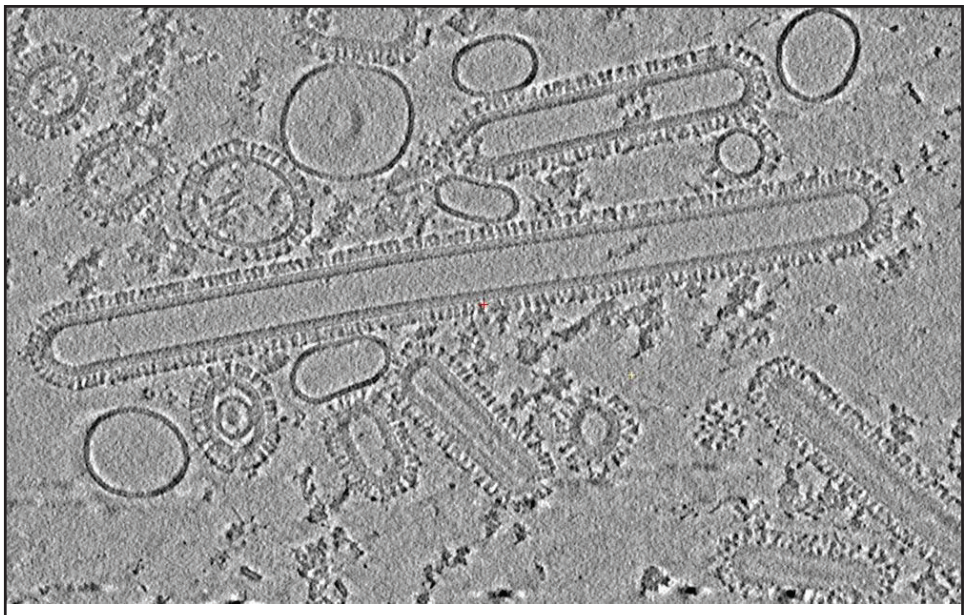
Influenza Hemagglutinin Spikes

A Very Challenging Example… Still in Progress!

(Data and Results Courtesy of Petr Chlanda)



Sample Data (binned 2X, NAD filtered, cropped)

Challenges and Features…

- Polymorphism
- HA Spikes
  - Small (4-5 nm diameter x ~13 nm long)
  - SNR is an issue
  - Trimeric, with C_3 symmetry
  - A crystal structure is available

An Overall Strategy

- Would like to align and average >10,000 spikes
- Too many to pick manually (at least for me!)
- Bootstrap Approach
  - Pick a manageable number of particles manually
  - Align these to generate an initial template
  - Template matching to pick additional particles
  - Screen candidates… *e.g.* by cross-correlation
  - Align and symmetrize

Where to Begin?

- For the initial average shall we use spikes
  A. from all viruses
  B. from all similarly shaped viruses
  C. from an individual spherical virus
  D. from an individual cylindrical virus

Where to Begin?

- Shall we initially average
  A. Spikes from all viruses
  B. Spikes from all similarly shaped viruses
  C. Spikes from an individual spherical virus
  D. Spikes from an individual cylindrical virus ✓
- *Divide and Conquer!*
- *Start with the big "cylindrical" virus*
- *Combine with results from other viruses later*
- *Big cylindrical virus has >100 spikes*

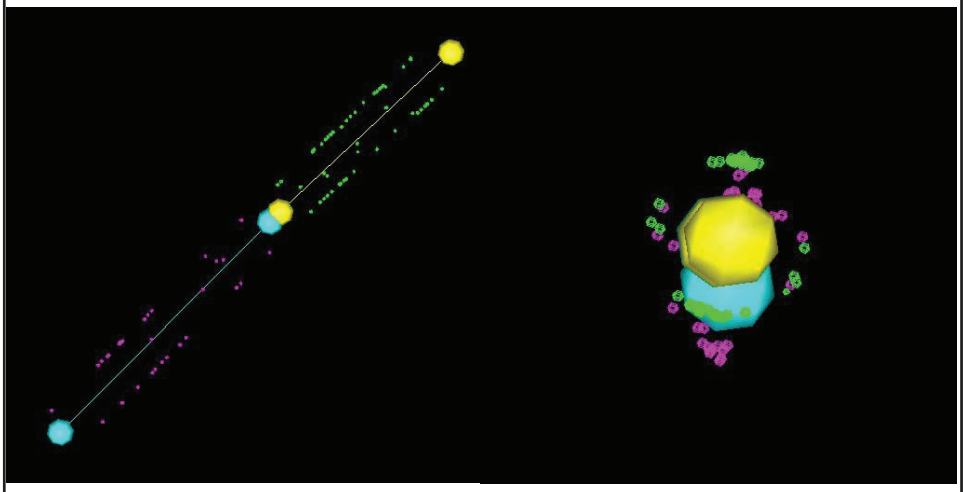
Modeling The Cylindrical Virus

- For initial modeling of the big virus and seeding candidates spikes , shall we use
  A. stalkInit with 2-point contours
  B. spikeInit and seedSpikes with a cylindrical model
  C. spikeInit and seedSpikes with a spherical model
  D. meshInit with hand drawn contours

Modeling The Cylindrical Virus

- For initial, manual modeling and seeding candidates spikes , shall we use
  A. stalkInit with 2-point contours
  B. spikeInit / seedSpikes with a cylindrical model ✓
  C. spikeInit / seedSpikes with a spherical model
  D. meshInit with hand drawn contours
- *Easiest and quite accurate*

Manually Picked Spikes (for SpikeInit)



Averaging the Manually Picked Spikes

- For the starting reference shall we use
  A. a single particle polar reference
  B. a single particle equatorial reference
  C. a multi-particle reference
  D. A reference generated by a no-search 1st iteration

Averaging the Manually Picked Spikes

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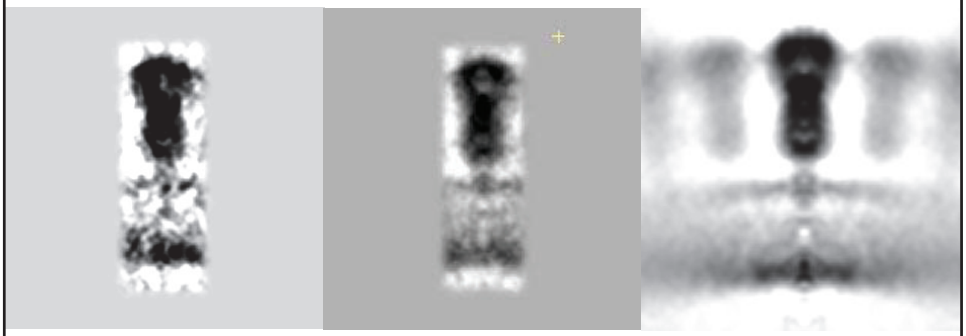
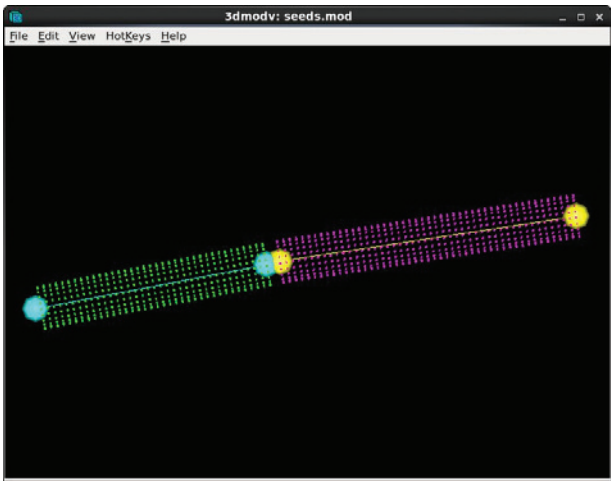
  D. A reference generated by a no-search 1st iteration
- *A polar reference seems more likely to preserve the C_3 rotational symmetry*

Results From Manually Picked Spikes (For Template Matching)



Original C_3 Symmetric C_{180} Symmetric

Seeding Candidate Spikes



How Densely to Seed Candidates?

- More candidates with closer spacing
  - Smaller search distances
  - Discard more candidates afterwards
- Fewer candidates with larger spacing
  - Larger search distances
  - Don't need to discard as many candidates
- Not clear *a priori* which will work better
- Goals: minimize reference bias & maximize speed

Preserving C_3 Symmetry

- Shall we
  A. Assume C_3 symmetry from the outset
  B. Assume nothing and let the data guide us

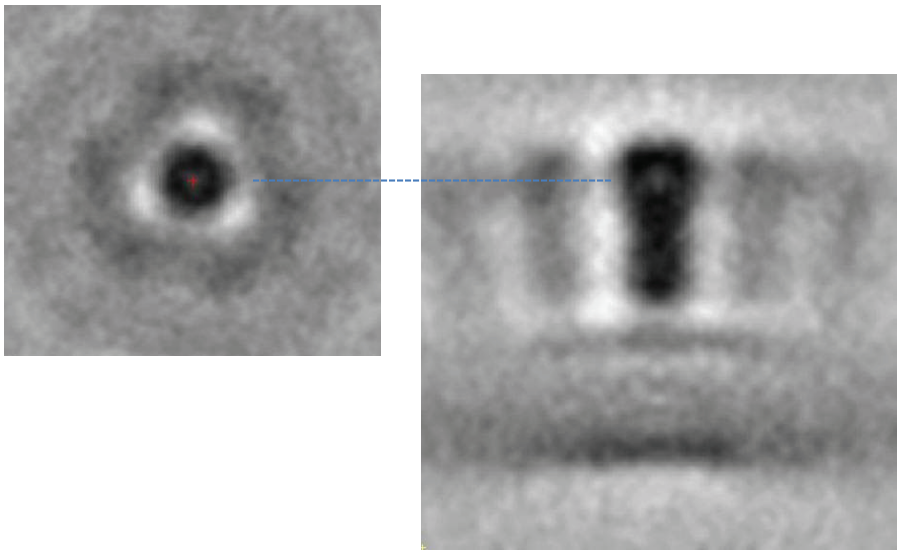
C_3 Symmetry

- Shall we
  A. Assume C3 symmetry from the outset ✓
  B. Assume nothing; let the data guide us
- *When practical (larger structures), I prefer B.*
- *In this case, it is not yet clear if symmetry will emerge spontaneously*
- *Will ultimately need to try both ways and see what works best*

C_3 Symmetry

- A hybrid approach
  - Use axial symmetry iterations early on
  - Impose C_3 symmetry with virtual particles only at later stages
- Still don't have enough data to know which approach is best for this case

Current Averages



Summary

- Consistent with known crystal structure
- Will need to see how results develop with more, smaller voxel size data
- Caution need to avoid reference bias
- If lost, C3 symmetry may be hard to recover
- Thanks to Petr for sharing his data and results
- Watch Petr's publications to see how this turns out!

Questions?