Combining Alignments / Averages

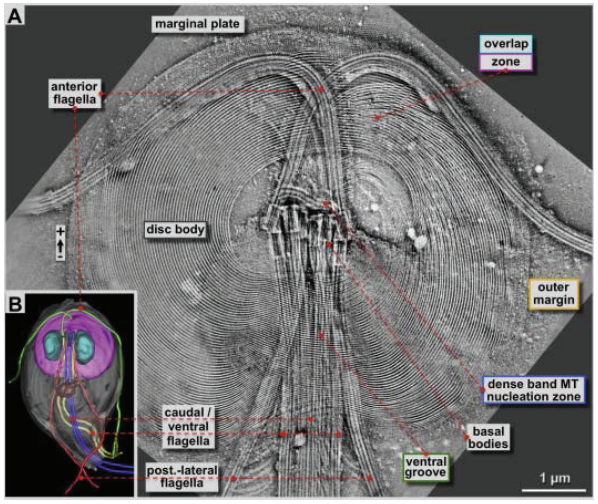
Divide and Conquer!

- Choose starting level: Tomograms? Subregions?
- Strategy:
  - Align and average starting at low(est) level
  - Combine hierarchically
- Finer grained… failures / errors less painful
- Allows incremental addition of new data
- Allow timely correction of some variations
  - Contrast (*e.g.* when combining tomograms)
  - Polarity (*e.g.* when combining microtubules)

Few or No New Tools Needed!

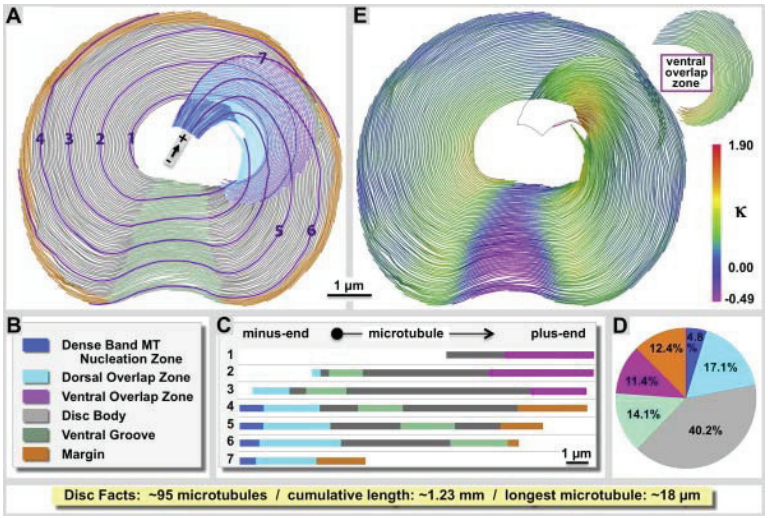
- Simple searches to find common alignment
  - *E.g.* as in gold standard FSC exercise
  - Can extend to multiple averages at once
- modifyMotiveList
- createAlignedModel
- Symmetrization is a type of combining
- May need IMOD to adjust tomogram contrast

Giardia Ventral Disk Example



Brown et al (2015) JSB **94**:38-48

Giardia Ventral Disk Example



Brown et al (2015) JSB **94**:38-48

Alignment Hierarchy

- Overall structure (for difference map)
  - Region (multiple tomograms)
    - Region (Individual tomogram)
      - Individual microtubule
        - Region (Individual microtubule)
- Check / correct consistency at "natural" level
  - Contrast (tomogram)
  - Voxel size (tomogram)
  - Imaging conditions (tomogram)
  - Polarity (microtubule)
  - Protofilament number (microtubule)

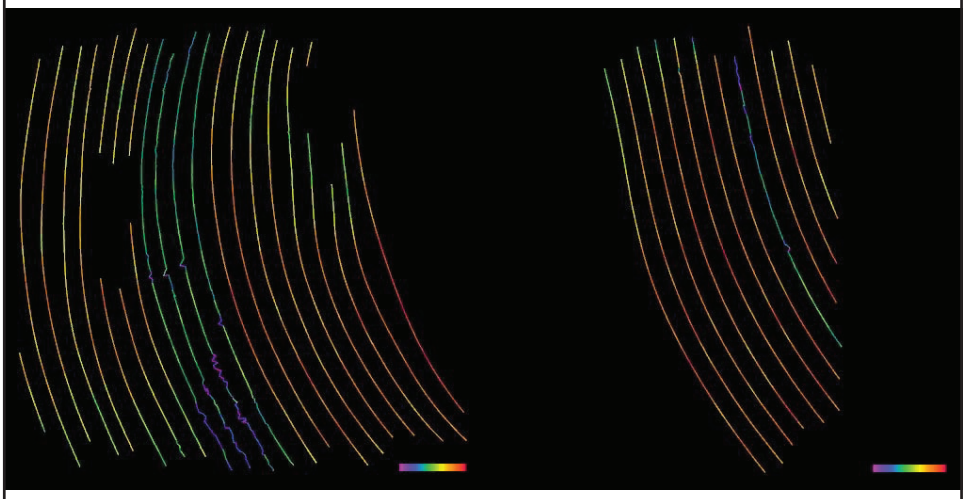
Strategy Will Vary with Application

- BPV: all tomos or single tomo or all tomograms?
- *In vitro* MTs: **single tube** or all tubes?
- Sperm Singlet Zone: **single tube** or all tubes?
- Giardia Ventral Disk: **region (each tomogram)** or region (all tomograms) or all regions and tomograms?

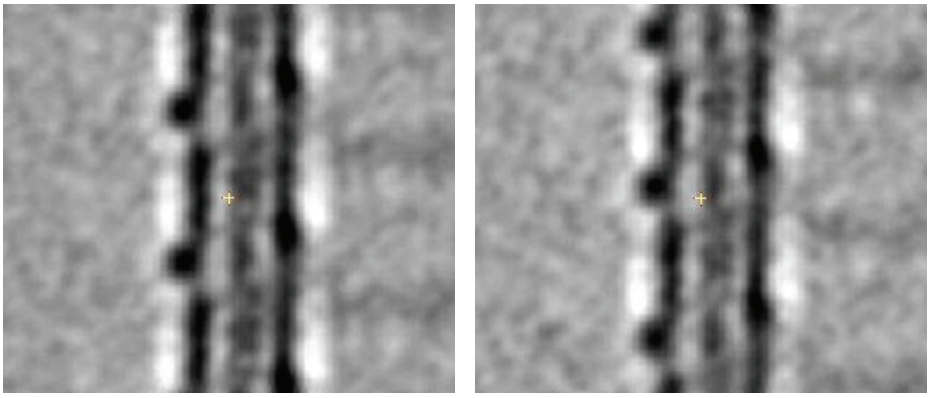
Sanity Check At Each Level!

- Run createAlignedModel
- View aligned models (scores and consistency)
- Can combine
  - Original models and aligned motive lists
    - Simplest approach
    - Preferred when current alignment is poor
  - Models / motive lists from createAlignedModel
    - Simpler when changing voxel size
    - May improve particle y axes estimates if alignment is good
- Clustering to check for heterogeneity

Sanity Checking: Bad Polarity

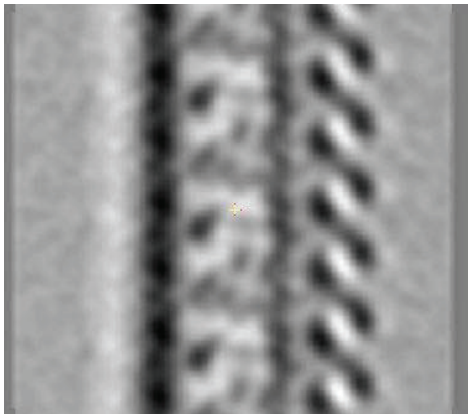


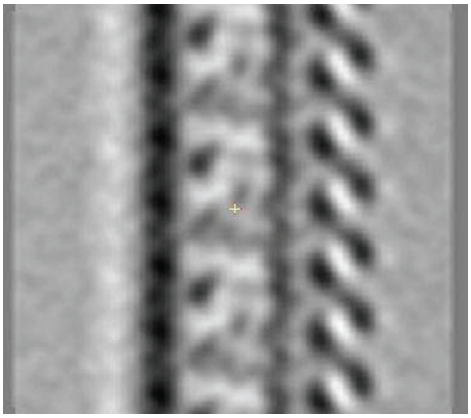
Clustering: Wrong Model Point Spacing



(Structure has both 8 and 16 nm Periodicity!)

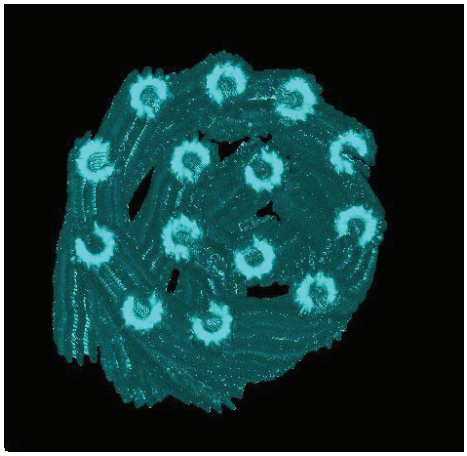
Clustering: Wrong Model Point Spacing



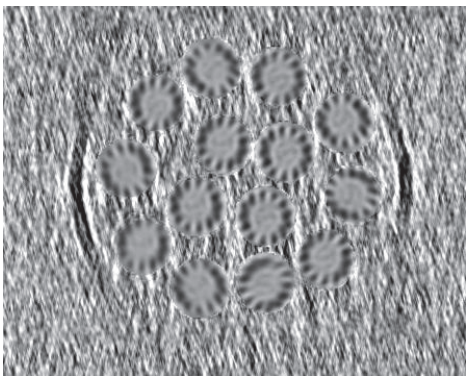


(Structure has both 8 and 16 nm Periodicity!)

Visualization: cloneModel / cloneVolume



Created with cloneModel



Created with cloneVolume

Questions?