Furthering our understanding of microtubule dynamic instability by CryoEM

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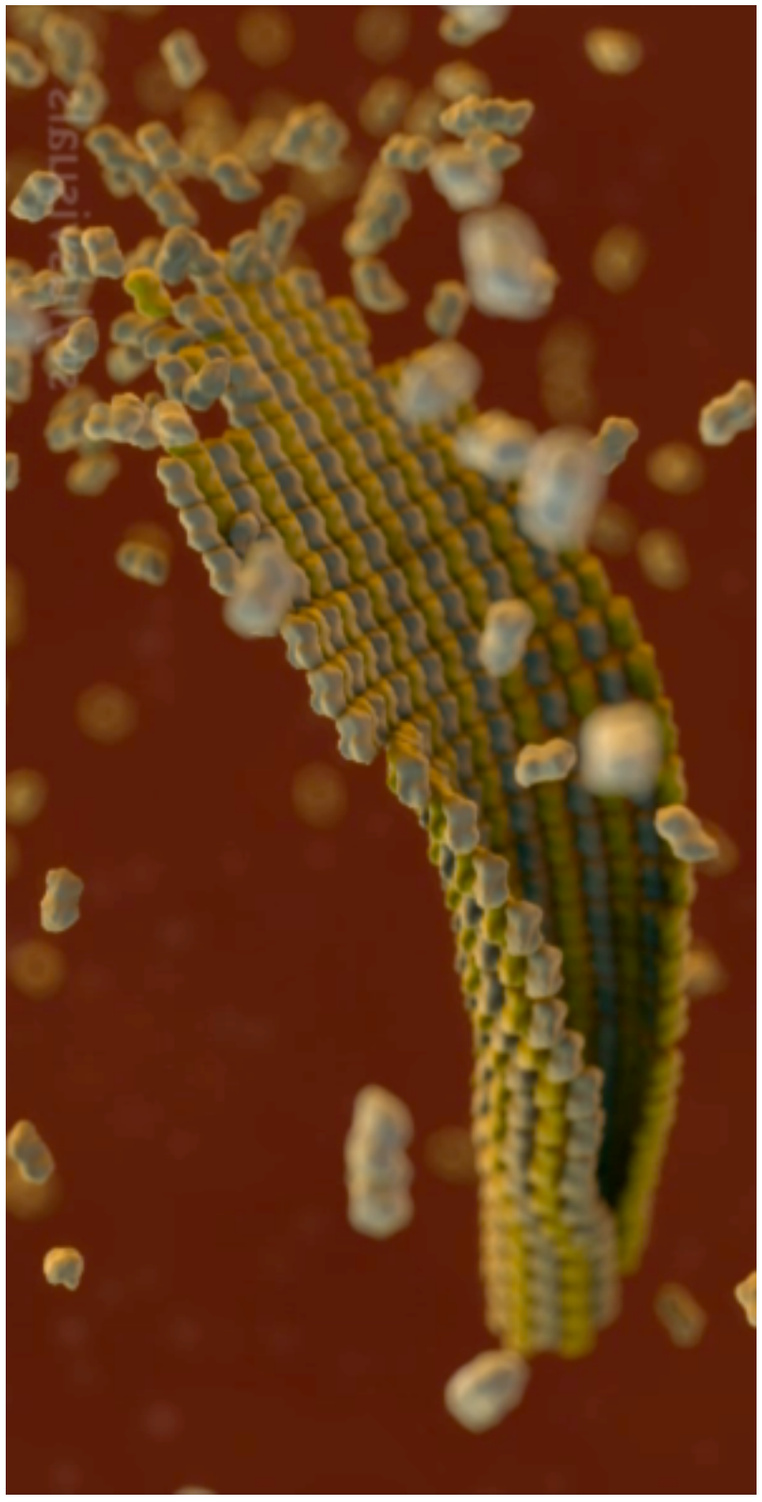
Greg Alushin

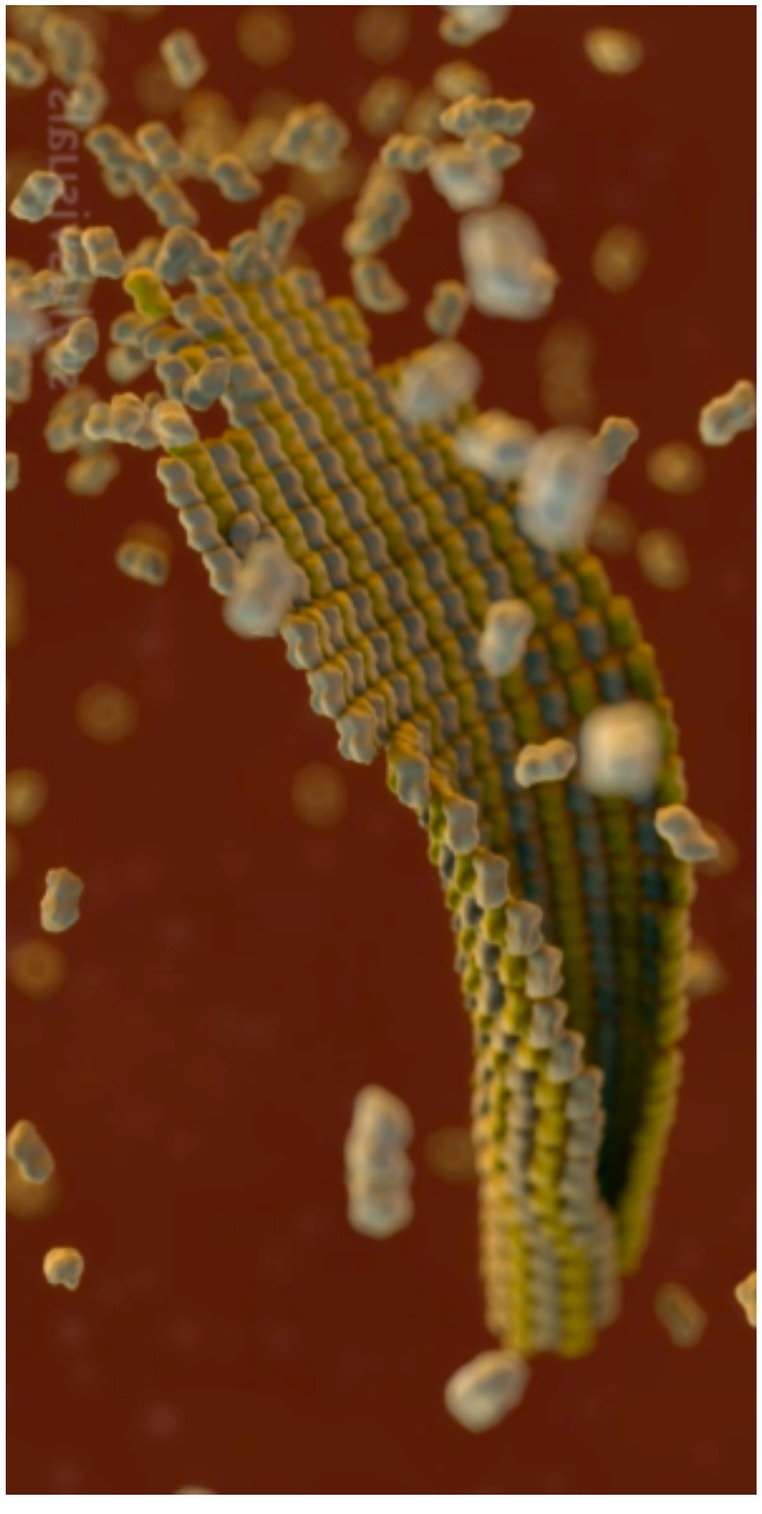
The Microtubule

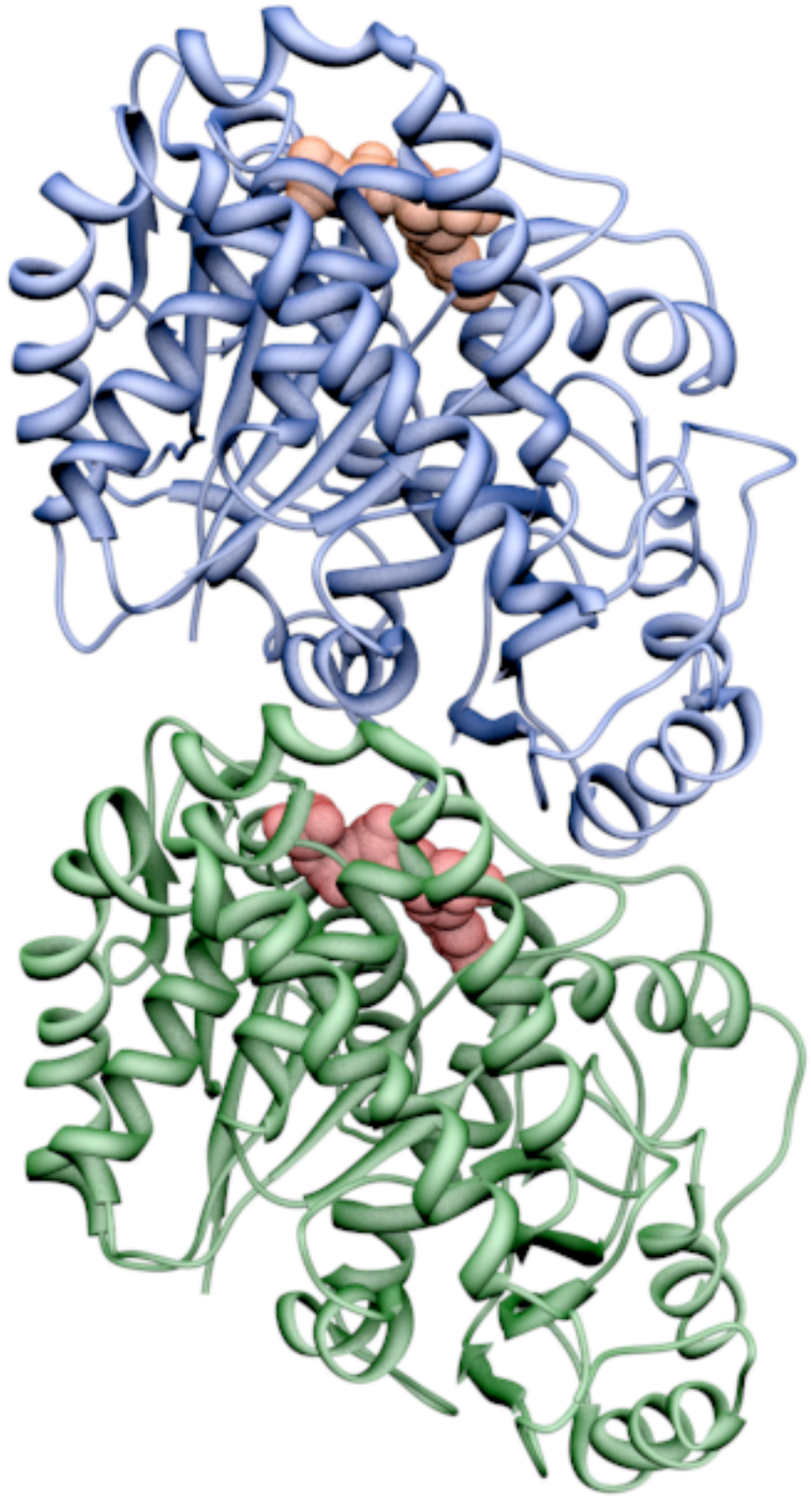


- Microtubules are among most important components of the cytoskeleton

- Fundamental part of many physiological processes:
  - intracellular transport
  - cell motility
  - cell polarization
  - cell division

The Tubulin Dimer - The Microtubule Building Block



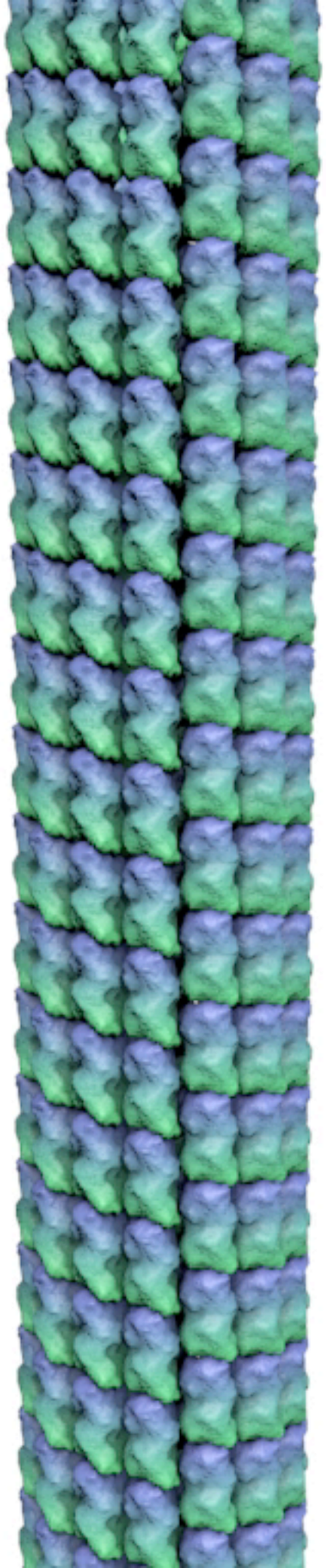


Beta Subunit

Alpha Subunit

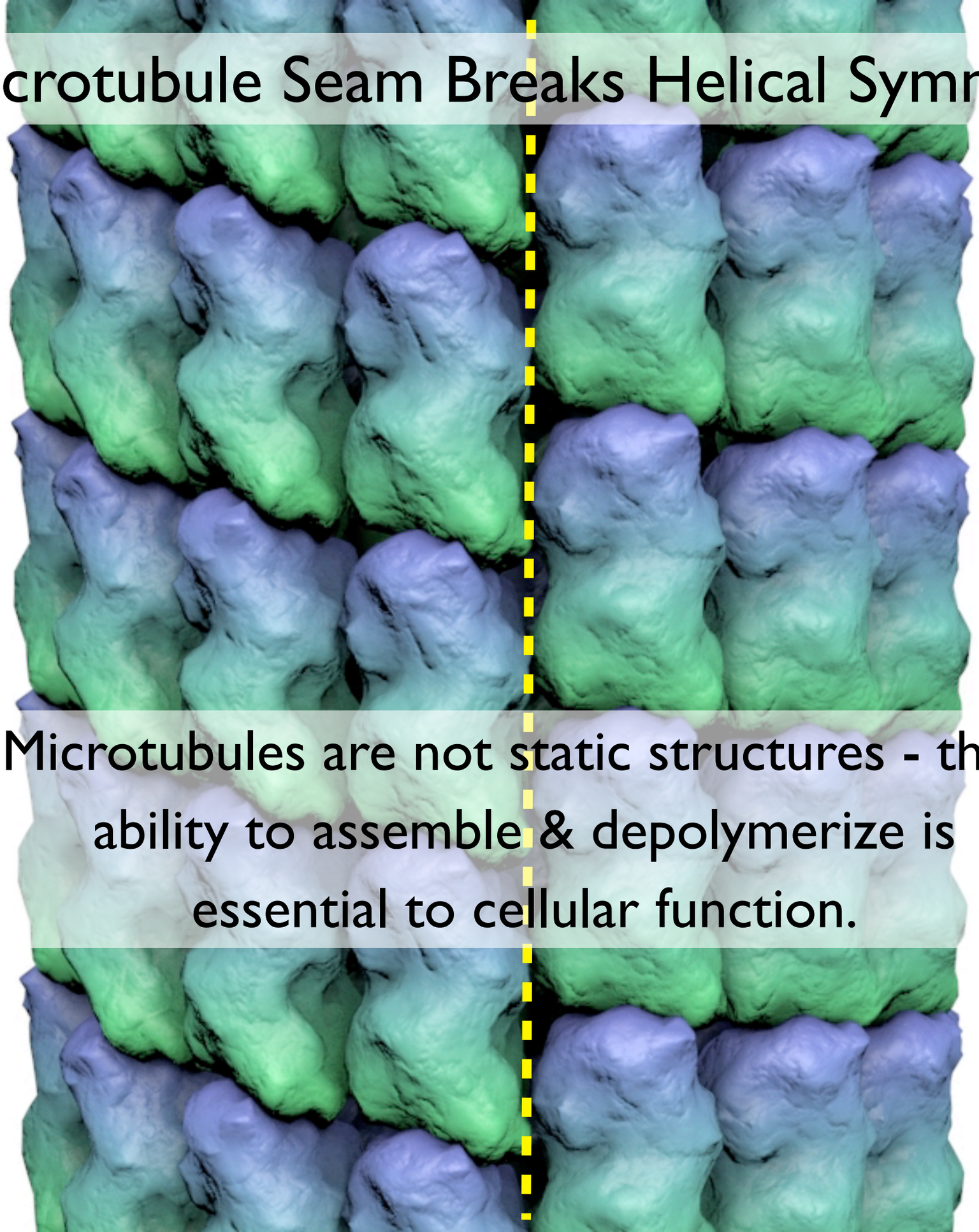
Tubulin dimers
assemble longitudinally

Protofilament



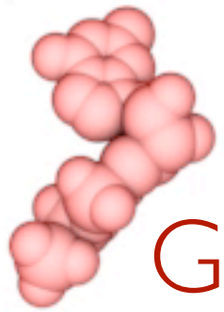
Microtubule

Microtubule Seam Breaks Helical Symmetry



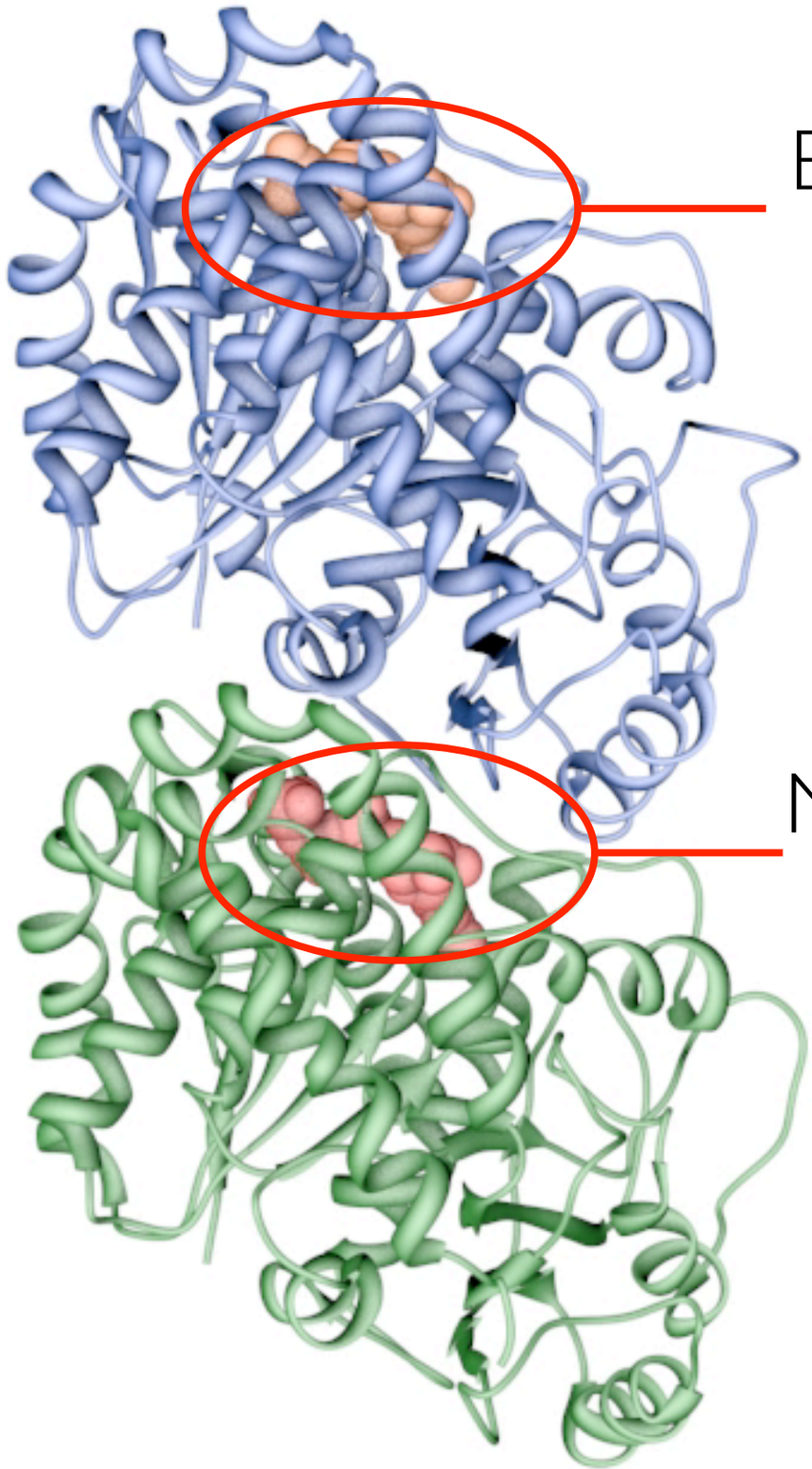
Microtubules are not static structures - their ability to assemble & depolymerize is essential to cellular function.

The Nucleotide Binding Pocket



GTP

Beta Subunit
(GDP)



Exchangeable site
(E-site)

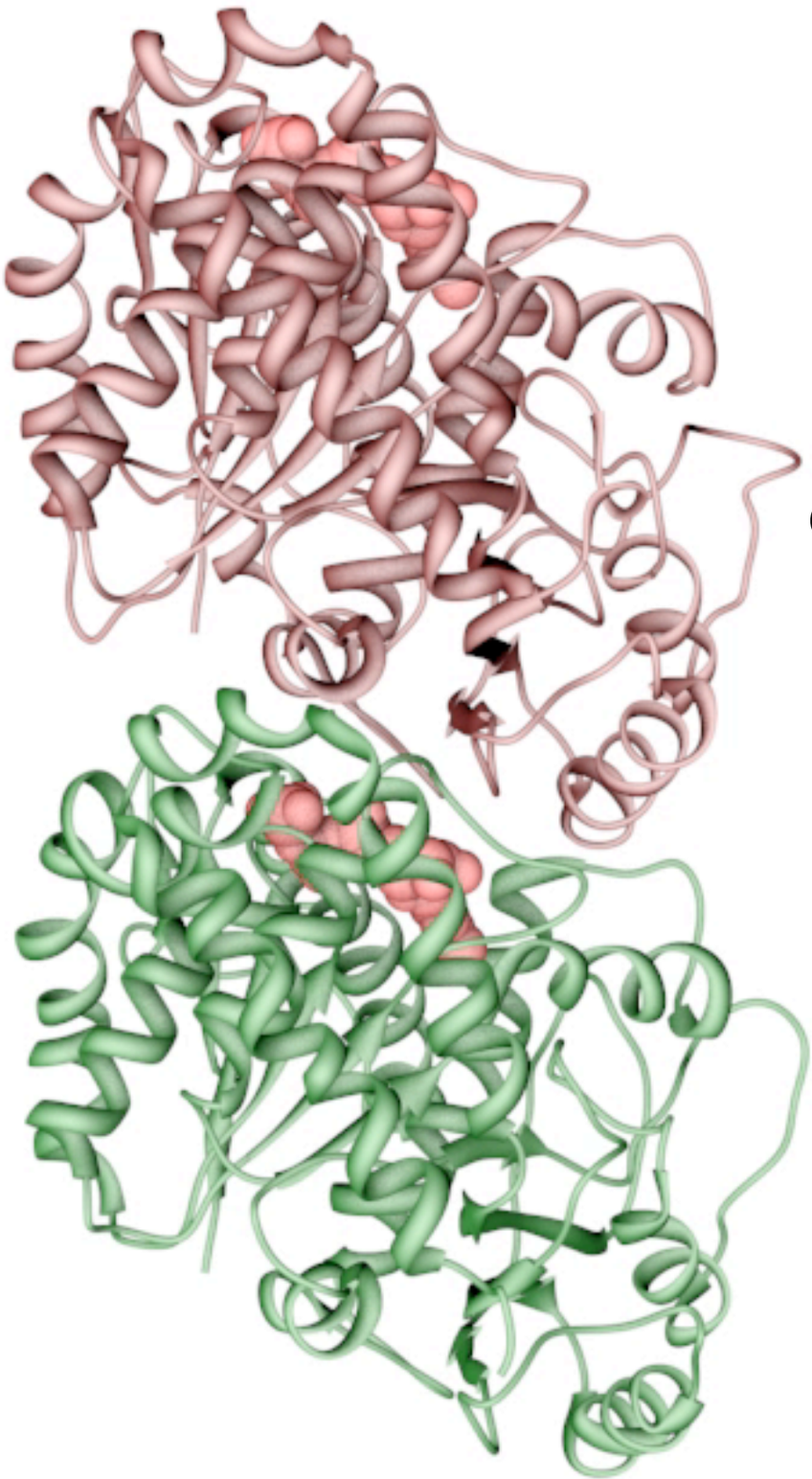
Free tubulin can exchange GDP for GTP

Non-exchangeable
site (N-site)

Alpha Subunit
(GTP)

The Nucleotide Binding Pocket

Beta Subunit (GTP)

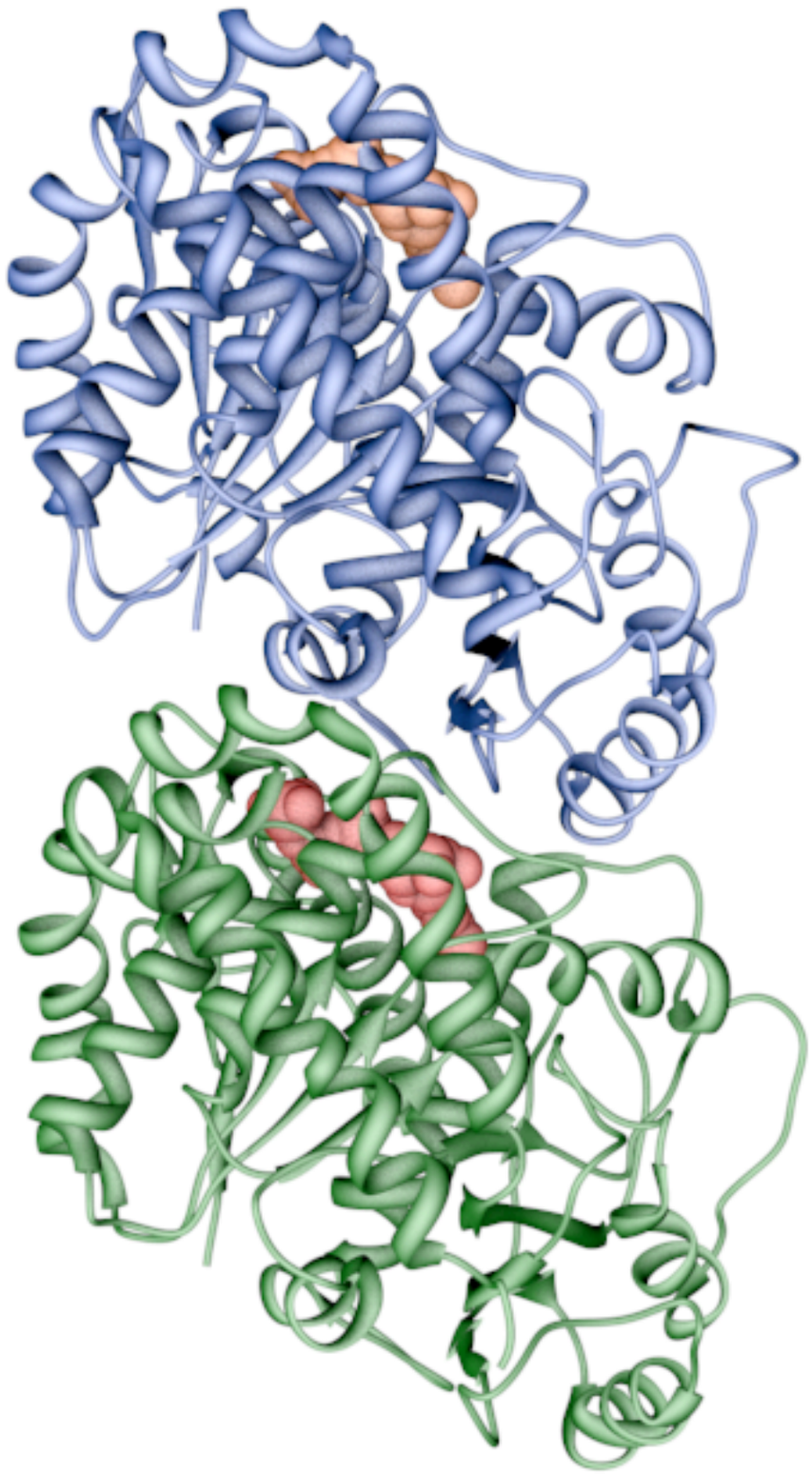


Alpha Subunit (GTP)

GTP is required at beta subunit for MT polymerization, creating strong inter-tubulin contacts

The Nucleotide Binding Pocket

Beta Subunit
(GDP)

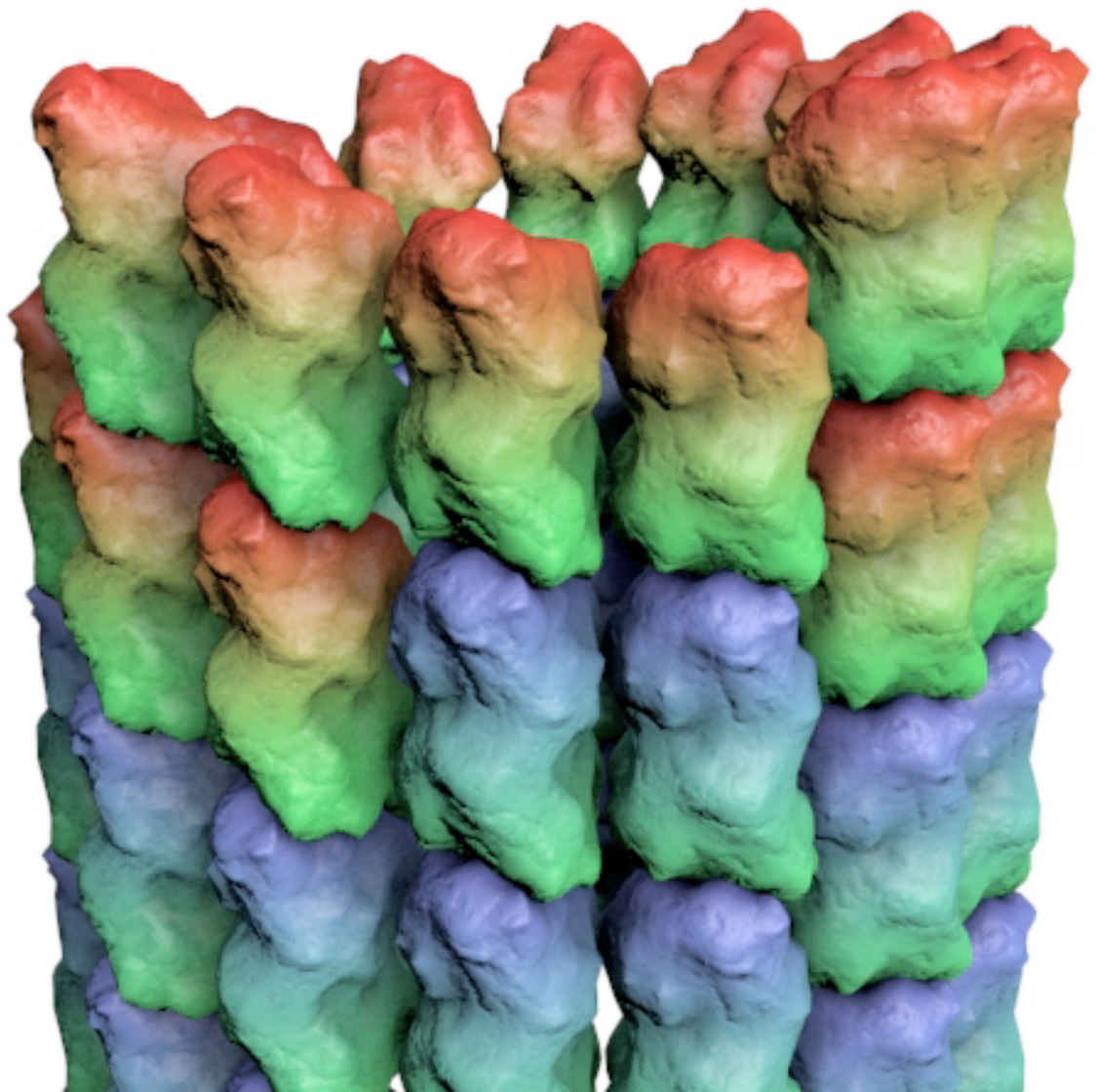


GTP hydrolysis to
GDP weakens the
inter-tubulin contacts

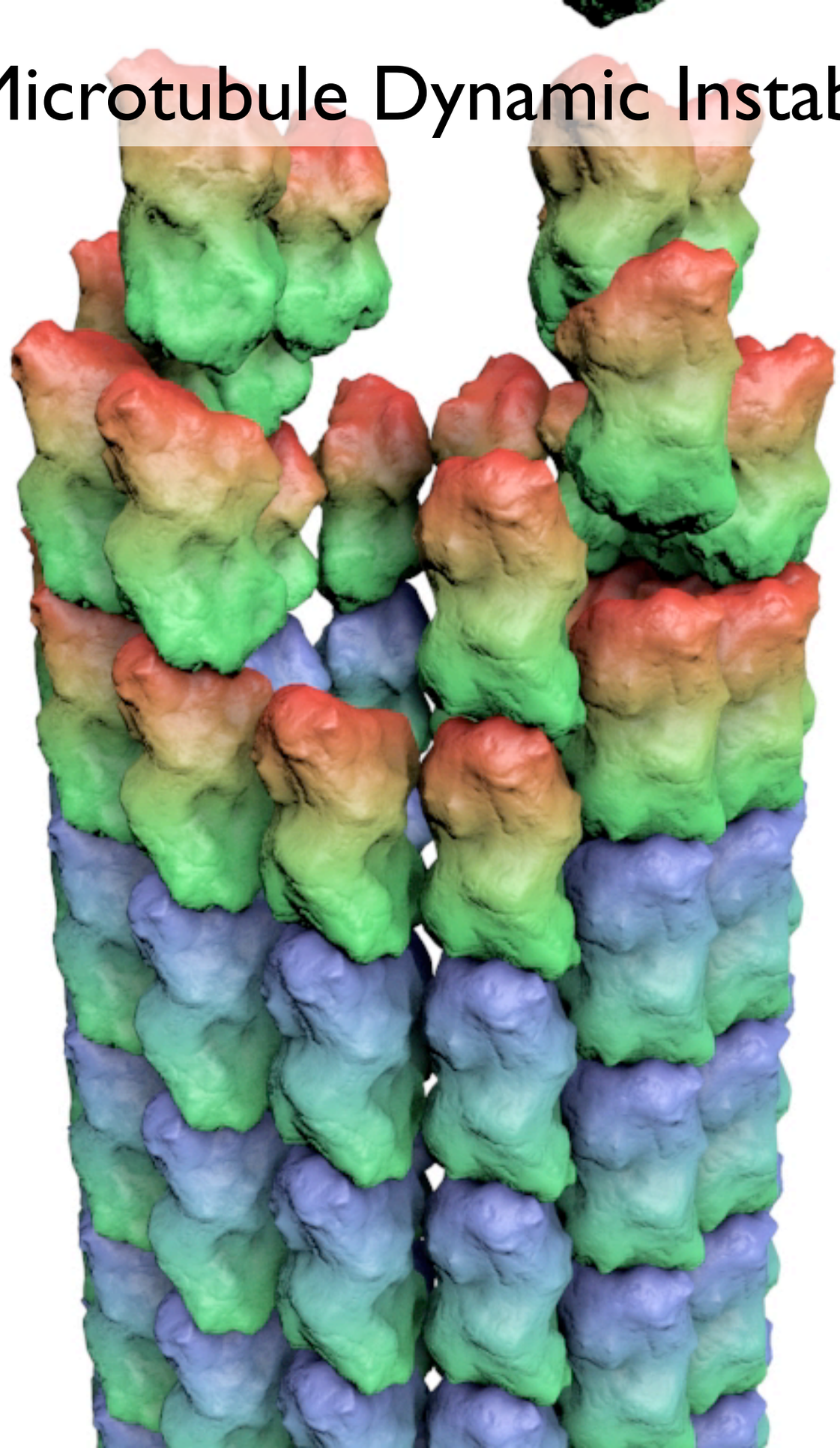
Alpha Subunit
(GTP)

Microtubule Dynamic Instability

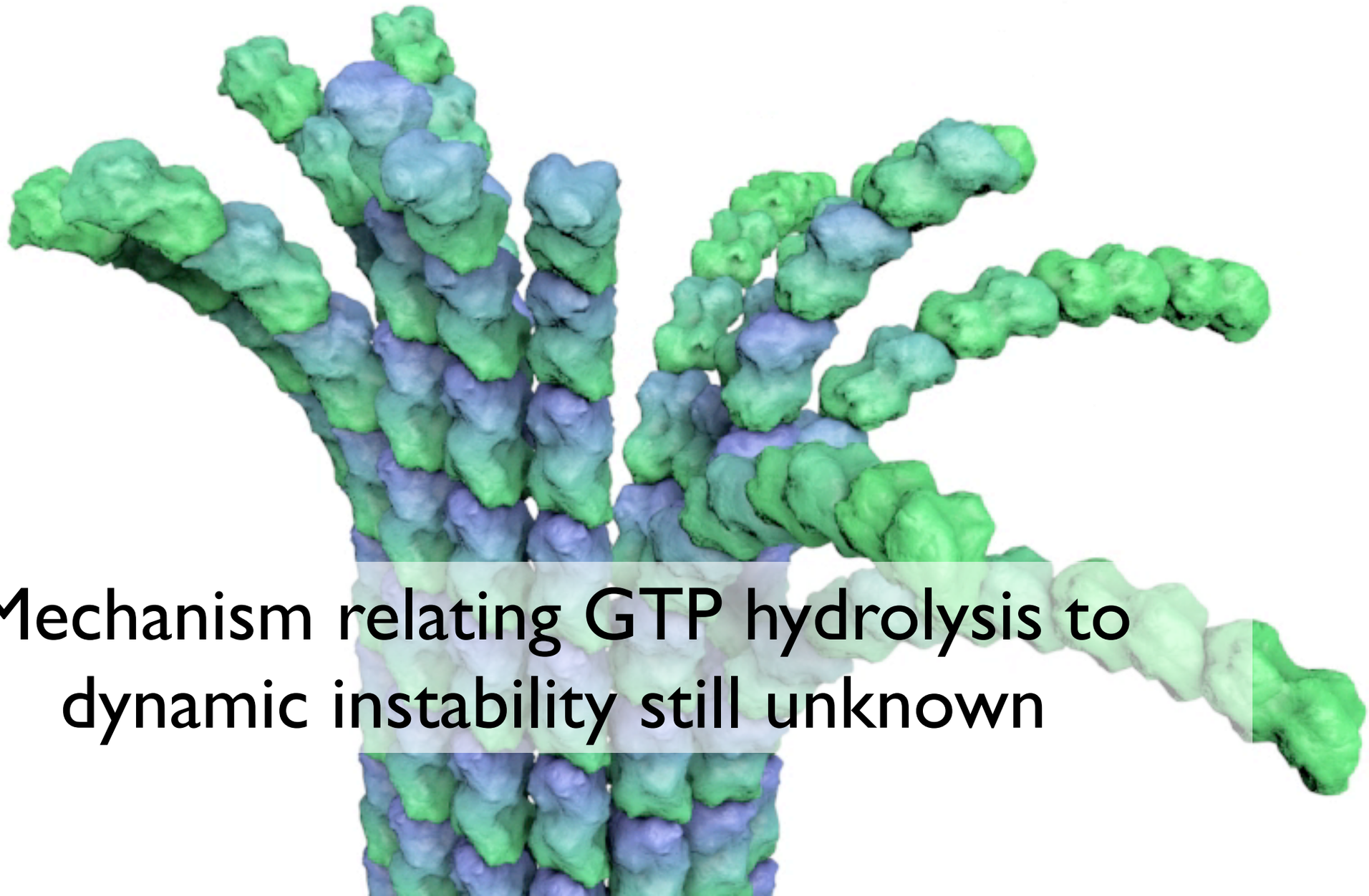
GTP "Cap" {



Microtubule Dynamic Instability

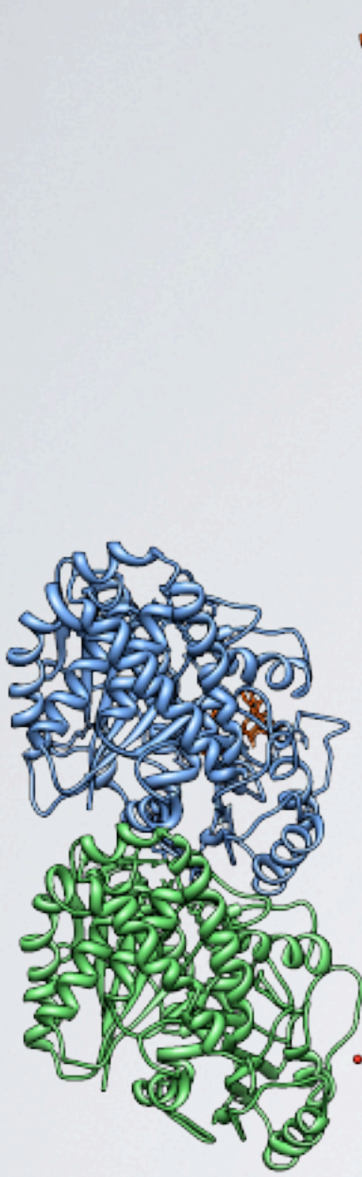


Microtubule Dynamic Instability

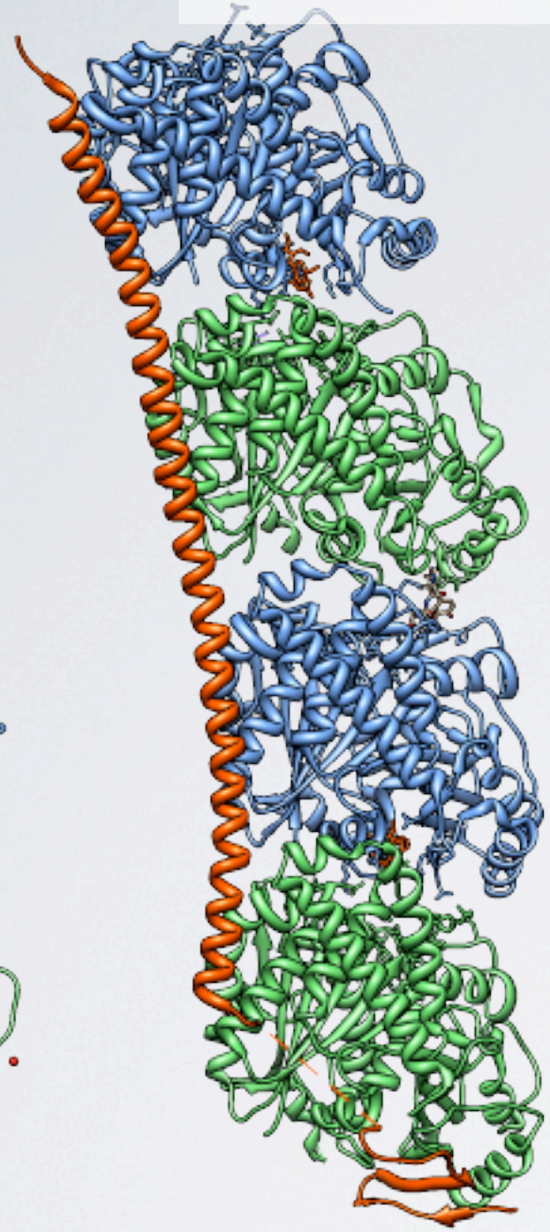


Mechanism relating GTP hydrolysis to dynamic instability still unknown

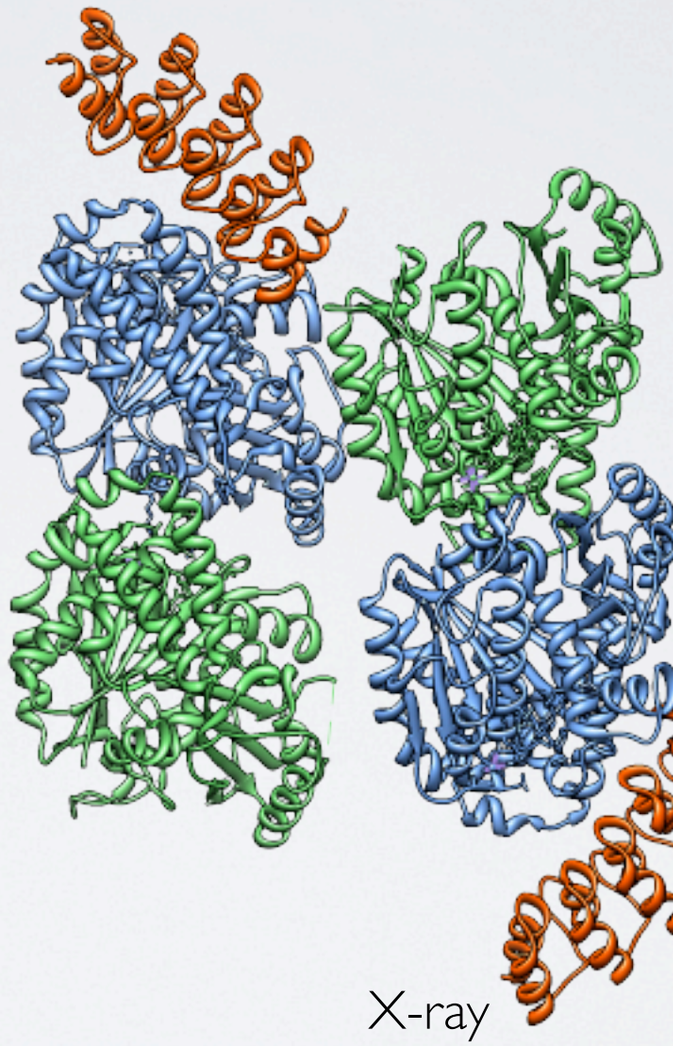
Atomic-Resolution Structures



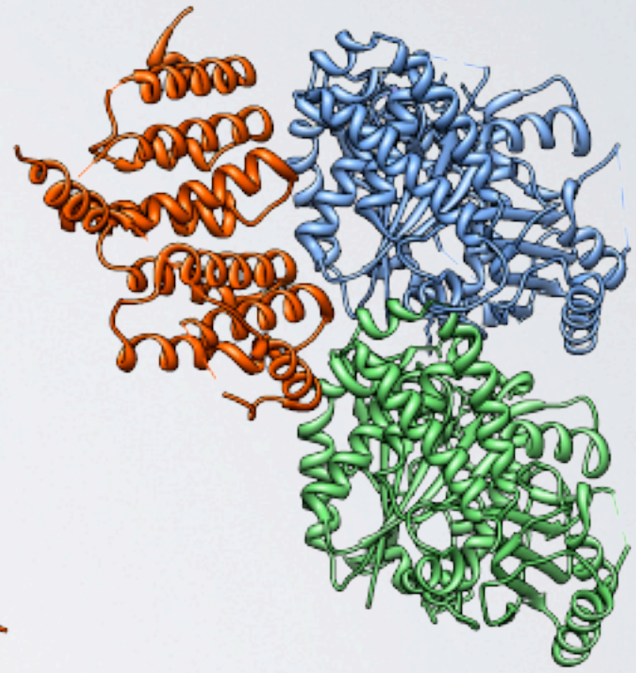
Electron
Crystallography
(1TUB,1JFF,1TVK)
Zinc-induced antiparallel
tubulin sheets +
stabilizing ligand



X-ray
Crystallography
(1FFX,1SA0,1Z2B,
3DU7,3HKB,3N2G,3RYC,
4F61,4UT5)
Polymers bound to
stathmin-like domains

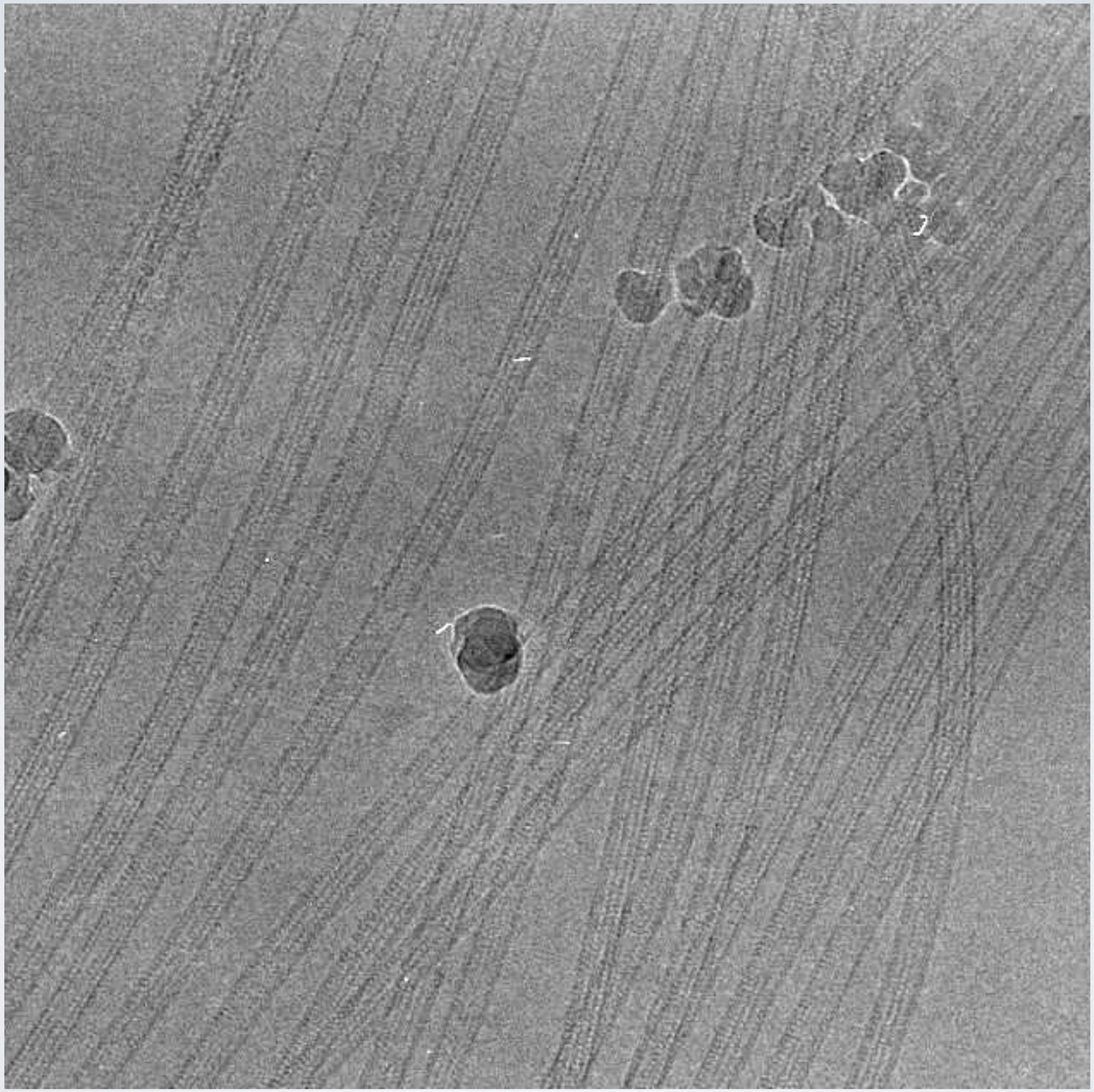


X-ray
Crystallography
(4DRX,4F6R)
DARPin-bound dimer

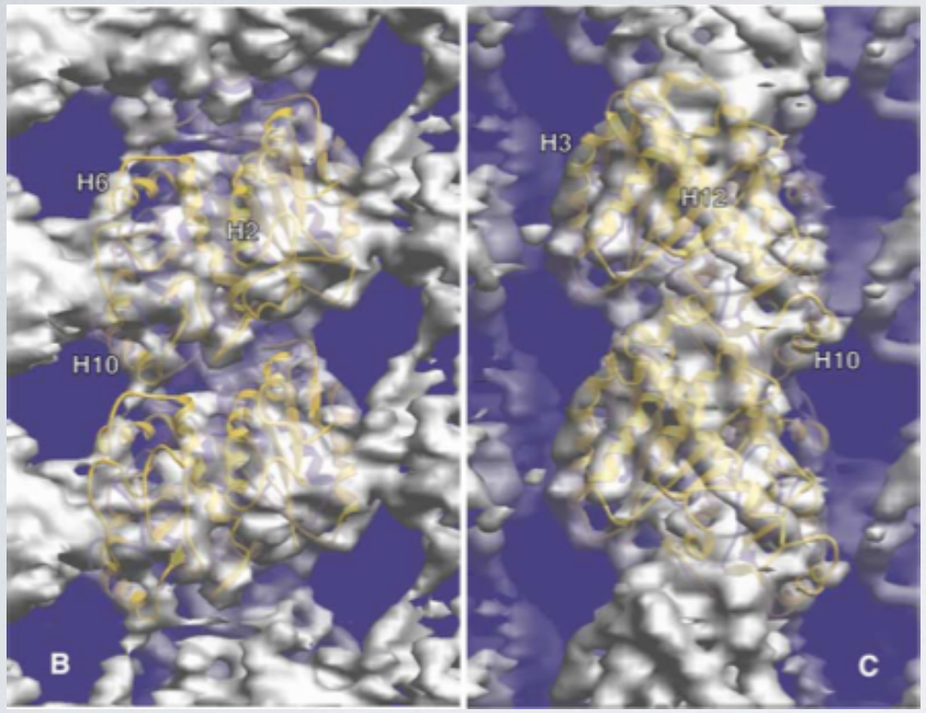


X-ray
Crystallography
(4FFB)
TOG-bound dimer

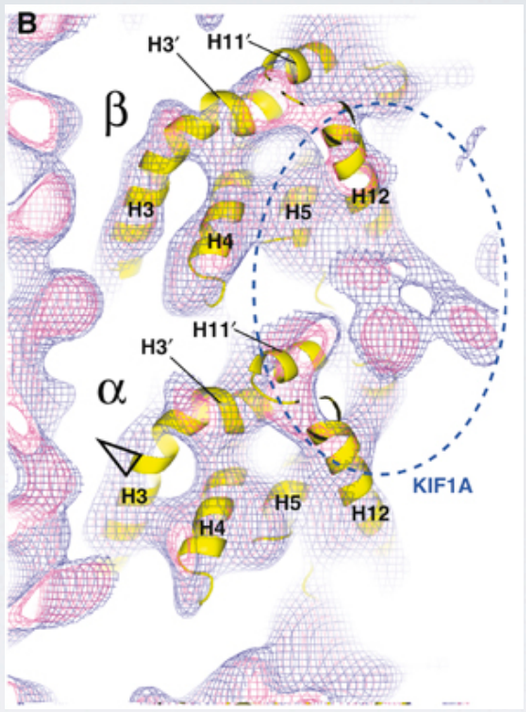
CryoEM of Microtubules



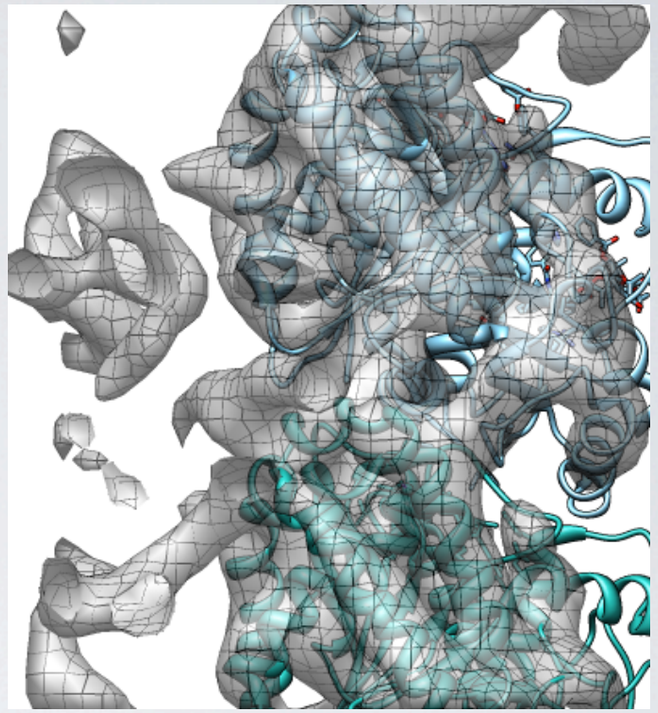
Subnanometer-Resolution CryoEM Structures



Li *et al*. Structure 2002 (9Å resolution)

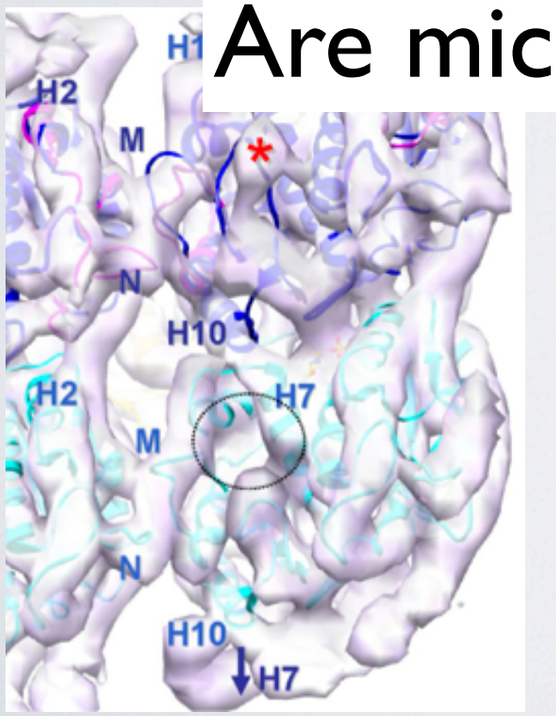


Kikkawa and Hirokawa
EMBO J 2006 (9.5Å resolution)

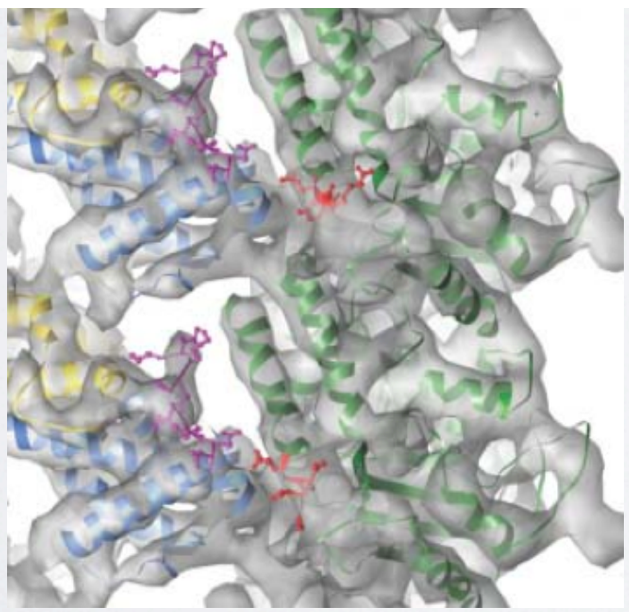


Sindelar and Downing
PNAS 2010 (8.5Å resolution)

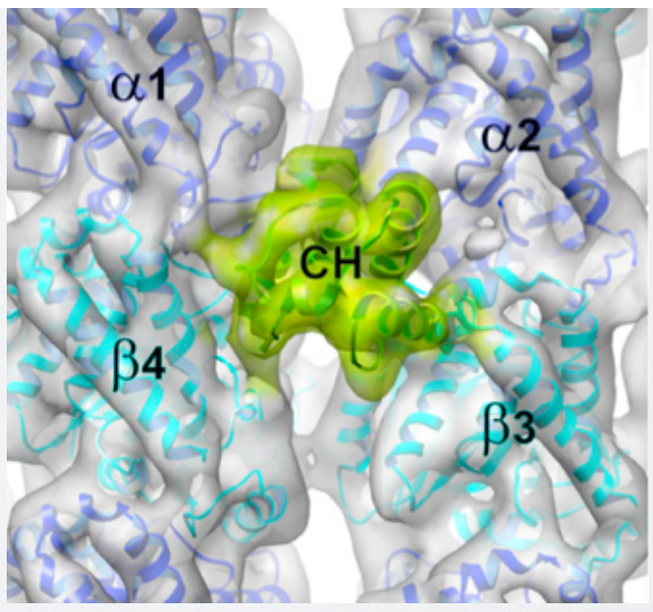
Are microtubules only ordered to 8Å resolution?



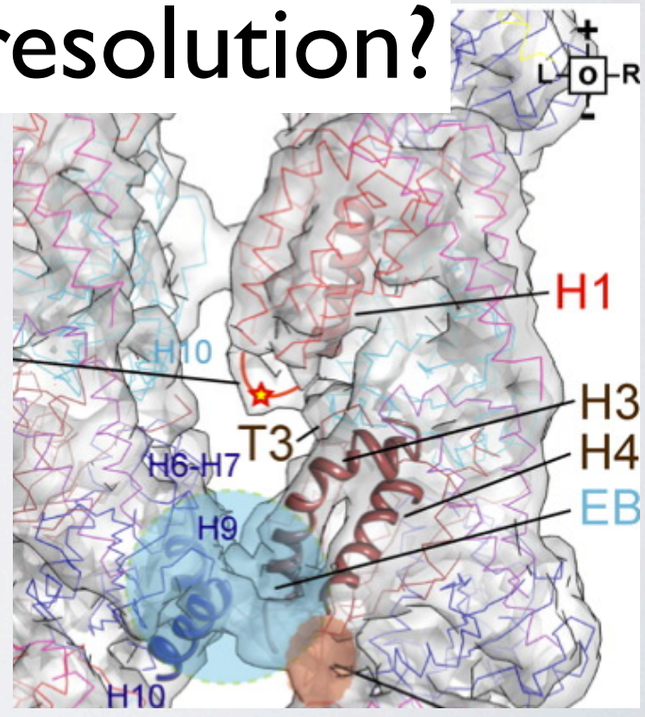
Fourniol *et al.* JCB 2010
(8Å resolution)



Alushin *et al*. Nature 2010
(8.6Å resolution)

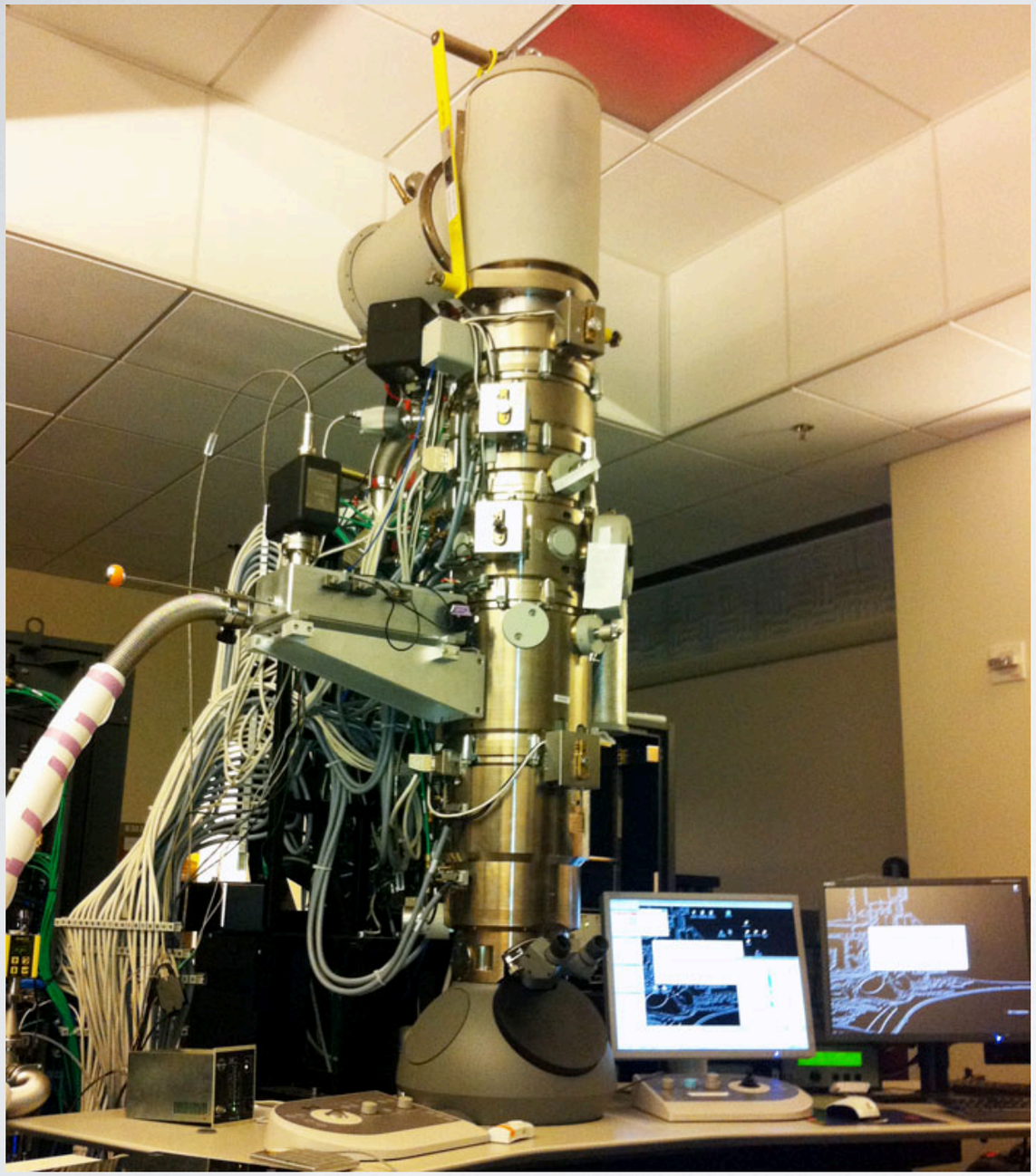


Maurer *et al*. Cell 2012
(8Å resolution)



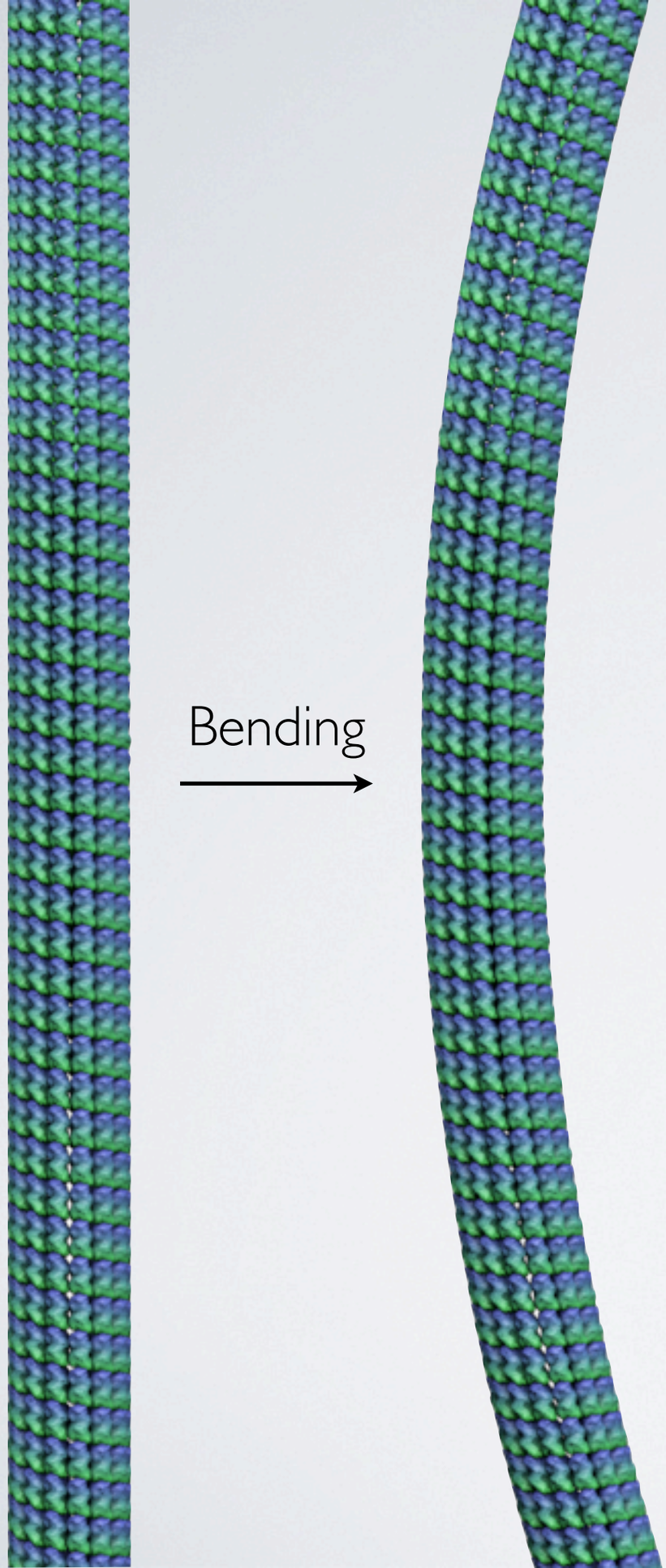
Yajima *et al*. JCB 2012
(9Å resolution)

FEI Titan EM (aka "The Beast")



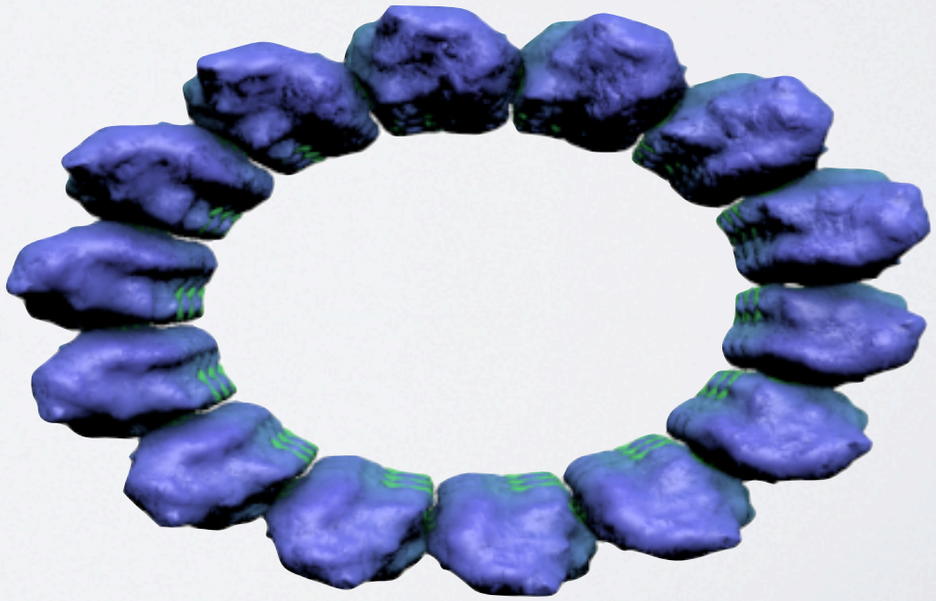
- C3 active, parallel illumination
- 300keV
- 2K CCD, no DD = film collection
- No Leginon = Tecnai Low Dose
- Side-entry holder
- "Weird State" feature!

Microtubule Distortions

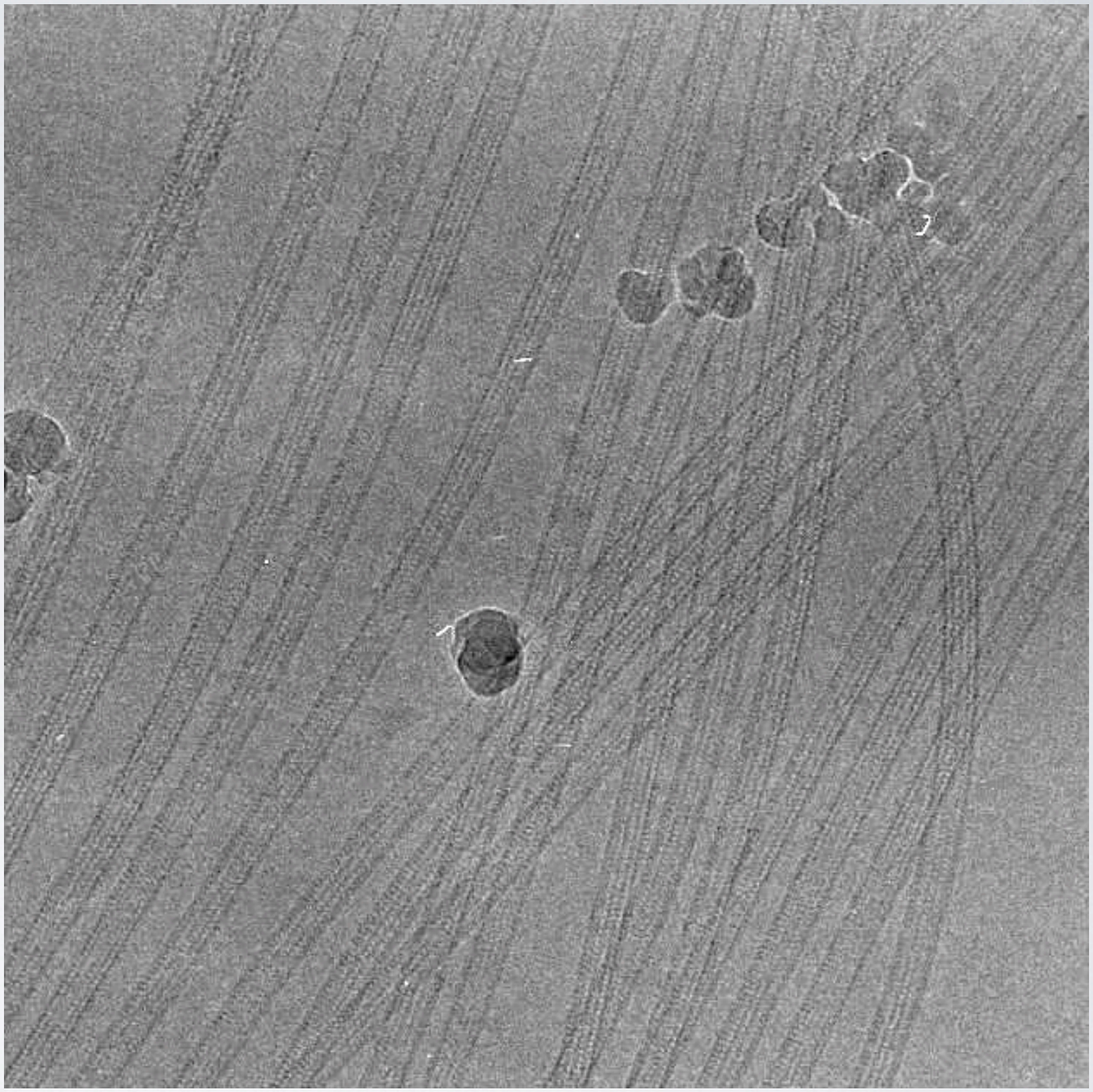


Bending

Flattening

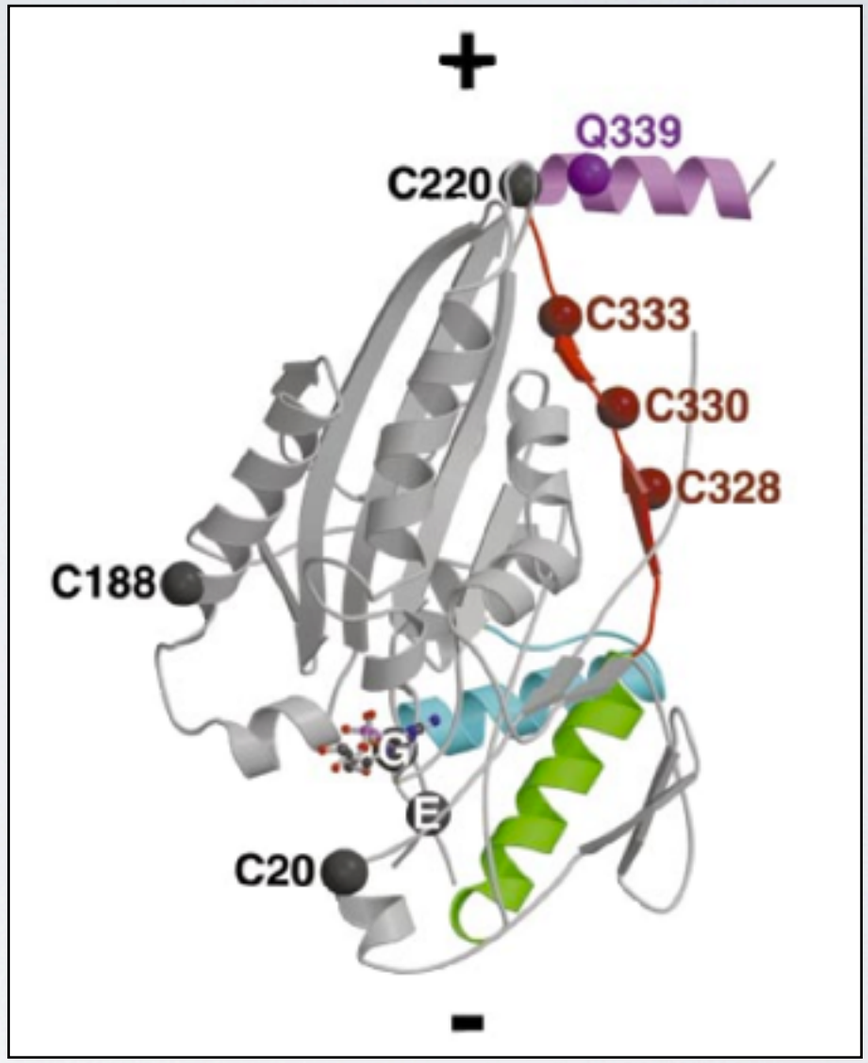


Distinguishing Alpha from Beta



In an EM micrograph, alpha tubulin is indistinguishable from beta tubulin

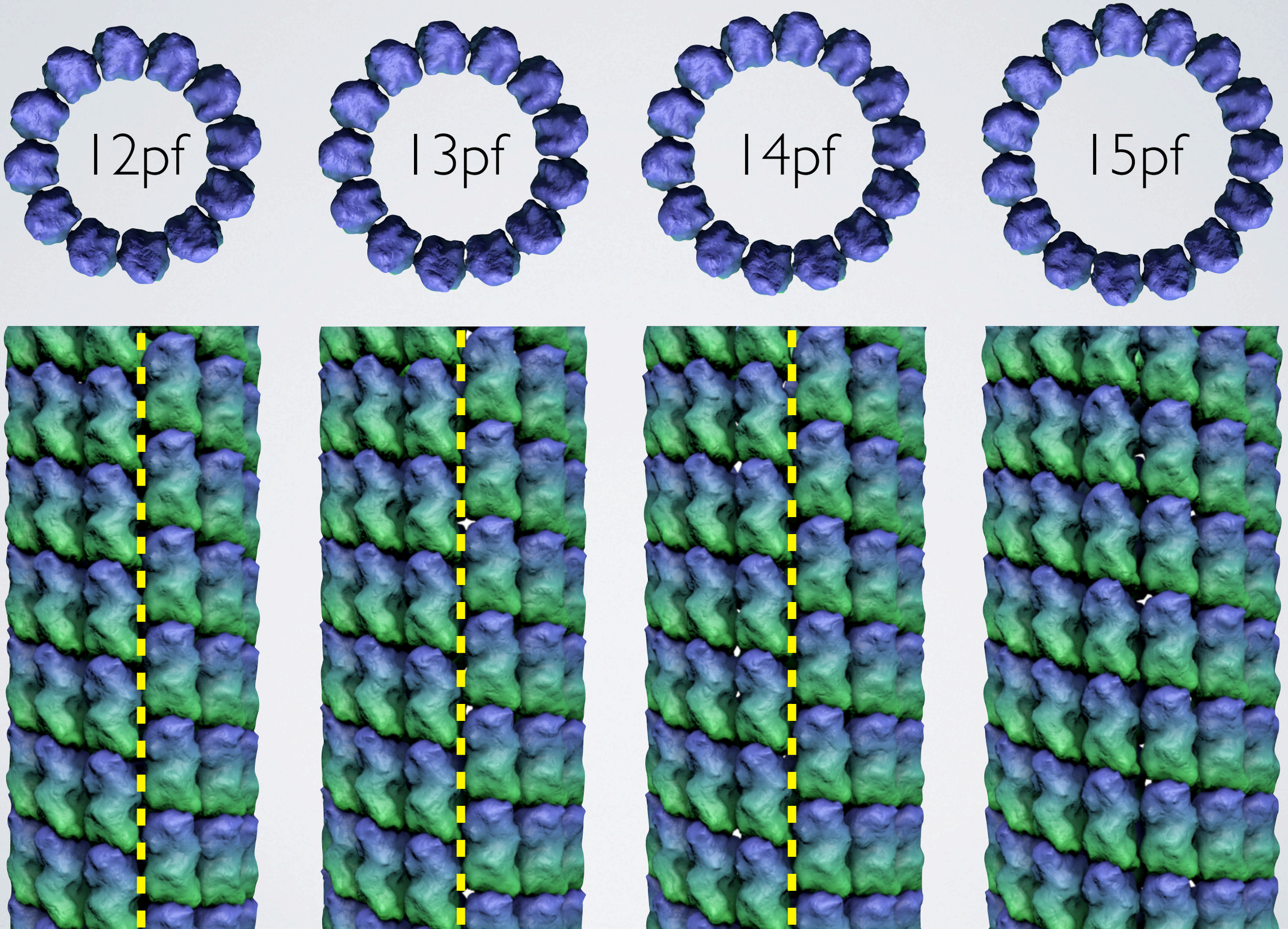
Human Kinesin Monomer

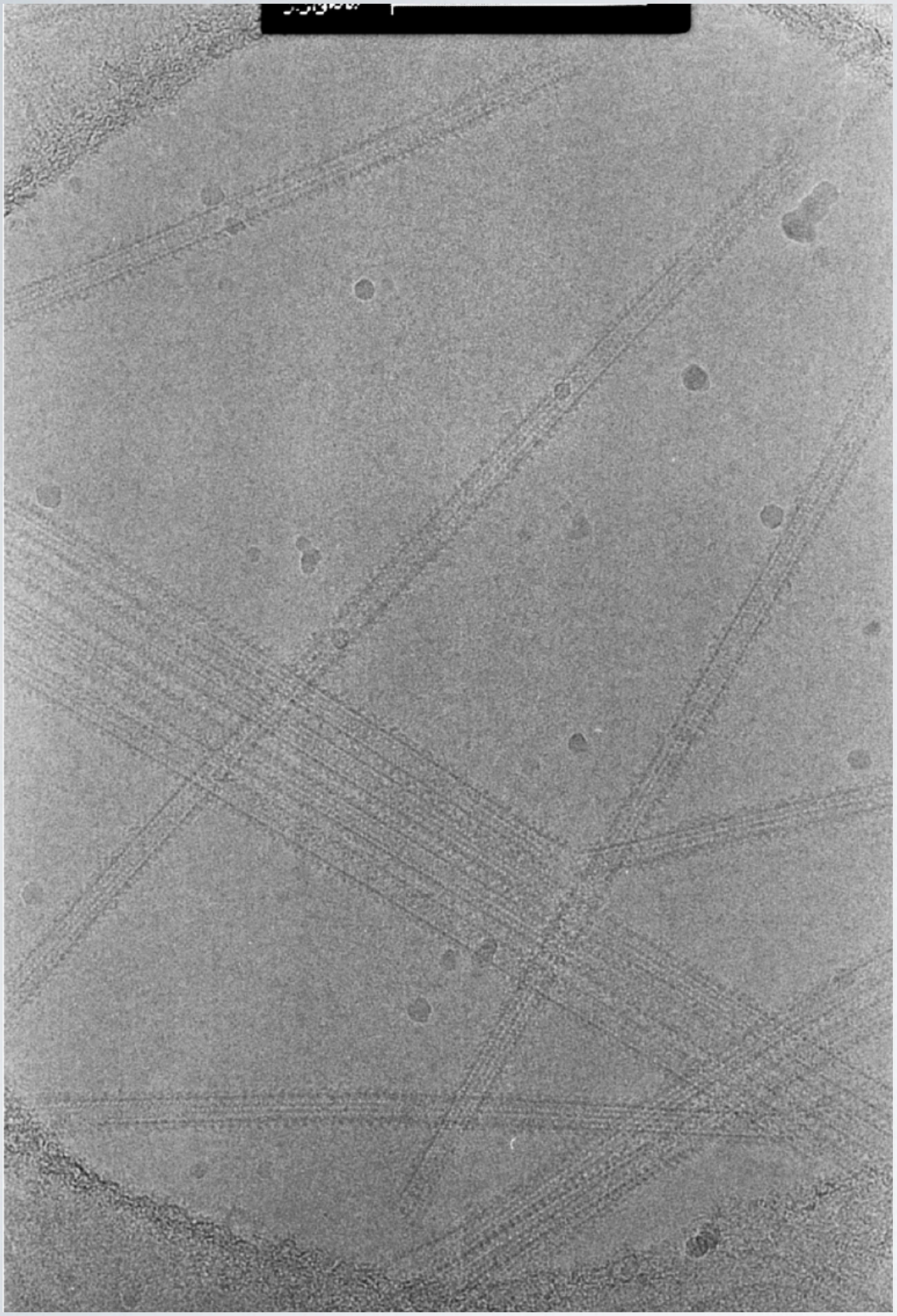


Rice *et al.* Nature, Dec 1999

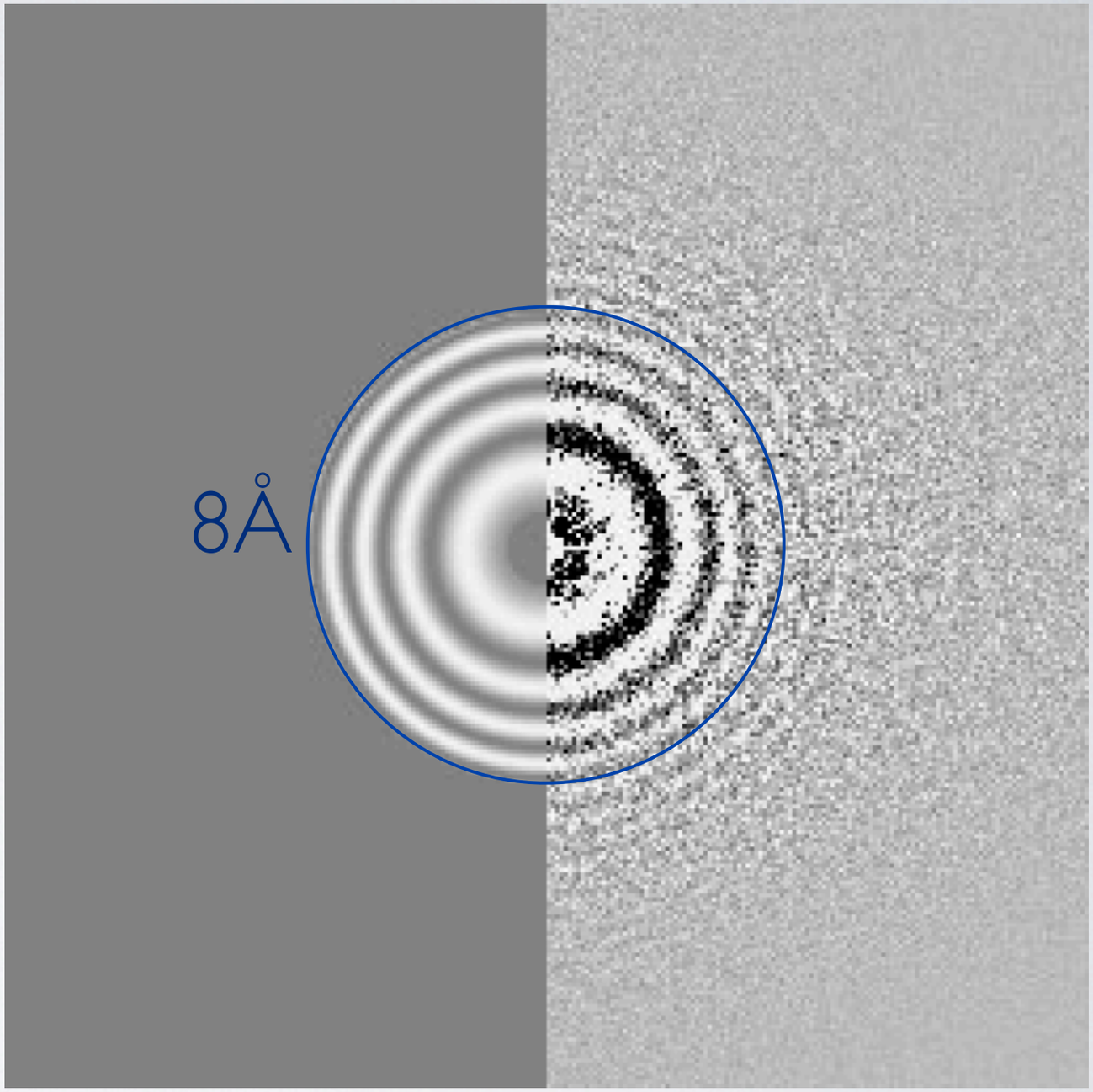
Mutation in switch II region inhibits ATP hydrolysis, stably binds to microtubules (plasmid from Vale lab, UCSF)

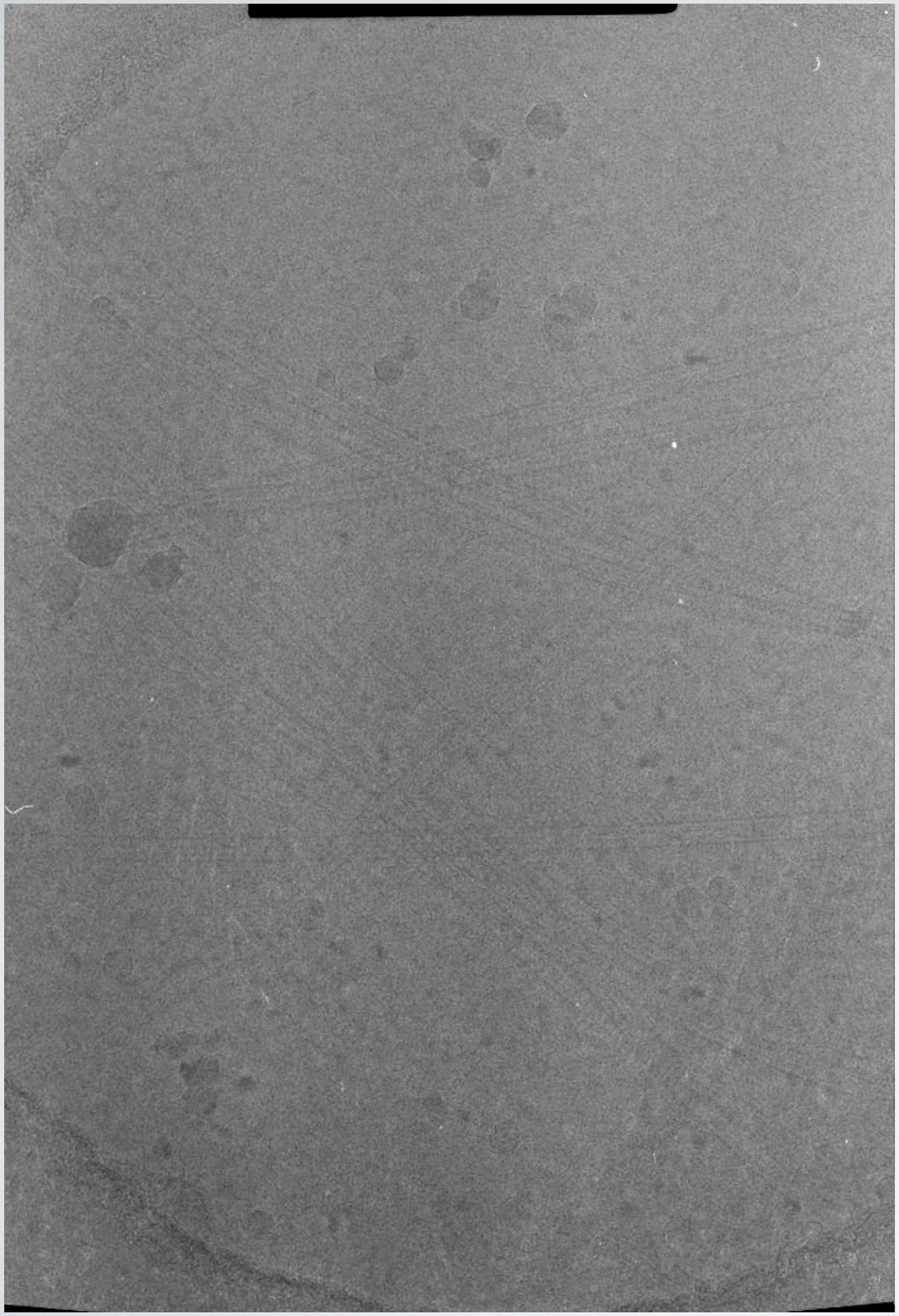
Heterogeneous Protofilament Symmetries & Seam



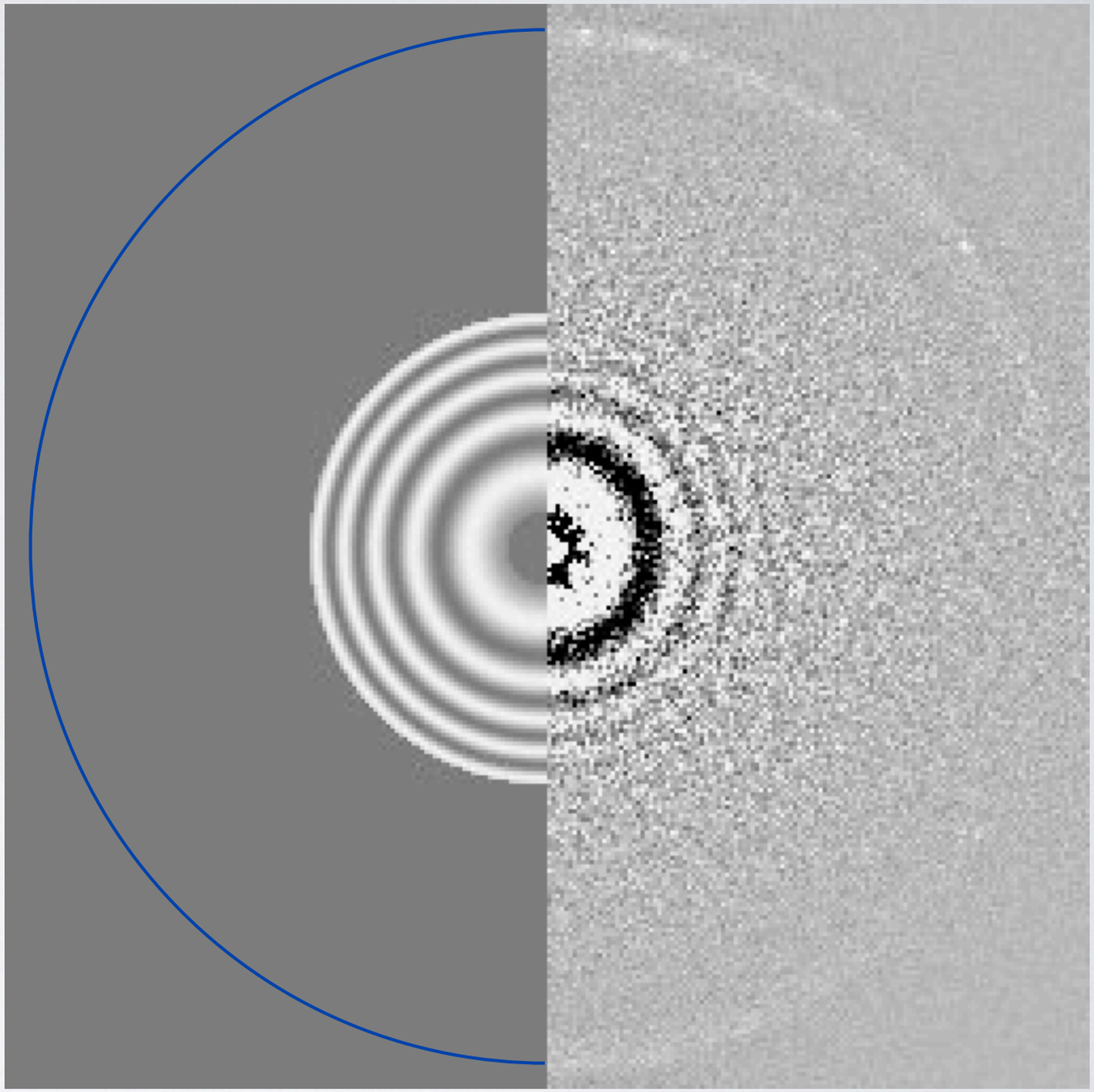


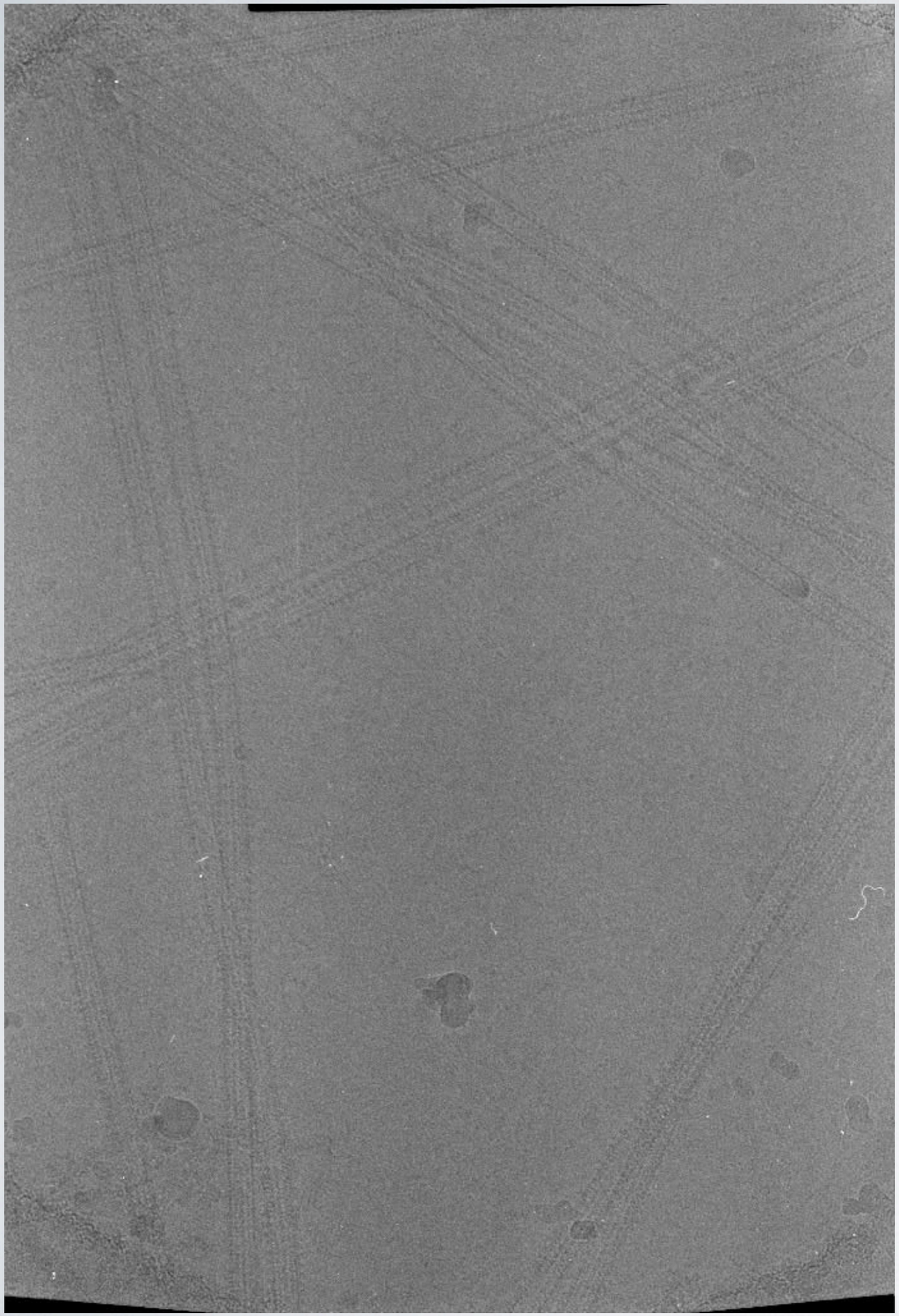
72000X (0.87Å/pixel)
25e^{-}/Å^{2}



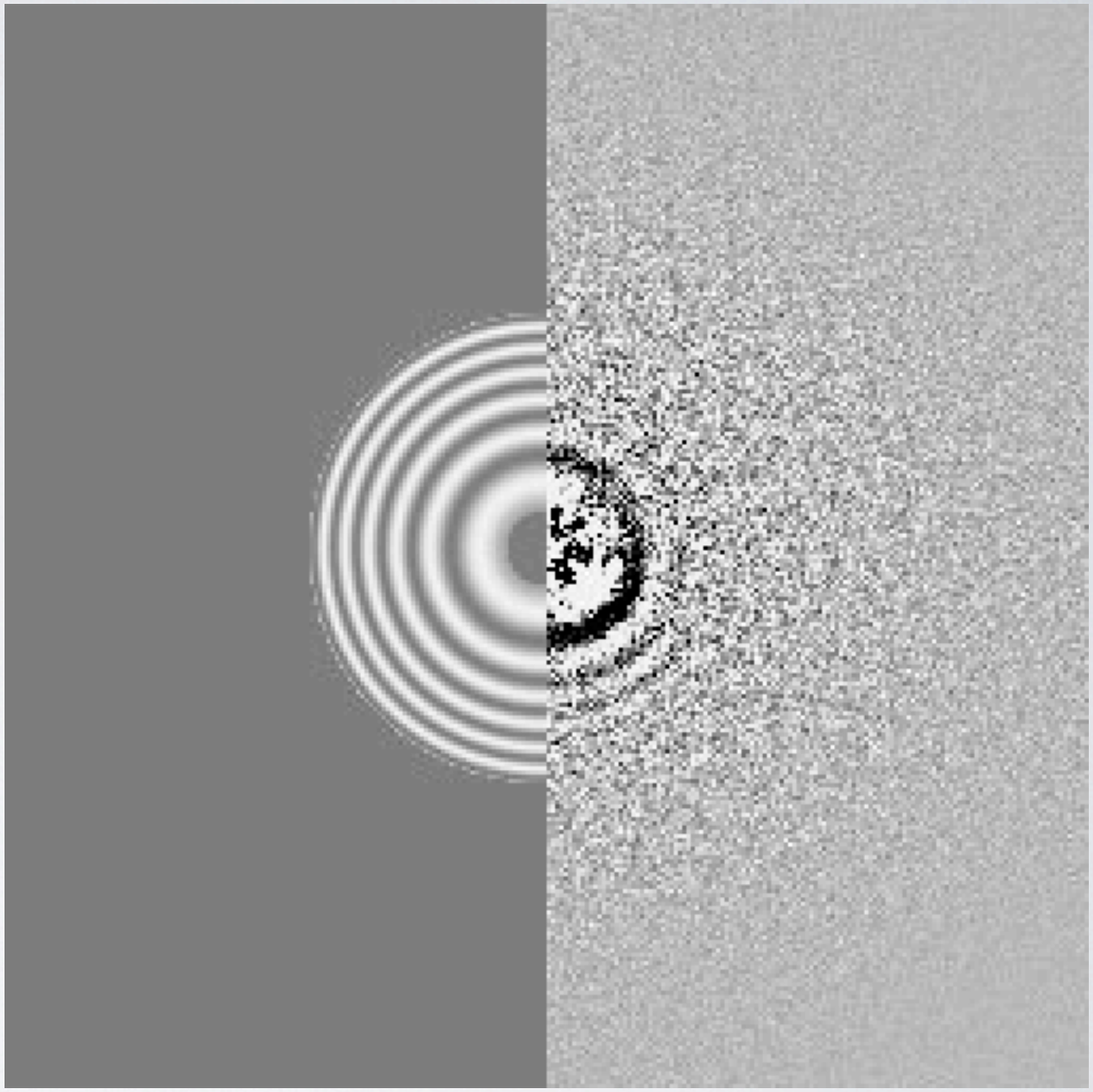


Ice ring at ~3.6Å

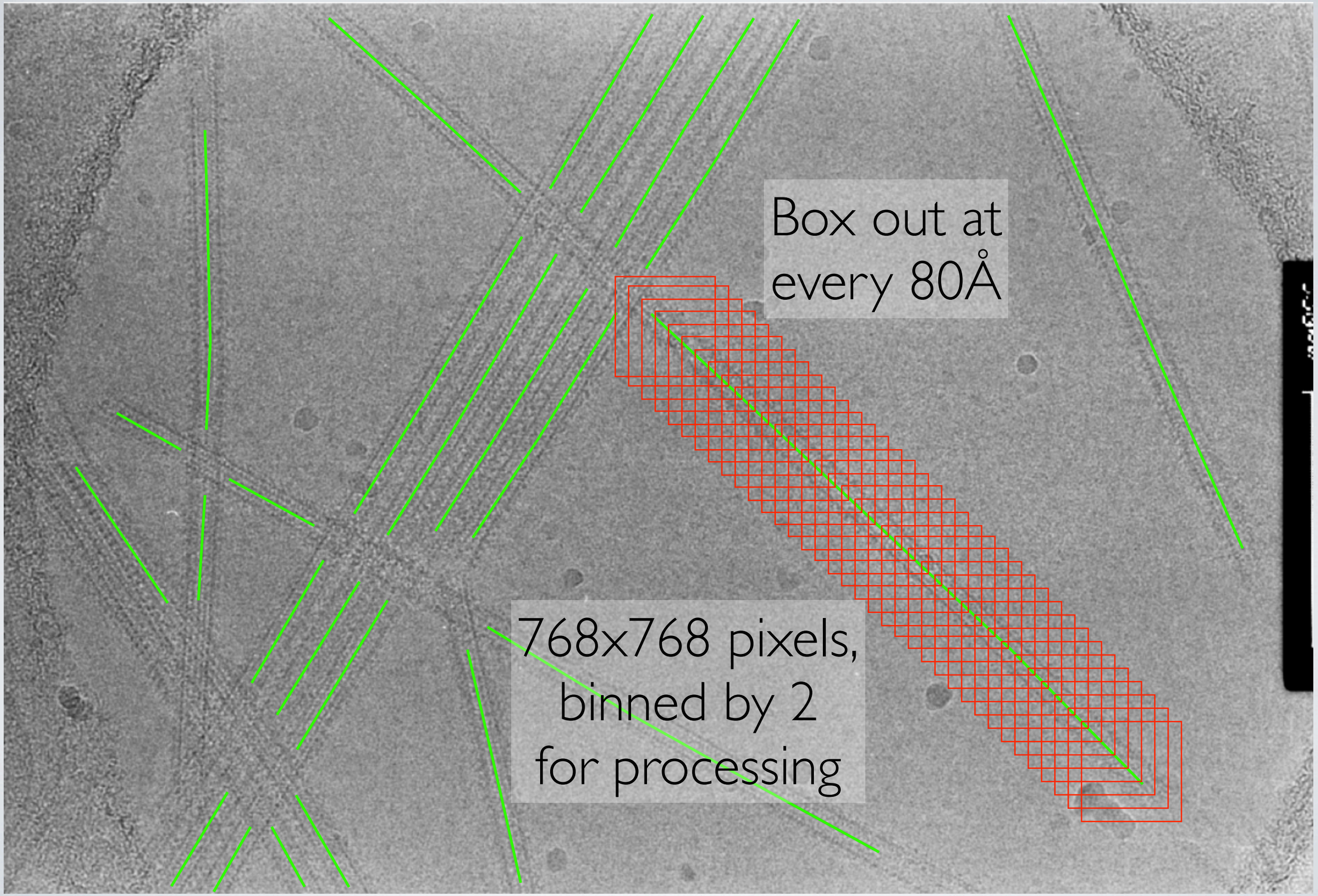


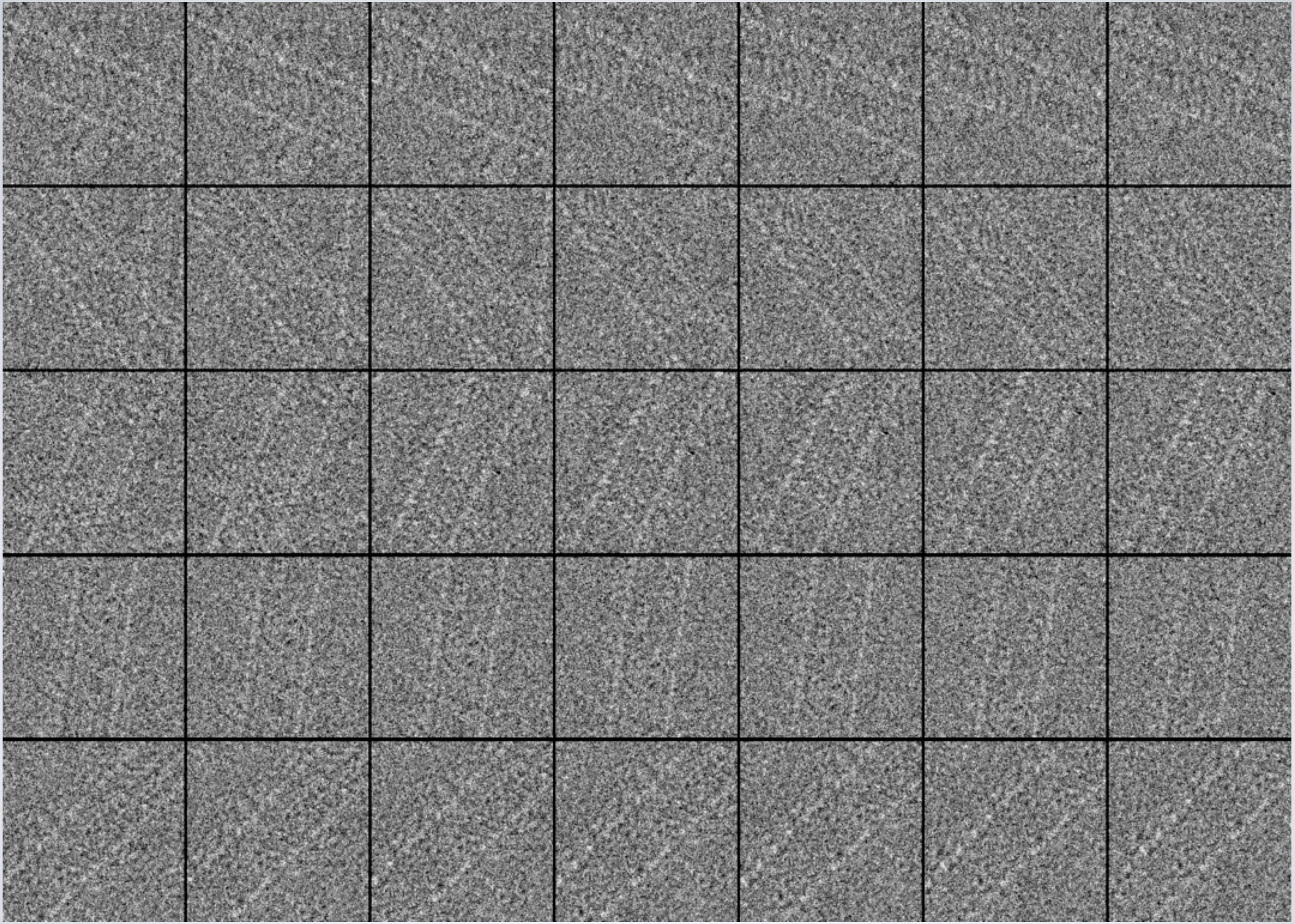


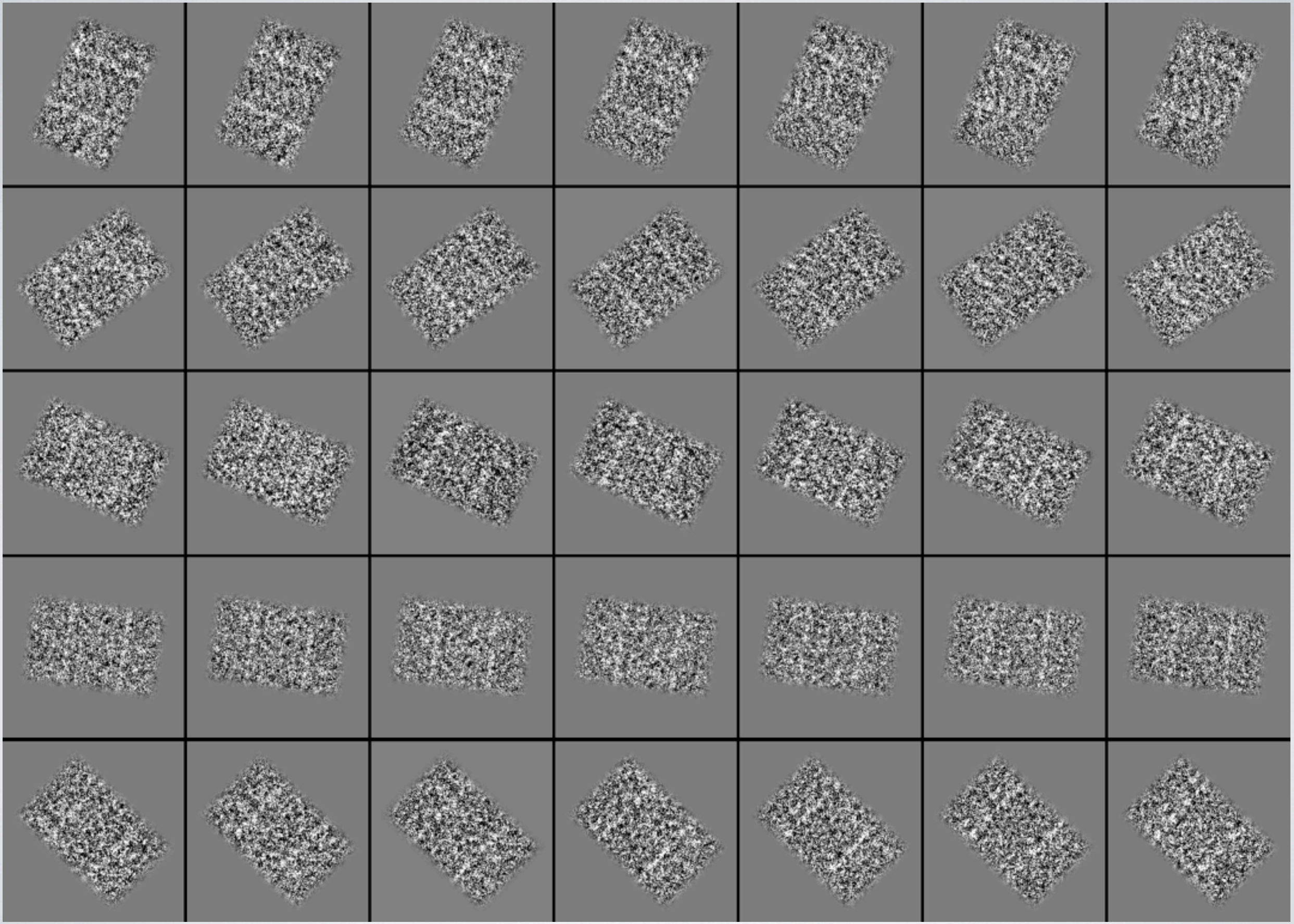
Remove images with drift/ beam induced motion



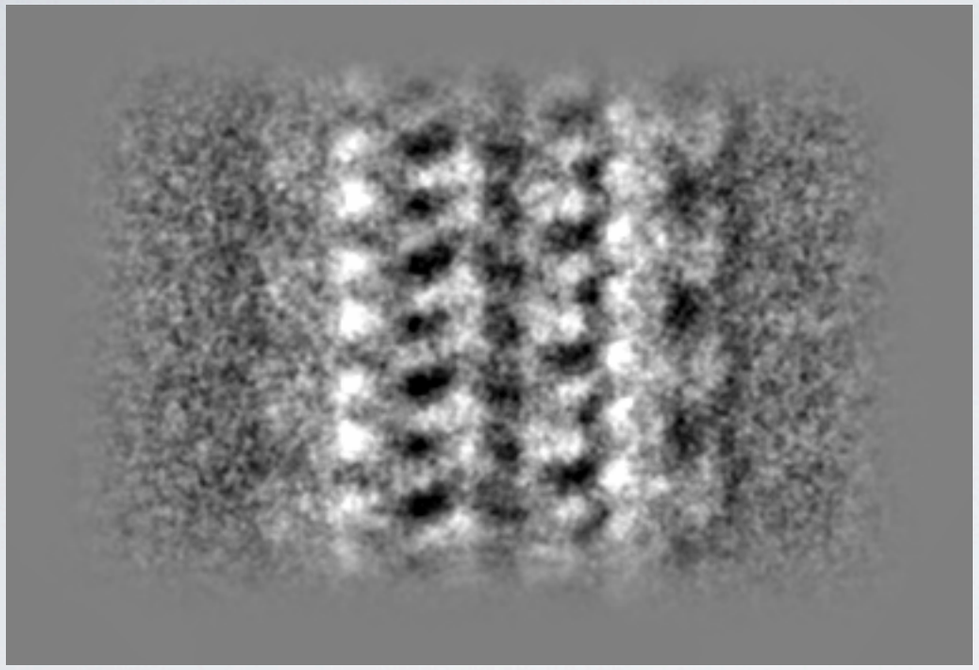
Pick MT Filaments

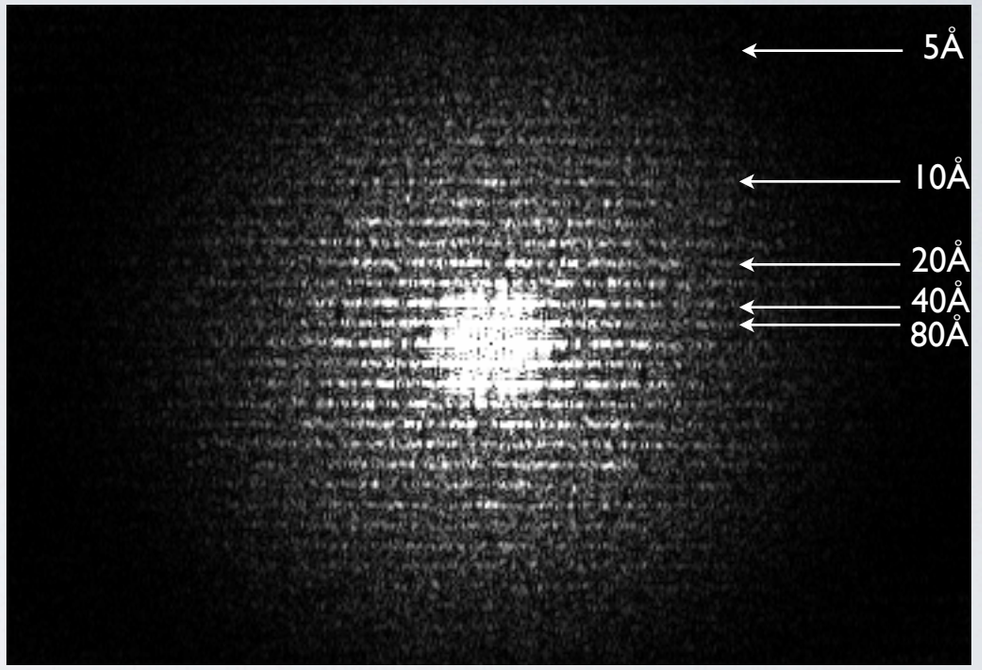




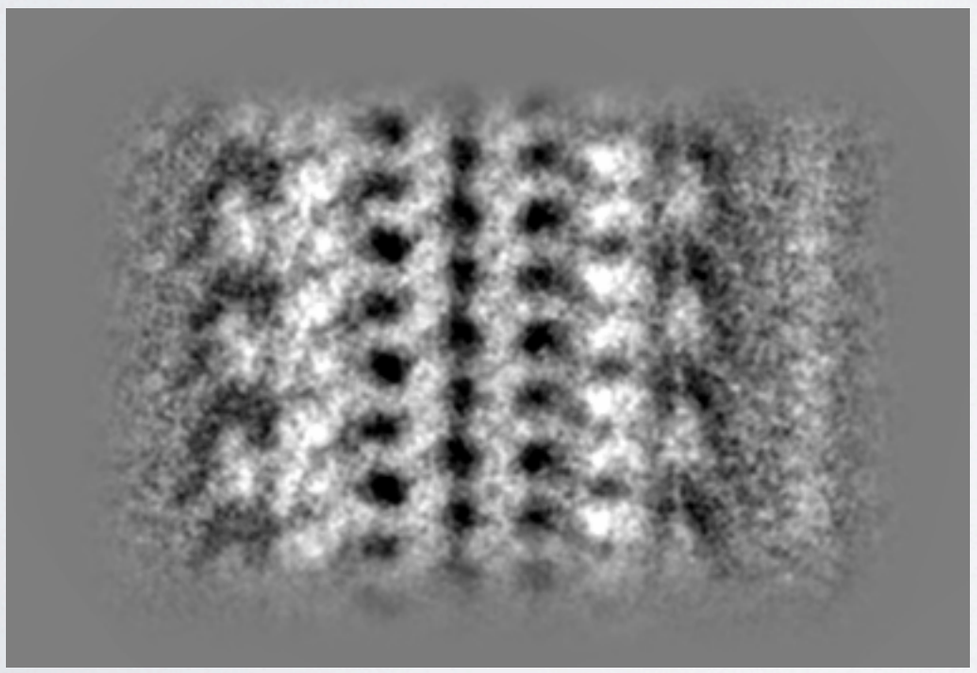


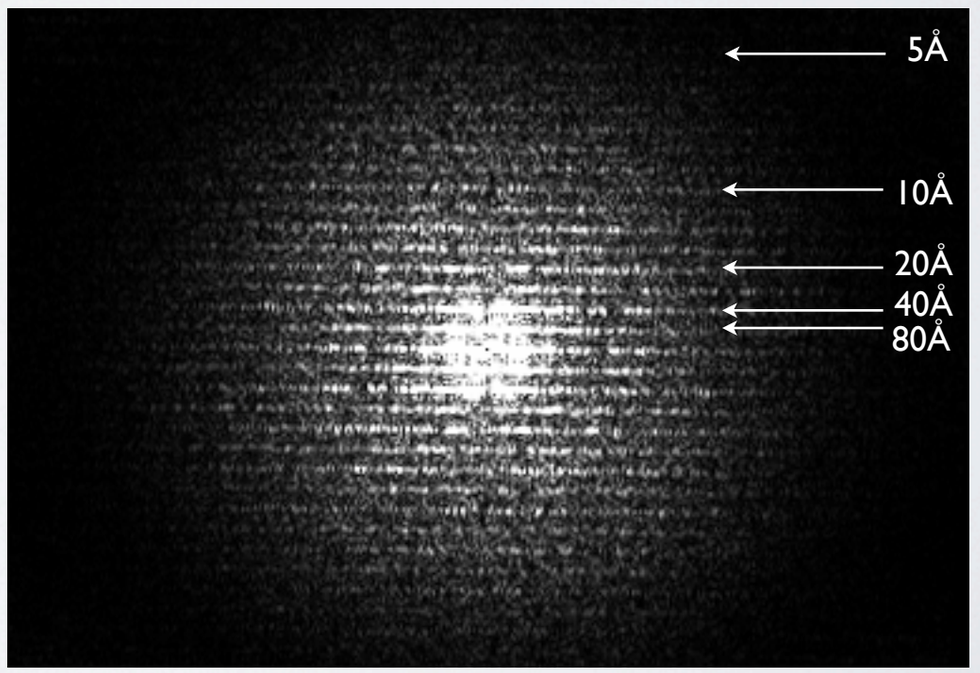
2D classification (IMAGIC MSA/MRA)



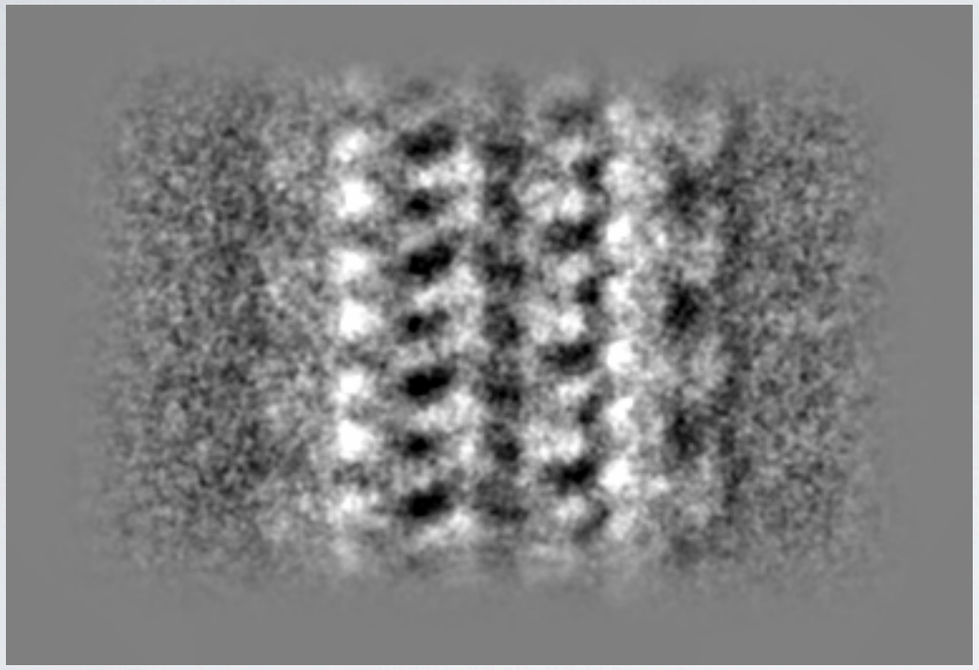


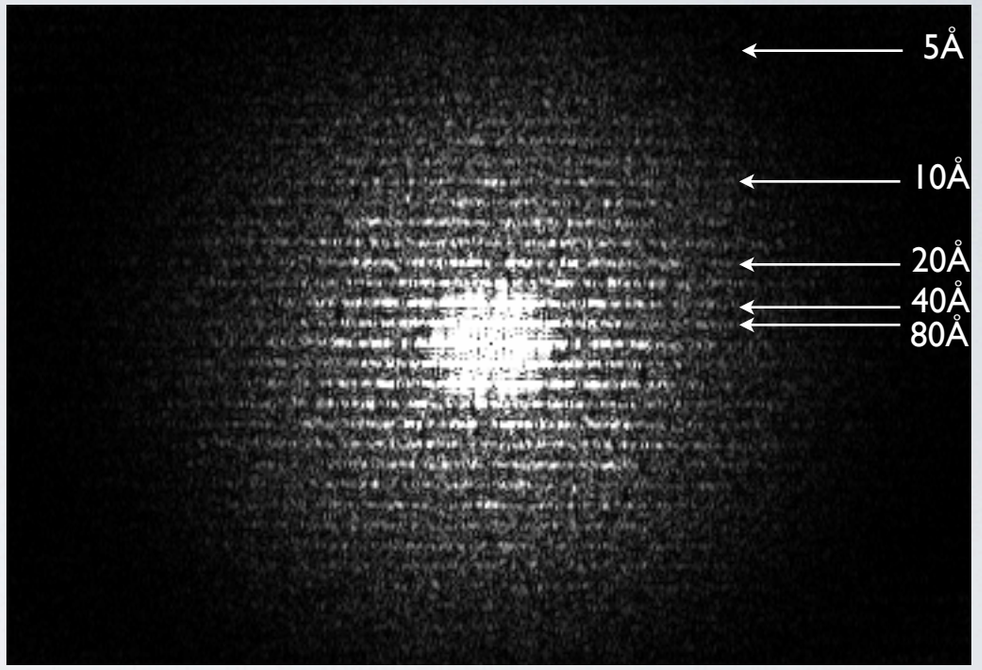
Layer lines visible out to ~5A resolution



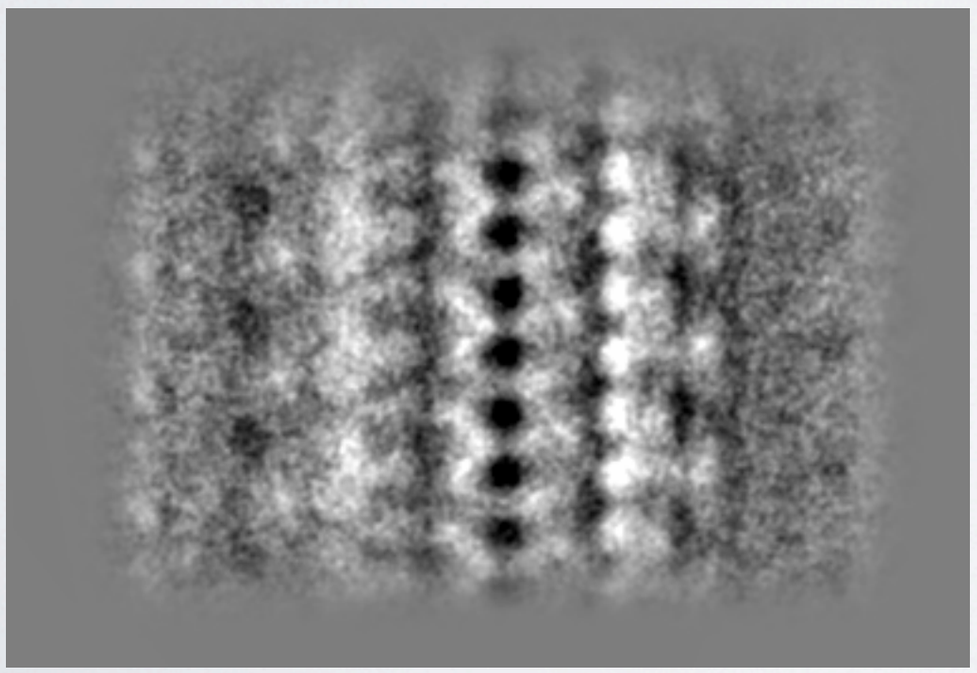


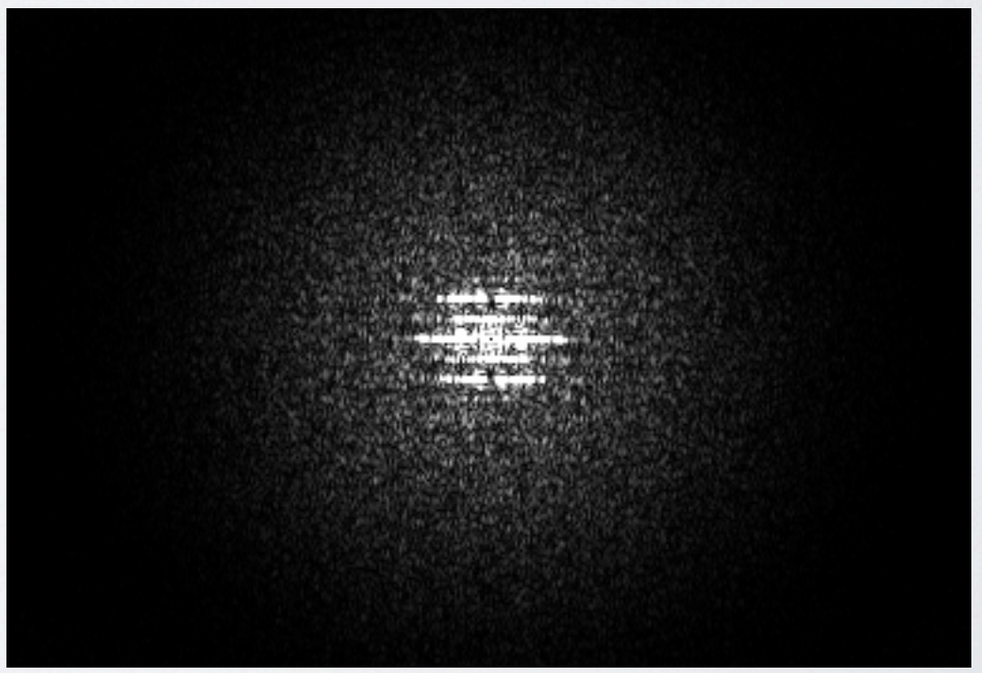
2D classification (IMAGIC MSA/MRA)



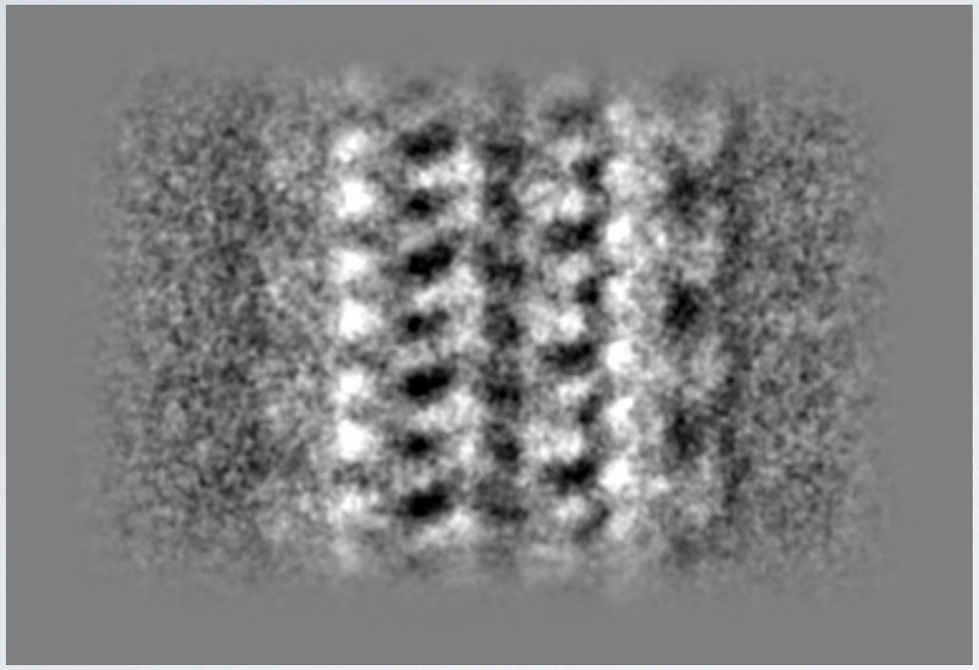


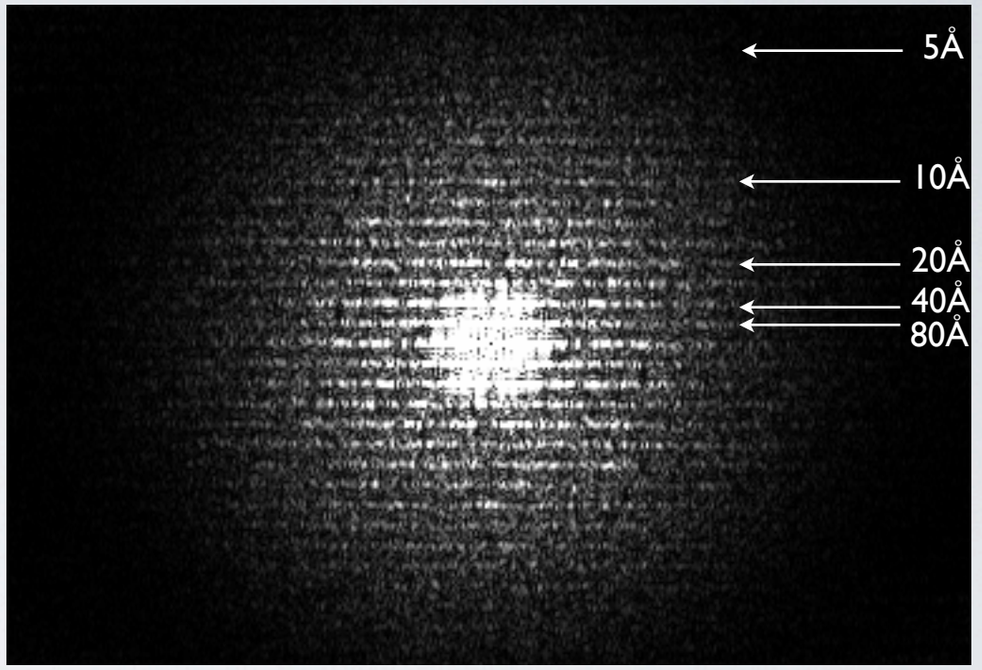
Remove low resolution particles



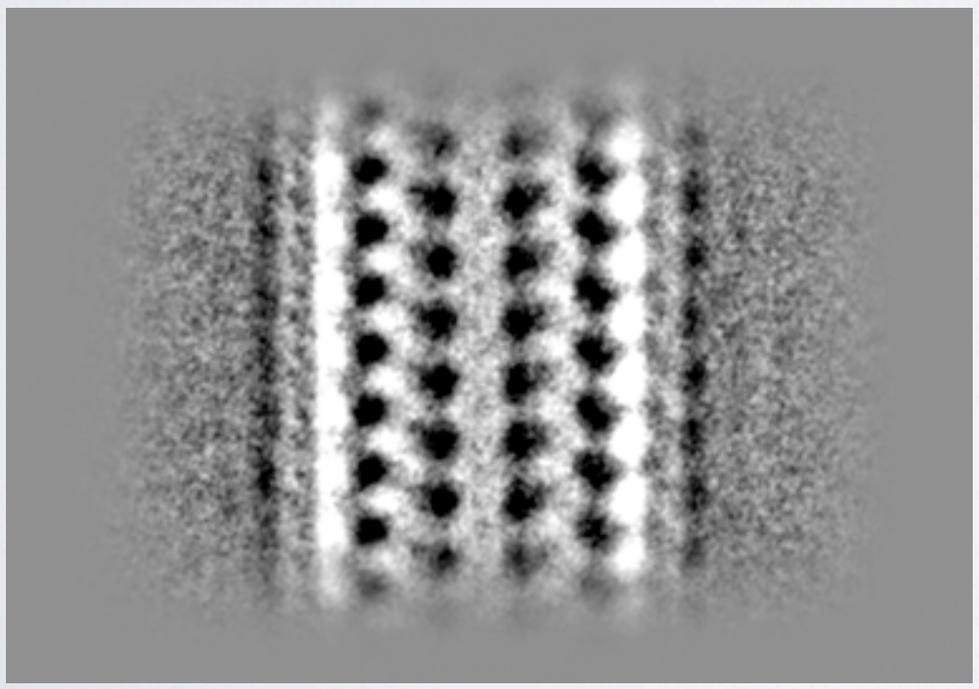


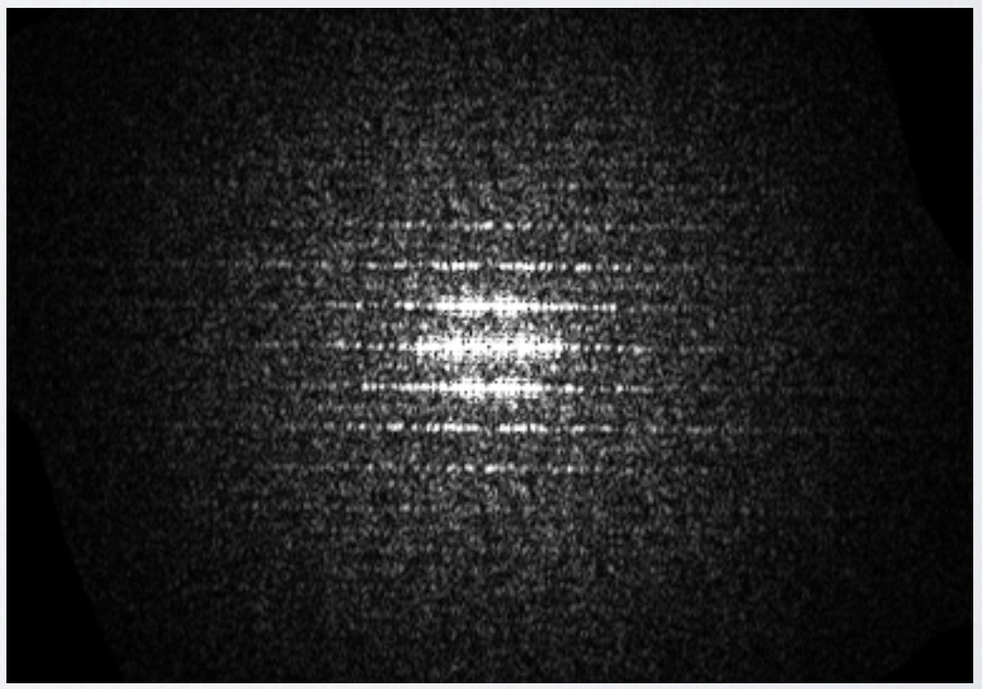
2D classification (IMAGIC MSA/MRA)



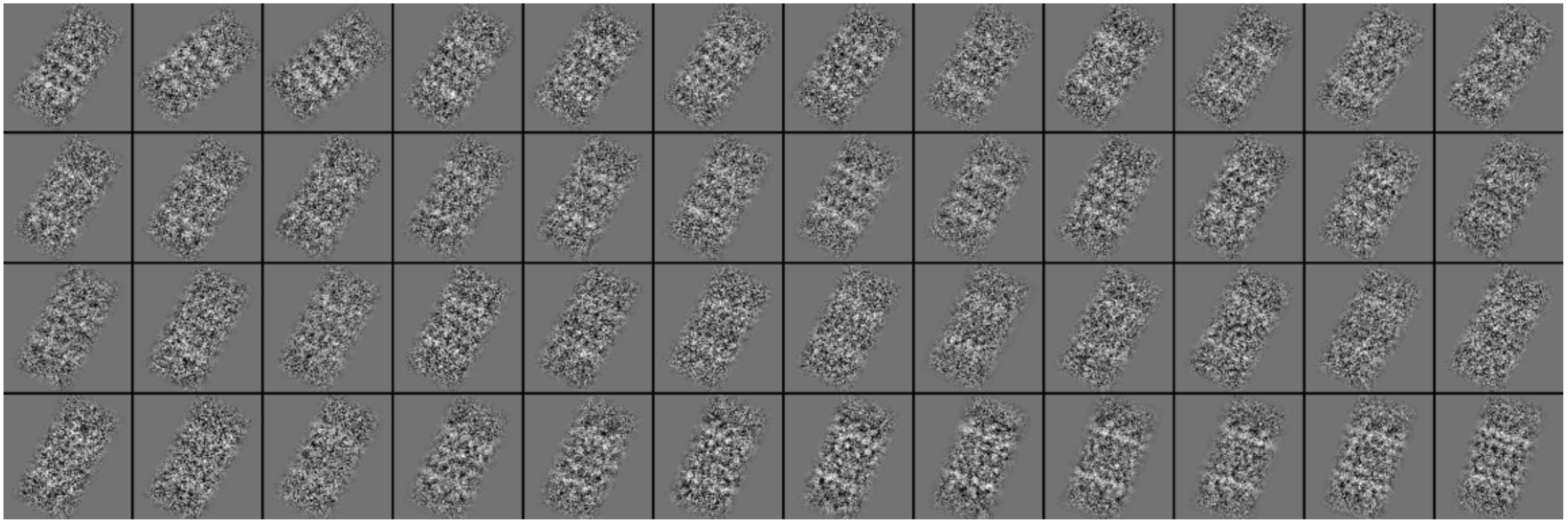


Remove particles missing kinesin, also 12pf & 15pf



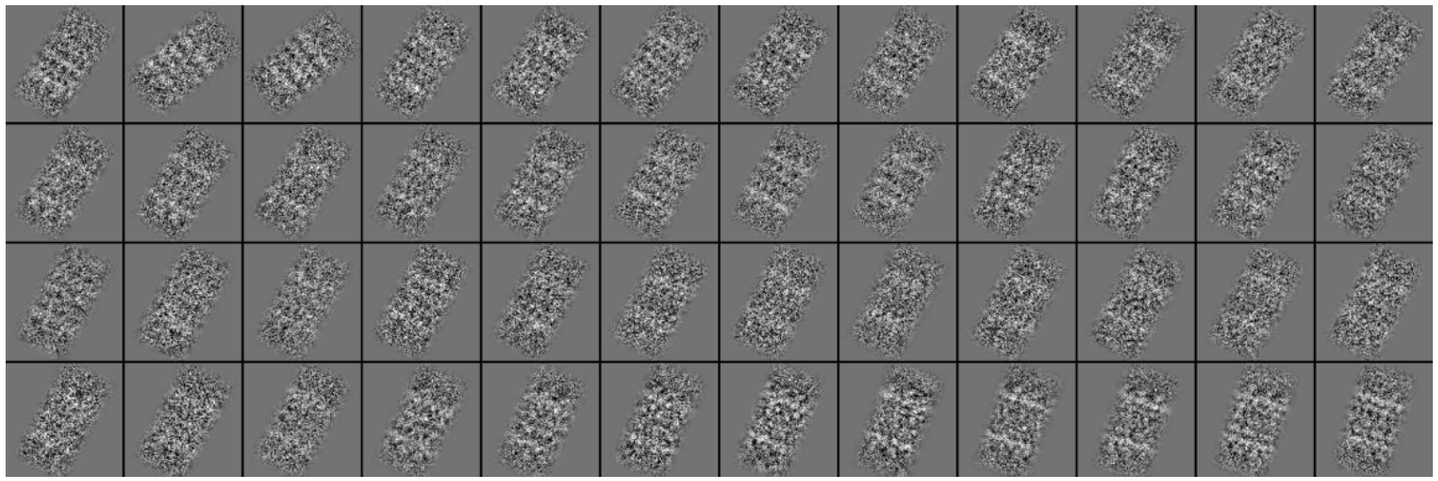


Refinement Scheme (EMAN2/SPARX Libs)

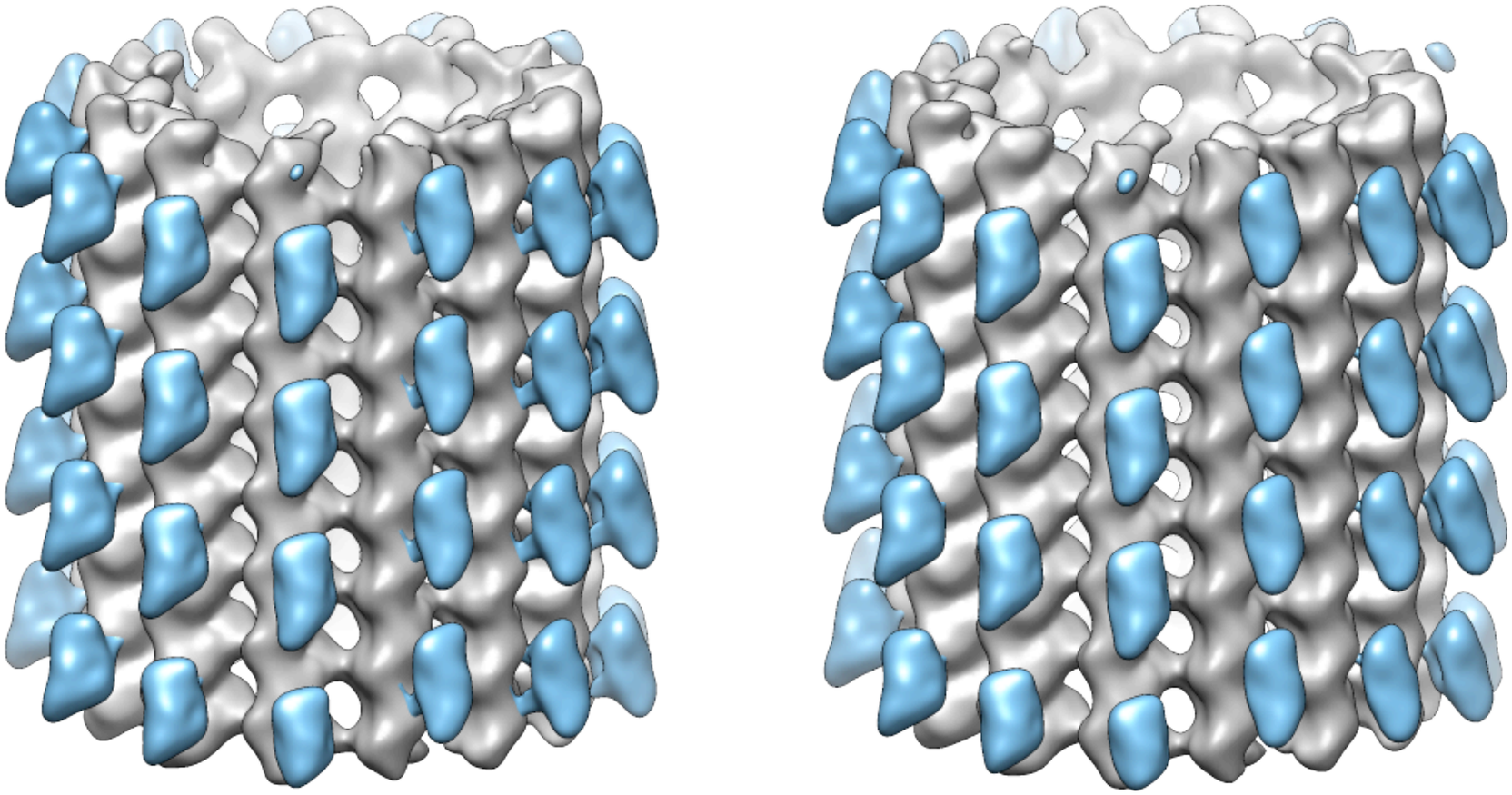


Masked particle
segments with mixed
protofilament numbers
(13 & 14pfs)

Refinement Scheme (EMAN2/SPARX Libs)

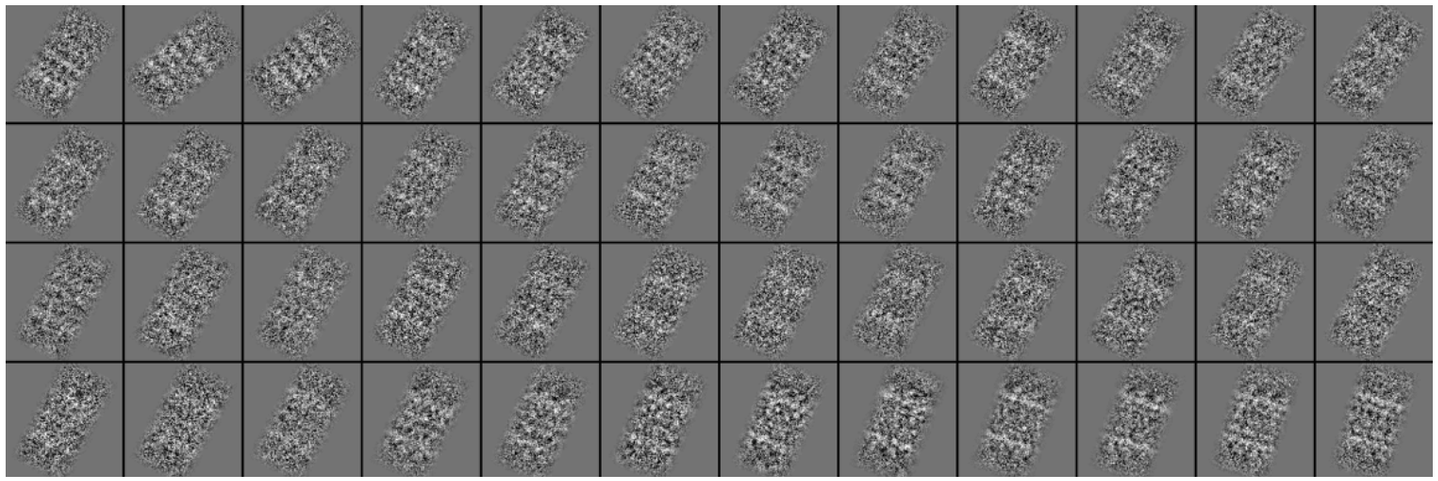


Masked particle segments with mixed protofilament numbers (13 & 14pfs)

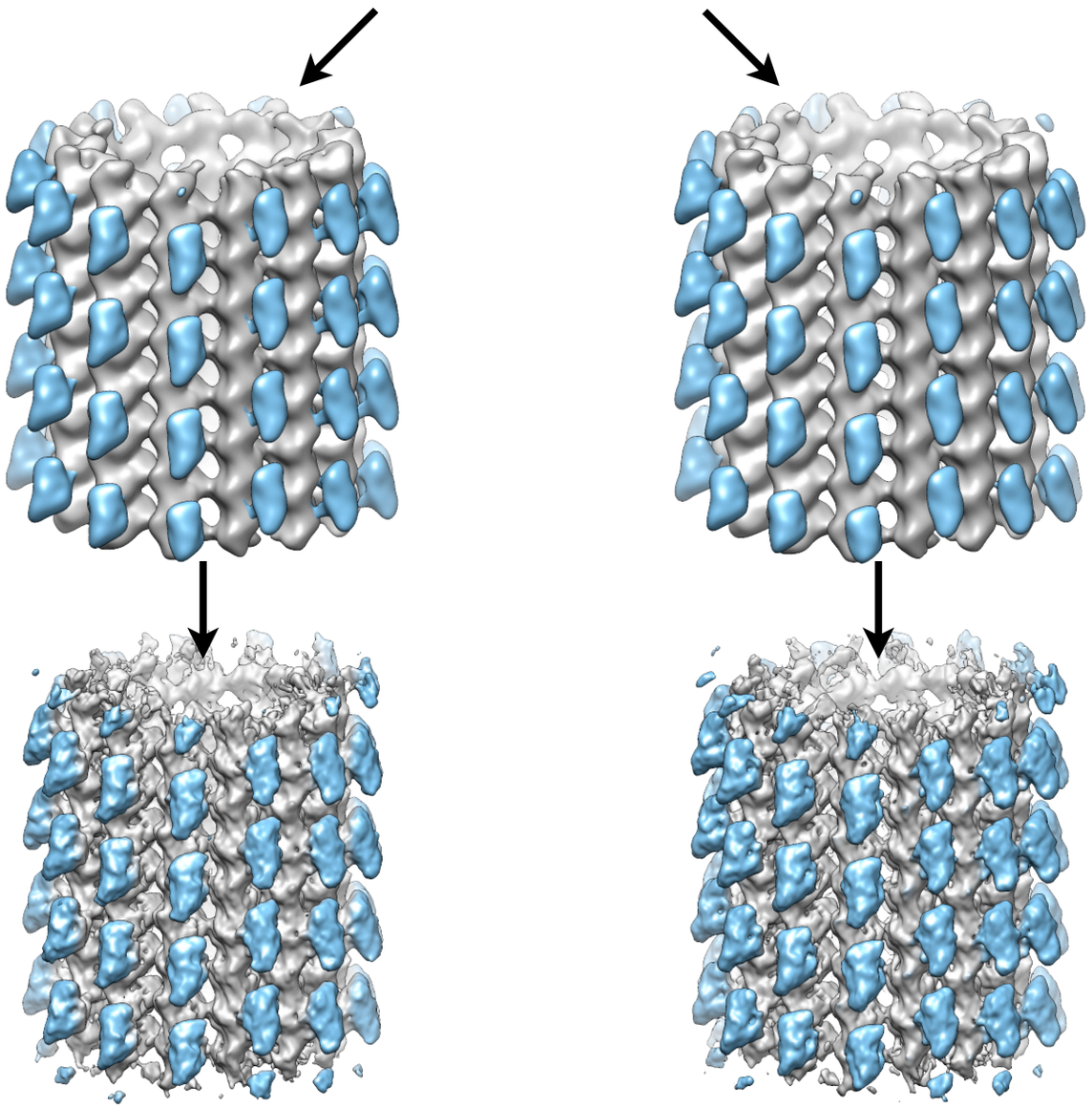


13 & 14pf initial models

Refinement Scheme (EMAN2/SPARX Libs)



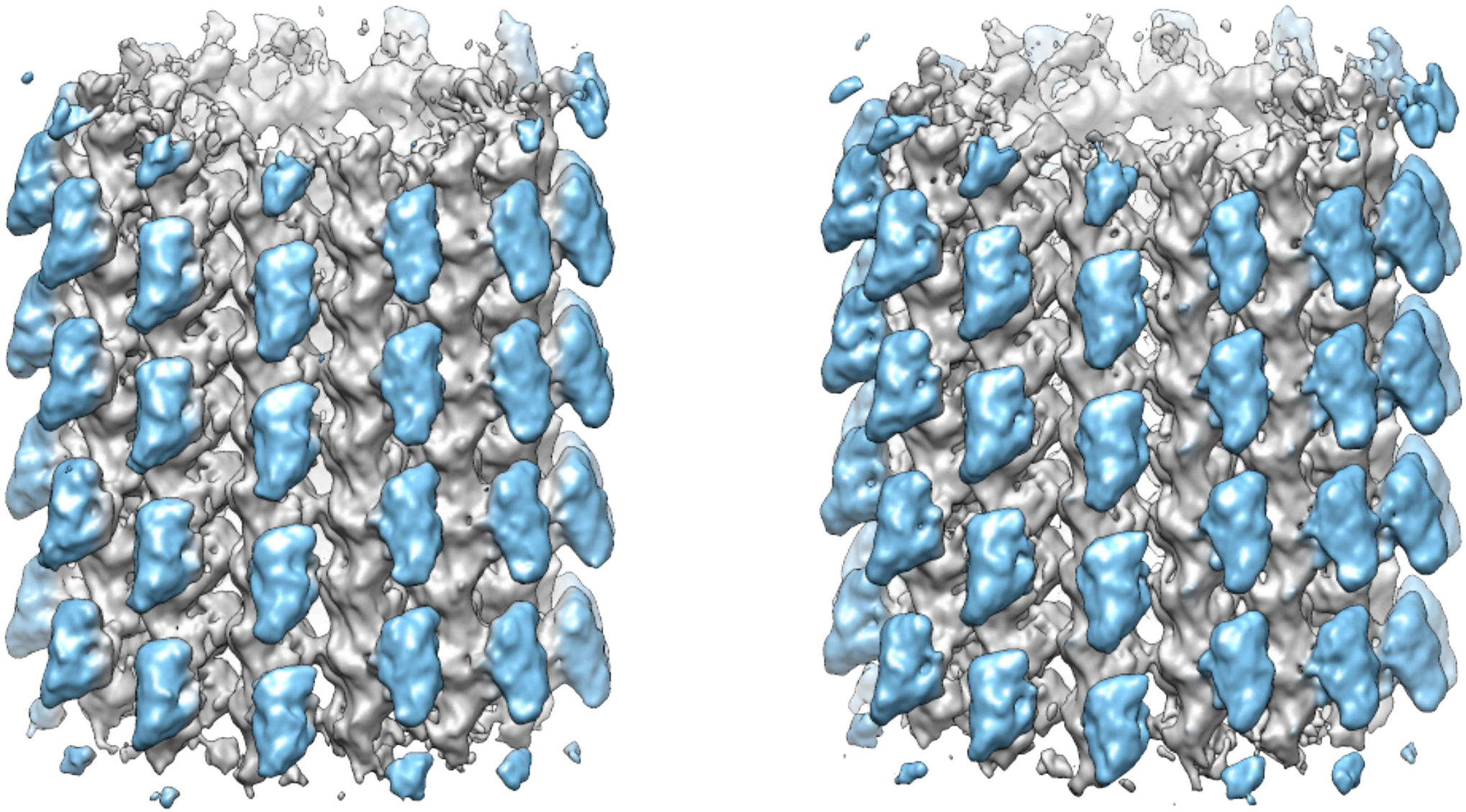
Masked particle segments with mixed protofilament numbers (13 & 14pfs)



Particles are sorted by multi-model projection matching using 13pf and 14pf models

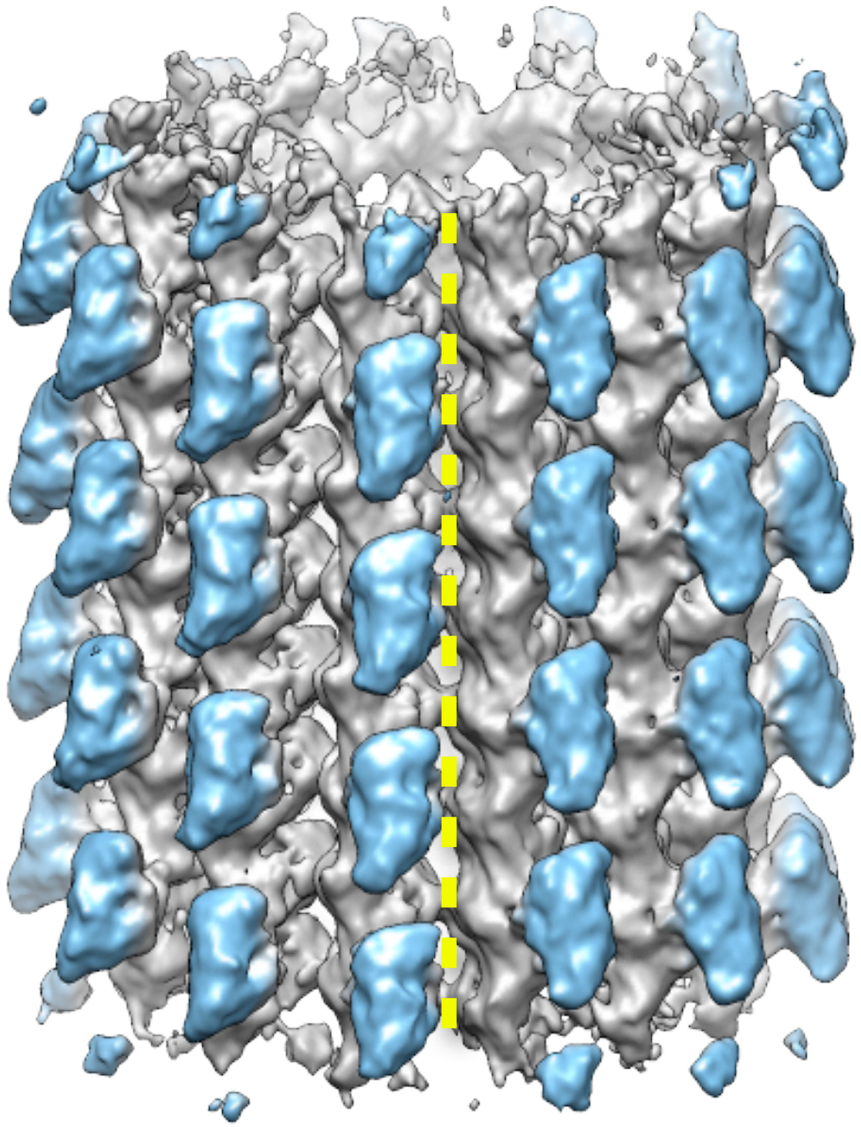
Asymmetric back projection of each pf symmetry

Low-resolution asymmetric densities

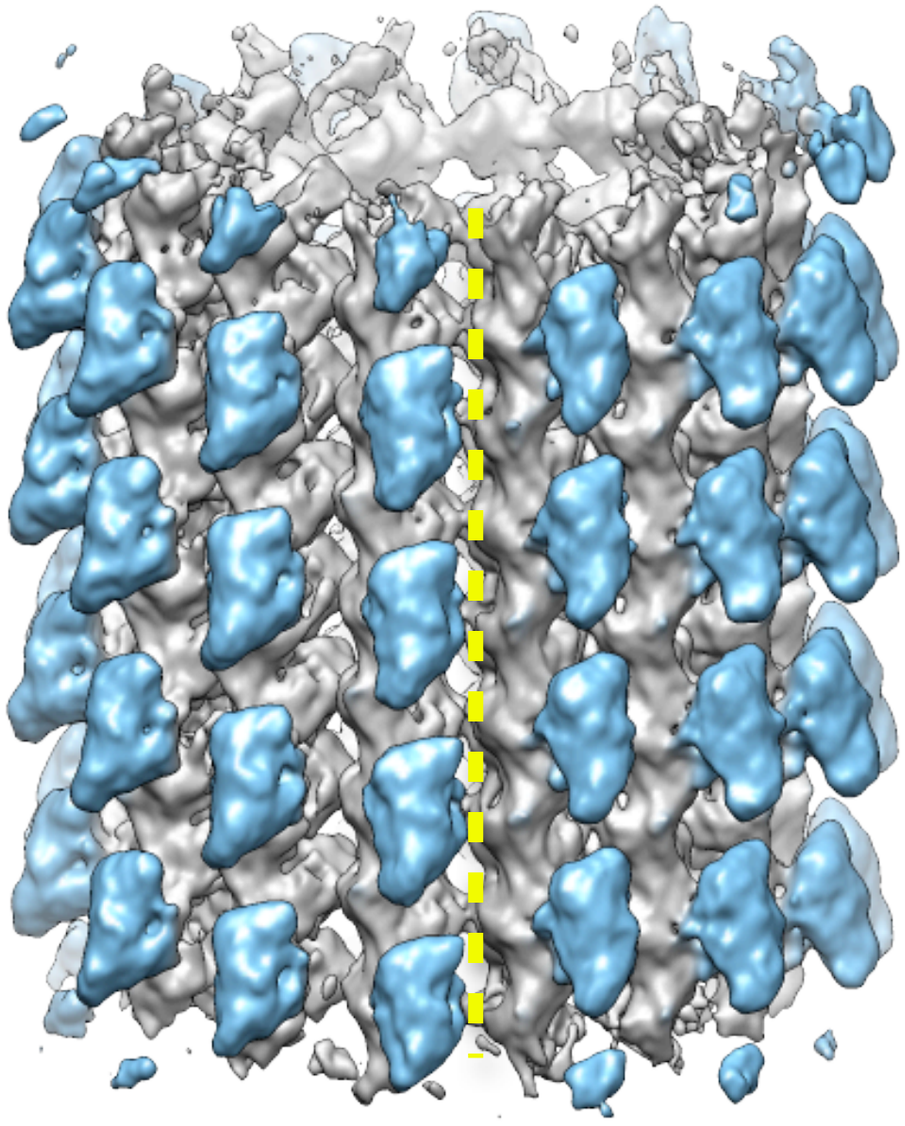


Determine helical symmetry of each pf number using only monomer density (Egelman's hsearch_lorentz)

Applying Pseudo-Symmetry

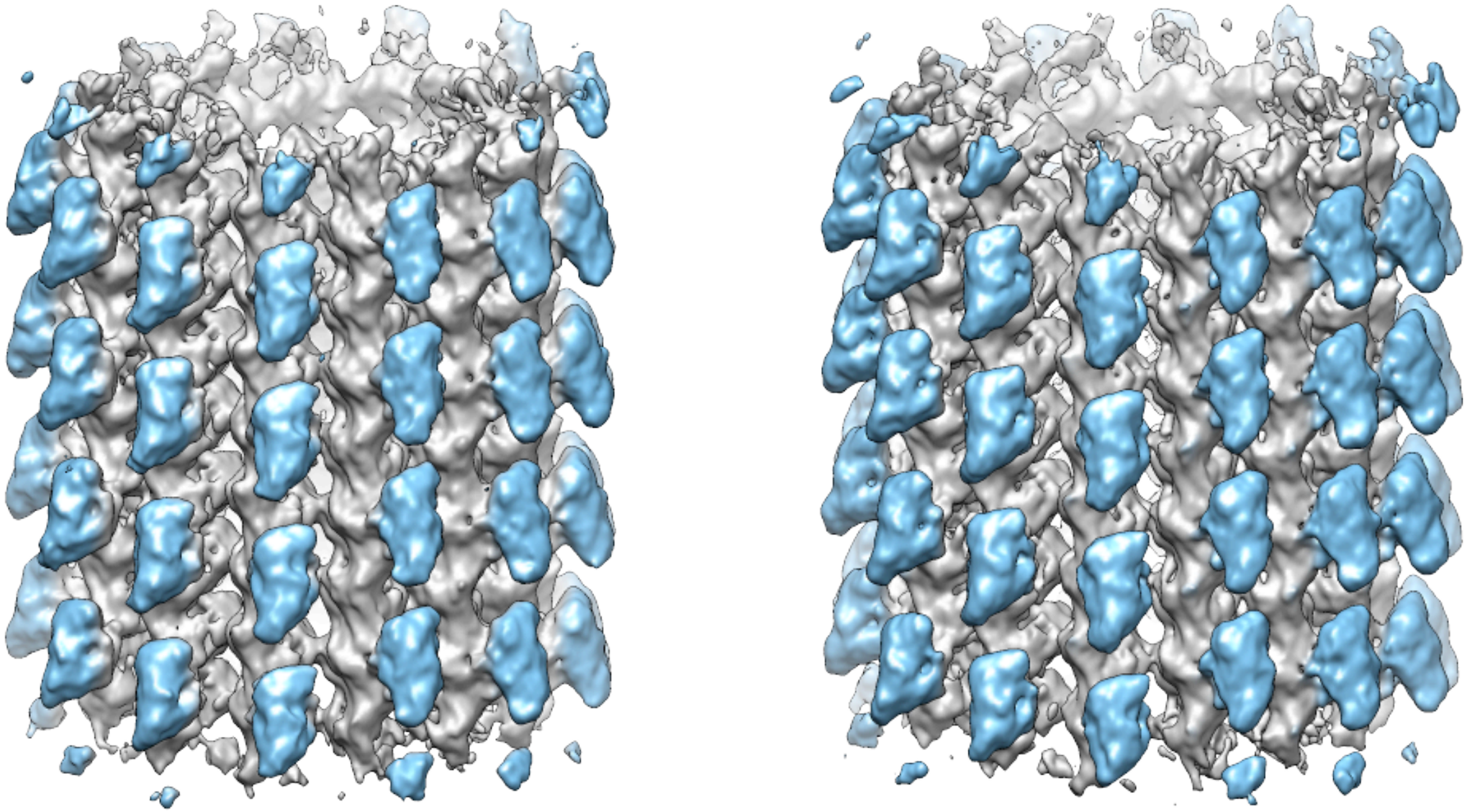


13 protofilament
turn = -27.67°
rise = 9.51Å



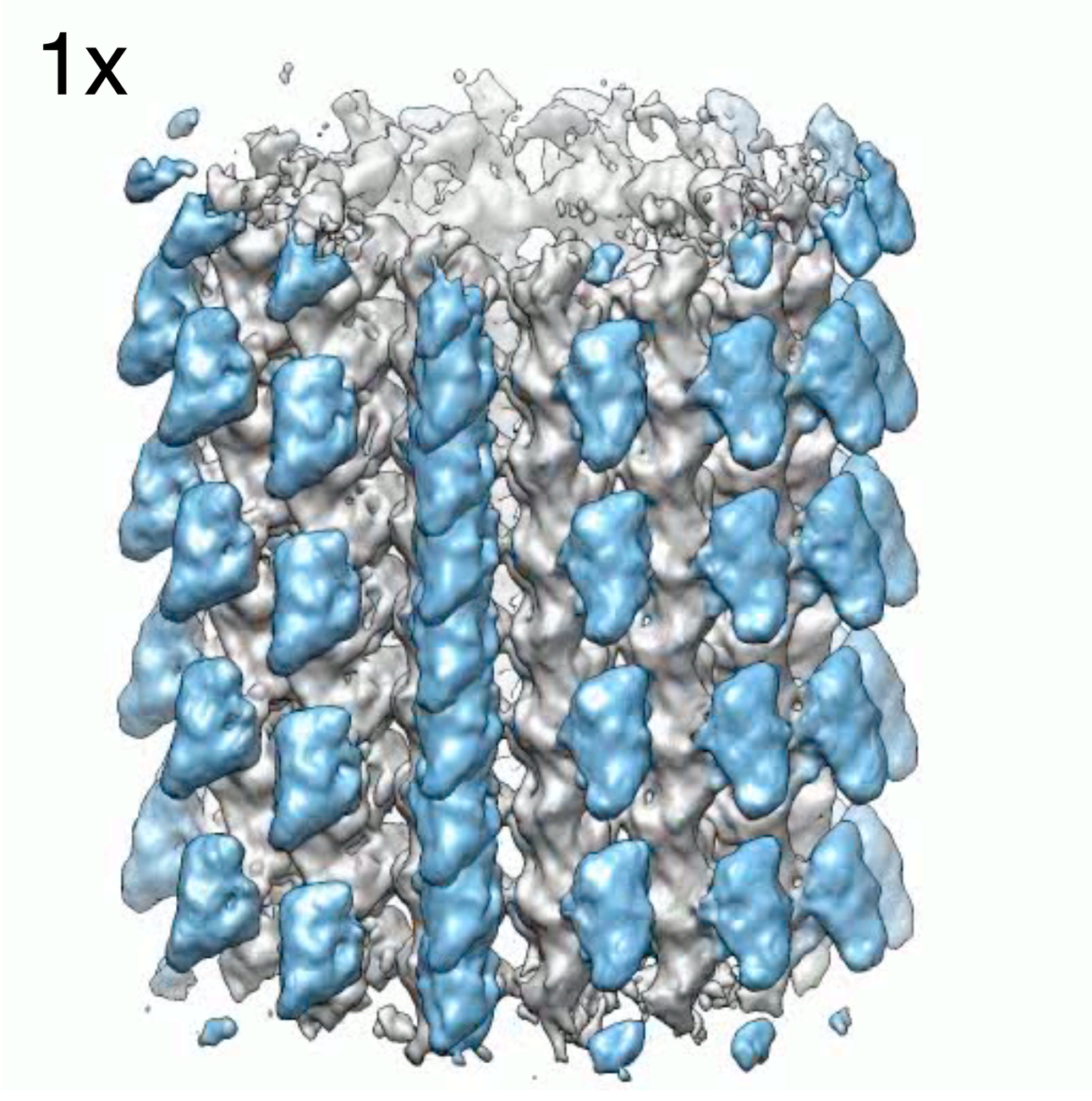
14 protofilament
turn = -25.75°
rise = 8.89Å

Applying Pseudo-Symmetry



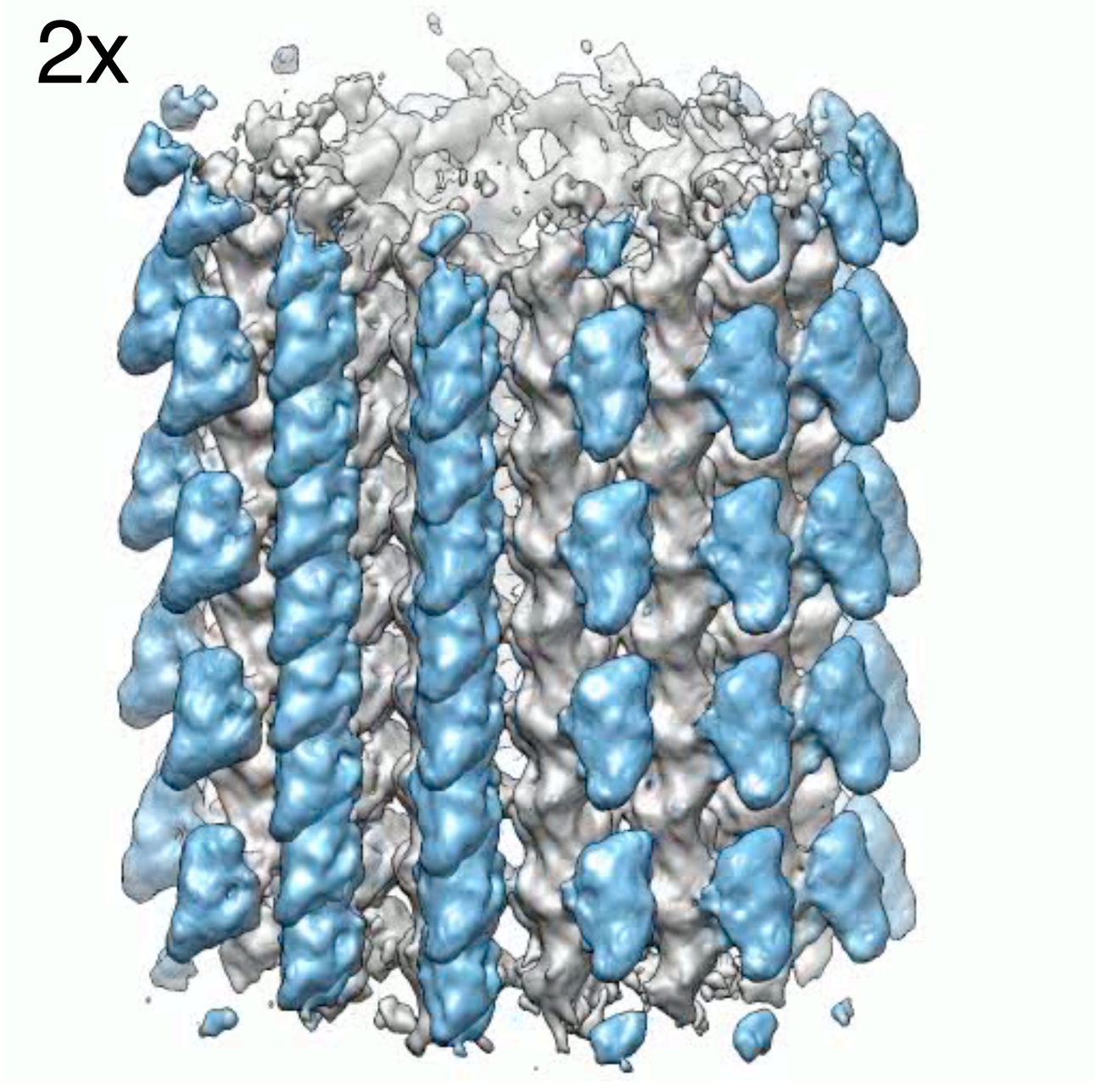
Average symmetry mates in Fourier space during back projection

Applying Pseudo-Symmetry (14pf example)



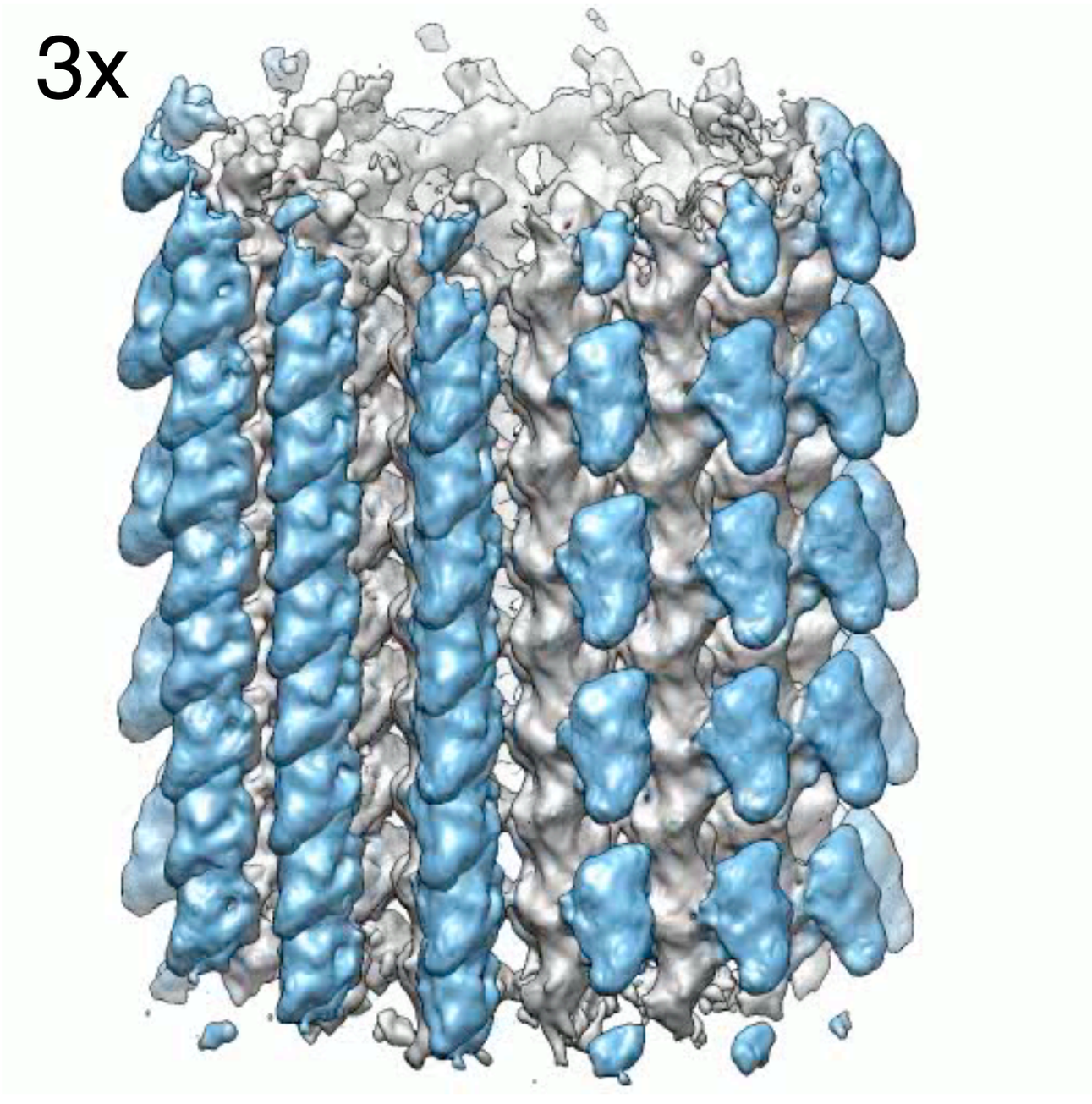
For each protofilament density, use the helical parameters to symmetrize the density with pf-1 symmetry mates

Applying Pseudo-Symmetry (14pf example)



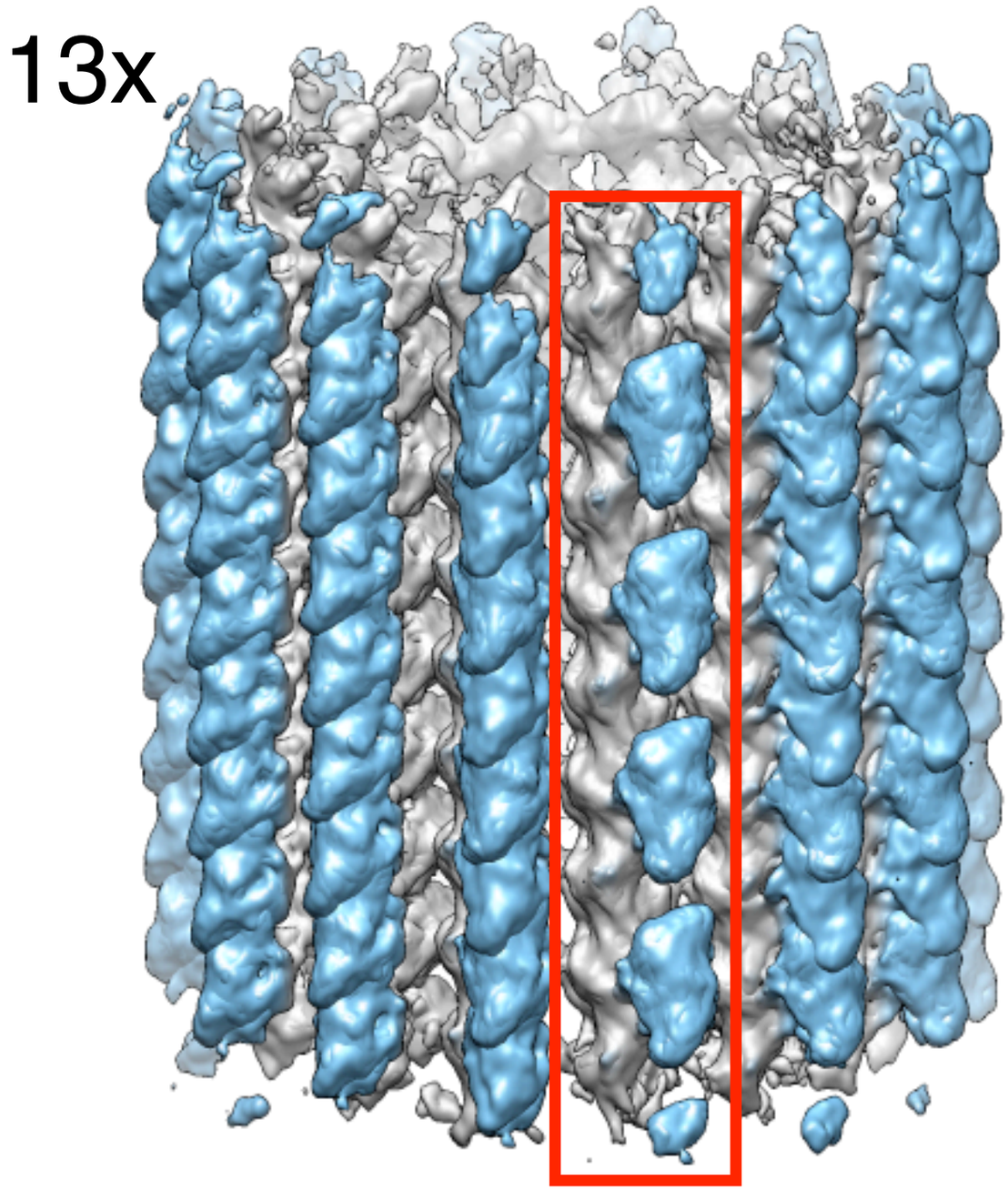
For each protofilament density, use the helical parameters to symmetrize the density with pf-1 symmetry mates

Applying Pseudo-Symmetry (14pf example)



For each protofilament density, use the helical parameters to symmetrize the density with pf-1 symmetry mates

Applying Pseudo-Symmetry (14pf example)

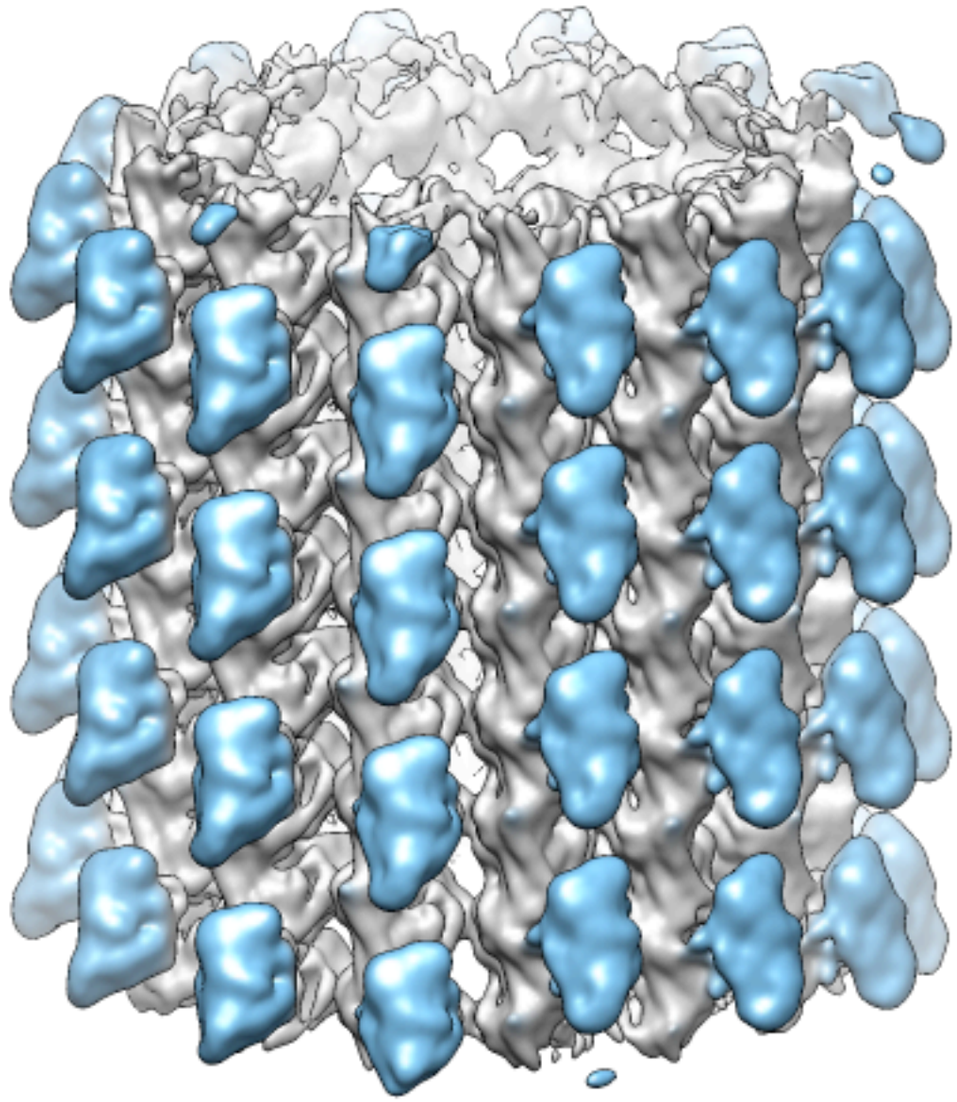


For each protofilament density, use the helical parameters to symmetrize the density with pf-1 symmetry mates

Applying Pseudo-Symmetry

Extract protofilament containing
symmetrized tubulin dimers

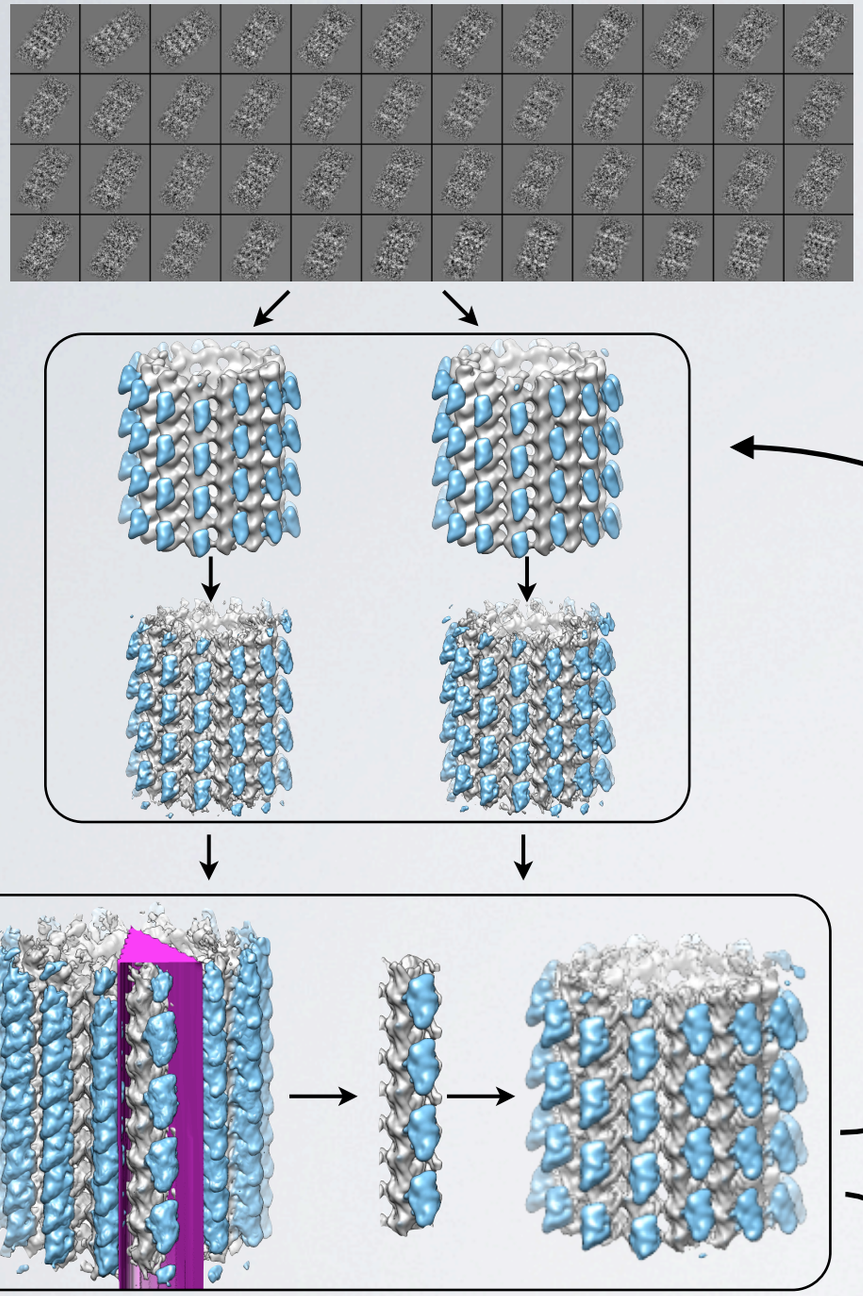
Generating Seamed Density

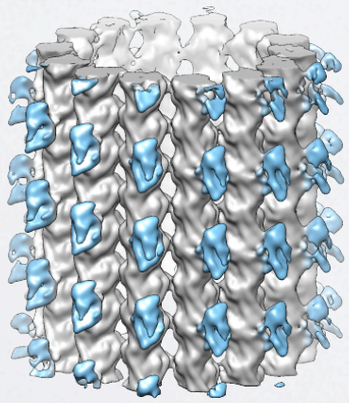


14 protofilament
turn = -25.75°
rise = 8.89Å

Regenerate 13 or 14-fold
microtubule with seam

Pseudo-Helical Microtubule Reconstruction





Particle segments with
mixed protofilament #'s

↓

Projection matching & back projection
using multiple pf models

↓

For each, find helical parameters
(Ed Egelman's hsearch_lorentz)

↓

Over symmetrize using helical
parameters (real space)

↓

Extract the "good" protofilaments &
create new models using helical params

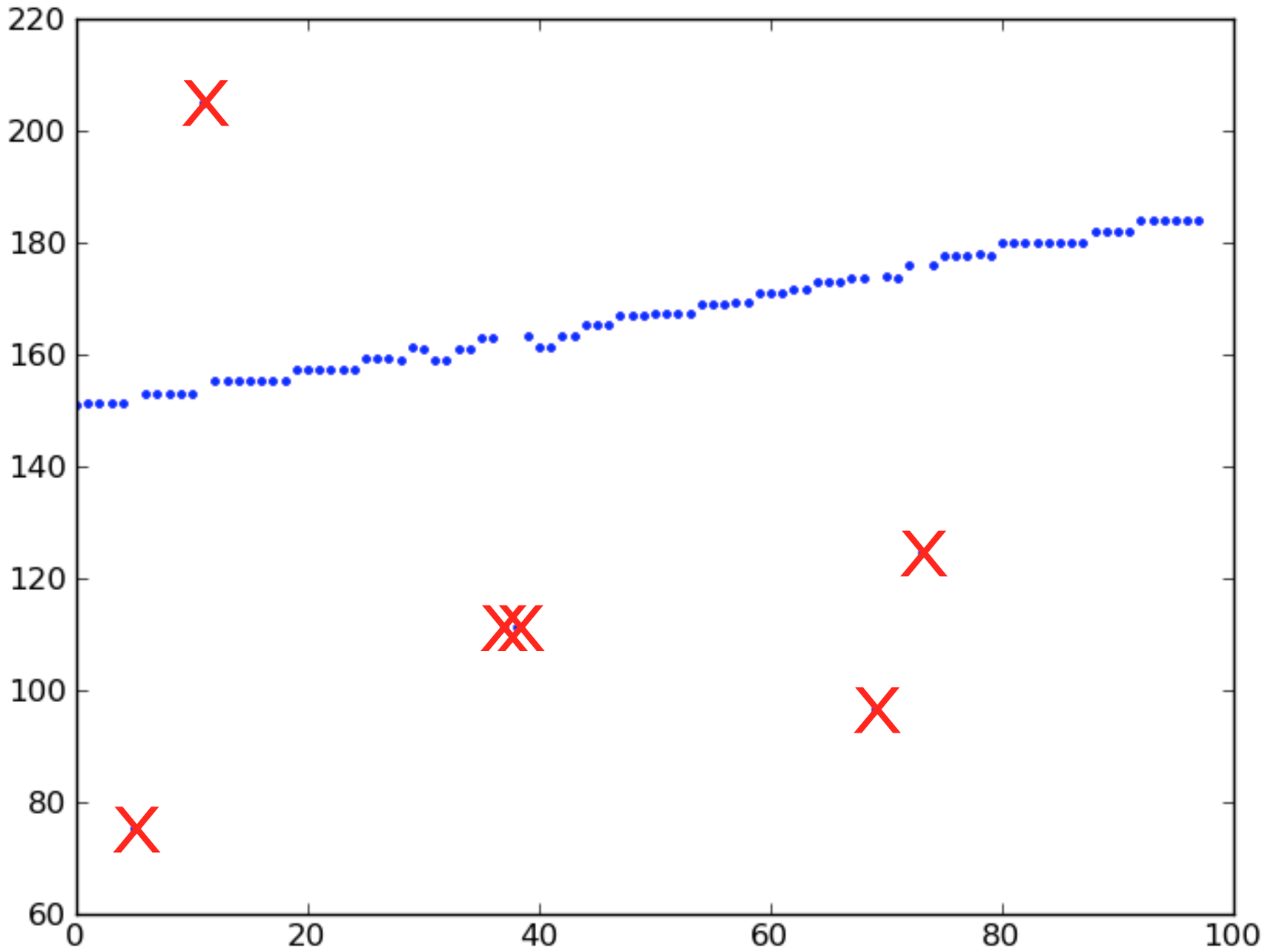
↓

Final refinement in FREALIGN with
same averaging & pf extraction

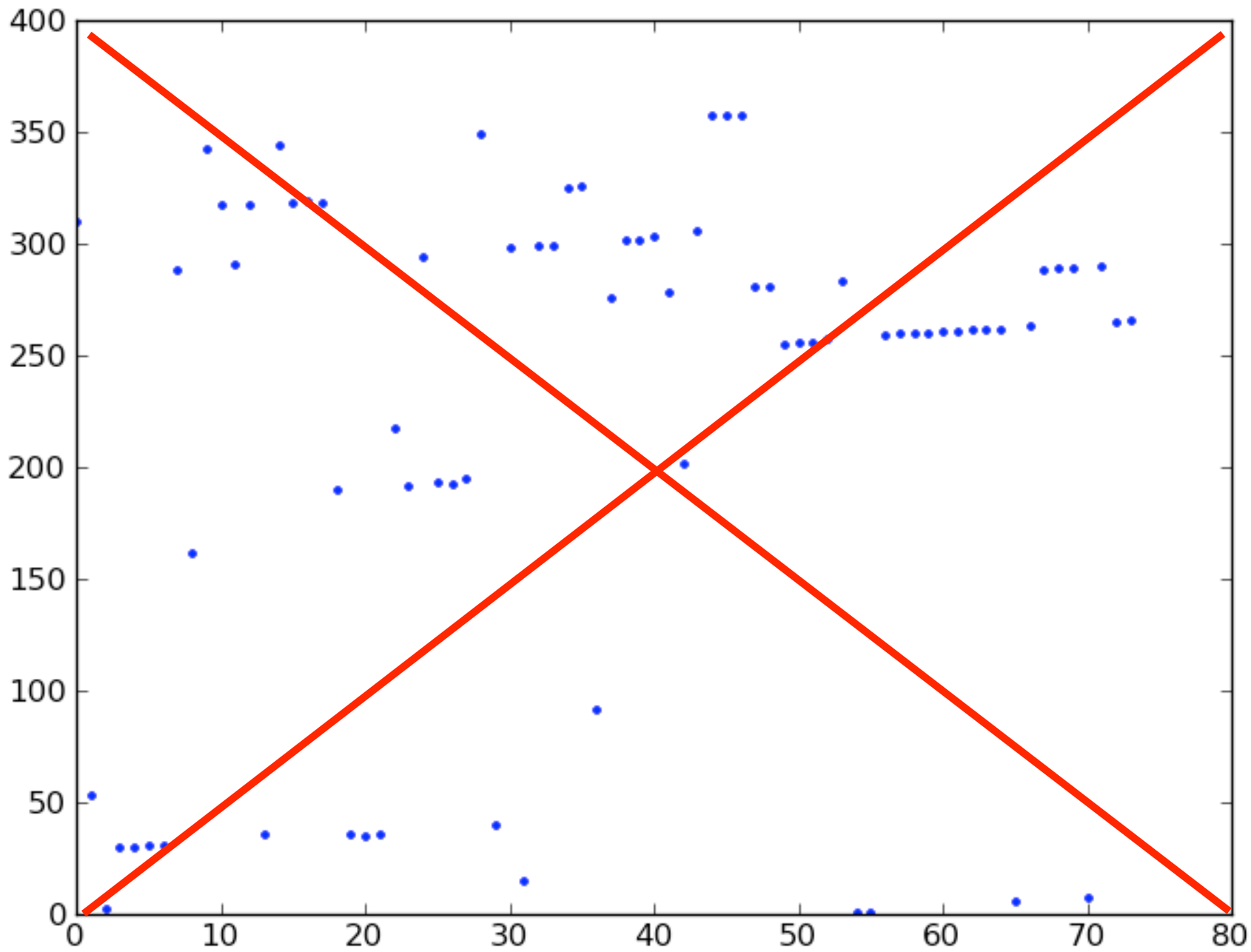
iterate

iterate

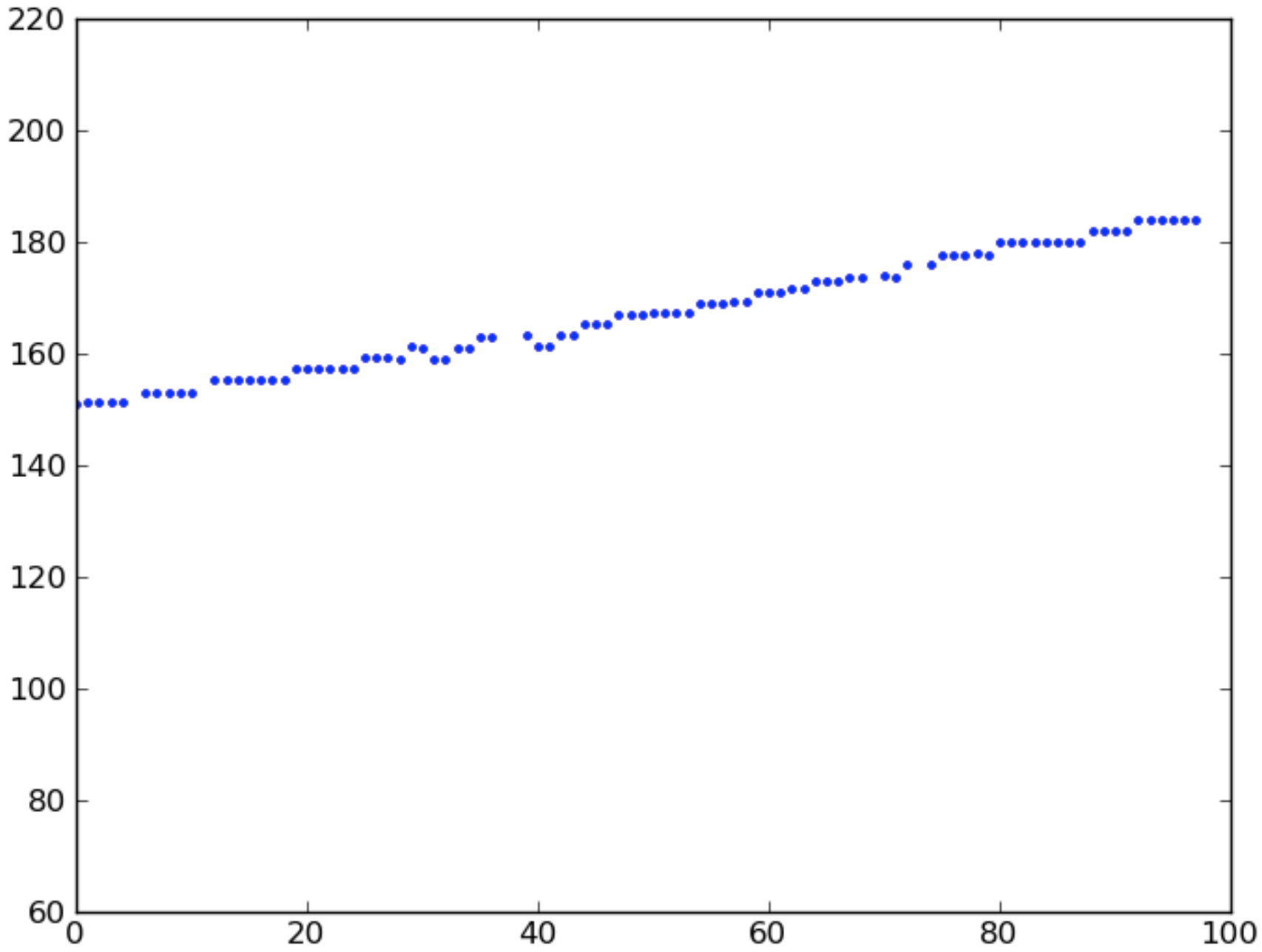
Assessing alignment with the seam



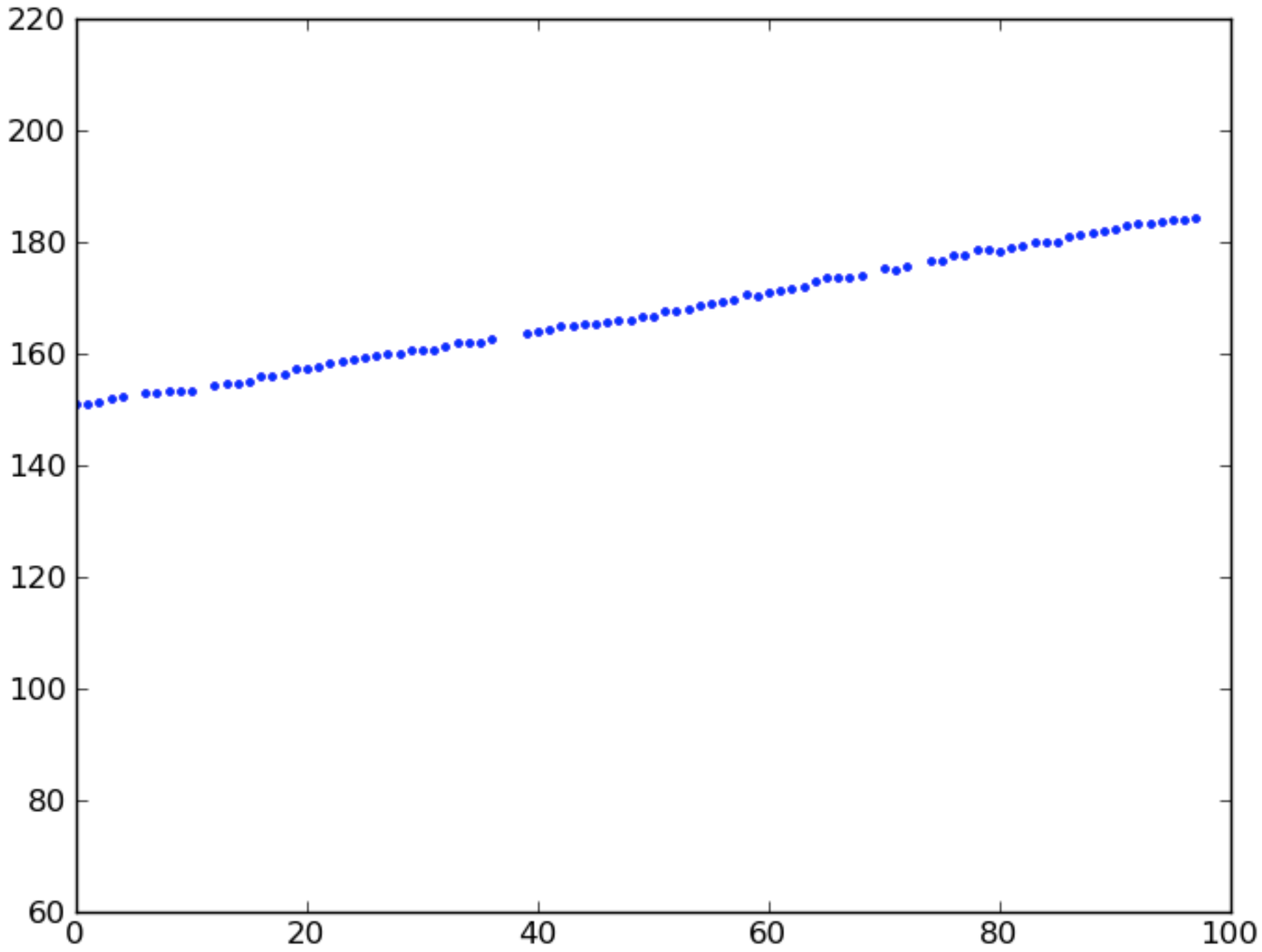
Removing “bad” microtubules



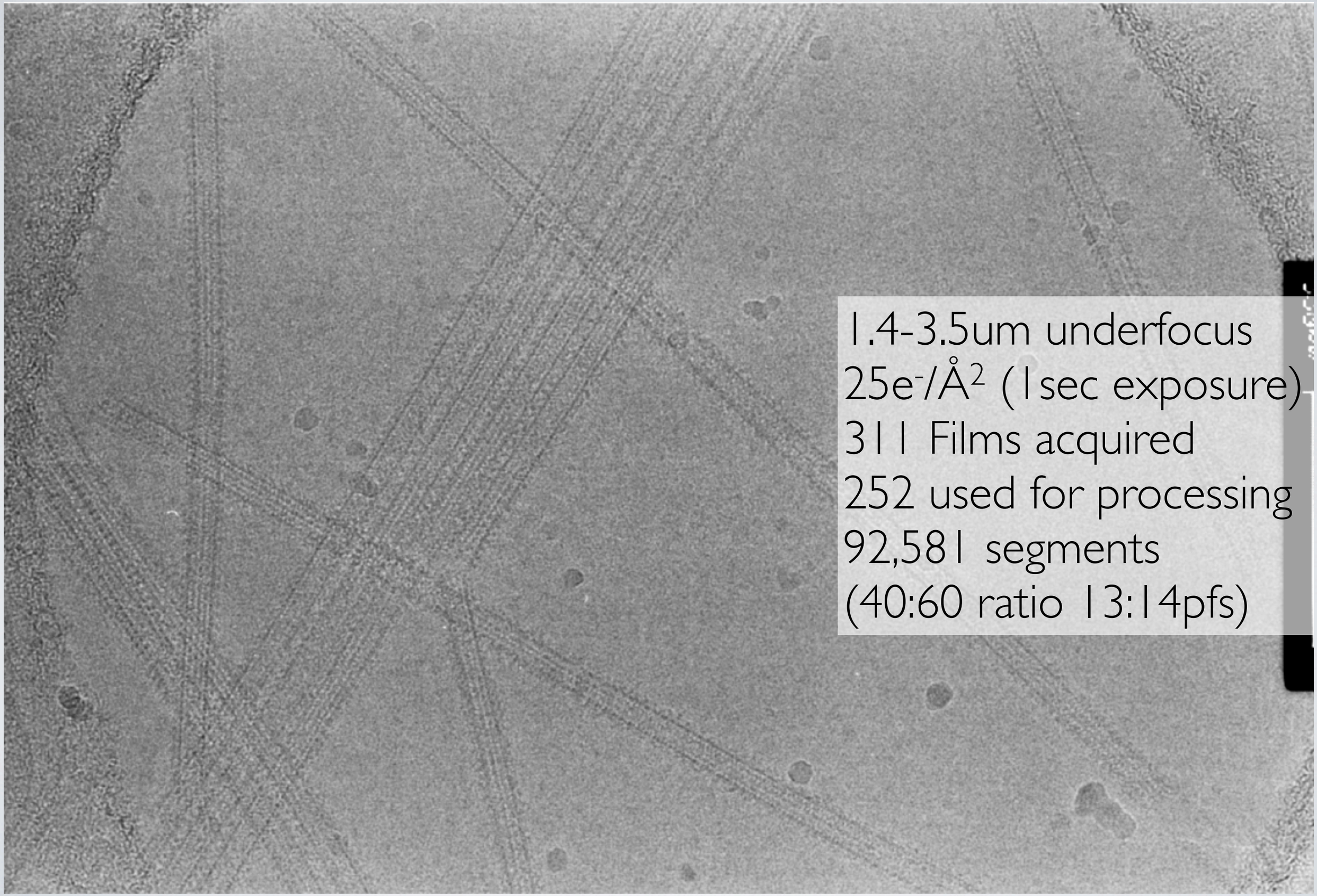
FREALIGN refinement



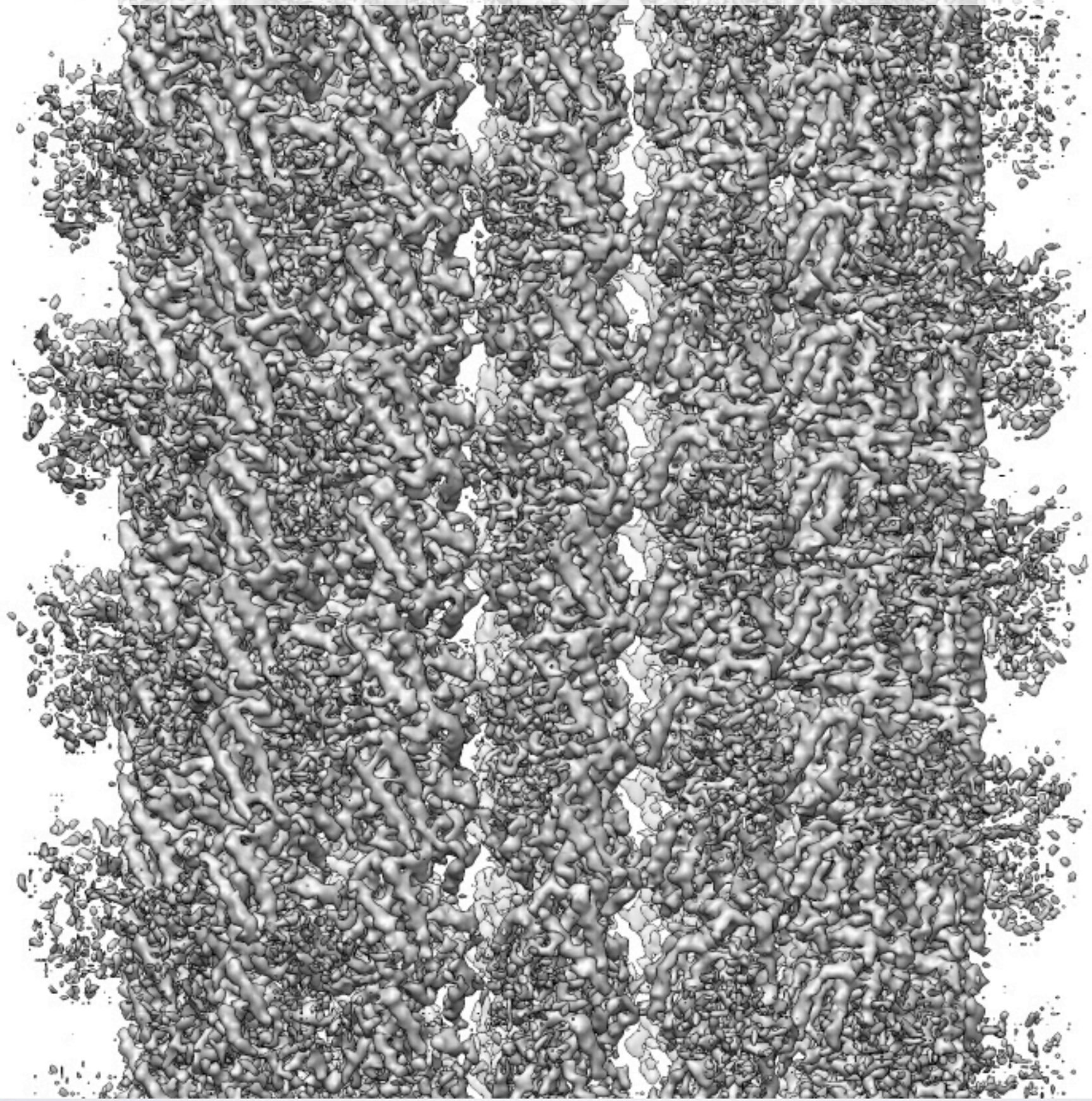
FREALIGN refinement



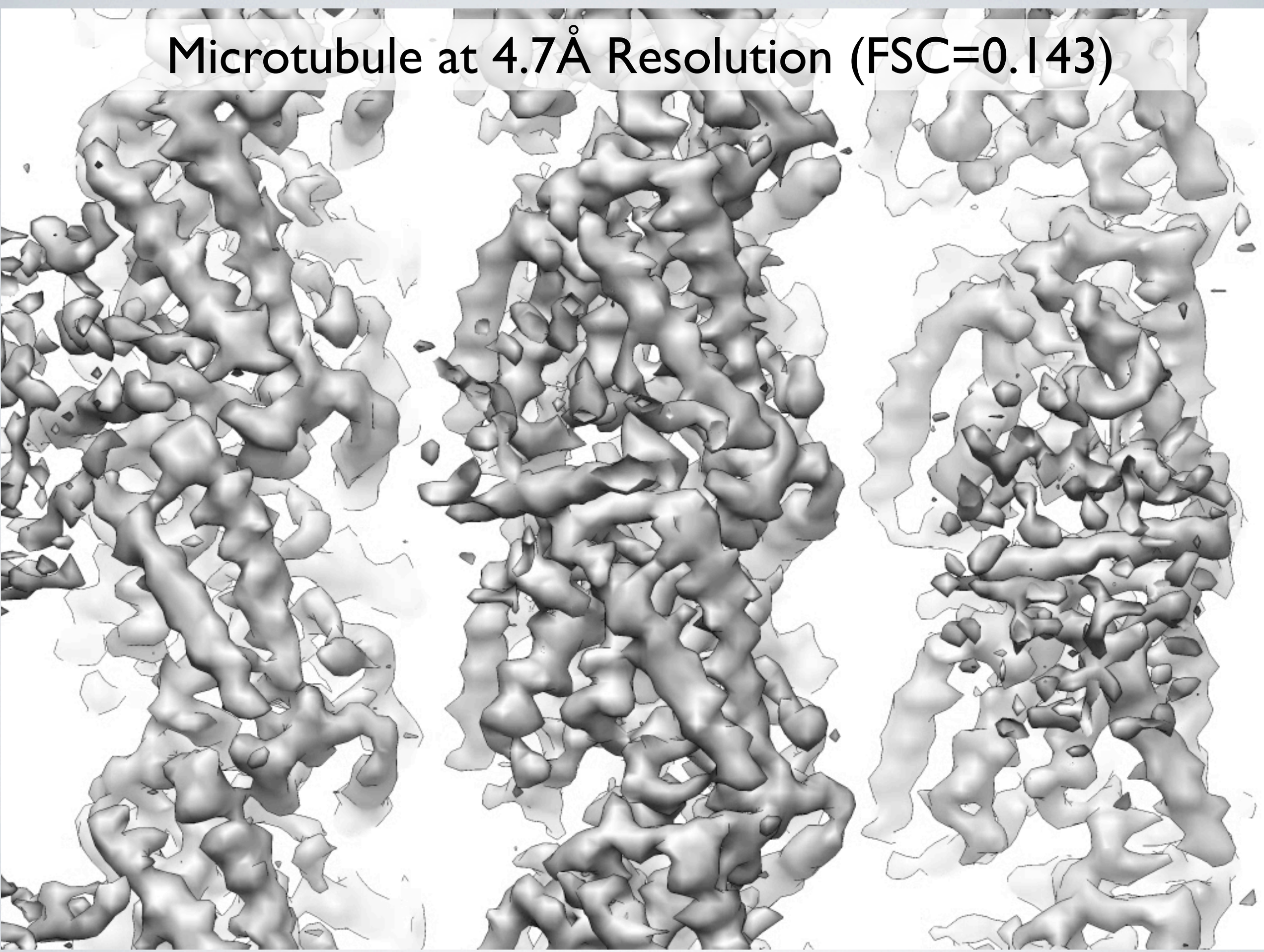
GMPCPP MTs + Kinesin



Microtubule at 4.7Å Resolution (FSC=0.143)

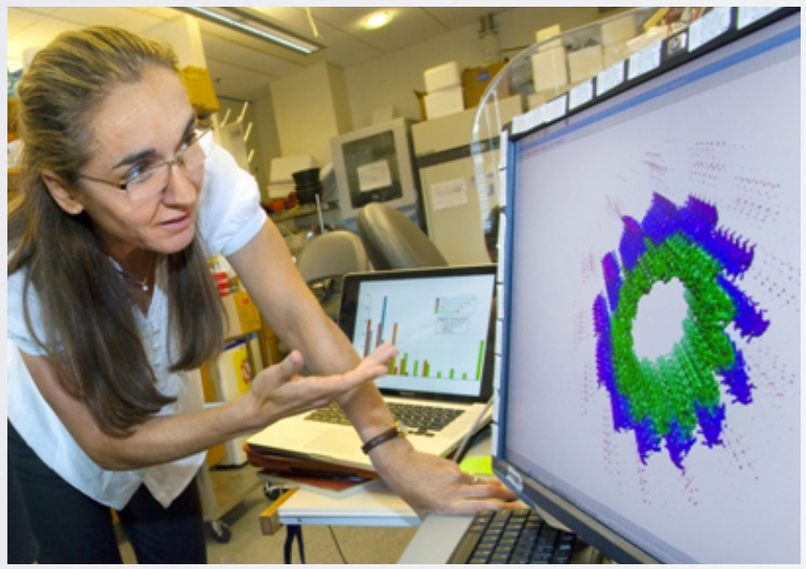


Microtubule at 4.7Å Resolution (FSC=0.143)



Acknowledgements

Greg Alushin (UCB)



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Paul Adams & Jeff Head (LBNL)

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BERKELEY LAB