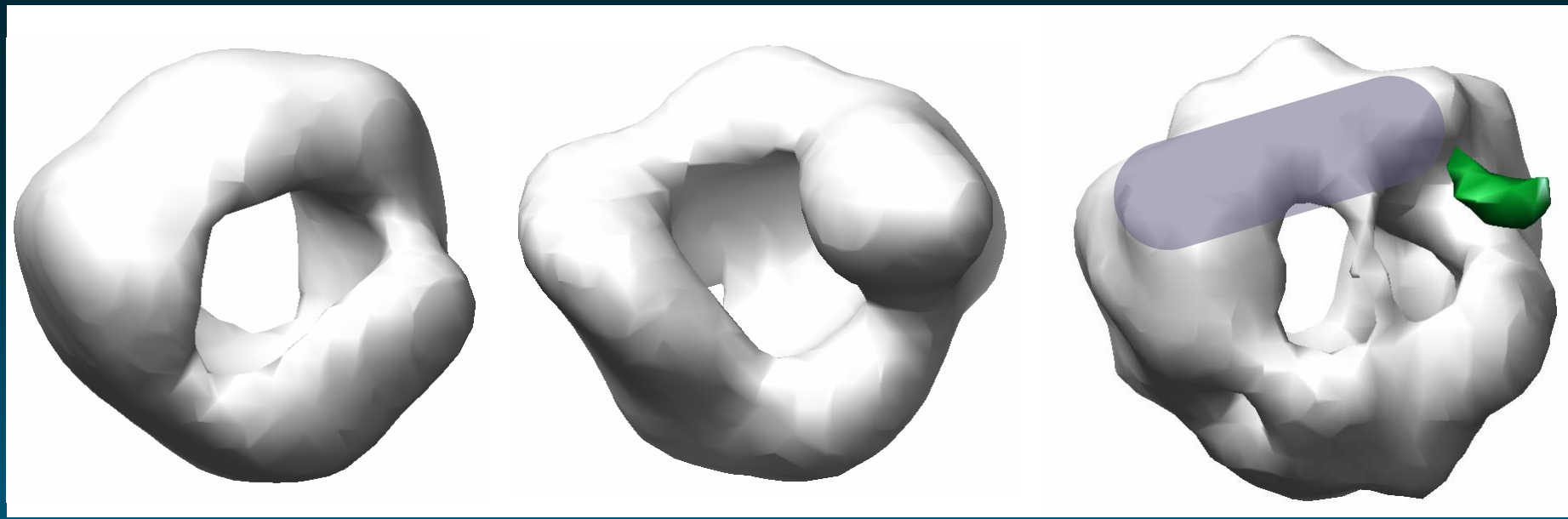


Detection of the N-terminal Tail

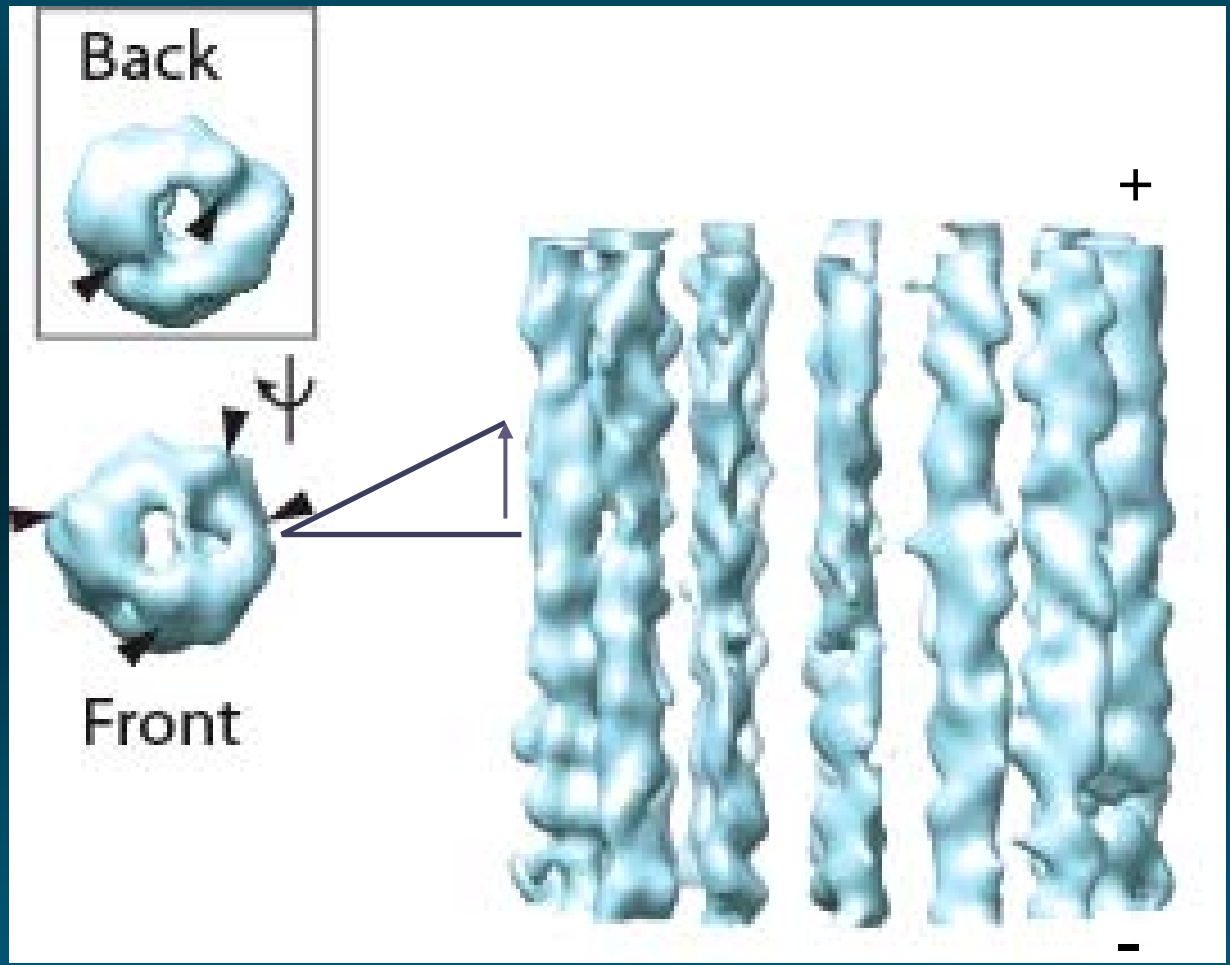
WT (30 Å)

+GFP N-terminal

t-map (Green)



Possible dynein motion



Conclusions

3D reconstruction was carried out.

- The axis of the ring is orthogonal to MT axis.
- Sub-domain assignment was done.

Acknowledgements

- Masahide Kikkawa
  (University of Texas, Southwestern Medical Center)
- Akihiro Narita (Spring8, Strategy, programming)
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