

# Single Particle Tomography

Classification

Alignment

Averaging

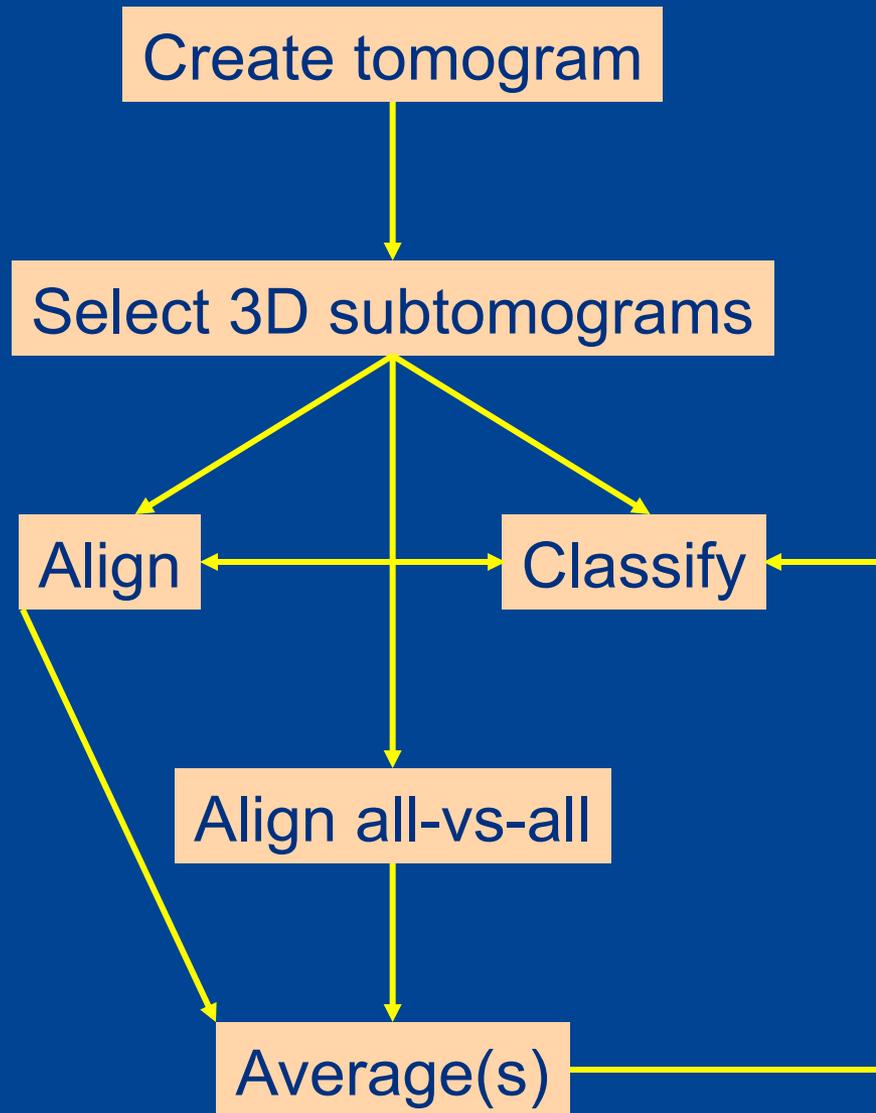
Michael Schmid (mschmid@bcm.edu)

Jesus Galaz-Montoya

John Flanagan

Steve Ludtke

# Flow Chart for processing subtomograms



# Key Points/Concepts

Effect of the Missing Wedge

Image considerations

filtering, masking

All-vs-all alignment as an option

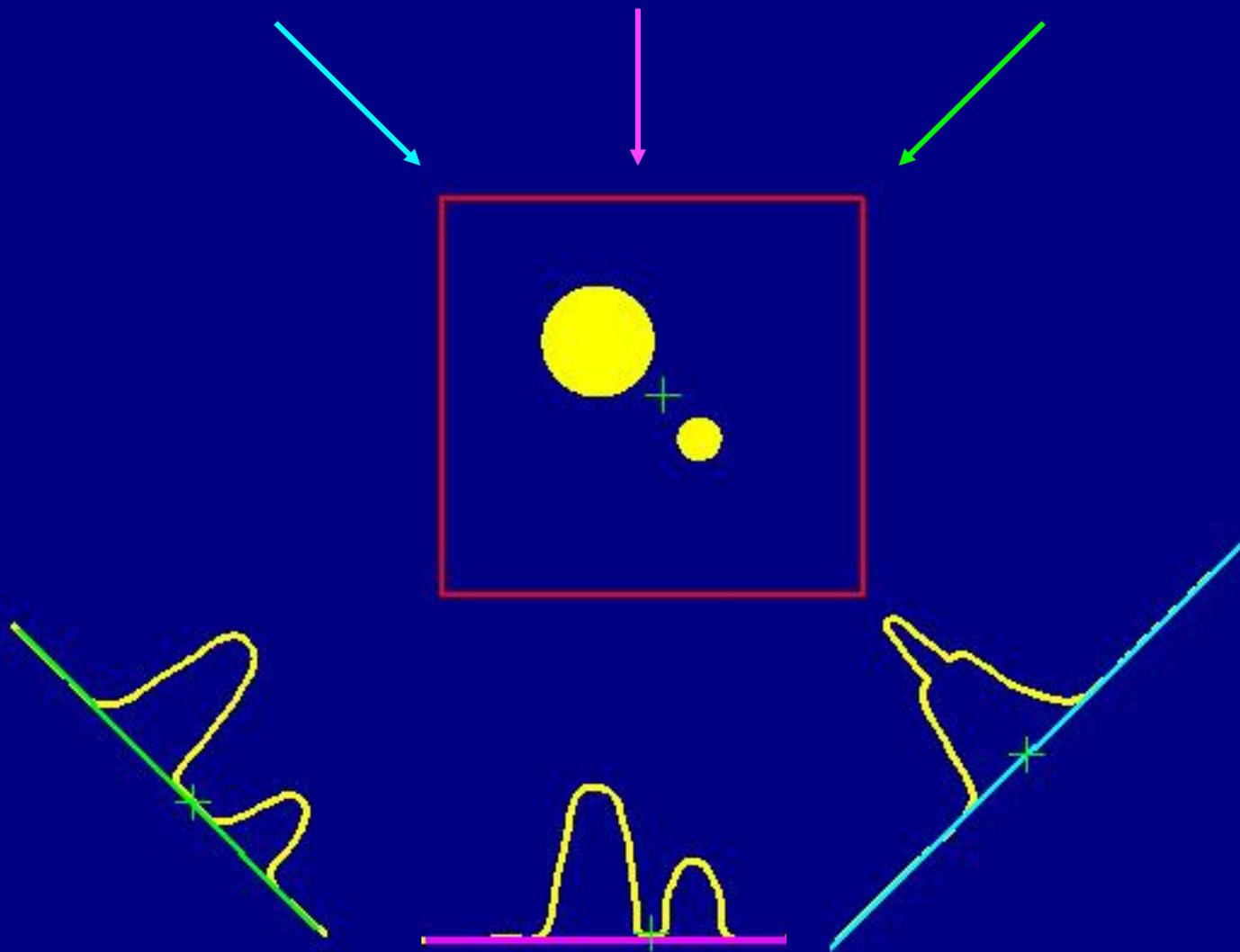
Optimizing search parameters for efficiency

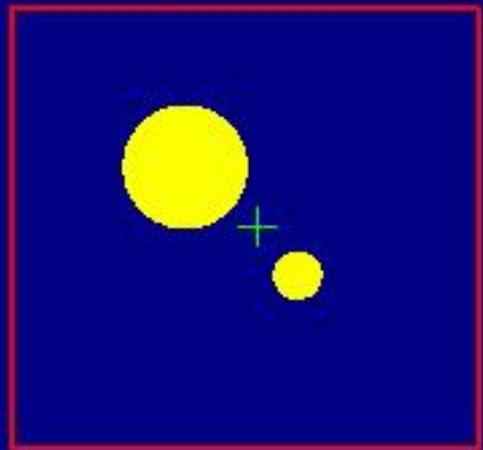
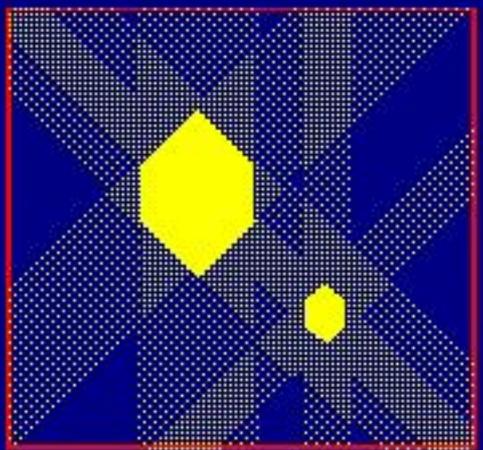
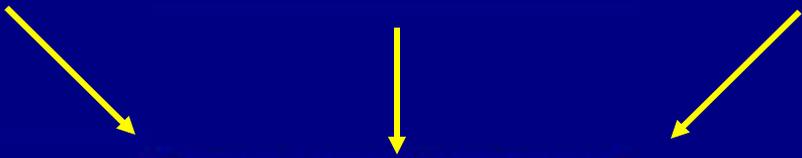
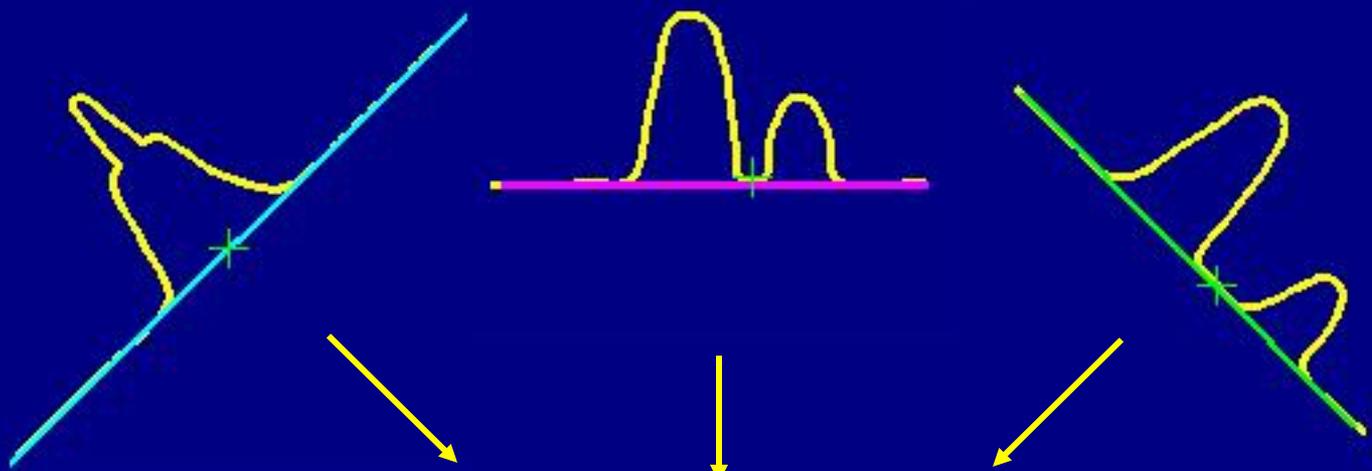
Examples

carboxysome,  $\epsilon 15$ , herpes pentonless capsid

# Effect of the missing wedge

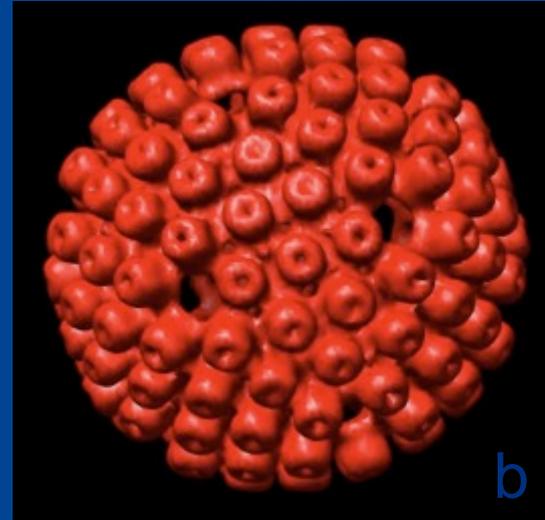
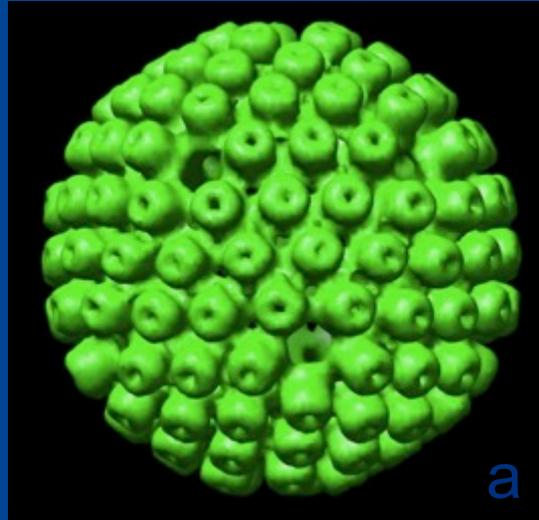
- Tomographic data is limited to  $\pm 70^\circ$  max tilts
- Distorts the reconstructions
- Makes mutual alignment difficult



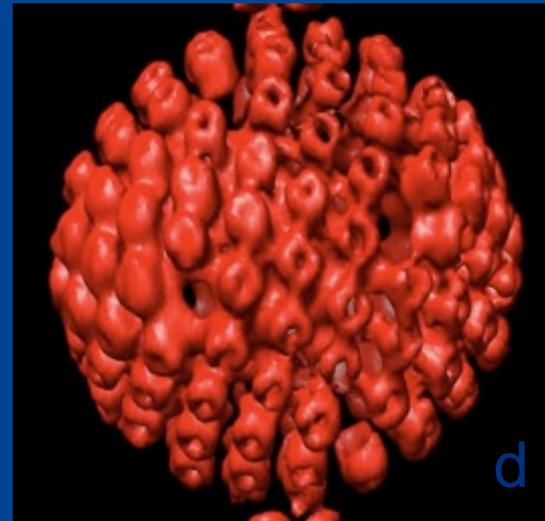
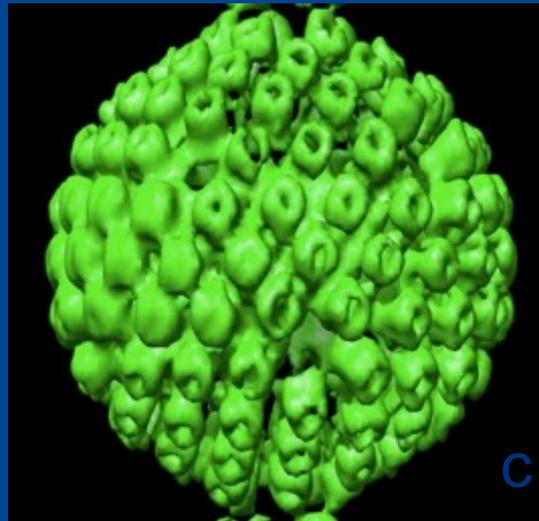


# Demonstration of the effect of the missing wedge

No missing wedge



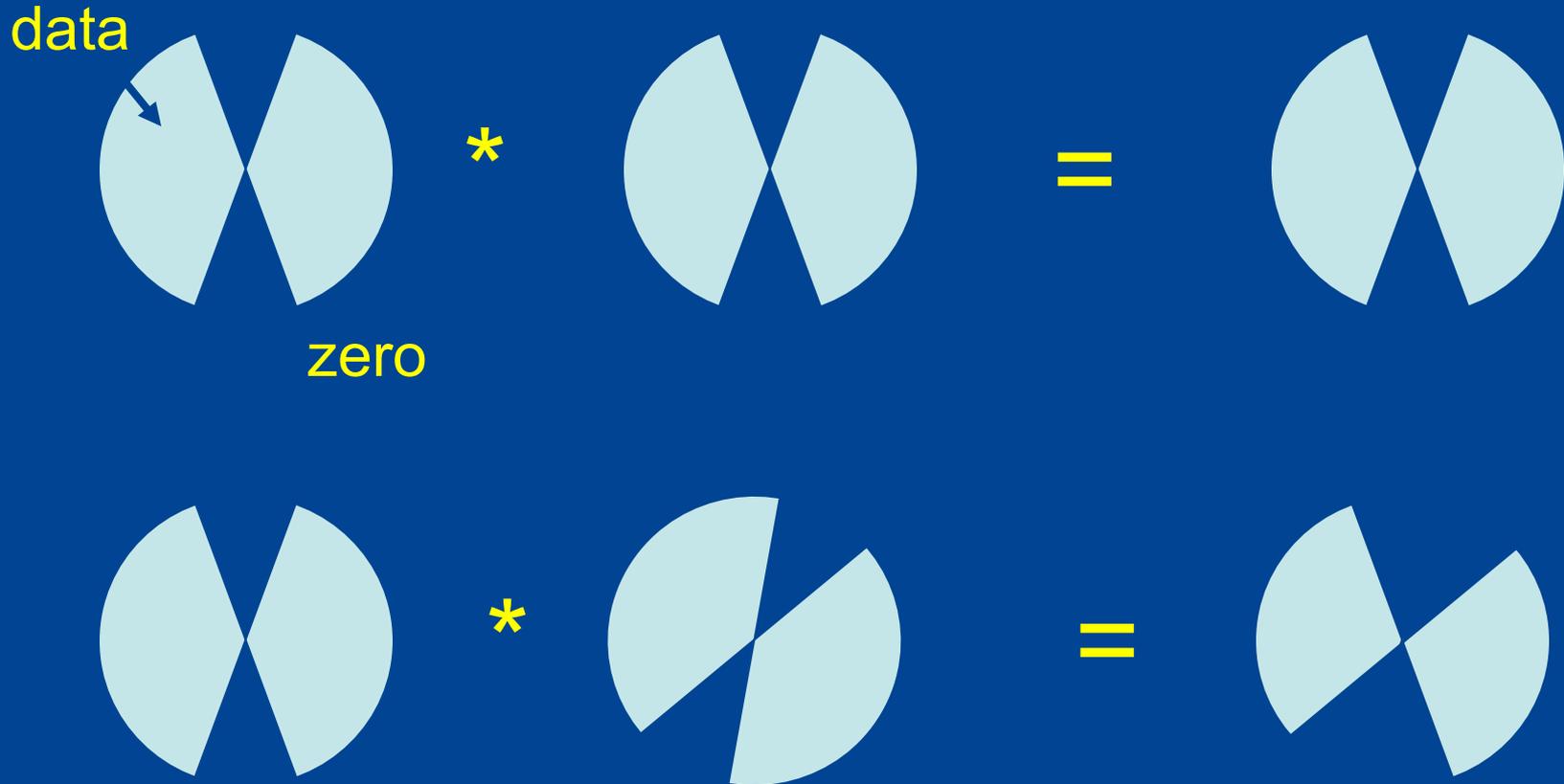
40% missing wedge  
(Equivalent to  $\pm 54^\circ$  tilt)



5-fold map

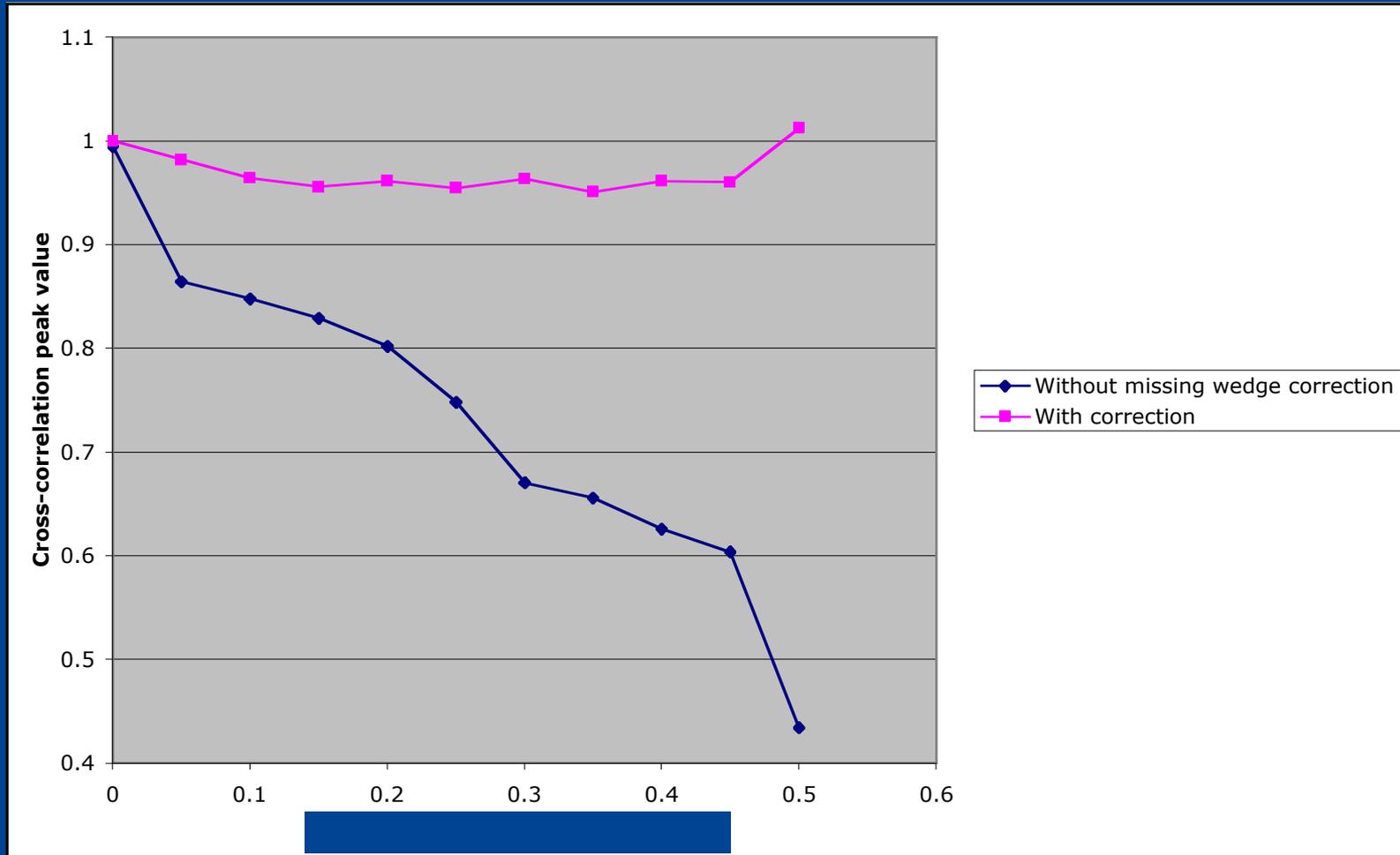
3-fold map

# The missing wedge in Fourier space during orientation cross-correlation search



The number of zeros in the complex product changes with orientation, and the more zeros, the lower the cross-correlation peak

## Effect of the zeros in complex product on cross-correlation peak height



One solution is to scale the cross-correlation peak by the reciprocal of the number of non-zeros in the complex product for that orientation

# Another more recent option

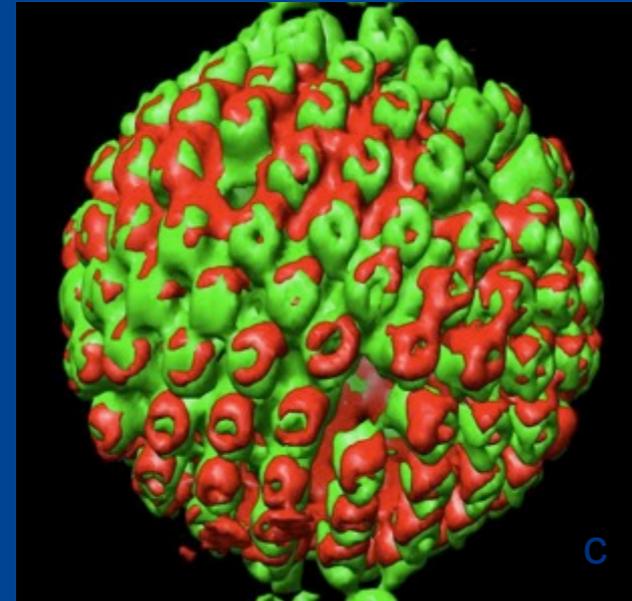
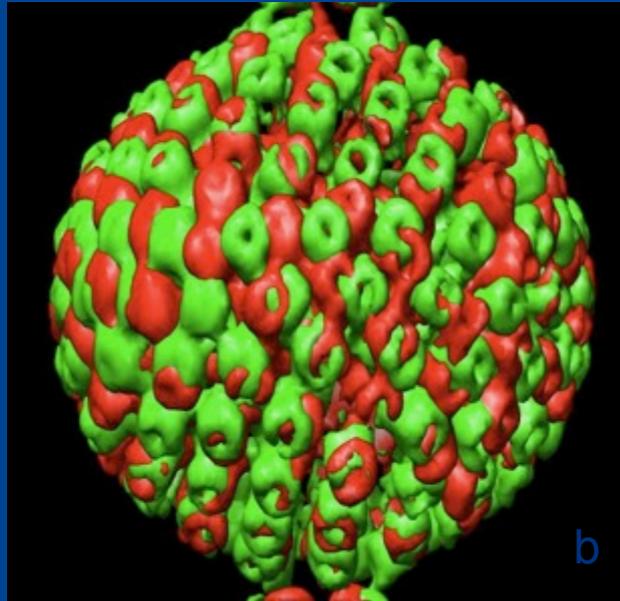
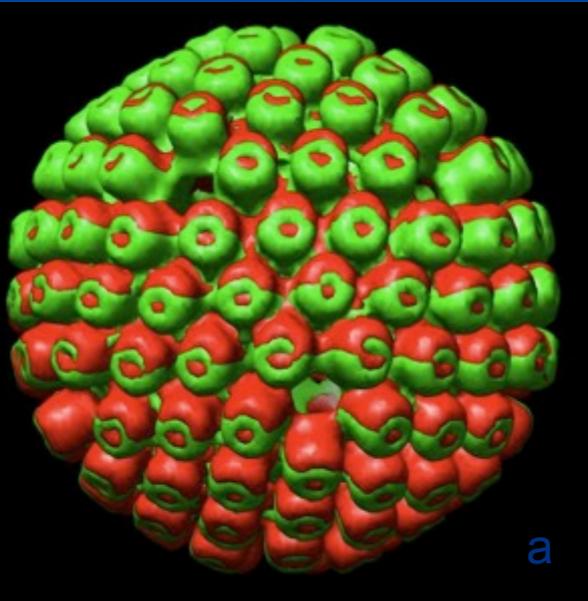
- At each orientation, normalize the cross-correlation map

- In real space, this makes the mean=0 and s.d.=1, so peak value is the # times s.d. by which the peak exceeds the average for that orientation

- In Fourier space this makes total power of the complex product equal for all orientations, which compensates for the missing wedge

# Mutual alignment of a 3-fold oriented map to the 5-fold for a $\pm 54^\circ$ tilt series

(exact correct answer =  $37.72^\circ$ ,  $18^\circ$ ,  $-18^\circ$ )



Alignment ( $5^\circ$  step size) of 3-fold maps to 5-fold maps

a- No Missing wedge ( $40^\circ$ ,  $20^\circ$ ,  $-20^\circ$  - RIGHT (to within  $5^\circ$  step size))

b- Missing wedge without compensation ( $5^\circ$ ,  $15^\circ$ ,  $-15^\circ$  - WRONG)

c- Missing wedge with compensation ( $40^\circ$ ,  $20^\circ$ ,  $-20^\circ$  - RIGHT)

This coarse search is close enough (for a and c) to be refined in a finer local search to the correct orientation.

However, b is too far away from the correct orientation.

# Improvement of alignment by accounting for the missing wedge in cross-correlation search

Right answer - ( $37.72^\circ$ ,  $18^\circ$ ,  $-18^\circ$  or 3-fold related\*)

Fraction missing	Tilt series equivalent	Ignoring effect of missing wedge	Accounting for missing wedge
.00	$\pm 90^\circ$	40, 20, -20	40, 20, -20
.05	$\pm 85.5^\circ$	40, 20, -20	40, 20, -20
.10	$\pm 81.0^\circ$	40, 20, -20	40, 20, -20
.15	$\pm 76.5^\circ$	40, 20, -20	40, 20, -20
.20	$\pm 72.0^\circ$	40, 20, -20	35, -105, 125*
.25	$\pm 67.5^\circ$	5, 20, -20	40, 15, -15
.30	$\pm 63.0^\circ$	5, 15, -15	35, 20, -20
.35	$\pm 58.5^\circ$	5, 15, -15	35, 20, -20
.40	$\pm 54.0^\circ$	5, 15, -15	40, 20, -20
.45	$\pm 49.5^\circ$	5, 15, -15	15, 75, -90
.50	$\pm 45.0^\circ$	5, 15, -15	10, -60, 50

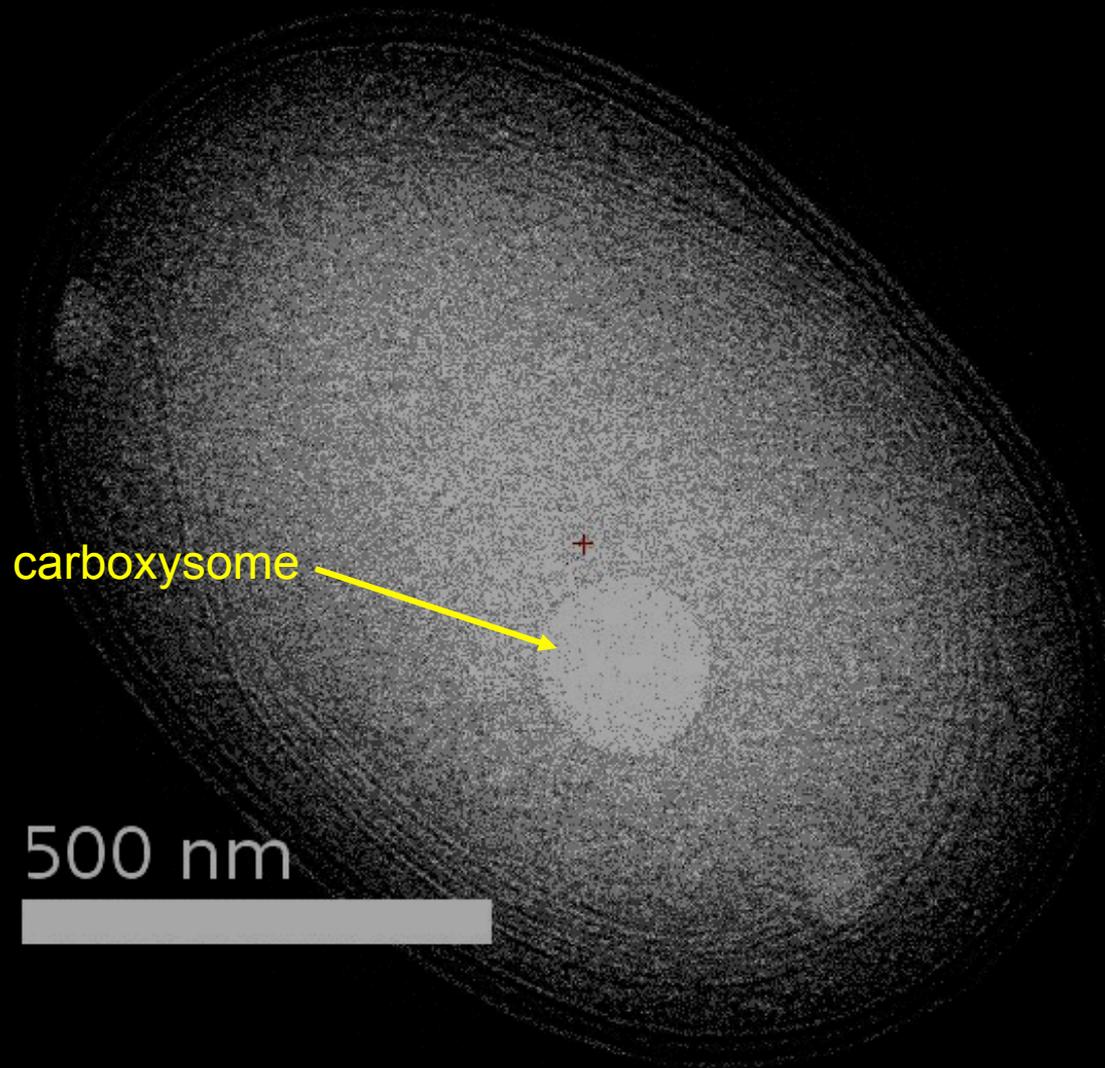
# Image Preparation Considerations

1. Contrast-flipping, initial rotation and hand-choice
  - Reconstruction is usually contrast-reversed
  - Some reconstructions do not have missing wedge along z
  - Some reconstructions have hand-flip
2. Filtering
  - Subtomograms are inherently low-res and high-noise, so alignment should not be overly optimistic ( $\sim 40\text{\AA}$  lowpass is appropriate)
  - Large-scale features of the object may allow even more aggressive filtering for initial alignment
3. Normalization
  - Useful for classification based on cross-correlation peak values
4. Masking
  - Spherical Zero mask insures correct “fill-in” function for rotated volumes
5. Always carry out resulting operations (rotation and translation) on the ORIGINAL files, not the low-passed, etc. versions!
6. Ultimate goal is to keep track of all transformations to be able to re-insert the average into the tomogram at the orientation of each instance of the structure

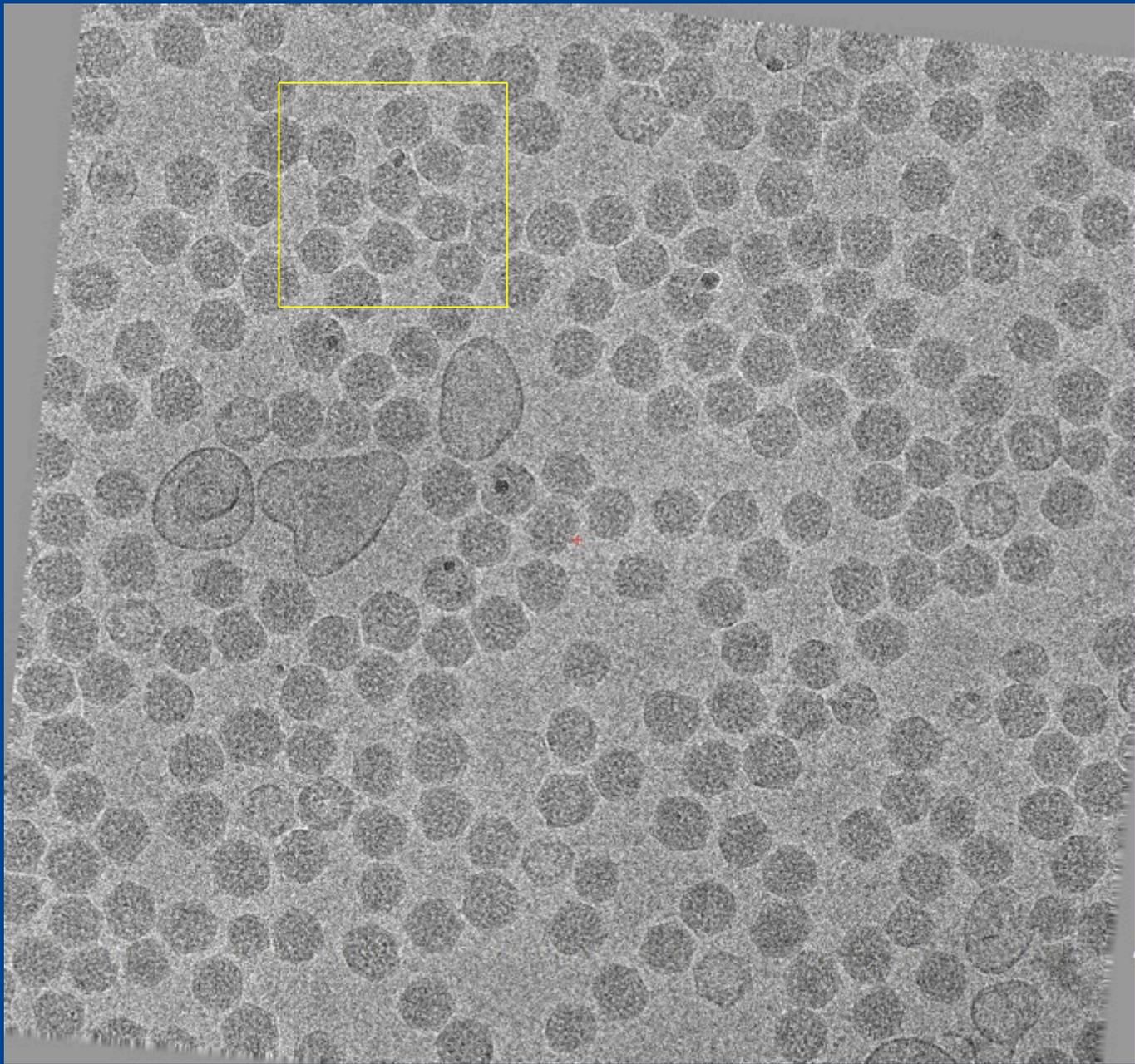
# Carboxysome

- Found in photosynthetic and chemoautotrophic bacteria
- "Polyhedral" bodies - ~100 nm diameter, thin angular shell, granular interior
- Contain RuBisCO - fixes CO<sub>2</sub>
- Regulated
- Size, shape, symmetry of carboxysome and arrangement of RuBisCO unknown
- What if we want to inventory macromolecular machines in the cell if we don't know much about them *a priori*

# Prochlorococcus



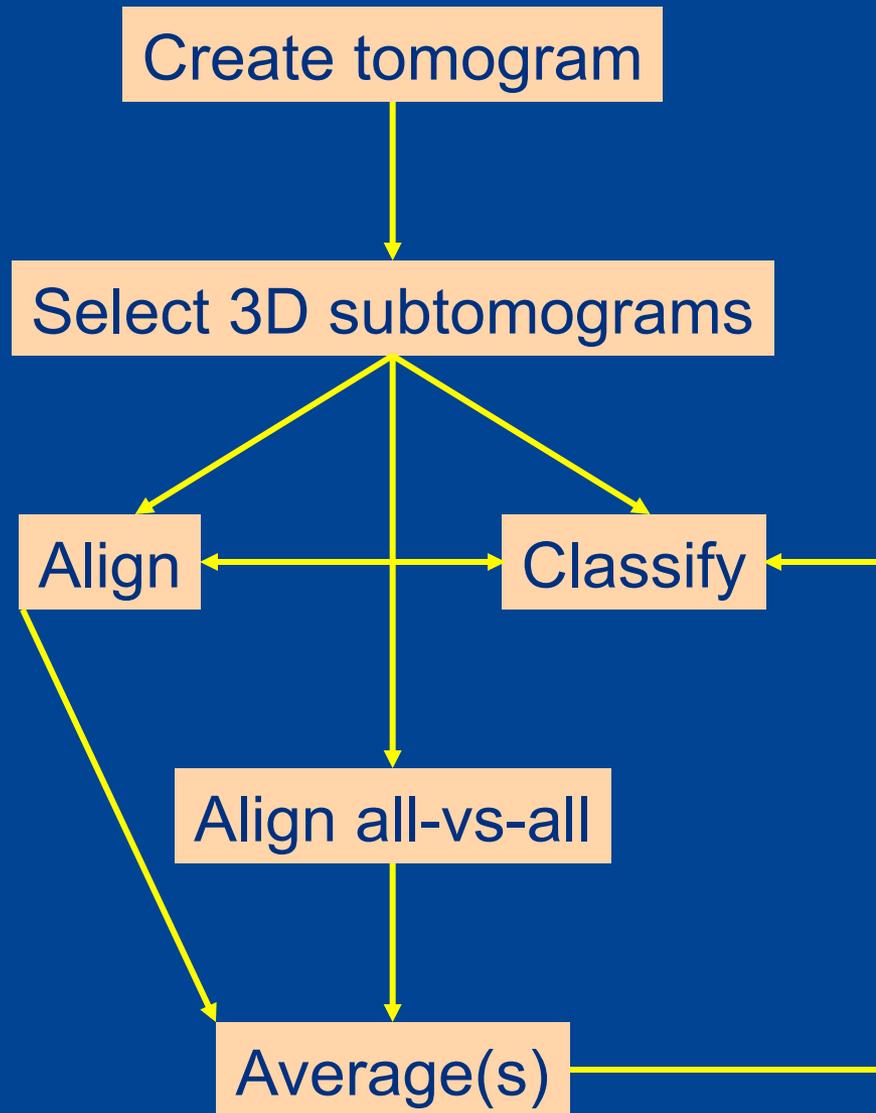
Mike Marsh



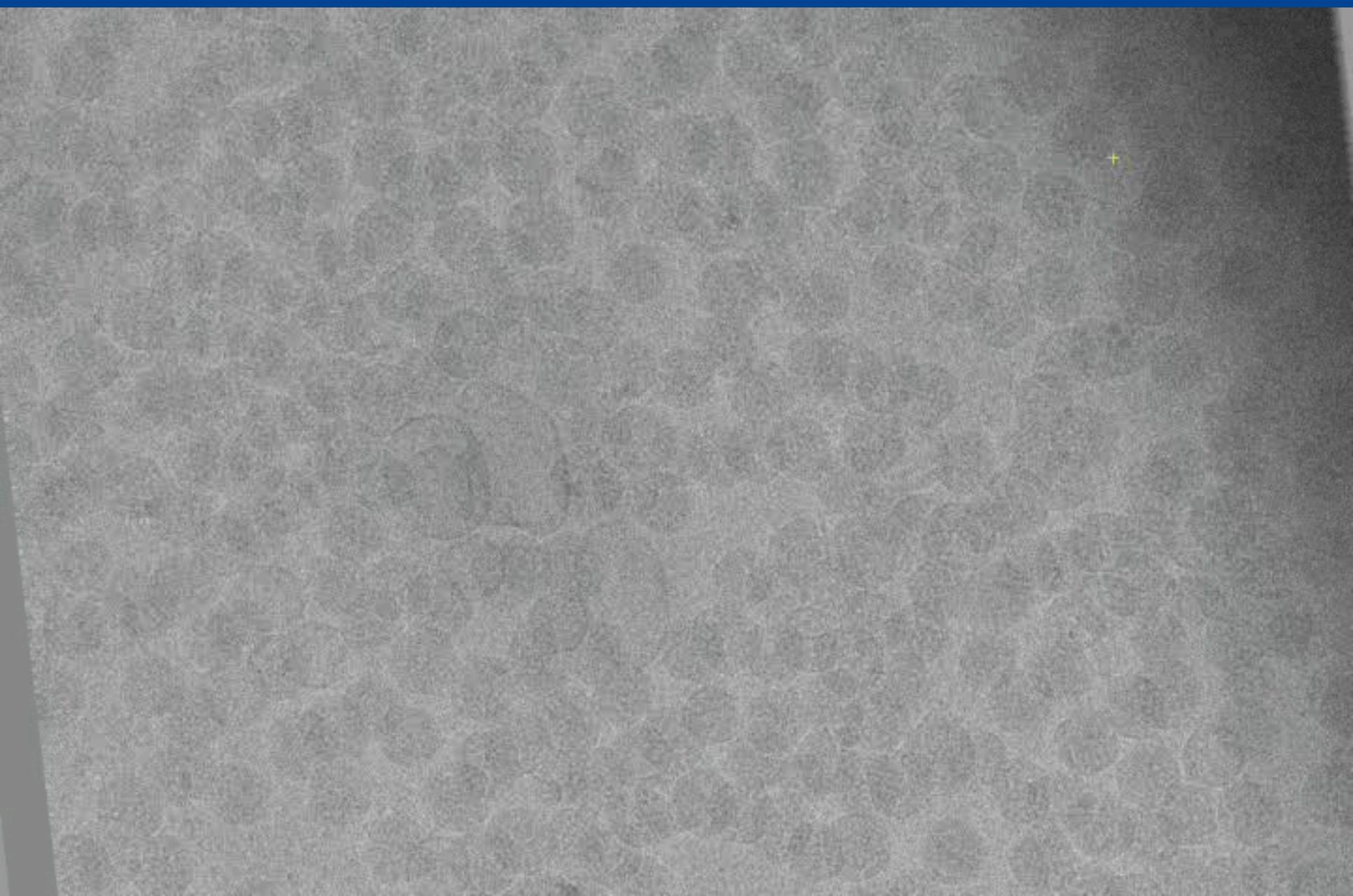
# Approach

- Conventional single particle processing with icosahedral symmetry using common lines did not work
- Our approach - averaging 3D subvolumes extracted from tomograms (subtomograms)
  - but subtomograms have a missing wedge in Fourier space the same shape as the missing wedge of the entire tomogram
- In the literature of post-tomographic averaging, subtomograms have been aligned against a 3D model template which does not have a missing wedge
- Size heterogeneity and unknown symmetry make it difficult to choose a starting model
- Therefore we chose to mutually align subtomograms to each other

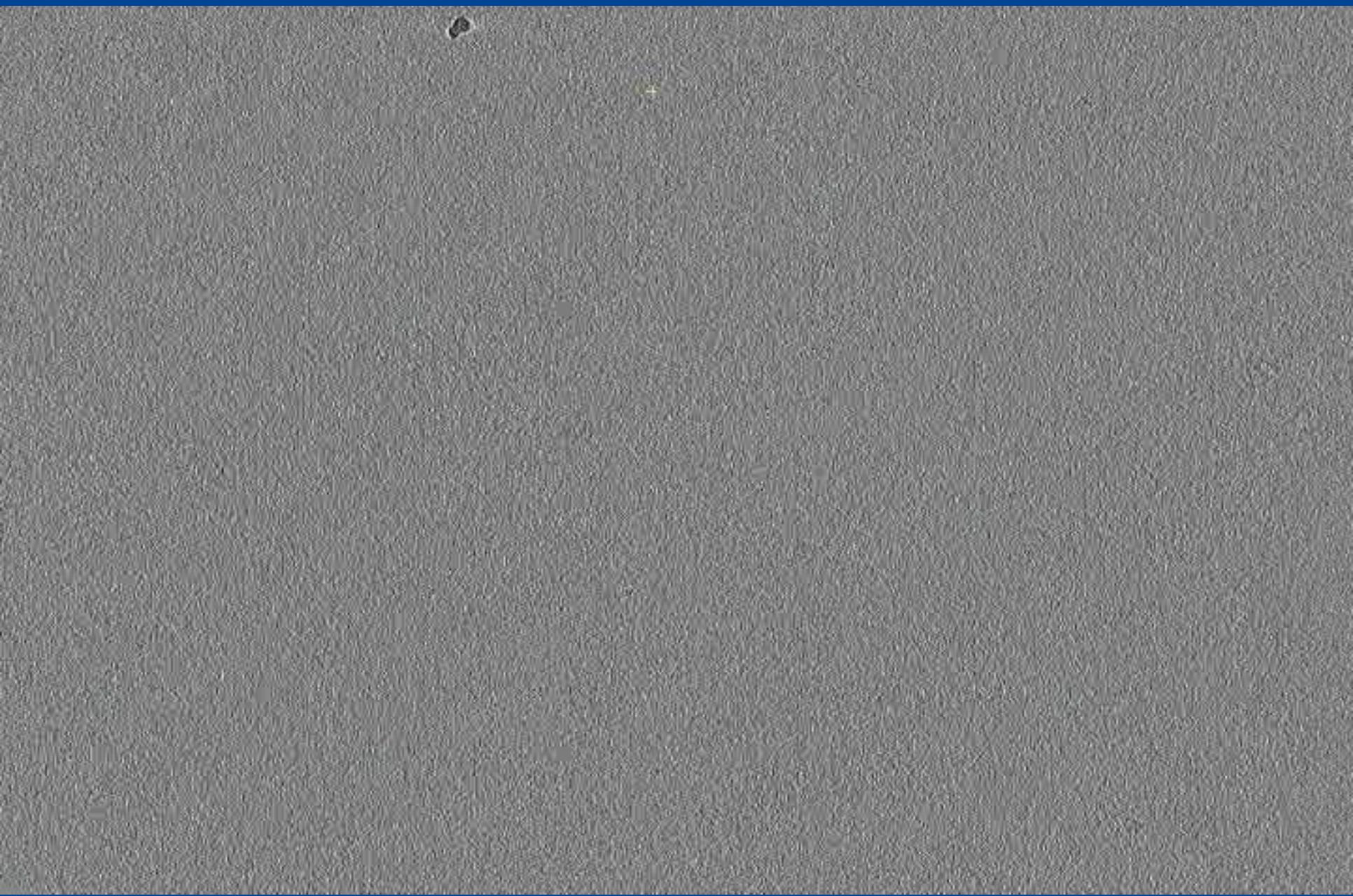
# Flow Chart for processing subtomograms

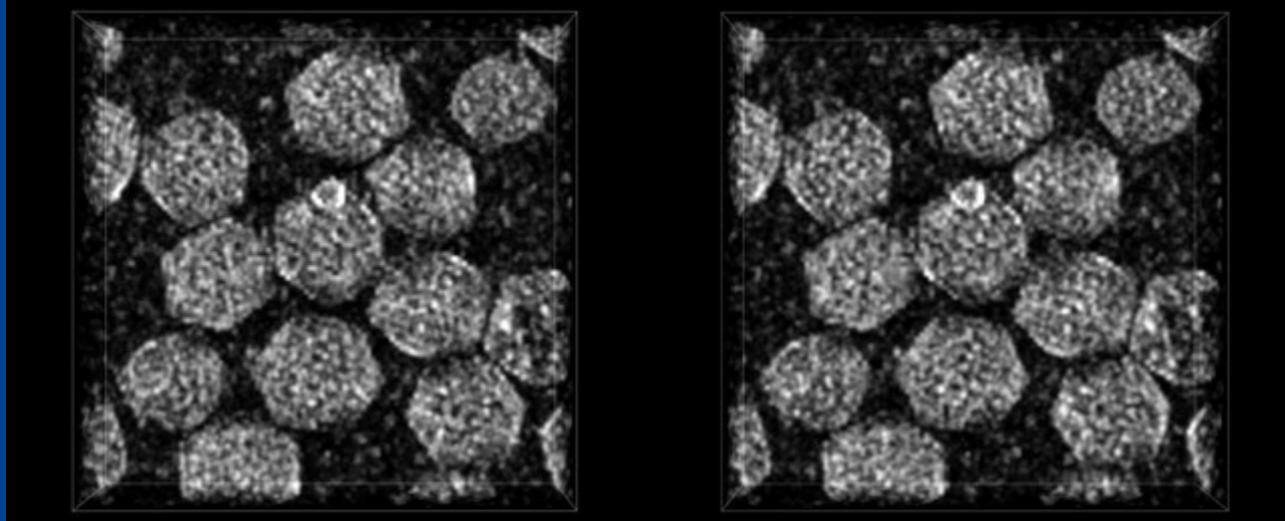
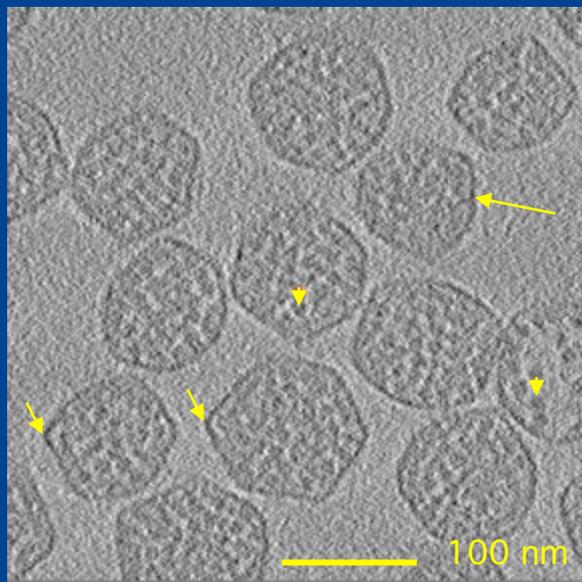


# Halothiobacillus carboxysome tilt series

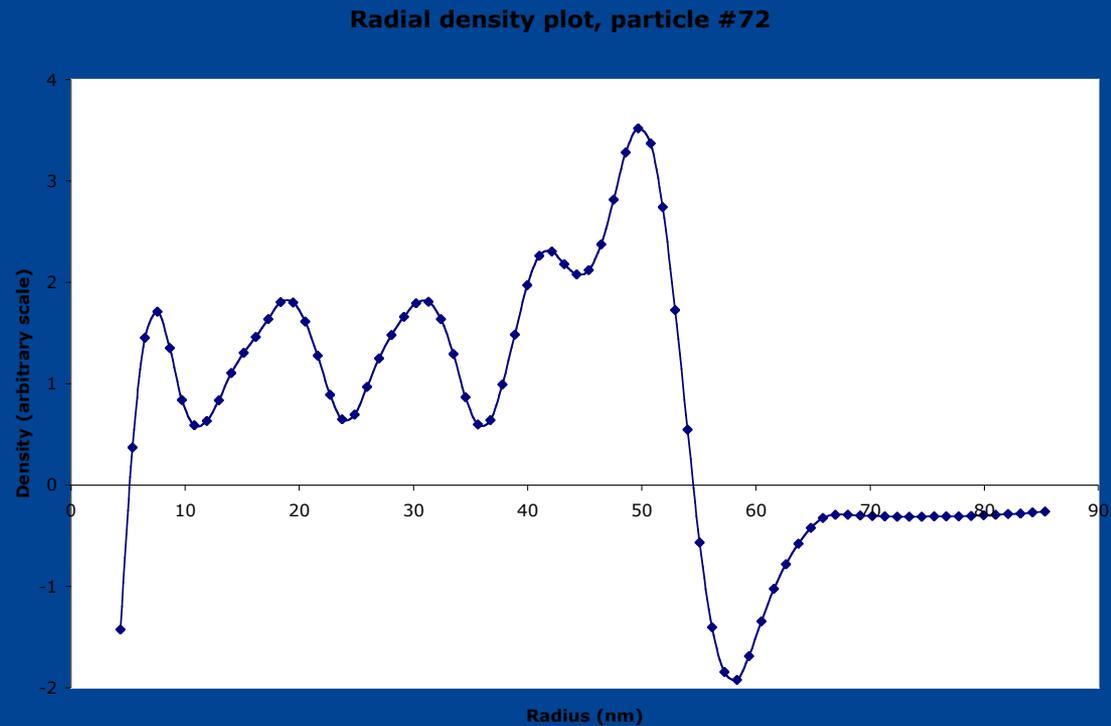


# Tomographic reconstruction

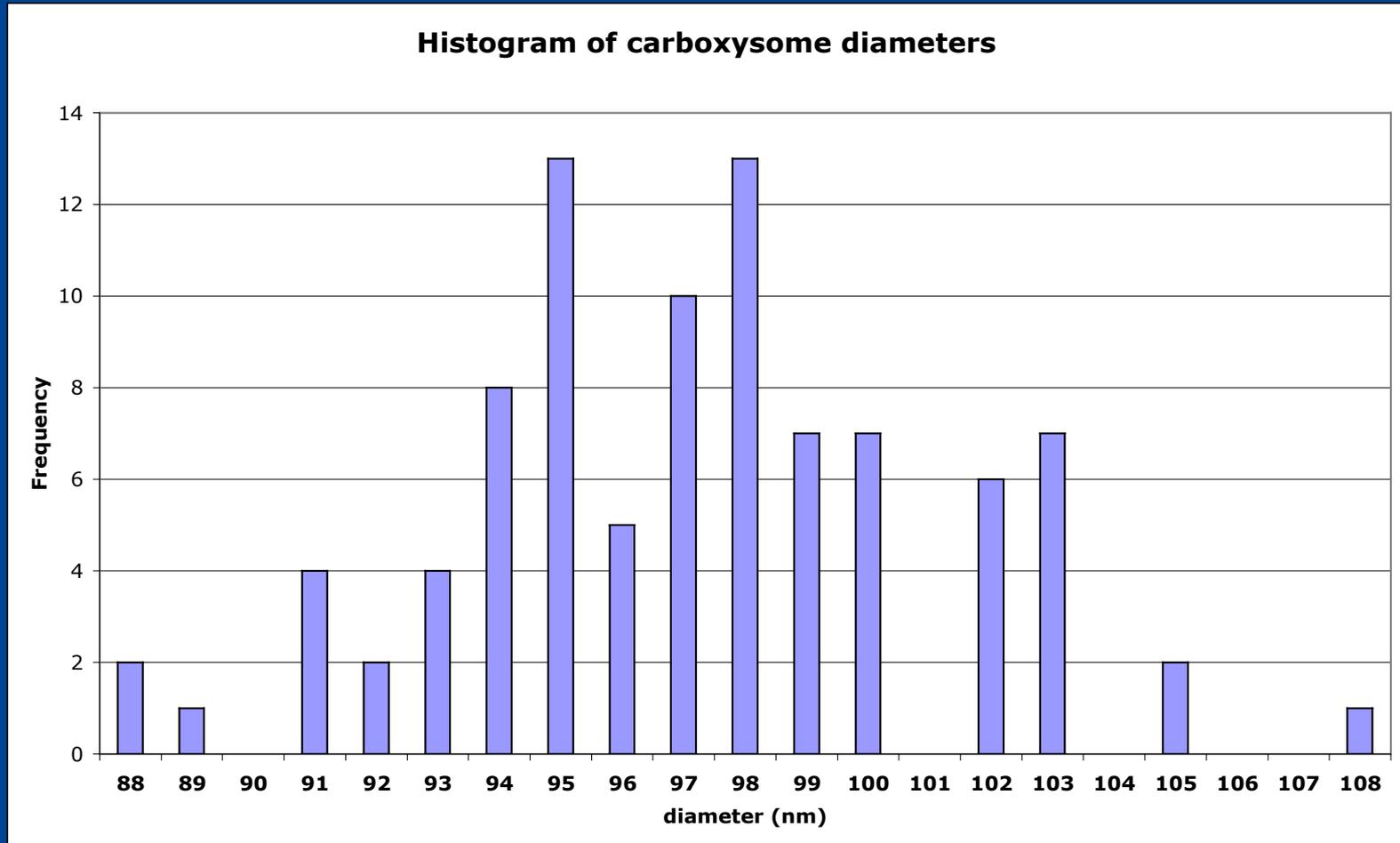




# Plot of density for 1 of 92 3-D Volumes



# Carboxysomes have size heterogeneity



# Reference-free 3-D alignment and averaging

Roughly split 3-D subvolumes  
into 9 diameter classes

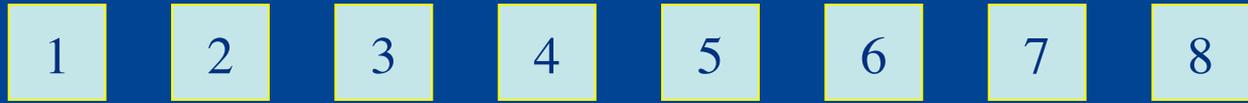
All-vs.-all mutual cross-correlation  
orientation alignment within each  
class, and also with the next larger  
and smaller diameter classes, shifting  
if necessary

Average best pairs of alignments  
These replace the original data pairs  
in new all-vs-all round



# Schematic

Cycle 1



Result 1



Avg 1



Cycle 2



Result 2



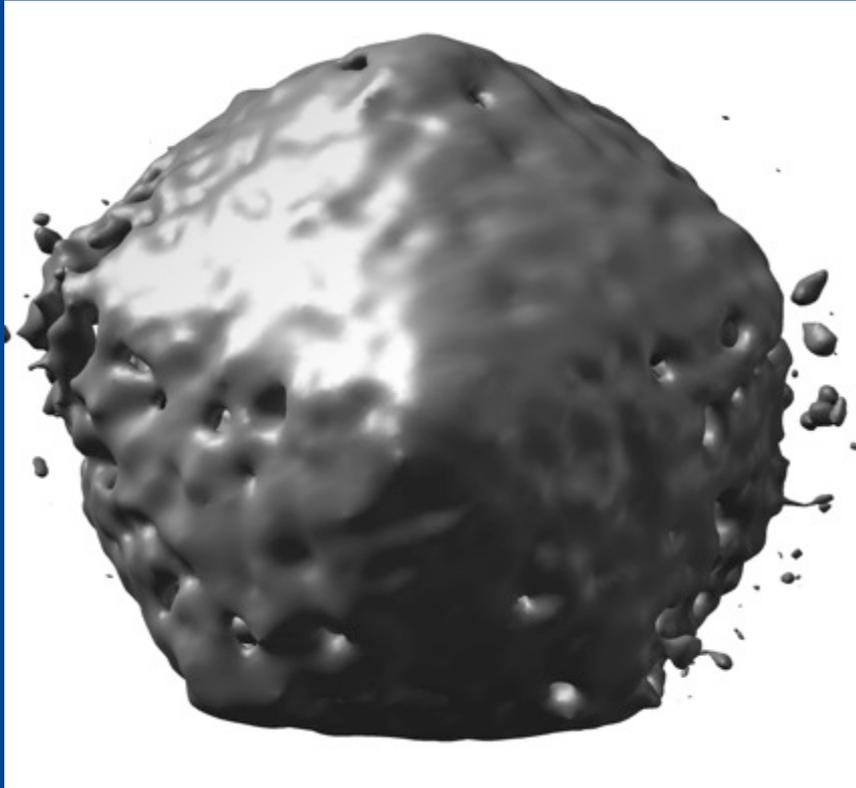
Avg 2



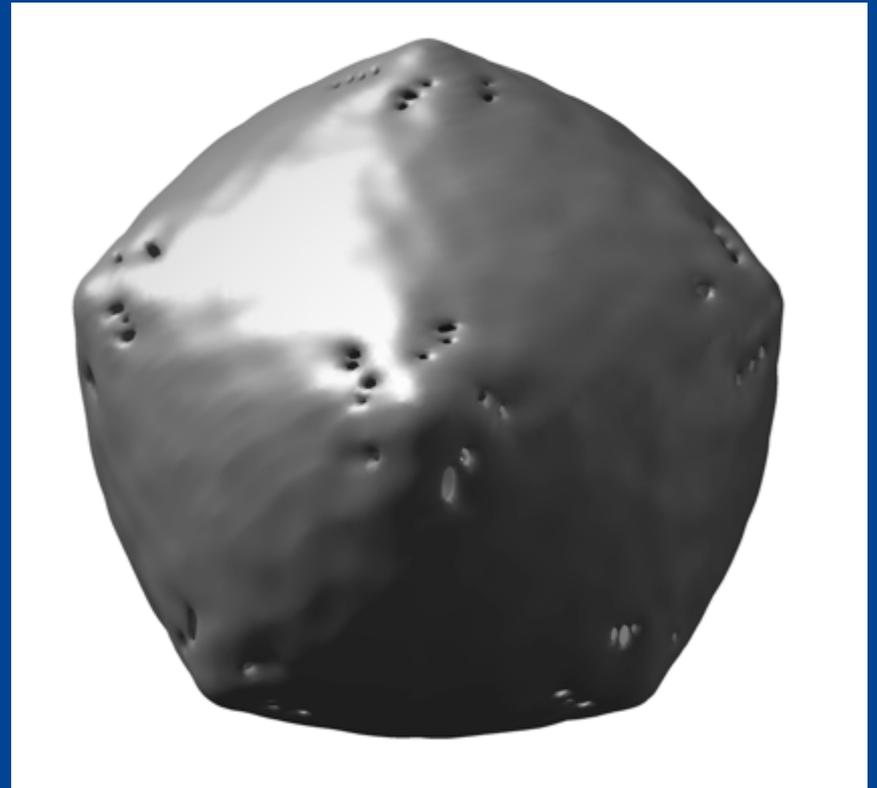
Cycle 3



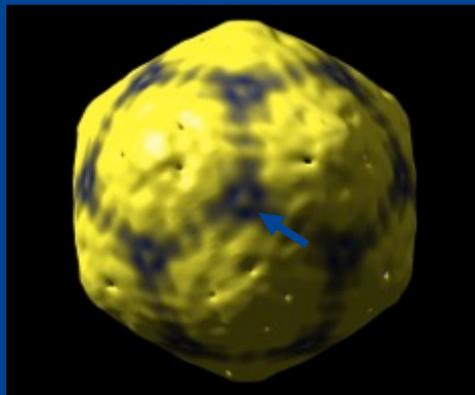
# Result of all-vs.-all mutual cross-correlation searching and averaging in size classes



Tomographic averaging - 100nm class -  
20 particles

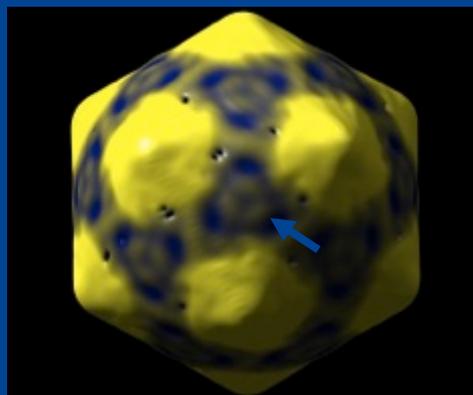


Tomographic plus icosahedral averaging



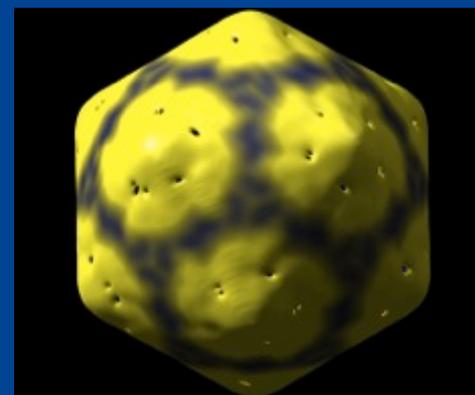
a

88nm



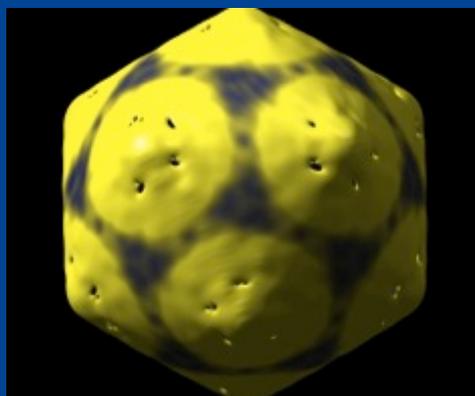
b

91nm



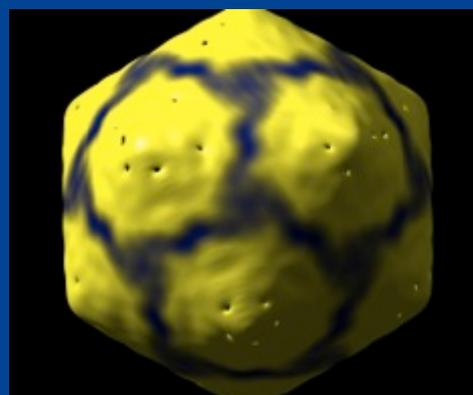
c

93 nm



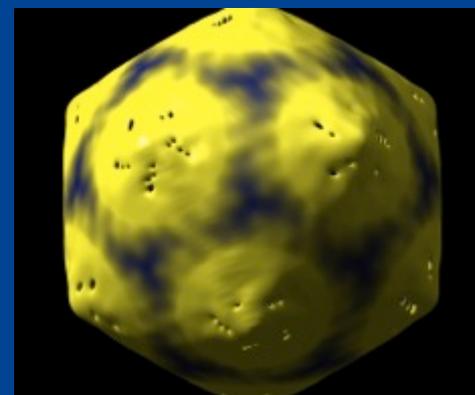
d

95 nm



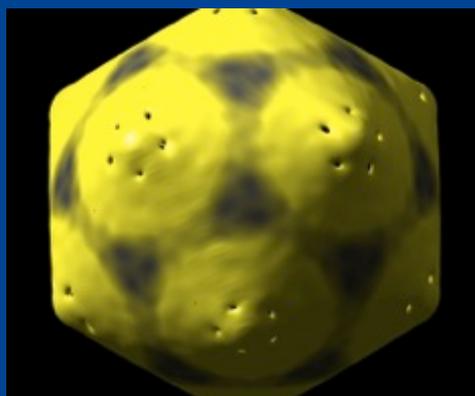
e

97 nm



f

100 nm

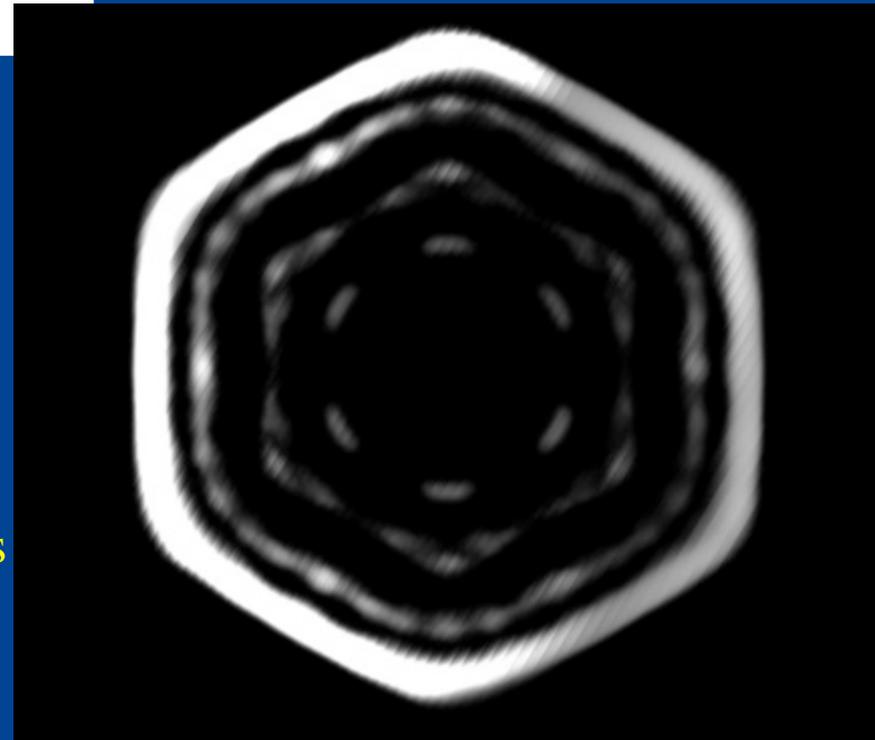
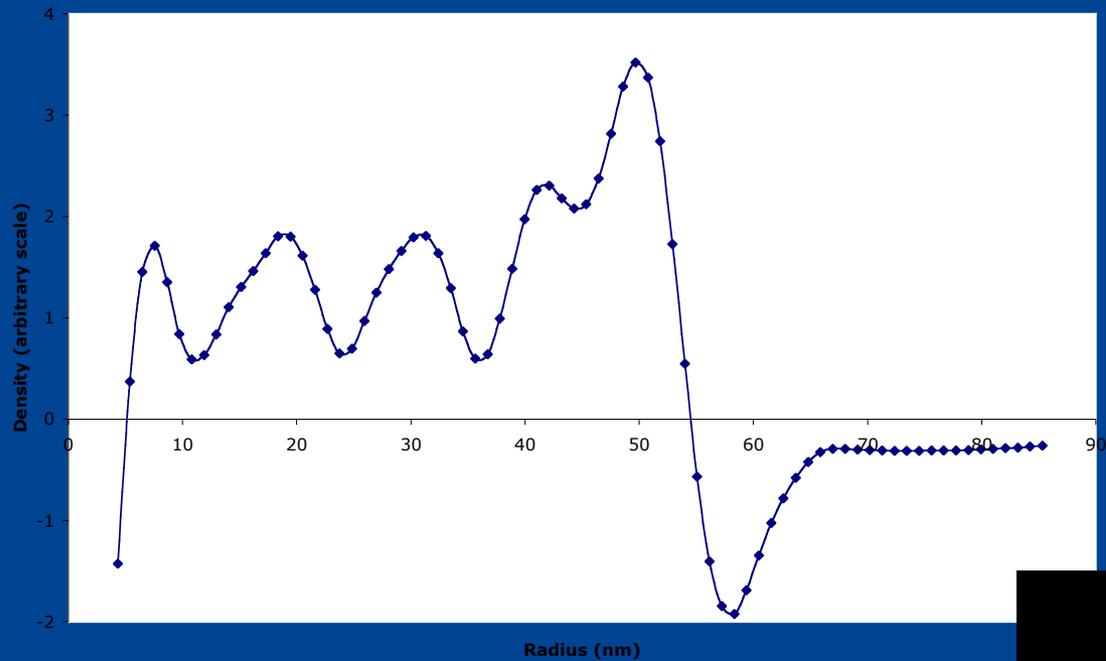


g

103 nm

Size classes

Radial density plot, particle #72



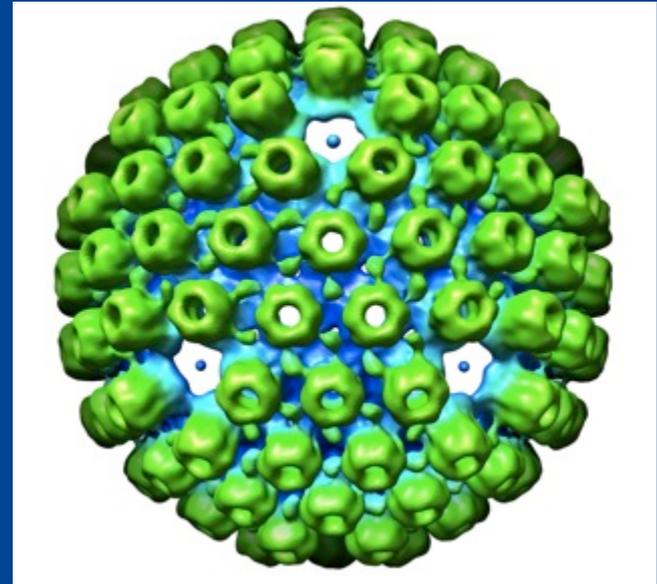
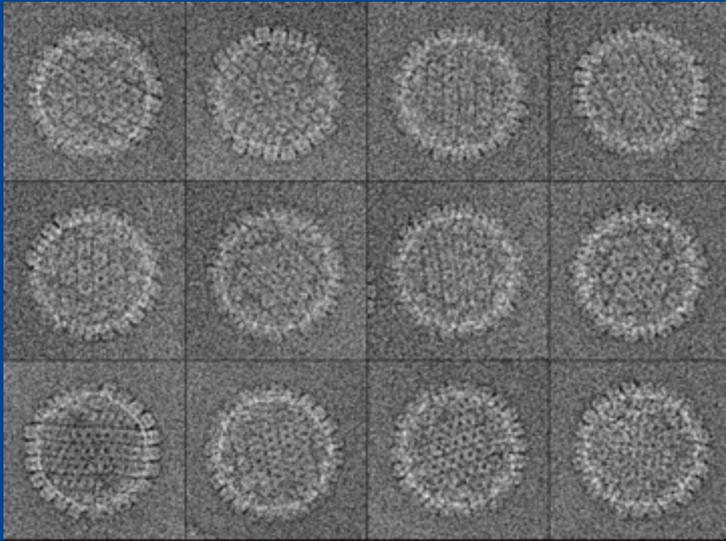
Central slice of averaged particle - 100nm class  
shell of average is higher density, interior  
densities do not have icosahedral symmetry.

# Conclusions

- Shell symmetry is icosahedral
- Size of carboxysome varies from 88 to 103nm - unusual for an icosahedral particle
- Shell protein arrangement varies with size
- RuBisCO organization in layers inside, but not regular, nor constant amount per particle
  
- Specialized processing needed for determining mutual orientation and for averaging of particles with missing wedge
  
- Schmid et al. (2006) J. Mol. Biol. (in press, online 09/14/06 )

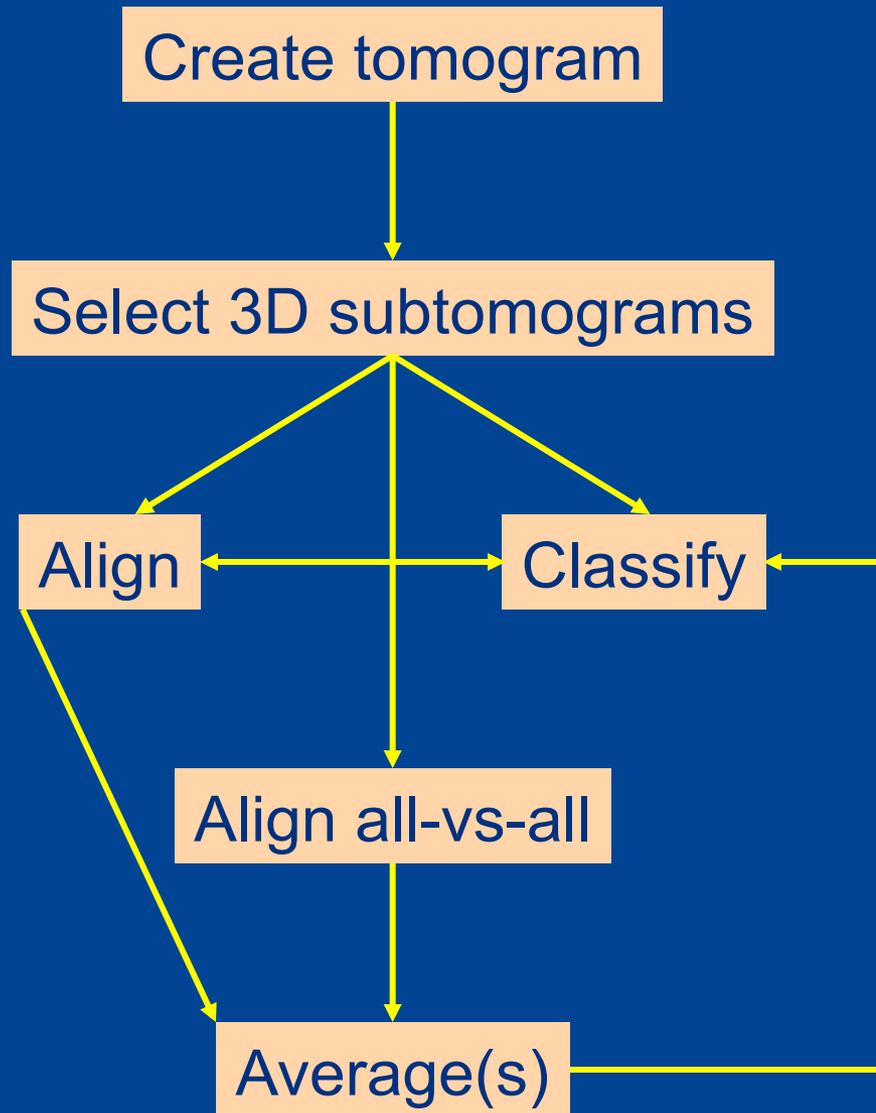
# HSV pentonless capsids

Produced by chemical treatment of capsids with urea - removes pentons, but not portal

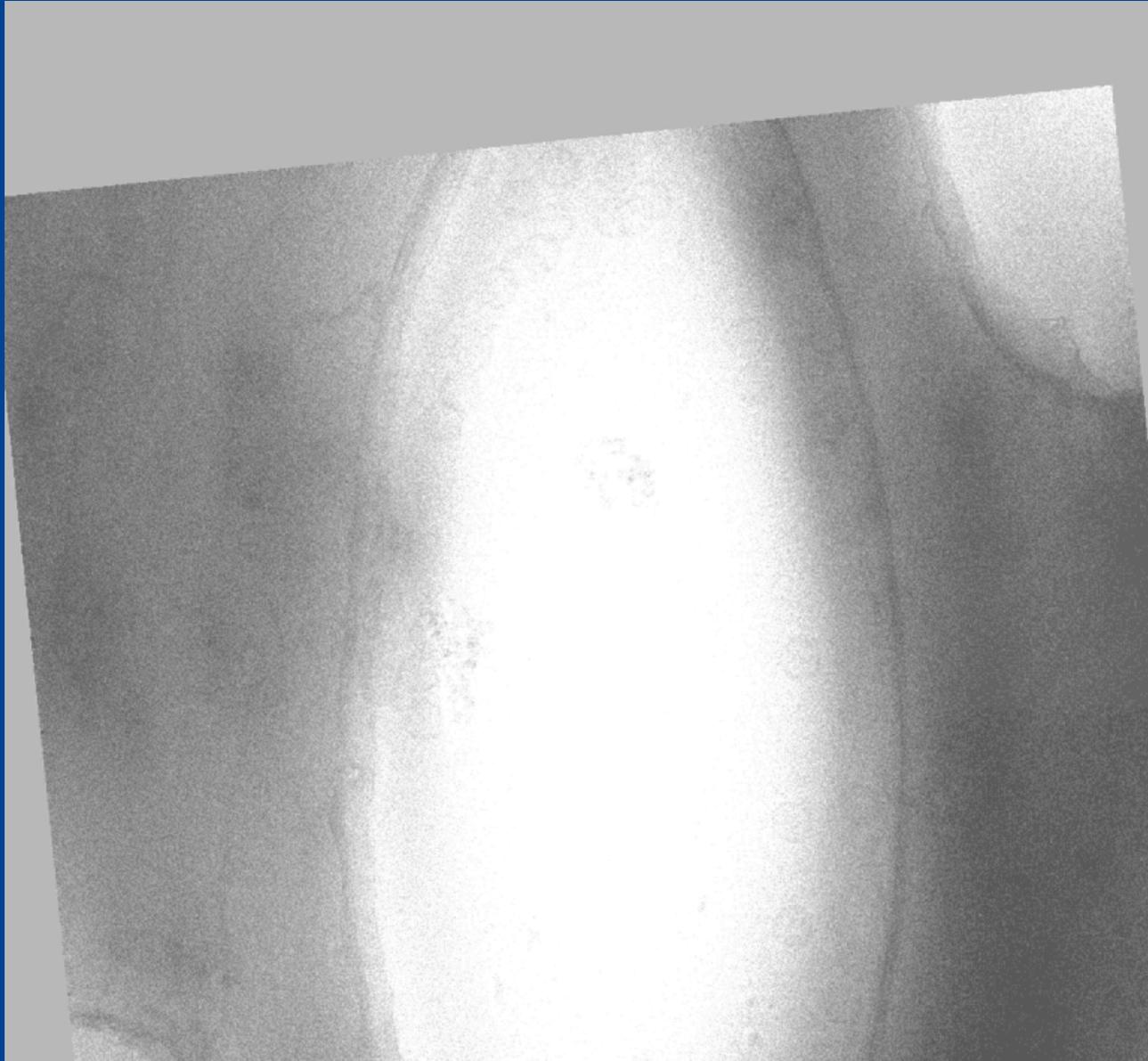


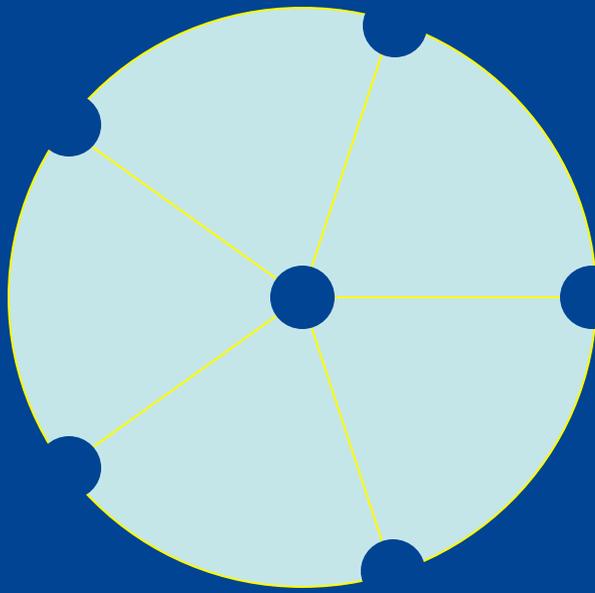
Icosahedral single particle reconstruction -  
Portal averaged away

# Flow Chart for processing subtomograms

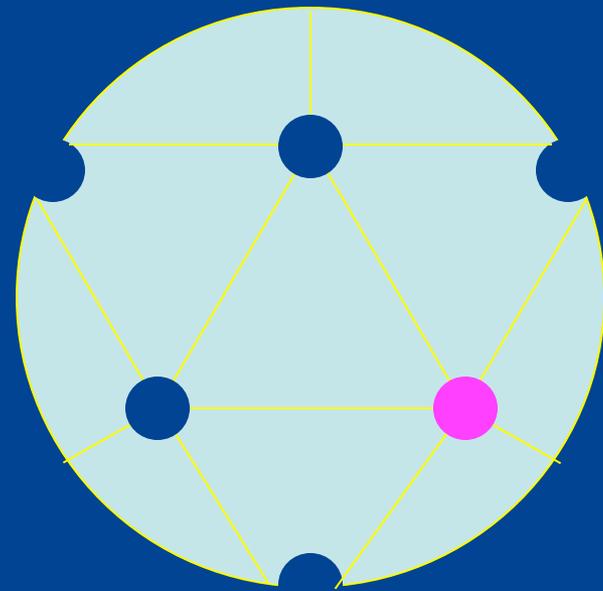


# Tilt Series of Herpes Pentonless capsid

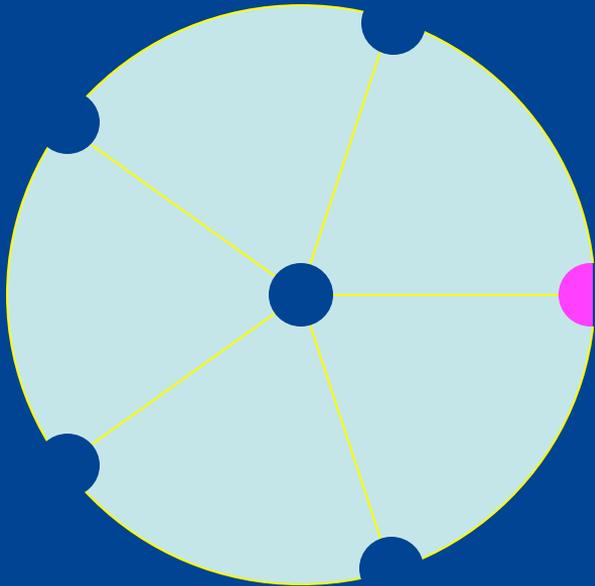




Icosahedral model



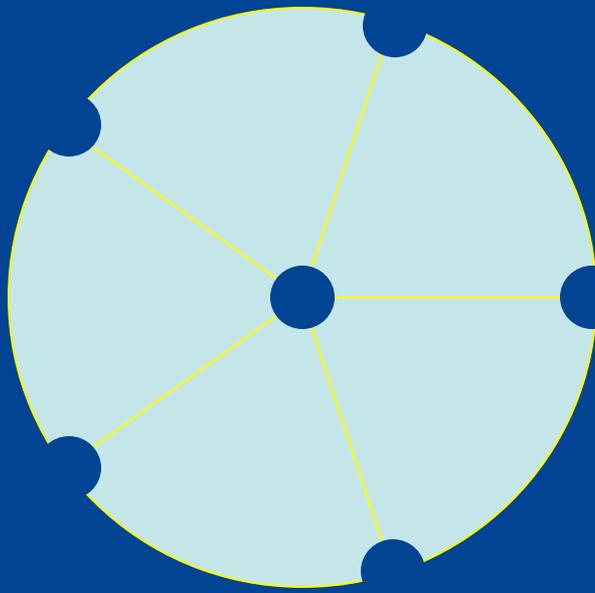
Tomographic reconstruction  
with unique portal



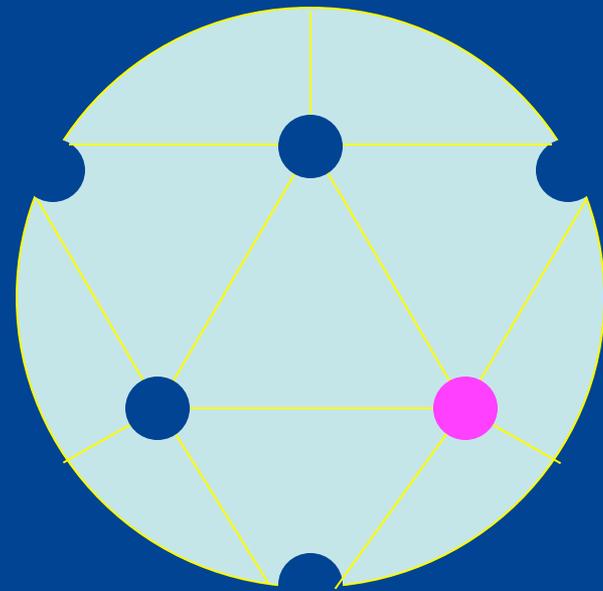
Tomographic aligned to model

# Alignment problem

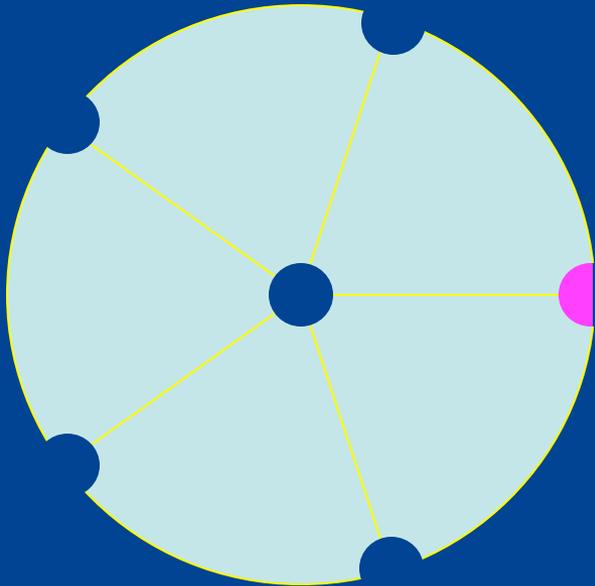
- The missing wedge causes densities to be different in different directions (from part 1)
- However, opposite vertices are affected equally by the missing wedge, so our solution was to compare the densities at opposite vertices; the one with the biggest *difference* in density was the portal vertex.



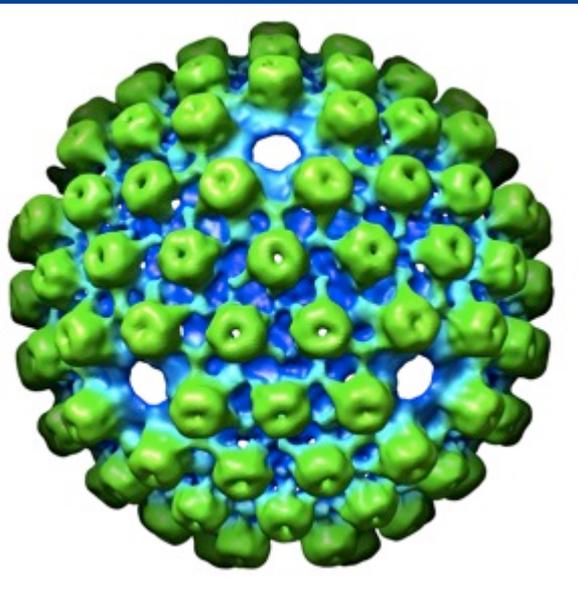
Icosahedral model



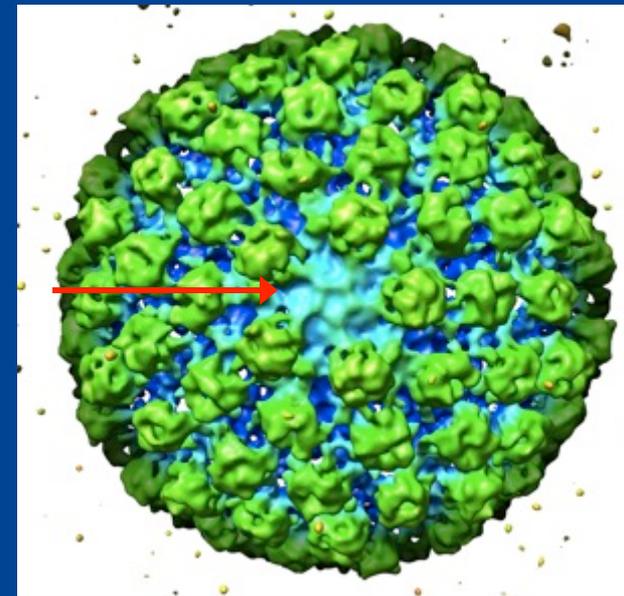
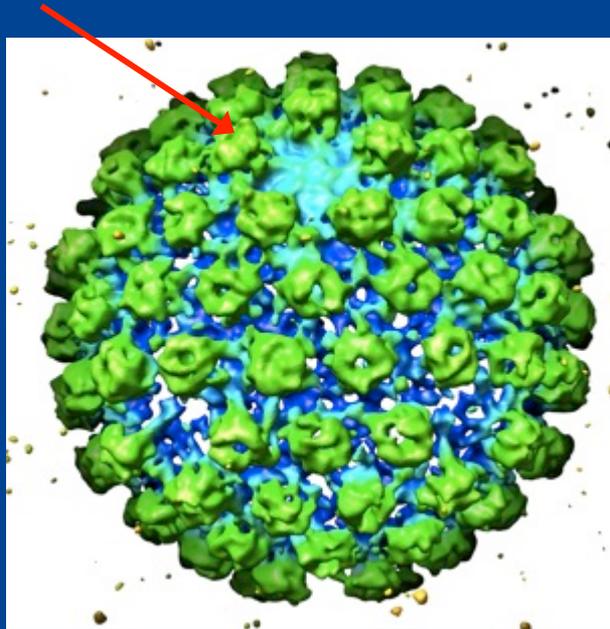
Tomographic reconstruction  
with unique portal



Tomographic aligned to model

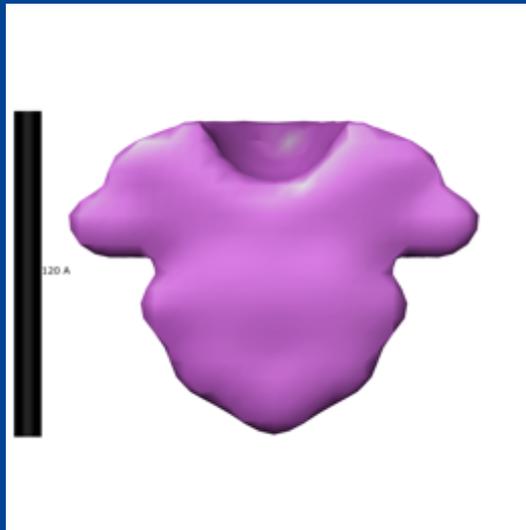


Tomographic icosahedral  
Compare with single  
Particle results

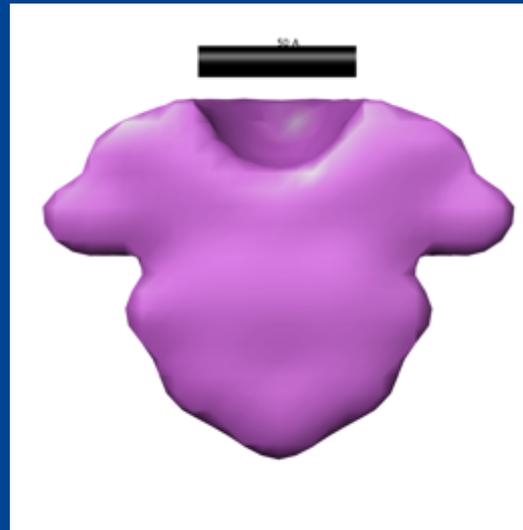


Portal vertices aligned,  
averaged and 5-fold  
symmetrized

# Difference map- 5-fold minus icosahedral average.



120A

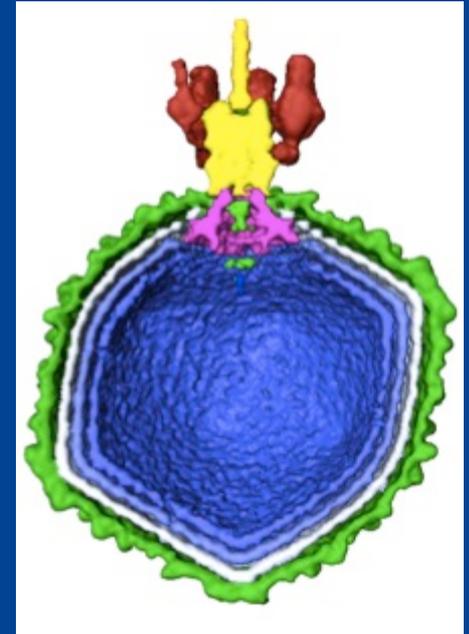
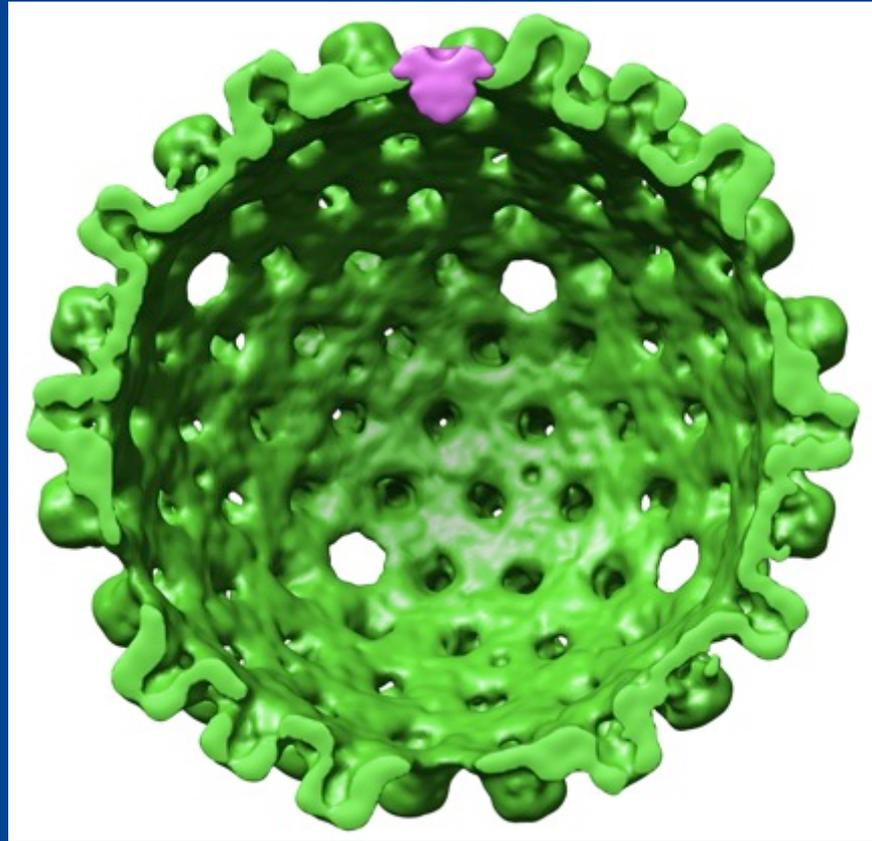
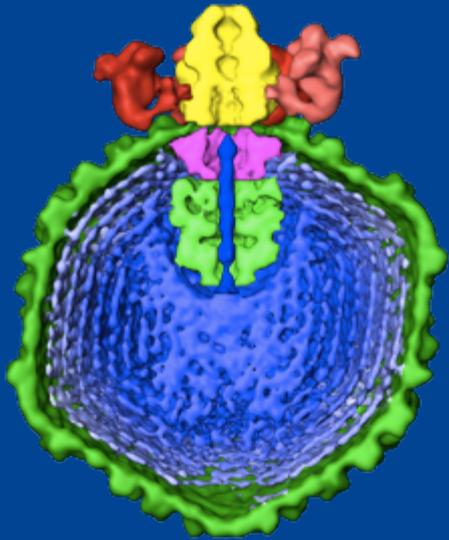


50A



150A

Difference map- 5-fold minus icosahedral average,  
cylindrically averaged, placed into icosahedral map



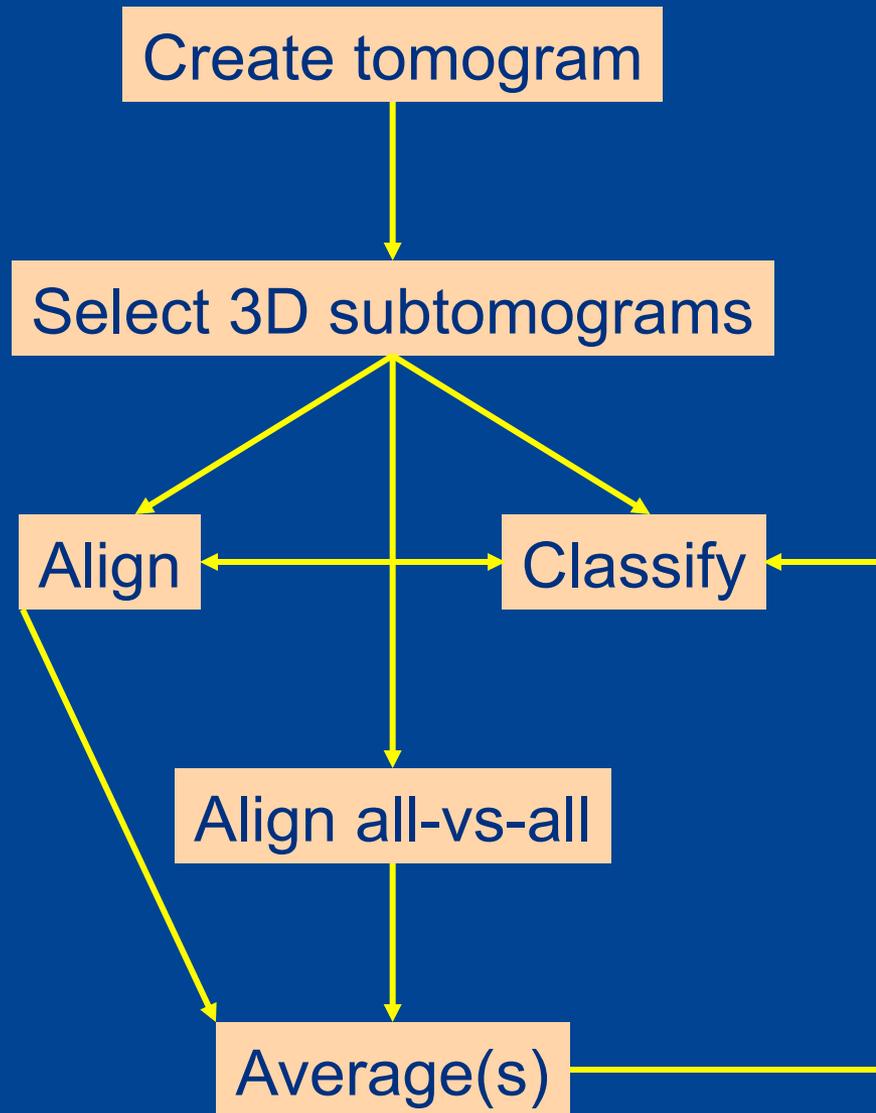
Epsilon Phage - Jiang et al.

P22 - Chang et al.

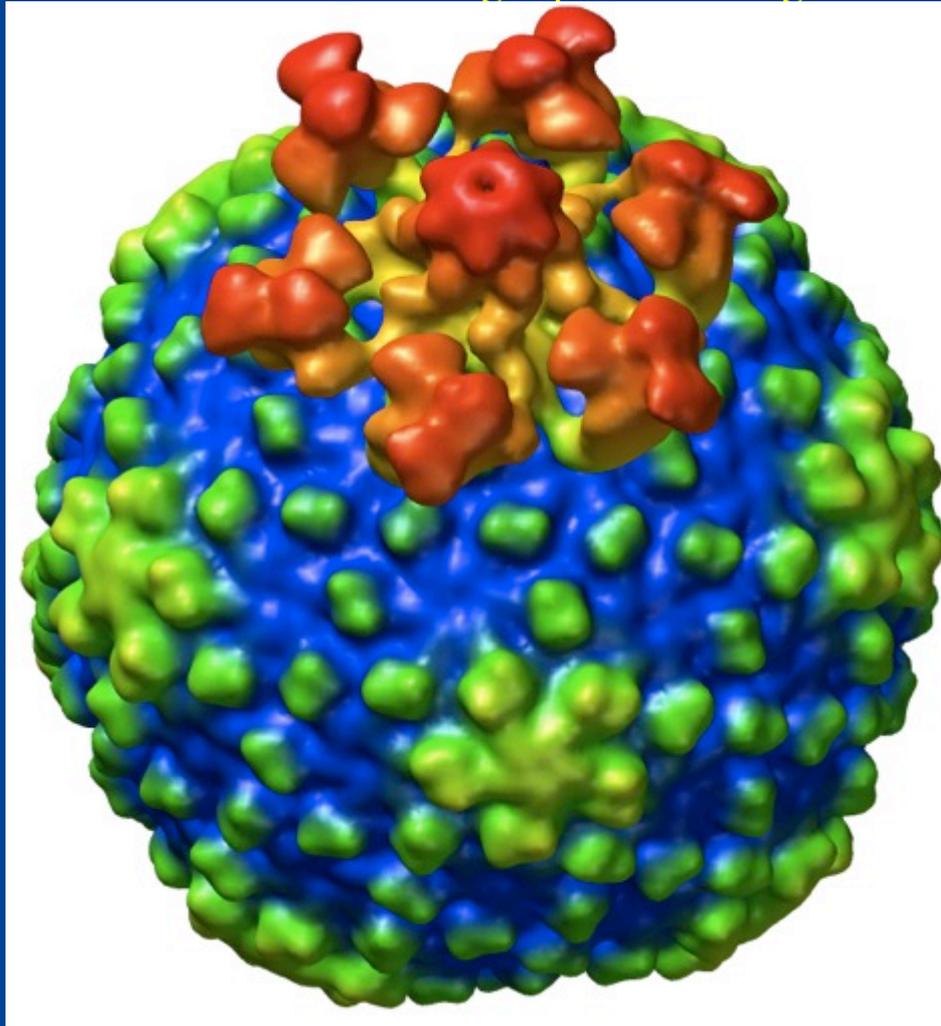
# Epsilon 15 tomographic averages

1. Align tomographic subvolumes to icosahedrally averaged model from single particle (after this, no model used)
2. Put each vertex in turn along z, average it c19 (cylindrical)
3. Put unique vertex along +z. (6 tail spikes still not aligned)
4. All-vs-all cross-correlation with 5 search orientations
5. Average best-correlating pairs, etc.

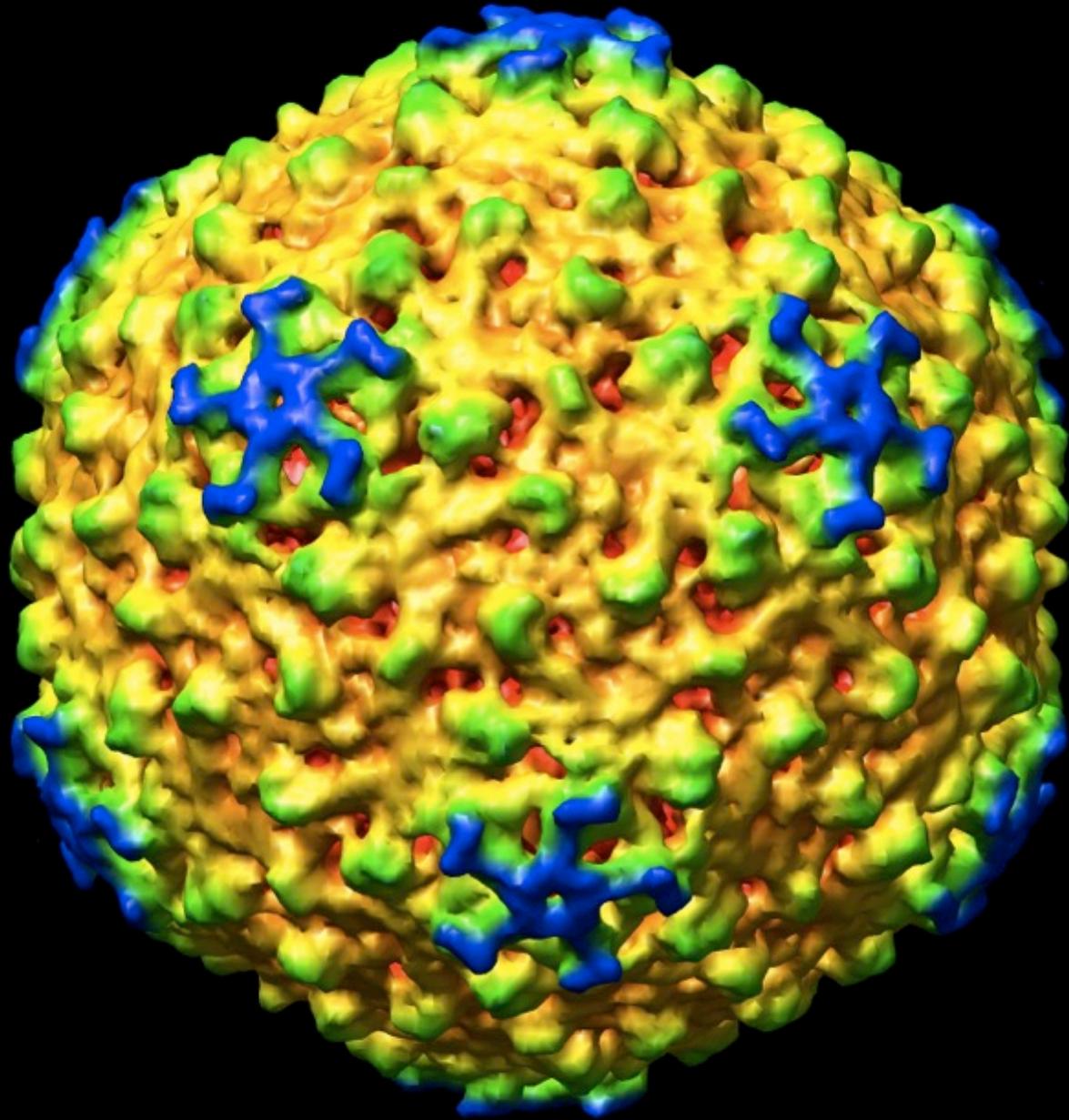
# Flow Chart for processing subtomograms



15000 single particle images

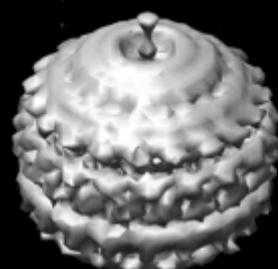
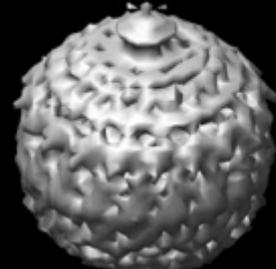
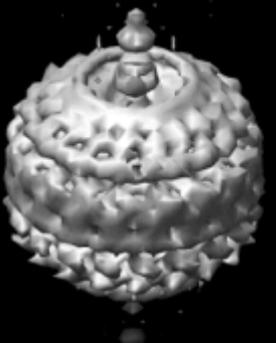
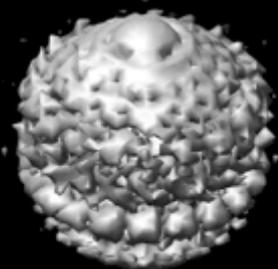
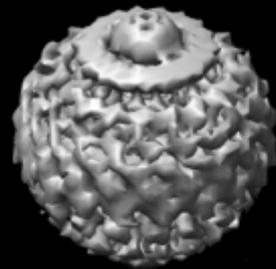
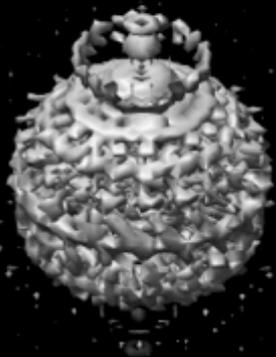


Jiang et al. (2006) Nature 439:612



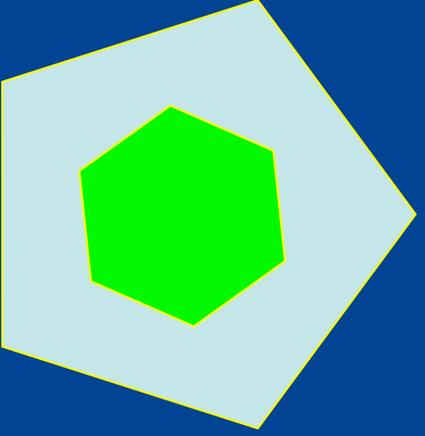
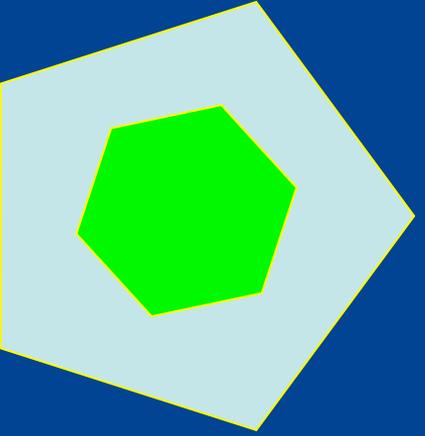
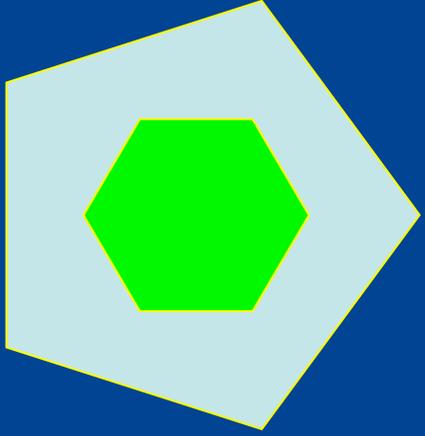
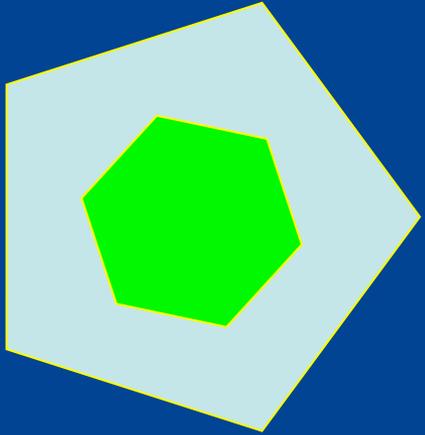
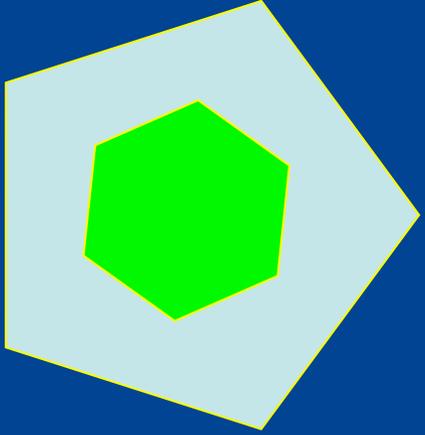
# Epsilon 15 tomographic averages

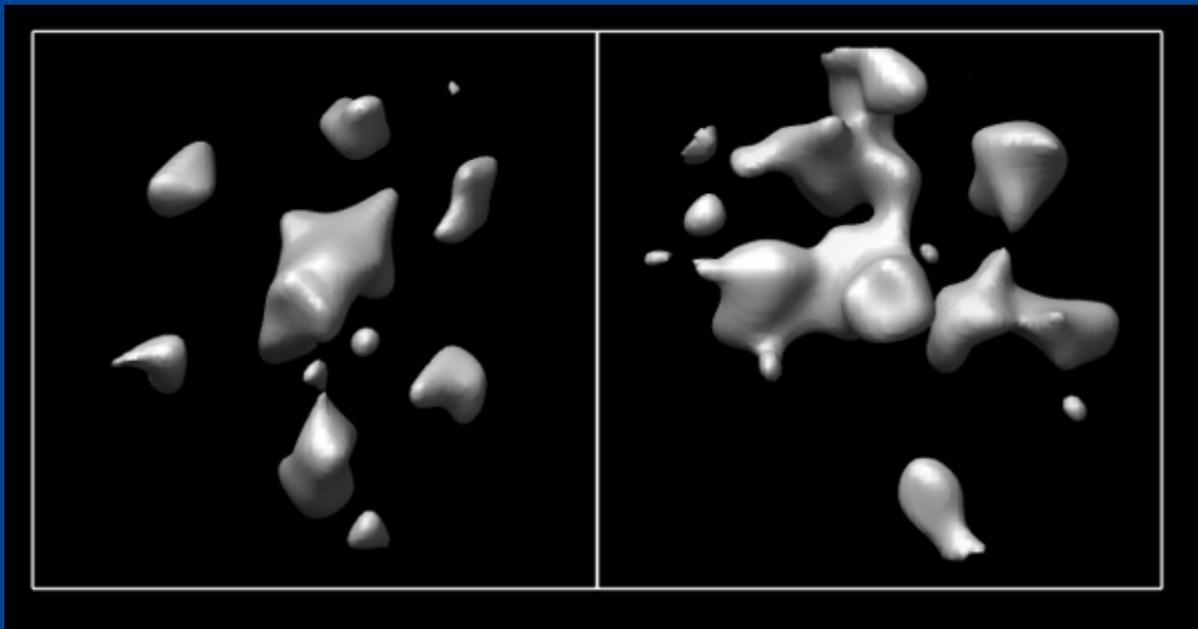
1. Align tomographic subvolumes to icosahedrally averaged model from single particle (after this, no model used)
2. Put each vertex in turn along z, average it c19 (cylindrical)
3. Put unique vertex along +z. (6 tail spikes still not aligned)
4. All-vs-all cross-correlation with 5 search orientations
5. Average best-correlating pairs, etc.



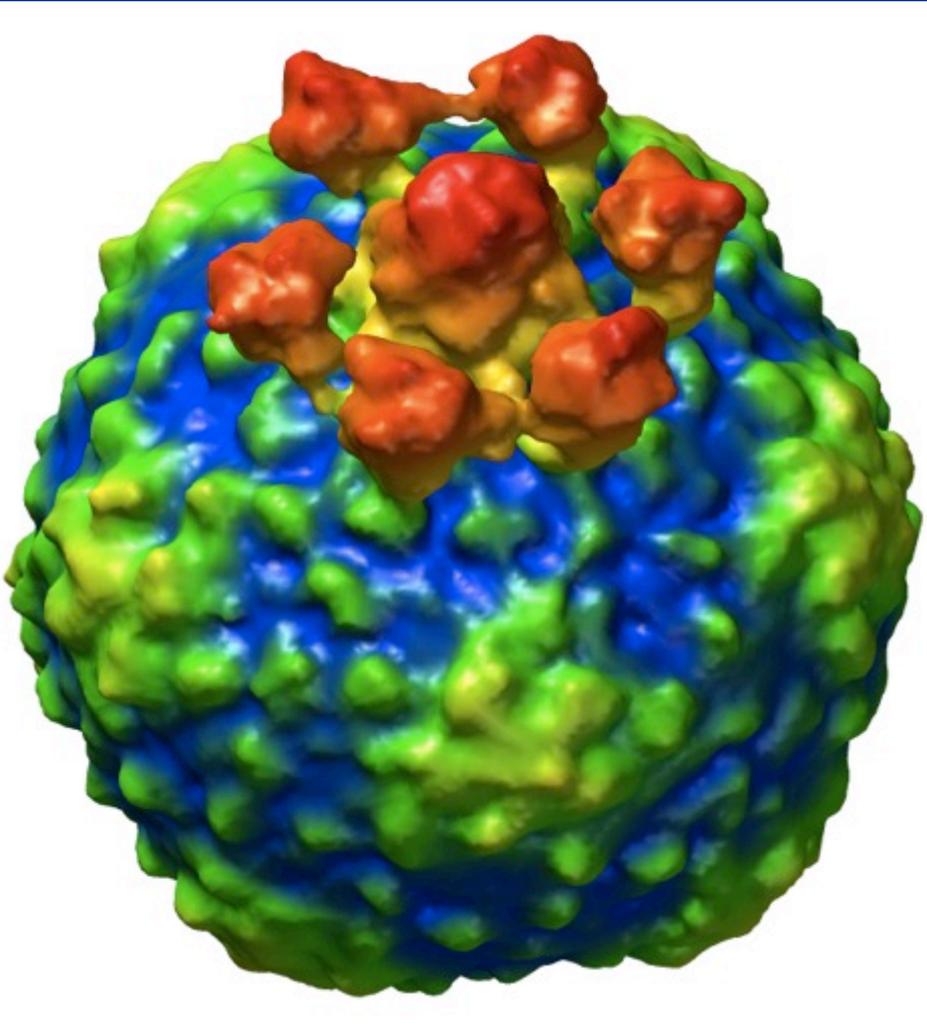
# Epsilon 15 tomographic averages

1. Align tomographic subvolumes to icosahedrally averaged model from single particle (after this, no model used)
2. Put each vertex in turn along z, average it c19 (cylindrical)
3. Put unique vertex along +z. (6 tail spikes still not aligned)
4. All-vs-all cross-correlation with 5 search orientations
5. Average best-correlating pairs, etc.

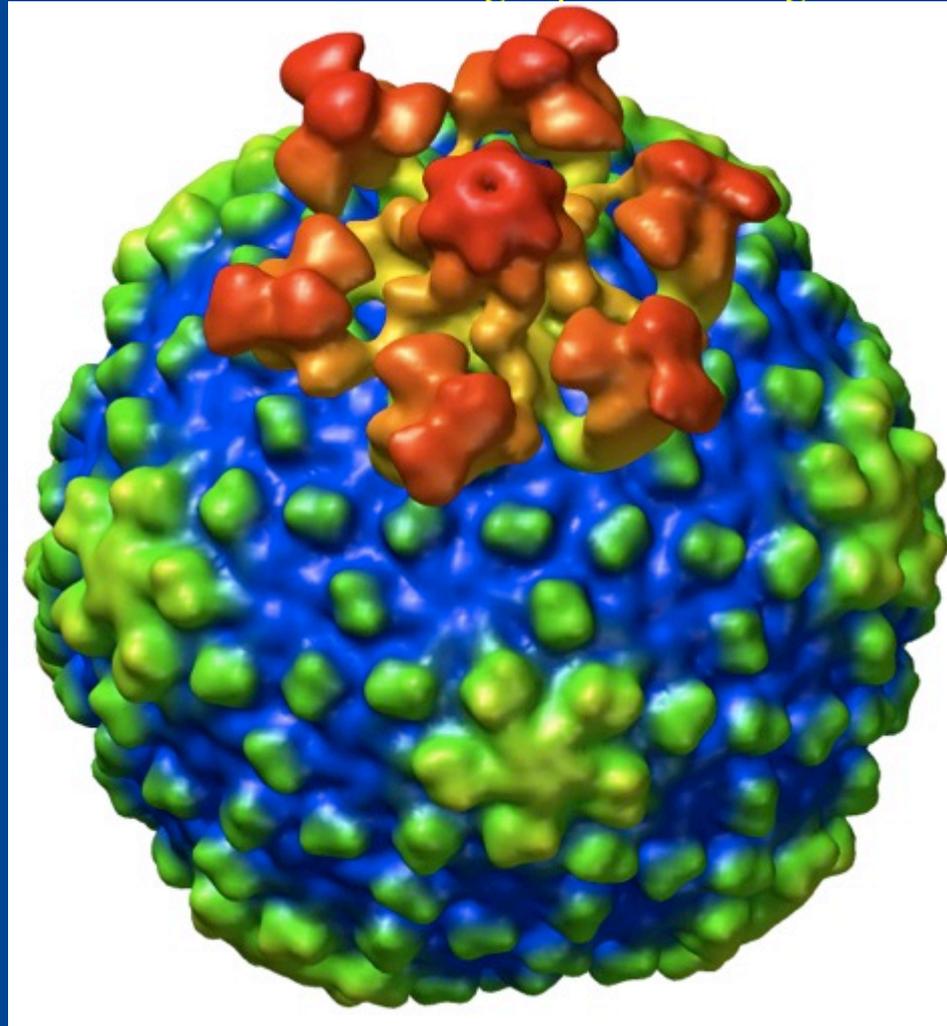




87 3D particles averaged with no symmetry



15000 single particle images



Schmid et al (unpublished)

Jiang et al. (2006) Nature 439:612

# Flow Chart for processing subtomograms

