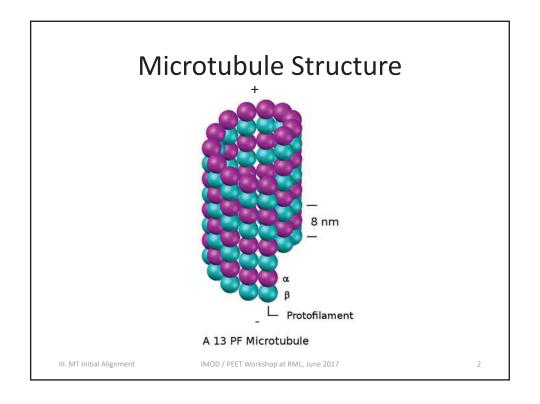
Aligning Filaments: Microtubules

III. MT Initial Alignment

IMOD / PEET Workshop at RML, June 2017



Microtubule Structure - Examples

- 13 PF
 - straight PFs (no supertwist), seam
- 14 PF
 - supertwist, seam
- 15, 16 PF
 - supertwist, no seam (helical)
- Alignment search strategies will vary!

III. MT Initial Alignment

IMOD / PEET Workshop at RML, June 2017

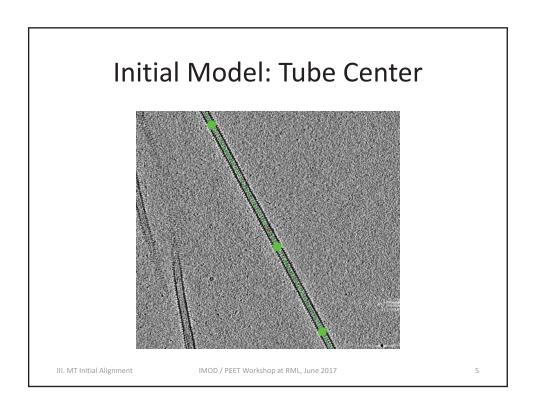
3

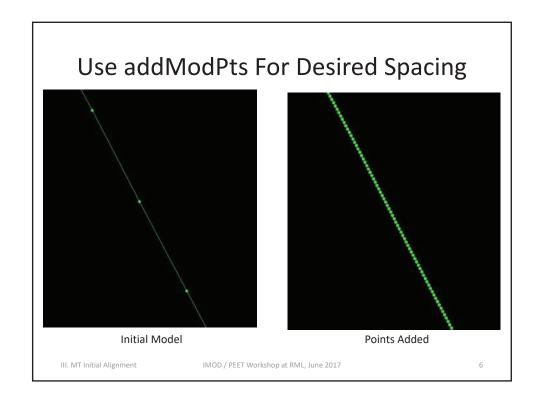
Modeling 8 nm Axial Repeat

- In all cases, want points every ~8 nm axially
- · Tedious and inaccurate to place manually
- Solution:
 - Handful of points define path of tube center
 - Run addModPoints to get desired spacing

III. MT Initial Alignment

IMOD / PEET Workshop at RML, June 2017





13 PF Search Strategy

- Straight (no supertwist), seam
- No axial randomization
- Align particle Y axes
- Missing wedge artifacts will be present
- Goal: visualize seam well enough to latter combine tubes with varying orientations to reduce / eliminate missing wedge artifacts

III. MT Initial Alignment

IMOD / PEET Workshop at RML, June 2017

7

15-16 PF Search Strategy

- Slight supertwist, helical 16 nm pitch (no seam)
- Align particle Y axes + axial randomization
- $360 / 15 = 24^{\circ} \phi$ (Phi) search range
- Will be able to exploit helical symmetry
- Can also use modTwist2EM as described next for twisted, non-helical tubes

III. MT Initial Alignment

IMOD / PEET Workshop at RML, June 2017

Other Numbers of PFs

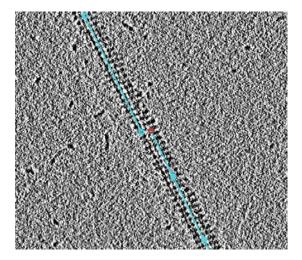
- Supertwist and seam
- · Twist can help reduce missing wedge artifacts
- How to handle the twisted seam?
 - Trace protofilaments (follow supertwist)
 - Object 1: points follow center of tube
 - Object 2: points follow protofilaments on surface
 - Contours correspond to protofilaments
 - Object 1 and 2 points in 1-to-1 correspondence (axial posn)
 - modTwist2EM generates an initial motive list which compensates for the supertwist
- Model twisted PFs before running addModPts (special addModPts mode for this case)

III. MT Initial Alignment

IMOD / PEET Workshop at RML, June 2017

9

Sample Model Following PFs



III. MT Initial Alignment

IMOD / PEET Workshop at RML, June 2017

Questions?

III. MT Initial Alignment

IMOD / PEET Workshop at RML, June 2017