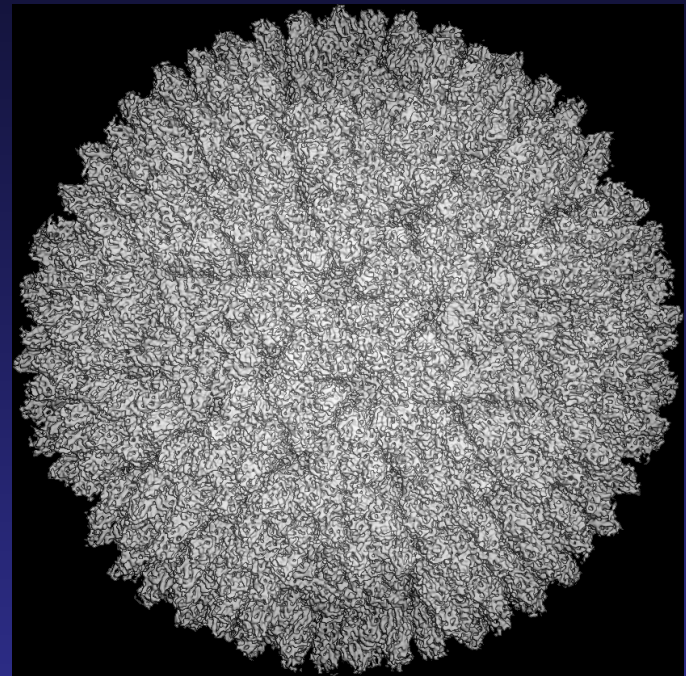
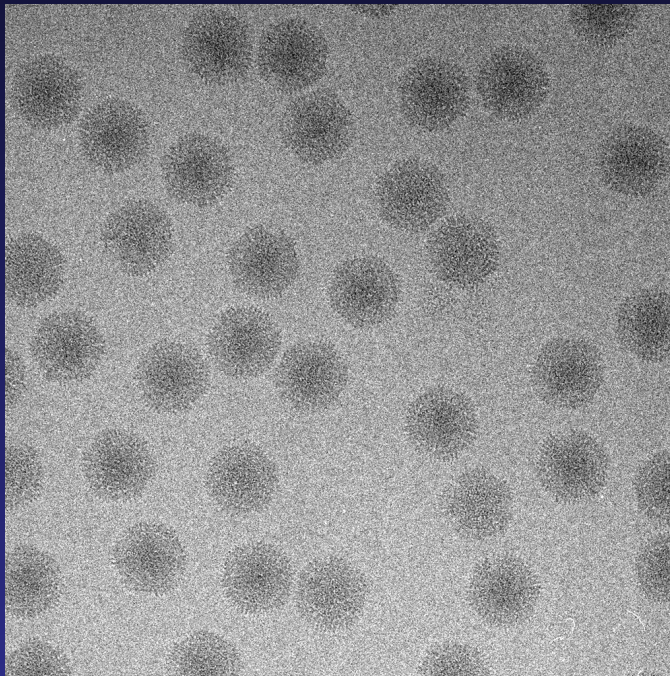


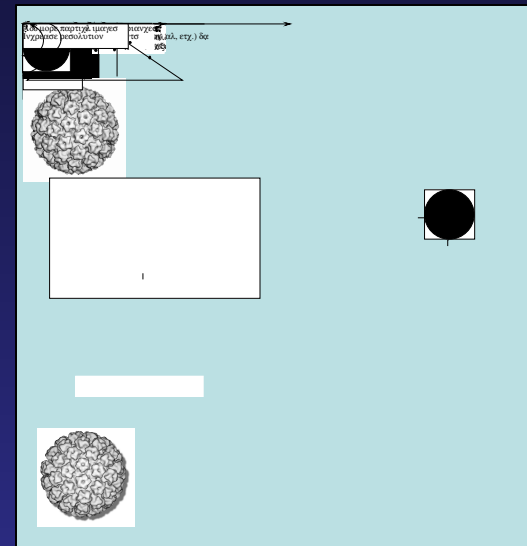
3D Reconstruction of Icosahedral Particles



3D Reconstruction of Icosahedral Particles

Outline

- Background
 - References; examples; etc.
- Symmetry
 - Icosahedral (532) point group symmetry
 - Triangulation symmetry
- “Typical” procedure (flow chart)
 - Digitization and boxing
 - Image preprocessing / CTF estimation
 - Initial particle orientation/origin search
 - Orientation/origin refinement
 - 3D reconstruction with CTF corrections
 - Validation (resolution assessment)
- Current and future strategies



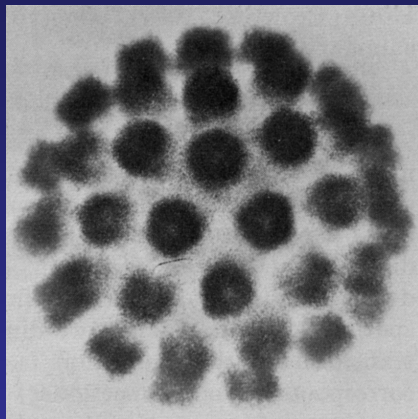
3D Reconstruction of Icosahedral Particles

REFERENCES

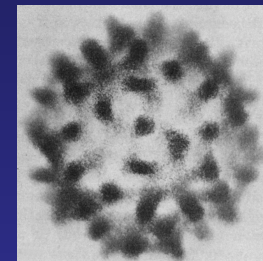
Crowther, R. A., Amos, L. A., Finch, J. T., DeRosier, D. J. and Klug, A. (1970) Three dimensional reconstructions of spherical viruses by Fourier synthesis from electron micrographs. *Nature* **226:421-425**

First 3D reconstructions of negatively-stained, spherical viruses:

Human wart virus



Tomato bushy stunt



500 Å

3D Reconstruction of Icosahedral Particles

REFERENCES

Crowther, R. A., DeRosier, D. J. and Klug, A. (1970) The reconstruction of a three-dimensional structure from projections and its application to electron microscopy. *Proc. Roy. Soc. Lond. A* **317:319-340**

Crowther, R. A. (1971) Procedures for three-dimensional reconstruction of spherical viruses by Fourier synthesis from electron micrographs. *Phil. Trans. R. Soc. Lond. B.* **261:221-230**

General principles of 3DR method

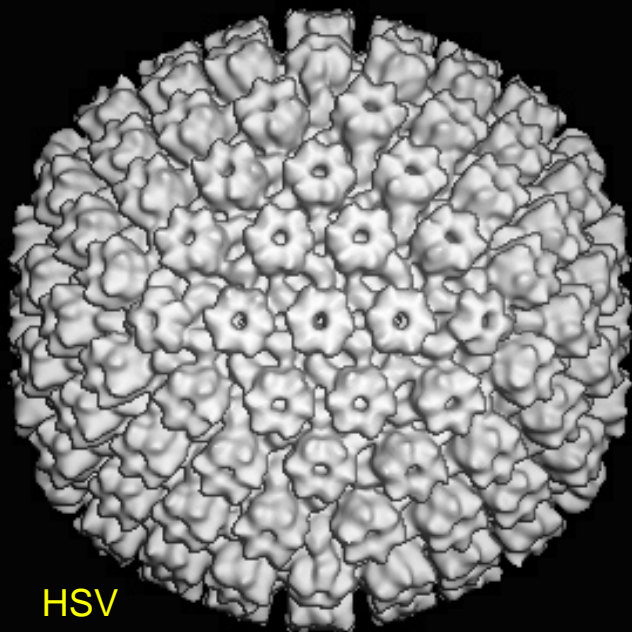
- Fourier-Bessel mathematics
- Common lines

3D Reconstruction of Icosahedral Particles

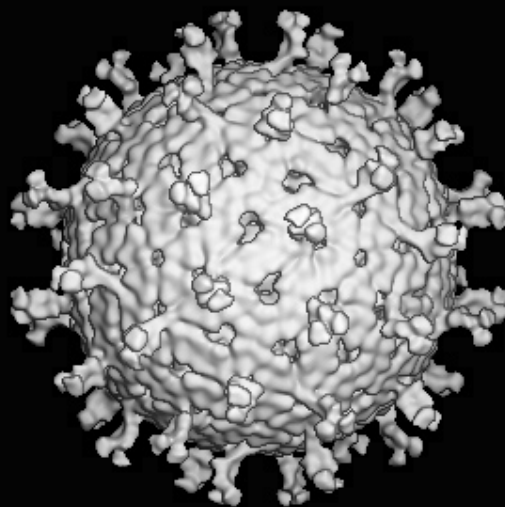
REFERENCES

- Reference list available as handout
- For die-hards:

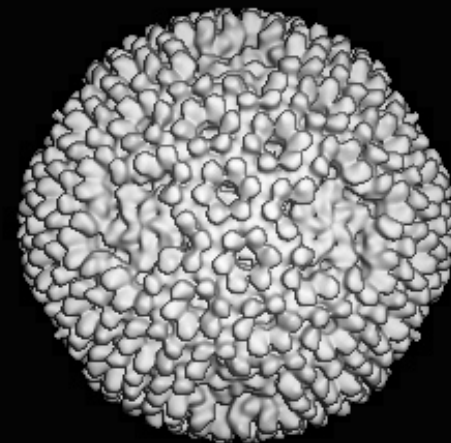
Baker, T. S., N. H. Olson, and S. D. Fuller (1999) Adding the third dimension to virus life cycles: Three-Dimensional reconstruction of icosahedral viruses from cryo-electron micrographs. *Microbiol. Molec. Biol. Reviews* **63:862-922**



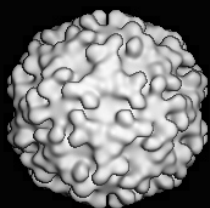
HSV



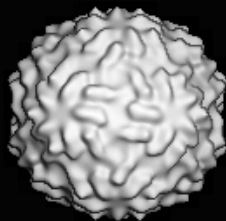
Rotavirus



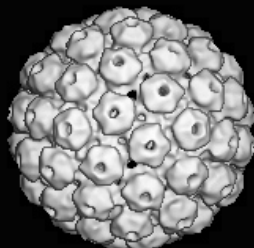
Reovirus



NvV



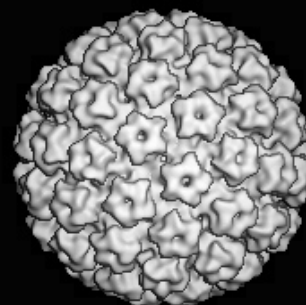
LA-1



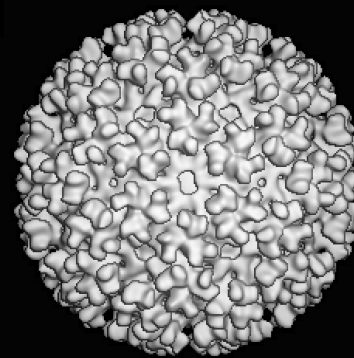
Polyoma



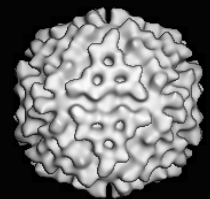
CaMV



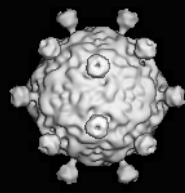
HPV



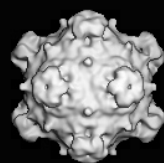
Ross River



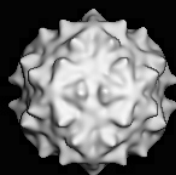
NβV



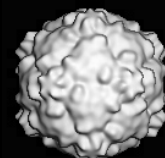
SpV-4



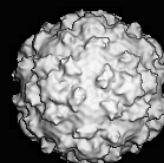
φX174



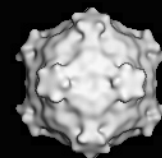
FHV



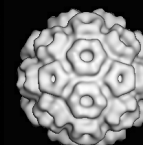
HRV-14



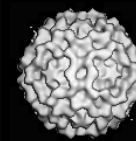
Polio



CPMV

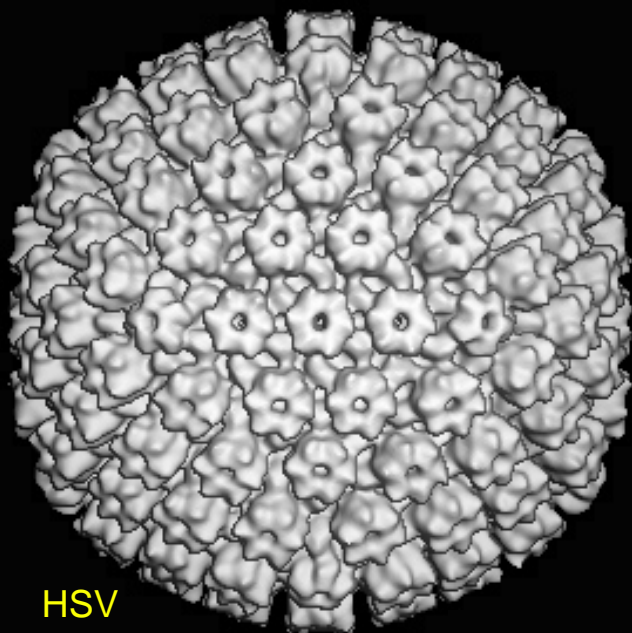


CCMV

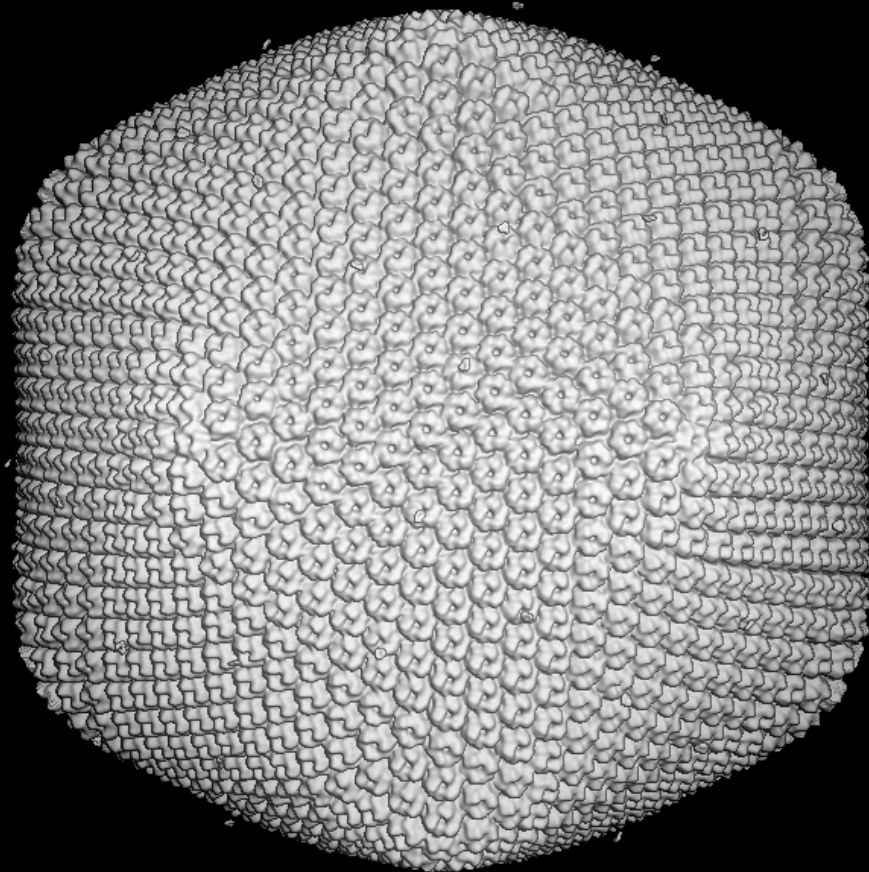


B19

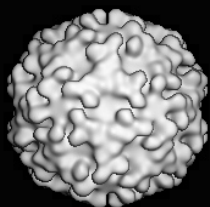
500 Å



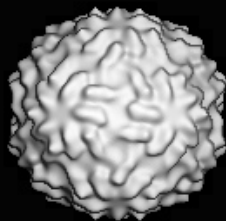
HSV



PBCV-1



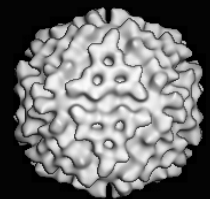
NφV



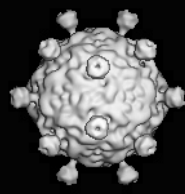
LA-1



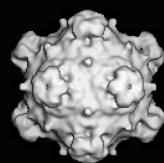
Polyoma



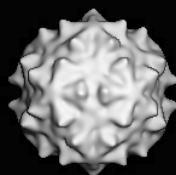
NβV



SpV-4



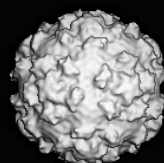
φX174



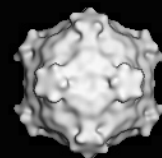
FHV



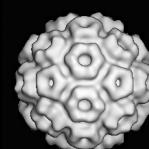
HRV-14



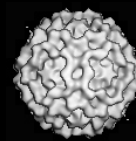
Polio



CPMV

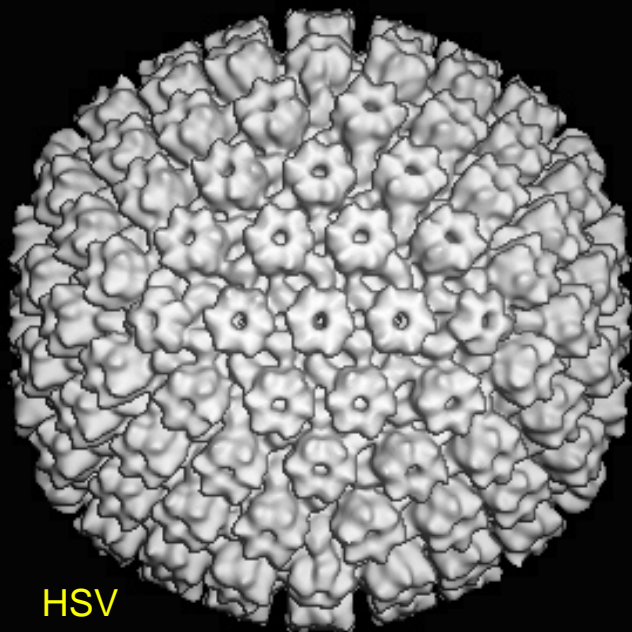


CCMV

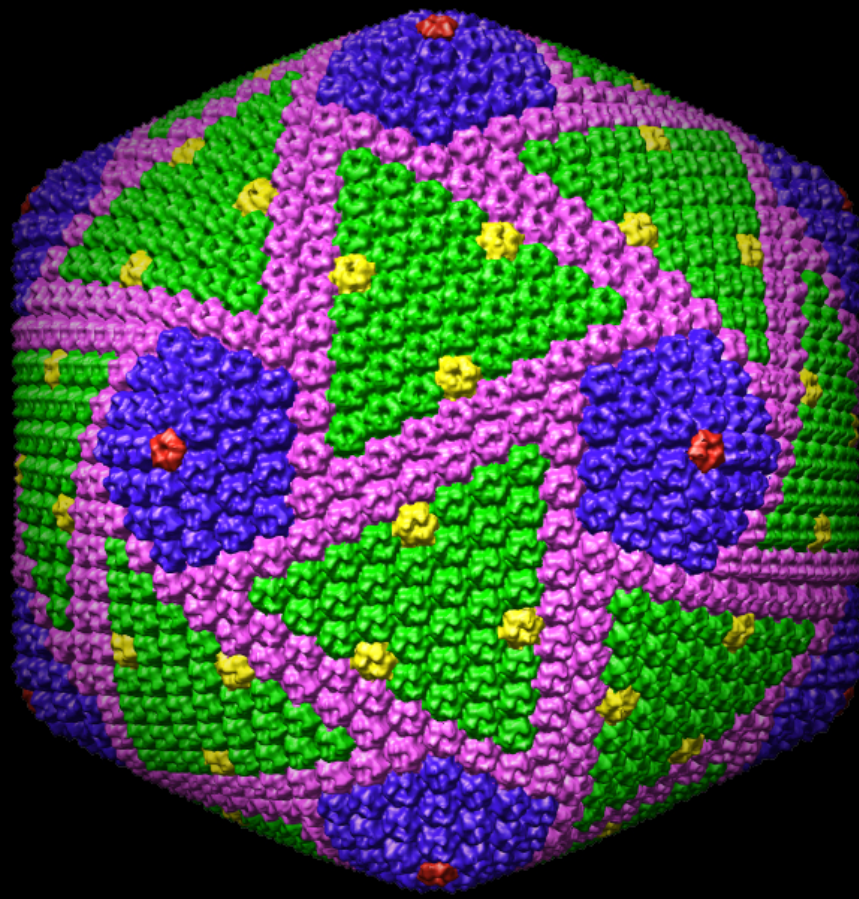


B19

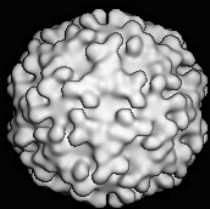
500 Å



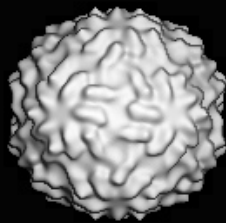
HSV



PBCV-1



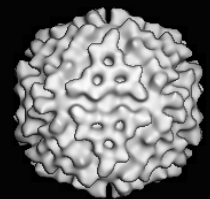
NCov



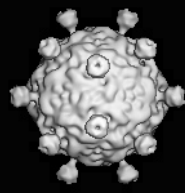
LA-1



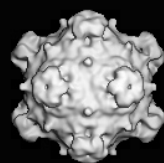
Polyoma



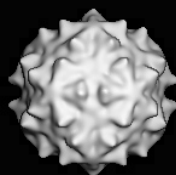
NCov



SpV-4



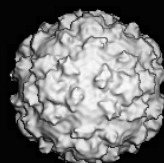
ϕ X174



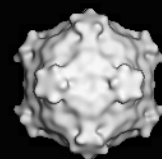
FHV



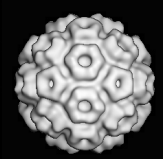
HRV-14



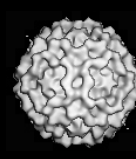
Polio



CPMV



CCMV



B19

500 Å

3D Reconstruction of Icosahedral Particles

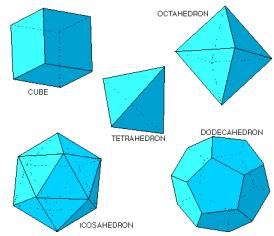
Outline

- Background
 - References; examples; etc.
- **Symmetry**
 - Icosahedral (532) point group symmetry
 - Triangulation symmetry
- “Typical” procedure (flow chart)
 - Digitization and boxing
 - Image preprocessing / CTF estimation
 - Initial particle orientation/origin search
 - Orientation/origin refinement
 - 3D reconstruction with CTF corrections
 - Validation (resolution assessment)
- Current and future strategies

3D Reconstruction of Icosahedral Particles

Symmetry

- ➔ 1. Icosahedral (532) point group symmetry
- 2. Triangulation symmetry



Regular Polyhedra (Platonic Solids)

There are just five platonic solids:

From **equilateral triangles** you can make:

with 3 faces at each vertex, a **tetrahedron**

with 4 faces at each vertex, an **octahedron**

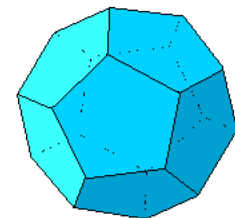
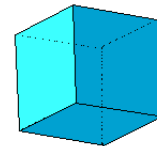
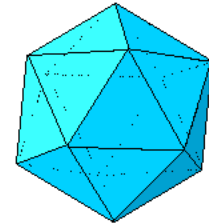
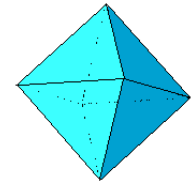
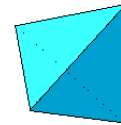
with 5 faces at each vertex, an **icosahedron**

From **squares** you can make:

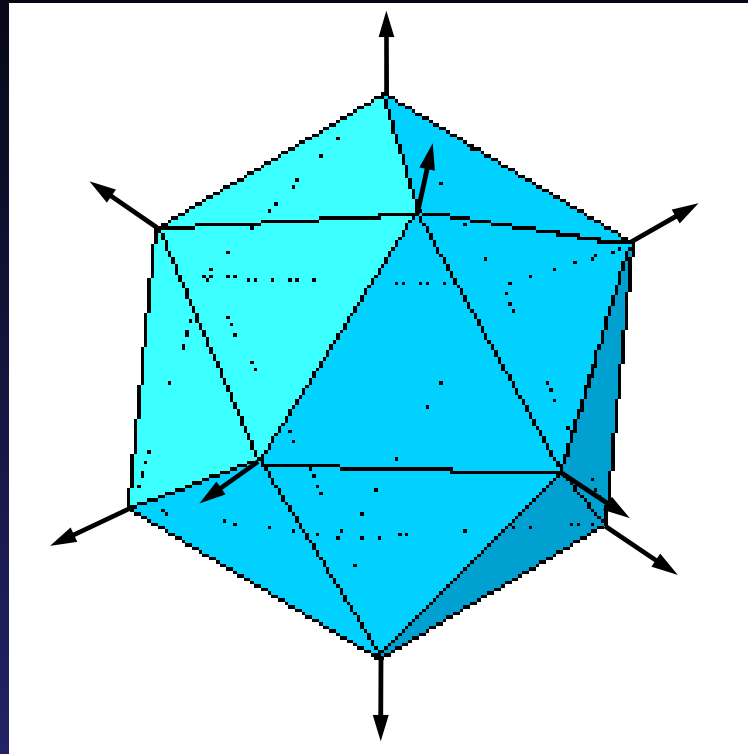
with 3 faces at each vertex, a **cube**

From **pentagons** you can make:

with 3 faces at each vertex, a **dodecahedron**

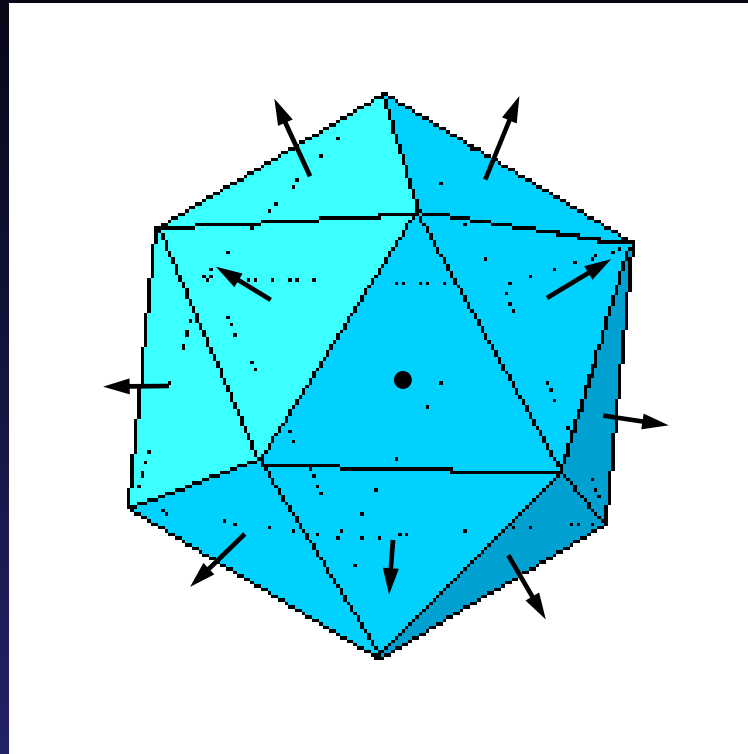


Icosahedral (532) Point Group Symmetry



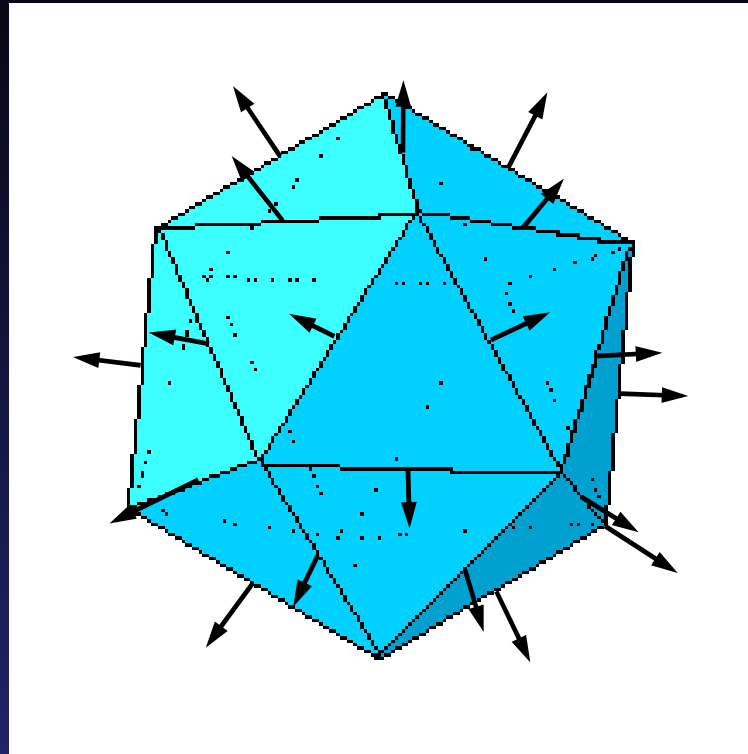
12 vertices (5-
fold)

Icosahedral (532) Point Group Symmetry



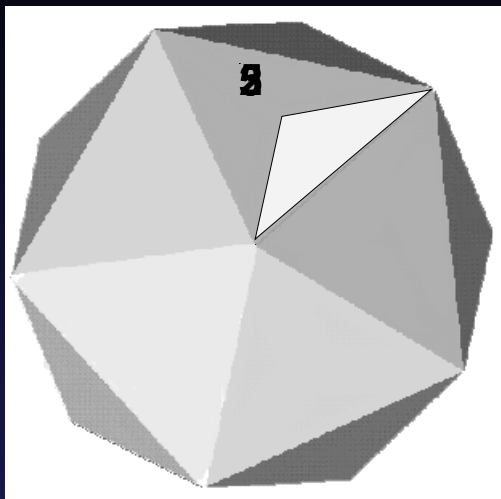
12 vertices (5-
fold)
20 faces (3-
fold)

Icosahedral (532) Point Group Symmetry

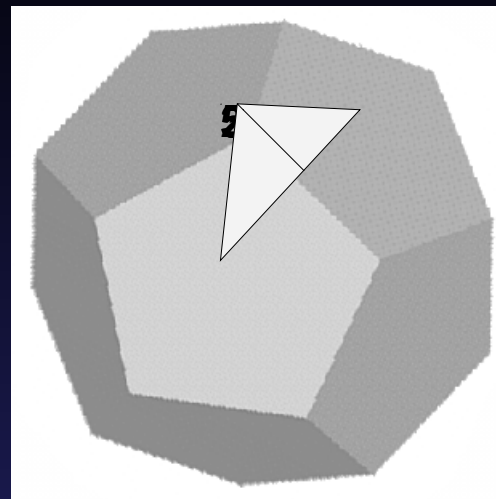


12 vertices (5-
fold)
20 faces (3-
fold)
30 edges (2-
fold)

Icosahedron



Dodecahedron



Different shapes, but both have 532 symmetry

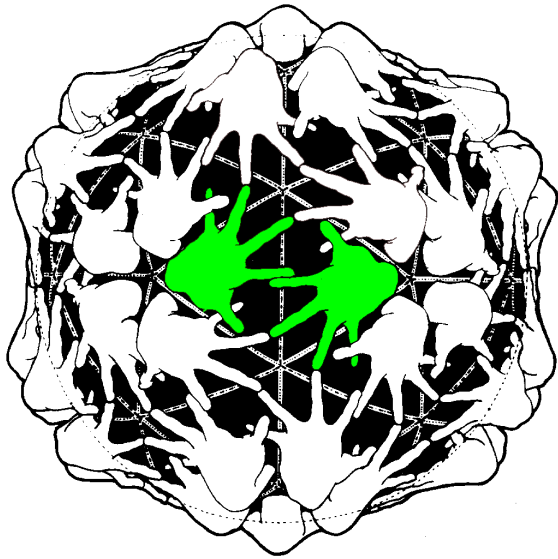
12 vertices, 20 faces, 30 edges
(6 5-folds, 10 3-folds, 15 2-folds)

20 vertices, 12 faces, 30 edges
(10 3-folds, 6 5-folds, 15 2-folds)

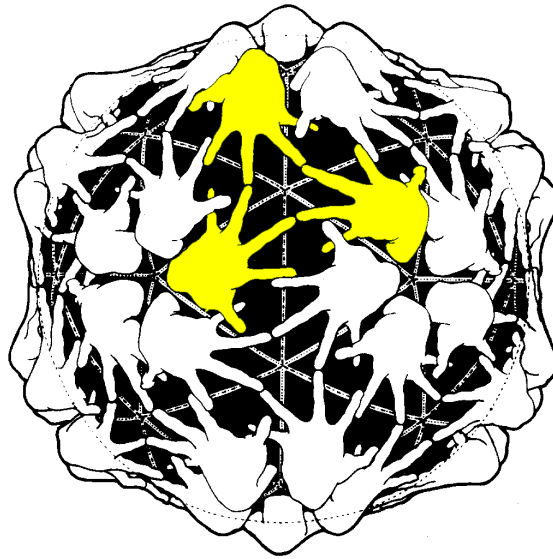
Asymmetric unit is $1/60^{\text{th}}$ of whole object

Object consists of 60 identical 'subunits' arranged with icosahedral symmetry

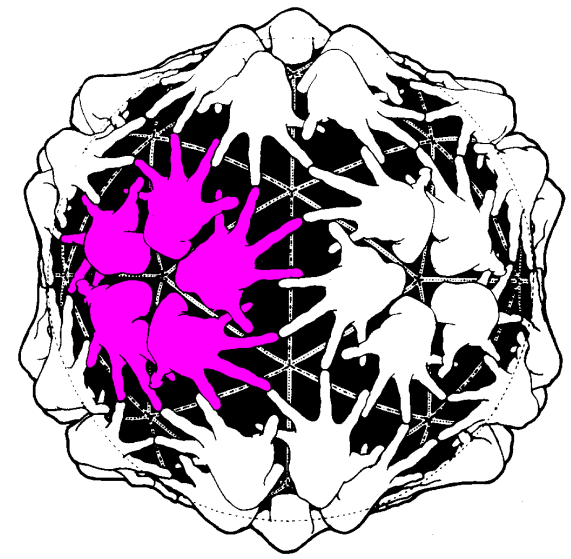
Icosahedral (532) Point Group Symmetry



30 dimers



20 trimers



12 pentamers

3D Reconstruction of Icosahedral Particles

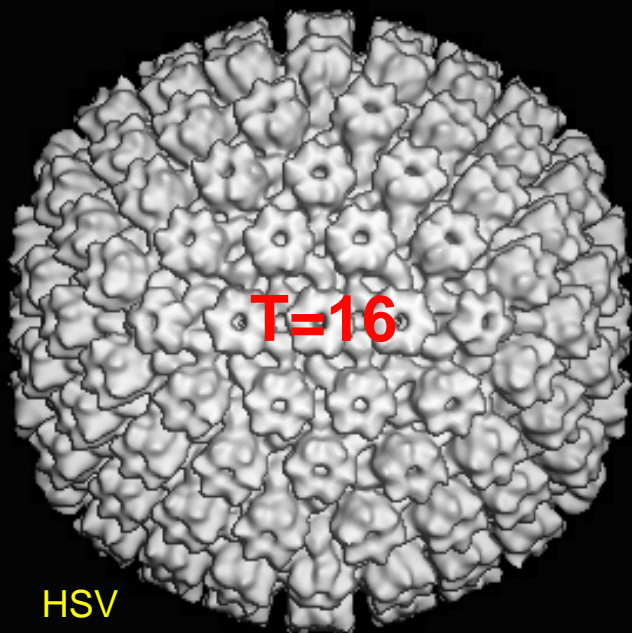
Symmetry

- ➔ 1. Icosahedral (532) point group symmetry
- ➔ 2. Triangulation symmetry

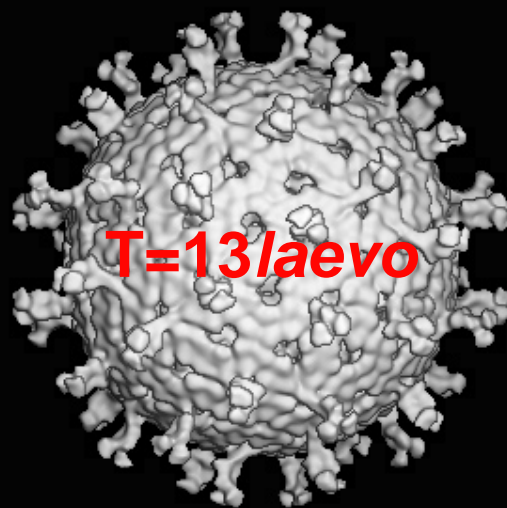
Purely mathematical concept (concerns lattices)

Real objects (e.g. viruses) with 532 symmetry often consist of multiples of 60 'subunits'

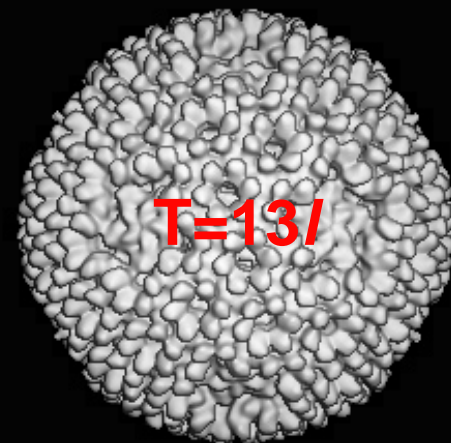
'Subunits' arranged such that additional, local or pseudo-symmetries exist



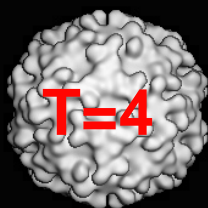
HSV



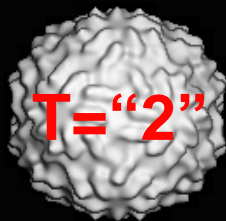
Rotavirus



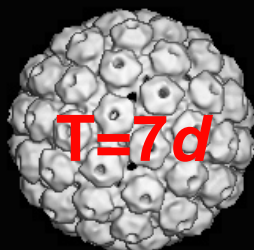
Reovirus



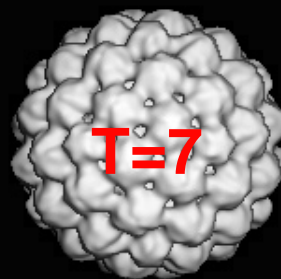
NoV



LA-1



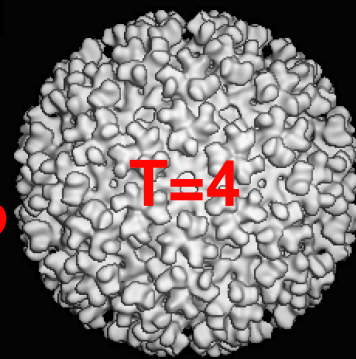
Polyoma



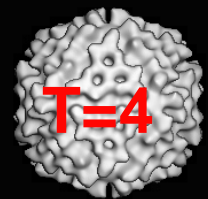
CaMV



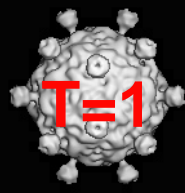
HPV



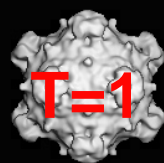
Ross River



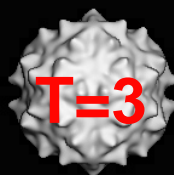
NβV



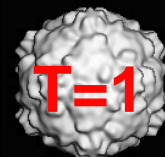
SpV-4



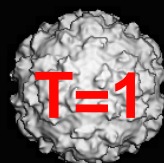
φX174



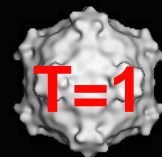
FHV



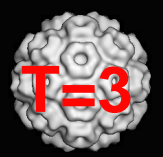
HRV-14



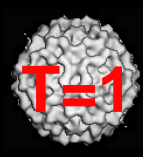
Polio



CPMV

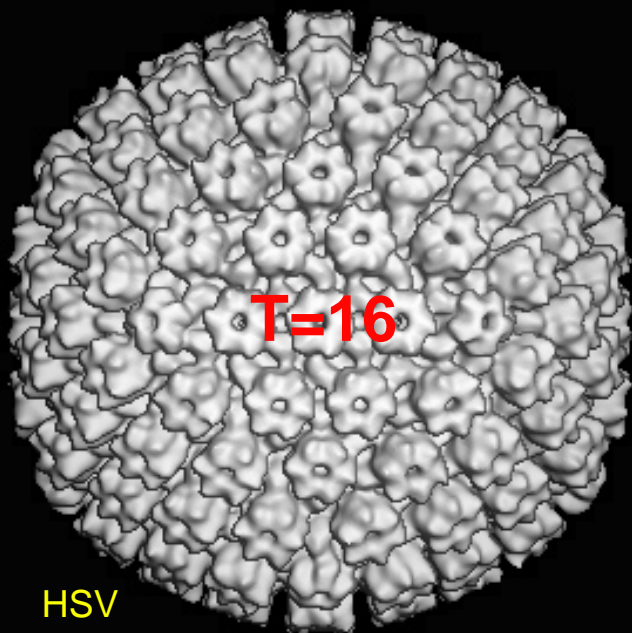


CCMV

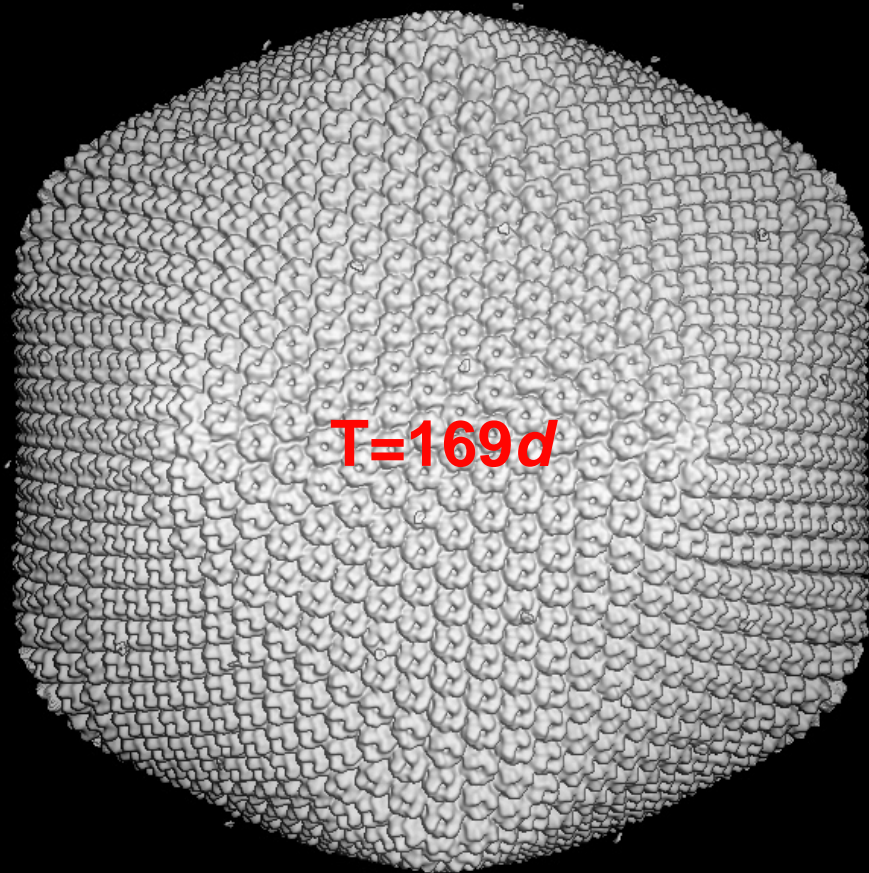


B19

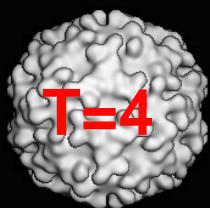
500 Å



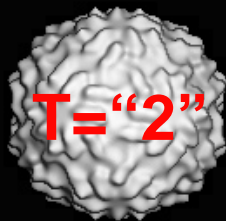
HSV



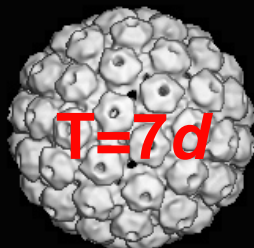
PBCV-1



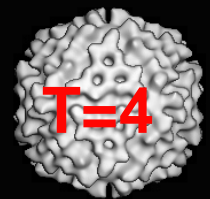
NoV



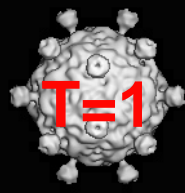
LA-1



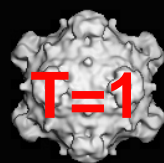
Polyoma



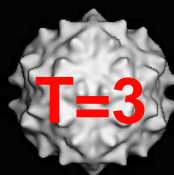
NβV



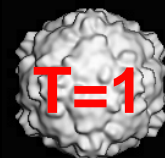
SpV-4



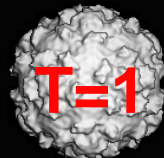
φX174



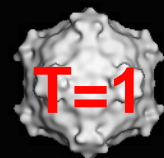
FHV



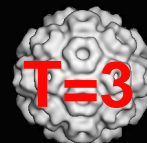
HRV-14



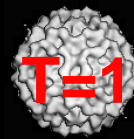
Polio



CPMV



CCMV



B19

500 Å

3D Reconstruction of Icosahedral Particles

Triangulation Number

Key Concept:

T symmetry is **NOT** incorporated into or enforced by the 3D reconstruction algorithms

Hence, T symmetry emerges as a result of a properly performed 3D reconstruction analysis

3D Reconstruction of Icosahedral Particles

Triangulation Number

Key Concept:

T symmetry is **NOT** incorporated into or enforced by the 3D reconstruction algorithms

In other words: What you determine is the structure of one asymmetric unit of the object

3D Reconstruction of Icosahedral Particles

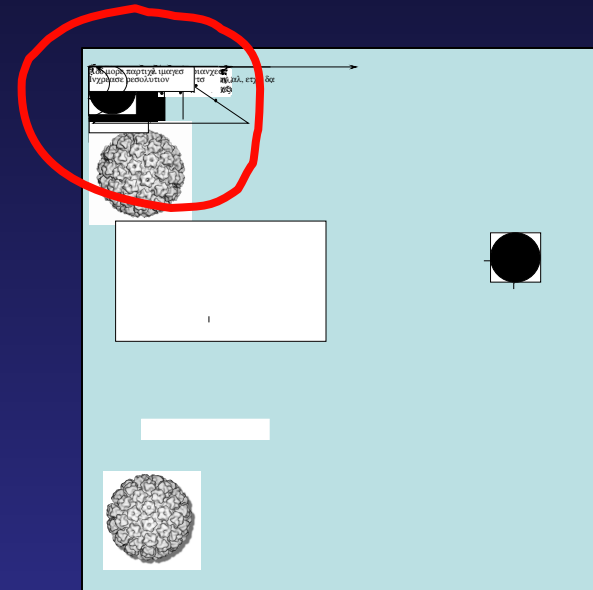
Two Basic Assumptions:

- Specimen consists of stable particles with 'identical' structures (else averaging is invalid)
- Programs test for and assume presence of icosahedral (532) symmetry

3D Reconstruction of Icosahedral Particles

Outline

- Background
 - References; examples; etc.
- Symmetry
 - Icosahedral (532) point group symmetry
 - Triangulation symmetry
- “Typical” procedure (flow chart)
 - Digitization and boxing
 - Image preprocessing / CTF estimation
 - Initial particle orientation/origin search
 - Orientation/origin refinement
 - 3D reconstruction with CTF corrections
 - Validation (resolution assessment)
- Current and future strategies



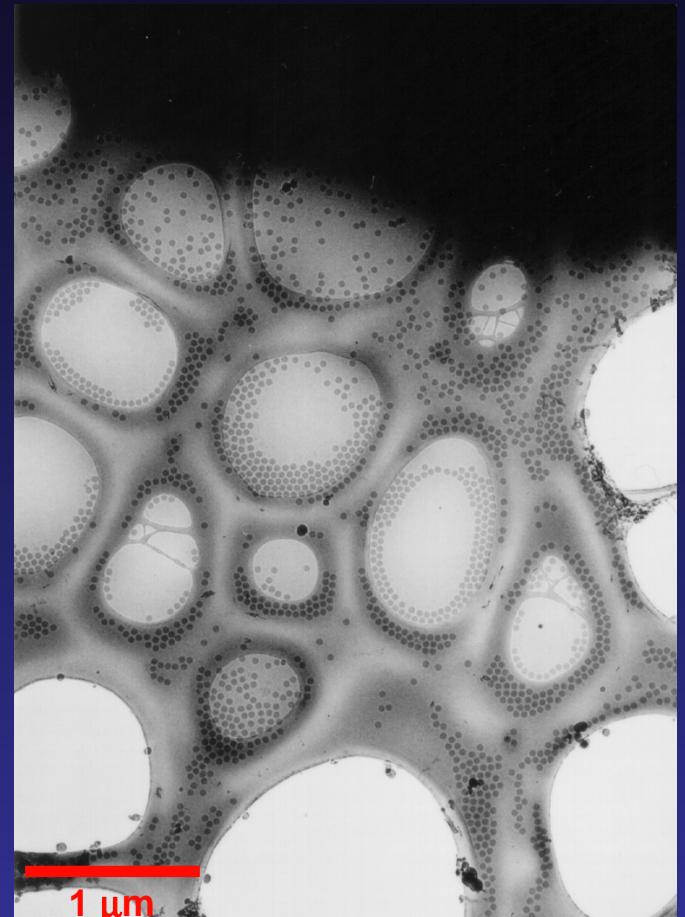
3D Reconstruction of Icosahedral Particles

Protocol

Electron Cryo-Microscopy

Sample : ~2-3 μl at 1-5 mg/ml

Specimen support: holey carbon film (1-2 μm)



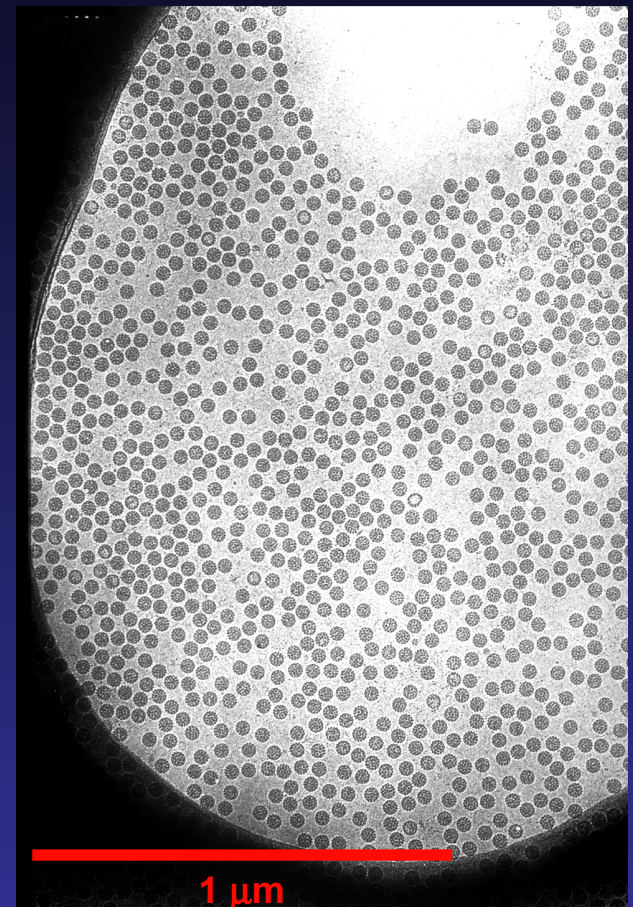
3D Reconstruction of Icosahedral Particles

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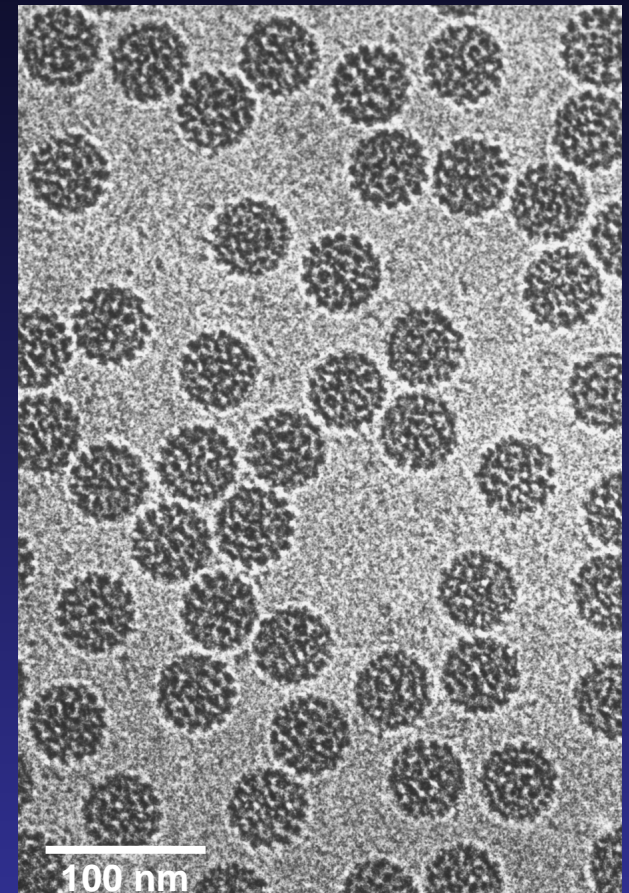
3D Reconstruction of Icosahedral Particles

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3D Reconstruction of Icosahedral Particles

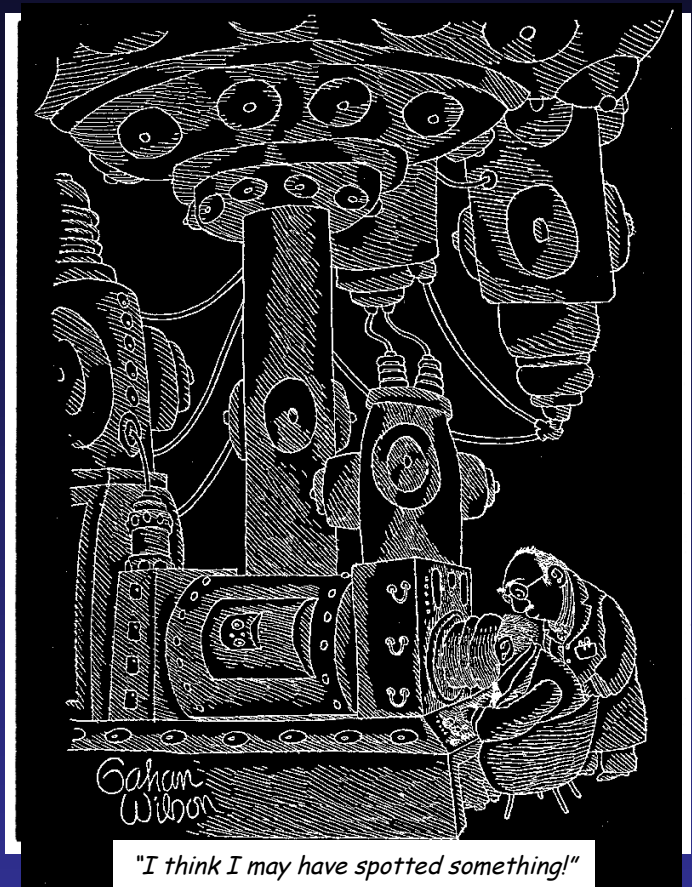
Protocol

Electron Cryo-Microscopy

Sample : ~2-3 μl at 1-5 mg/ml

Specimen support: holey carbon film (1-2 μm)

Microscope: 200-300 keV with FEG



3D Reconstruction of Icosahedral Particles

Protocol

Electron Cryo-Microscopy

Sample : ~2-3 μl at 1-5 mg/ml

Specimen support: holey carbon film (1-2 μm)

Microscope: 200-300 keV with FEG

Defocus range: 1-3 μm underfocus

Dose: 10-20 $\text{e}^-/\text{\AA}^2$

Film: SO-163 (12 min, full strength)

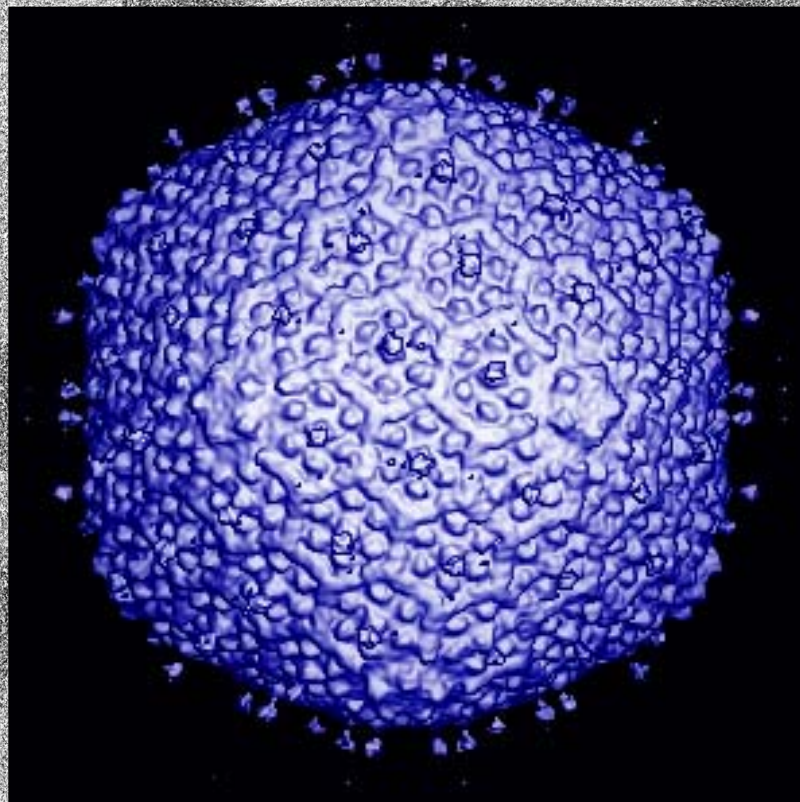
Micrographs: 50-100-->1000s(?)

Particles: 10^3 - 10^4 --> 10^5 ----> 10^6 (?)

Target resolution: 10 - 6 \AA --> 4 \AA (?)



FEI Tecnai F30 Polara

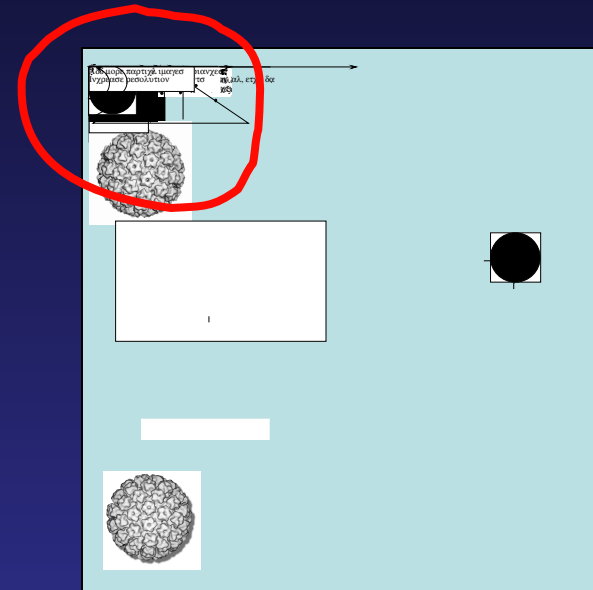


100 nm

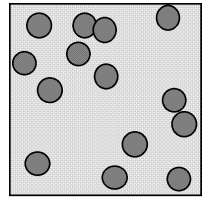
3D Reconstruction of Icosahedral Particles

Outline

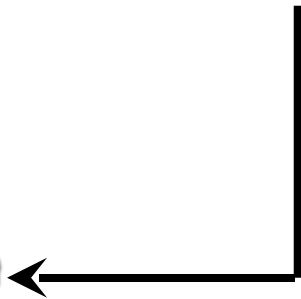
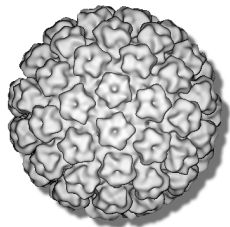
- Background
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 - Image preprocessing / CTF estimation
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 - Orientation/origin refinement
 - 3D reconstruction with CTF corrections
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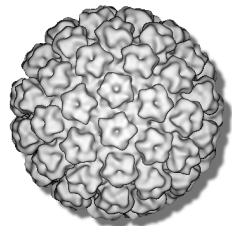
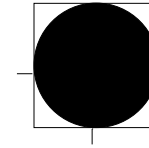
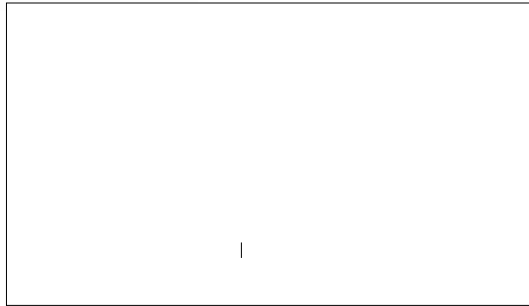
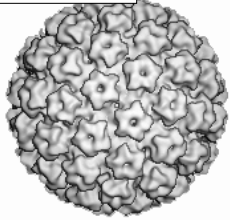
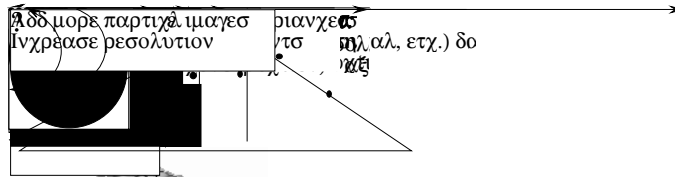
Icosahedral Particle Image Reconstruction Scheme



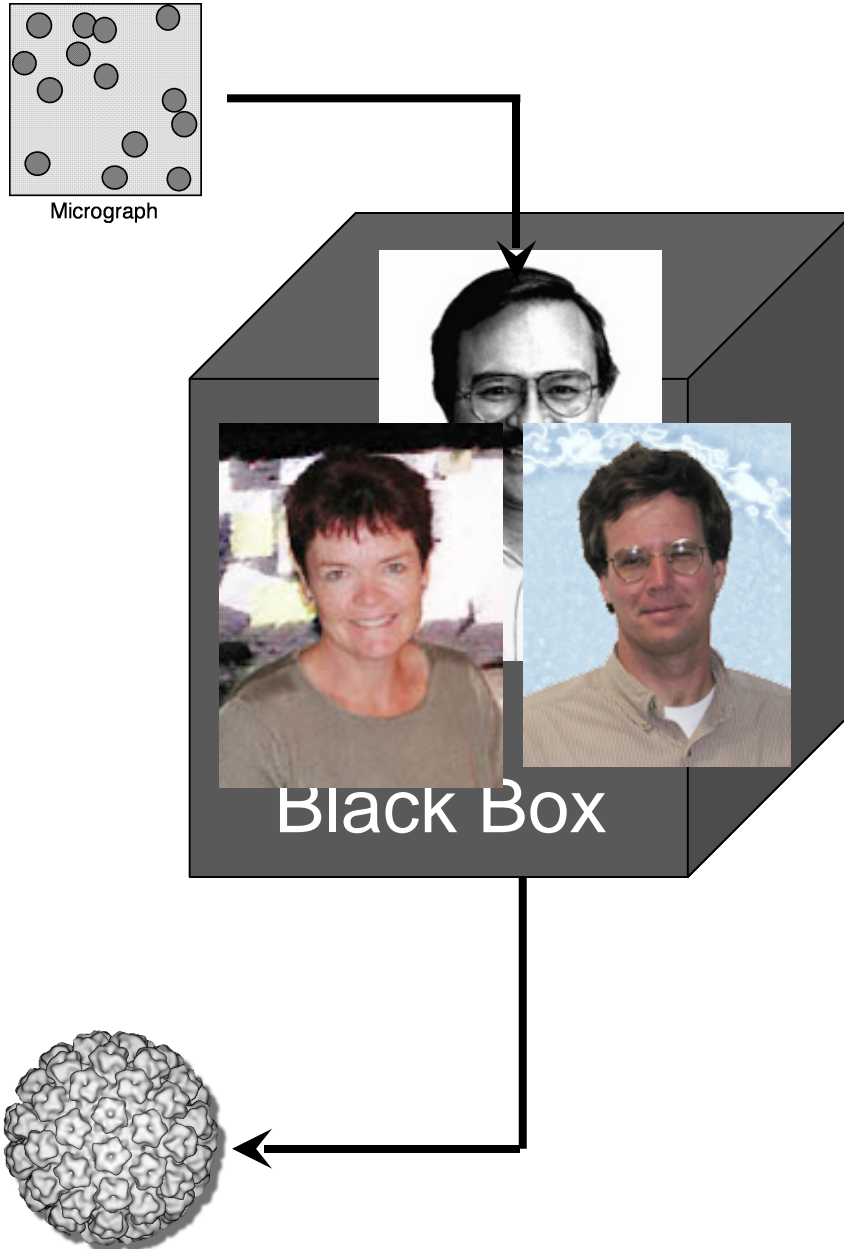
Micrograph



Icosahedral Particle Image Reconstruction Scheme

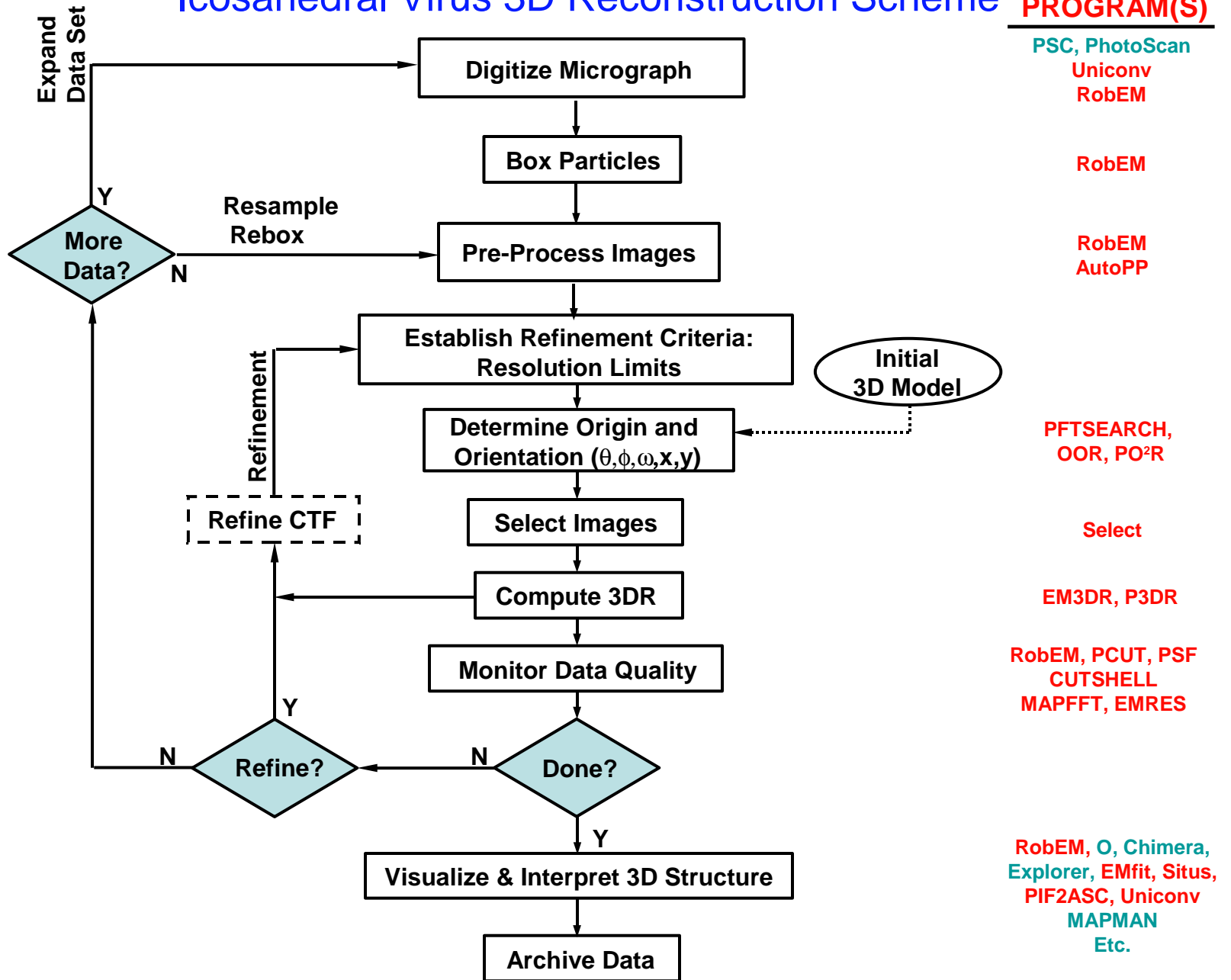


Icosahedral Particle Image Reconstruction Scheme

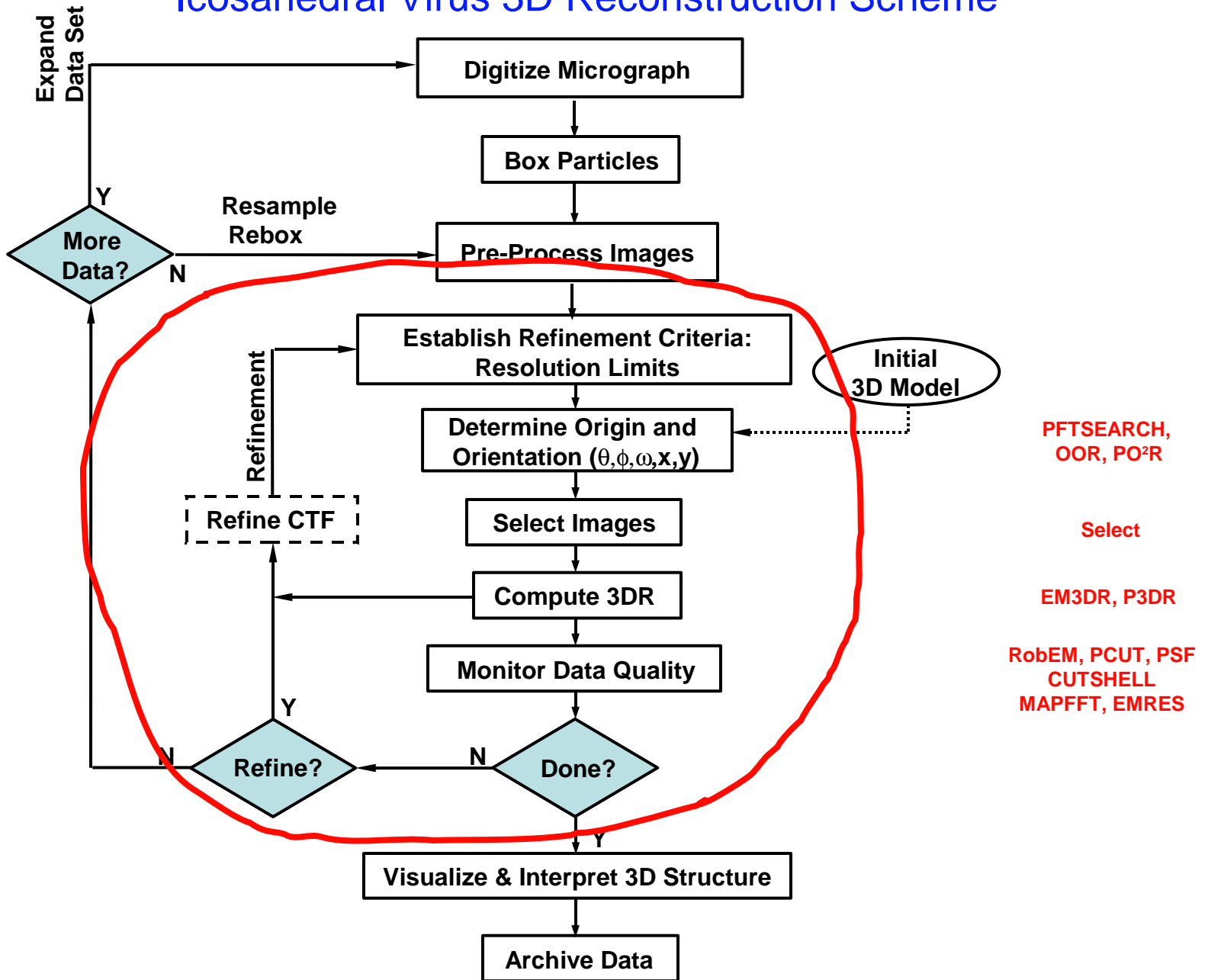


Icosahedral Virus 3D Reconstruction Scheme

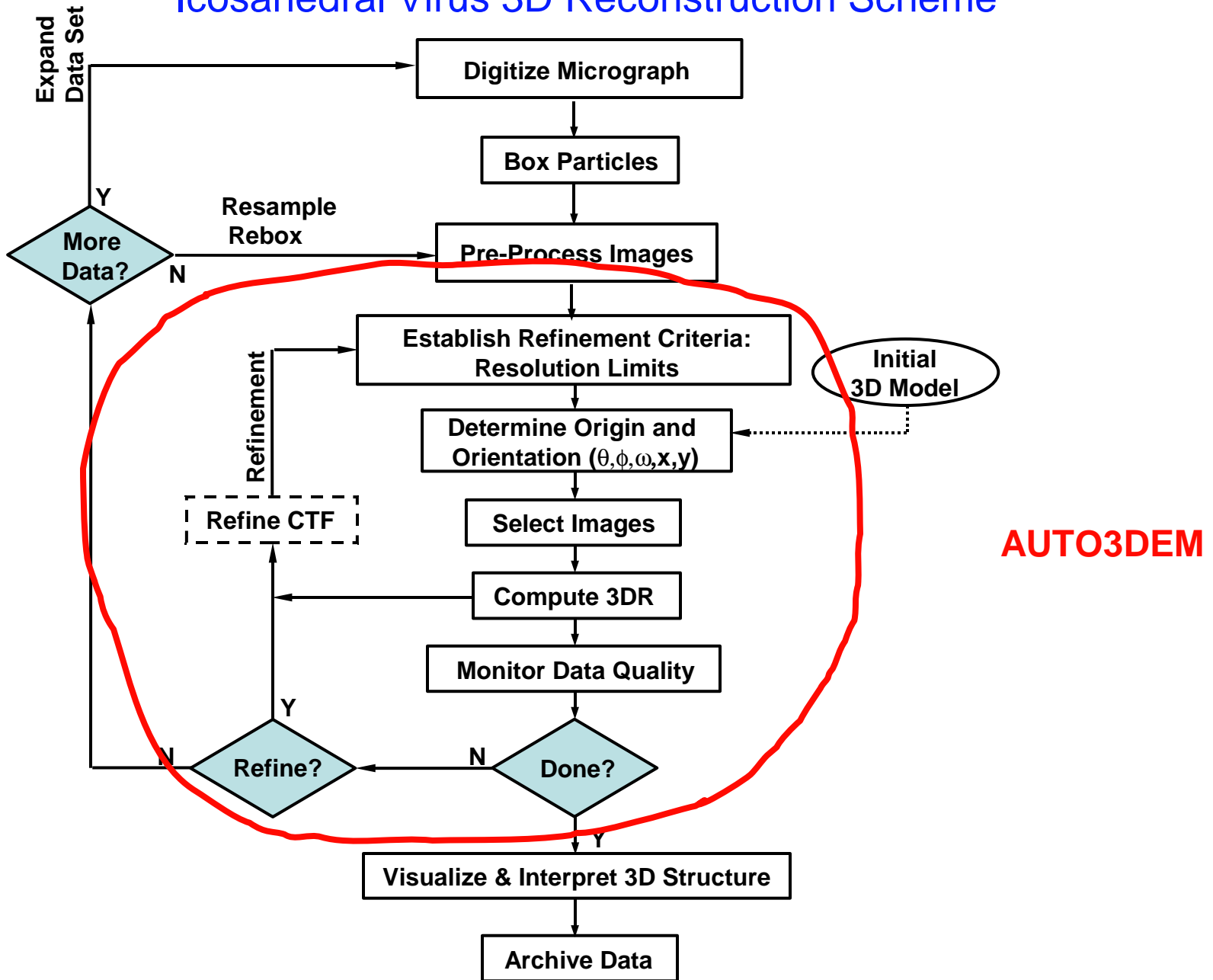
PROGRAM(S)



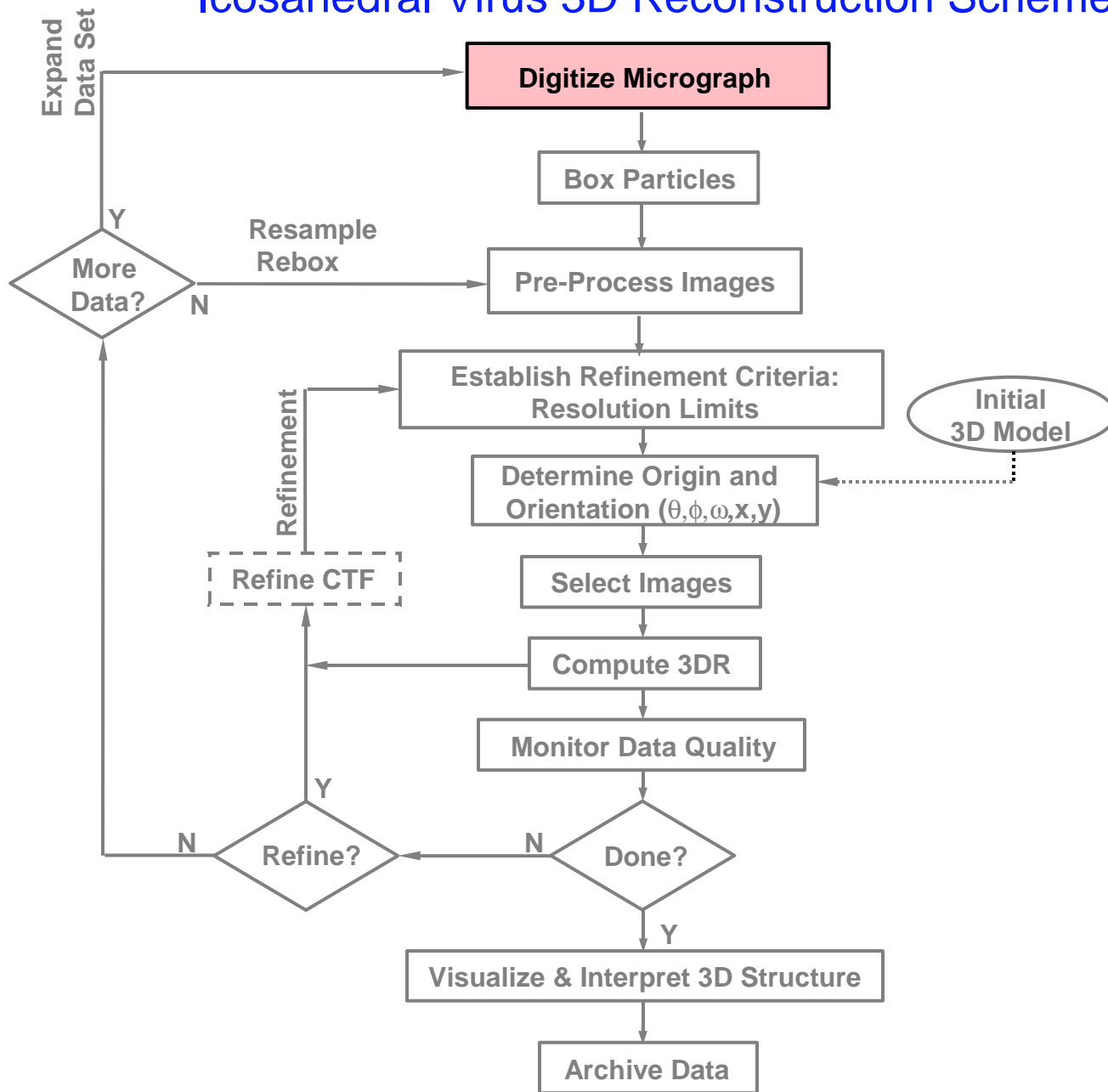
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



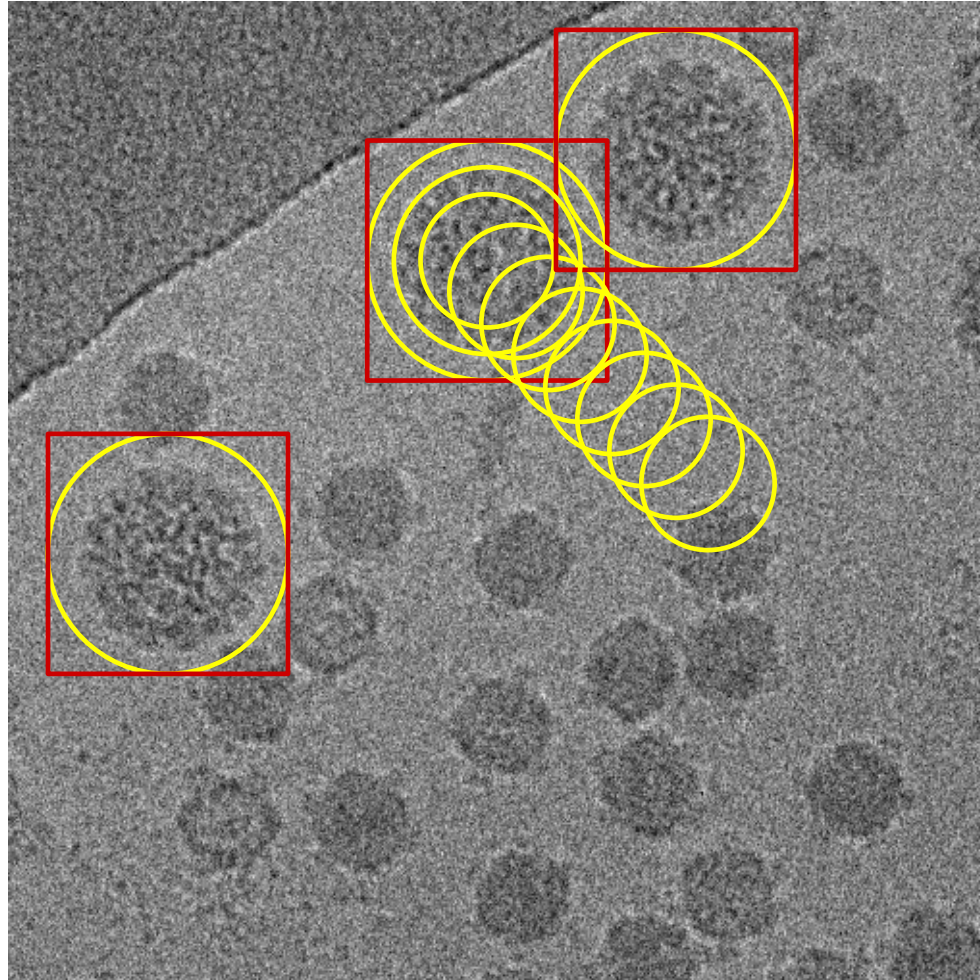
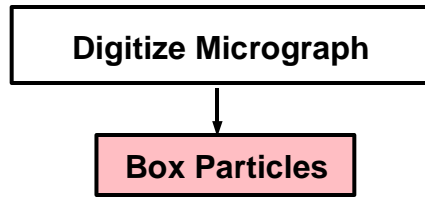
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

Icosahedral Virus 3D Reconstruction Scheme

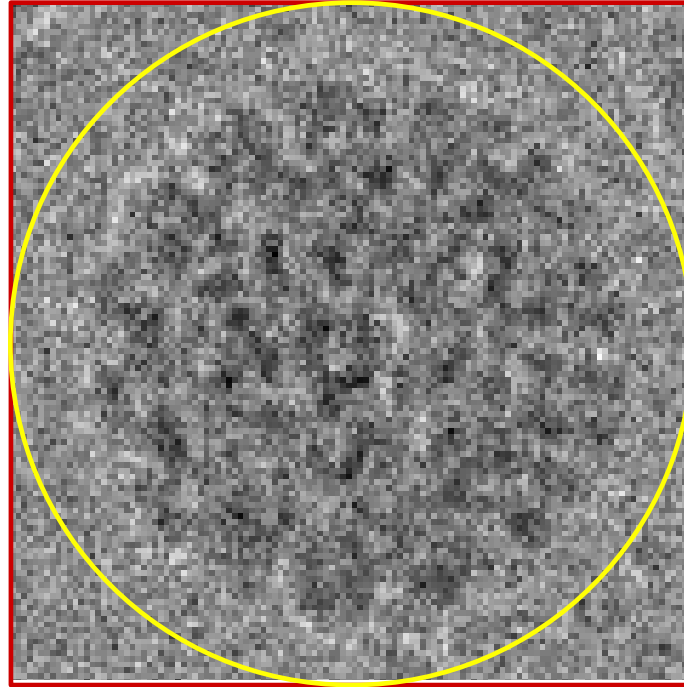


Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

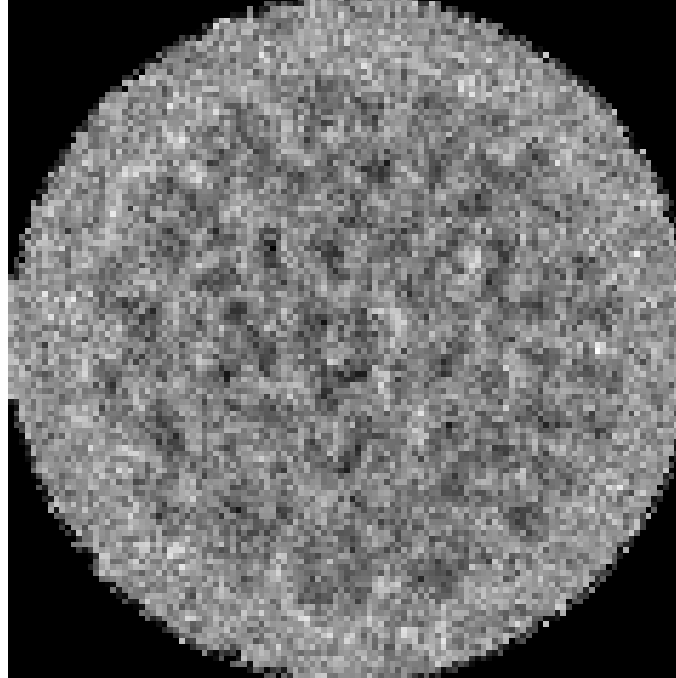
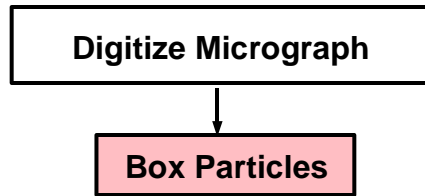


Box Particles



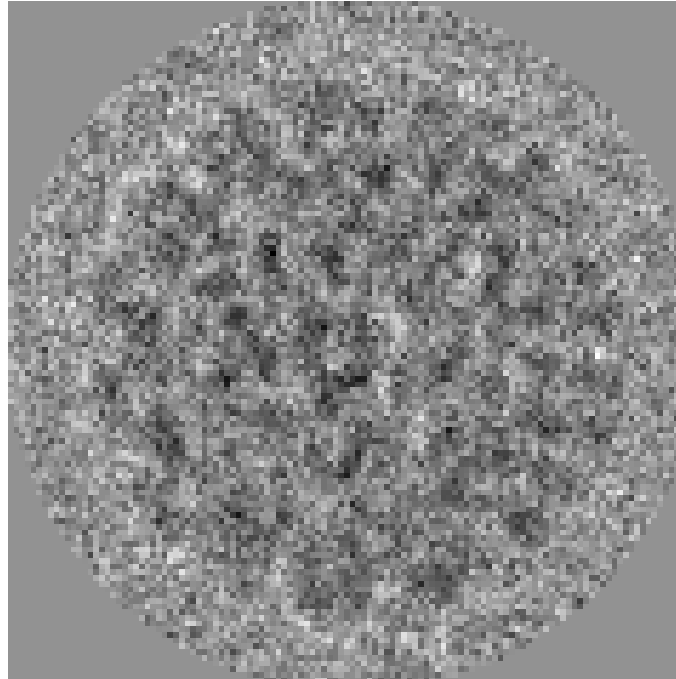
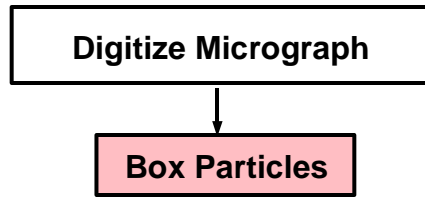
Extracted

Icosahedral Virus 3D Reconstruction Scheme



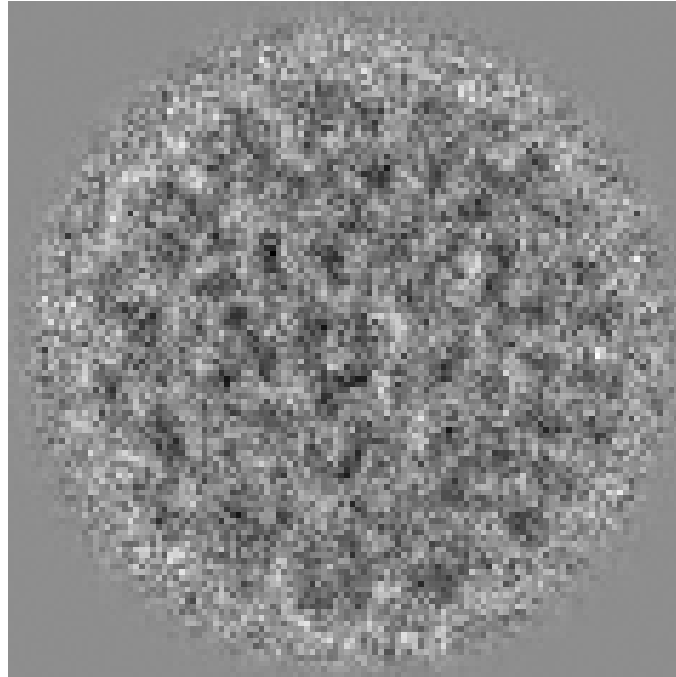
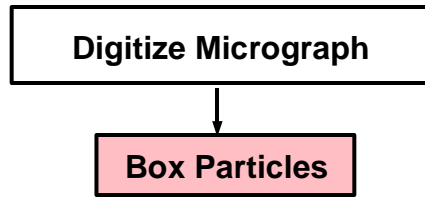
Masked

Icosahedral Virus 3D Reconstruction Scheme



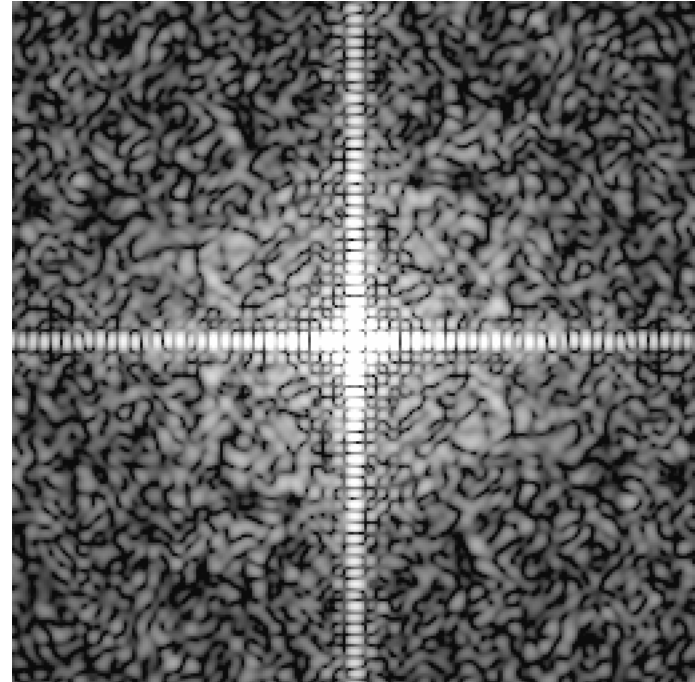
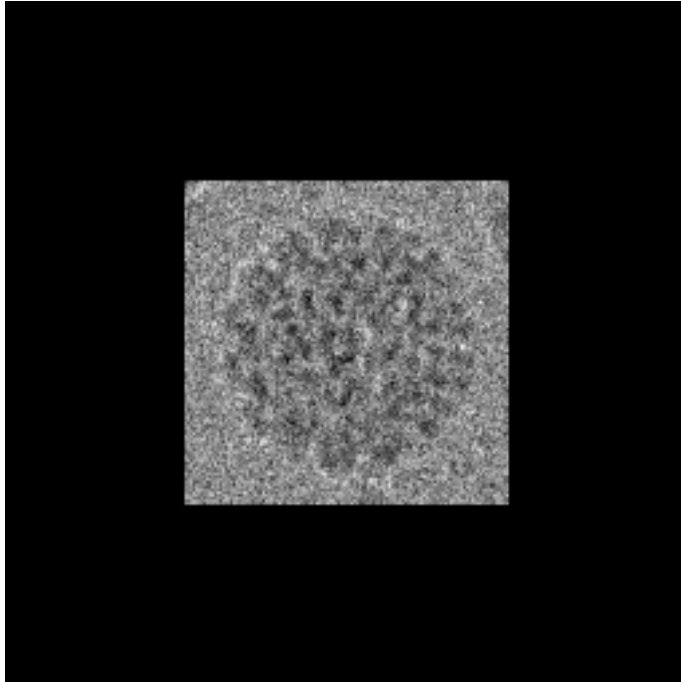
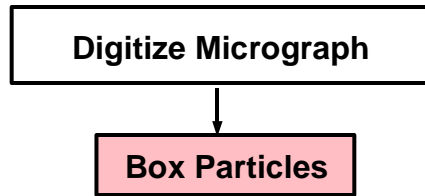
Floated

Icosahedral Virus 3D Reconstruction Scheme



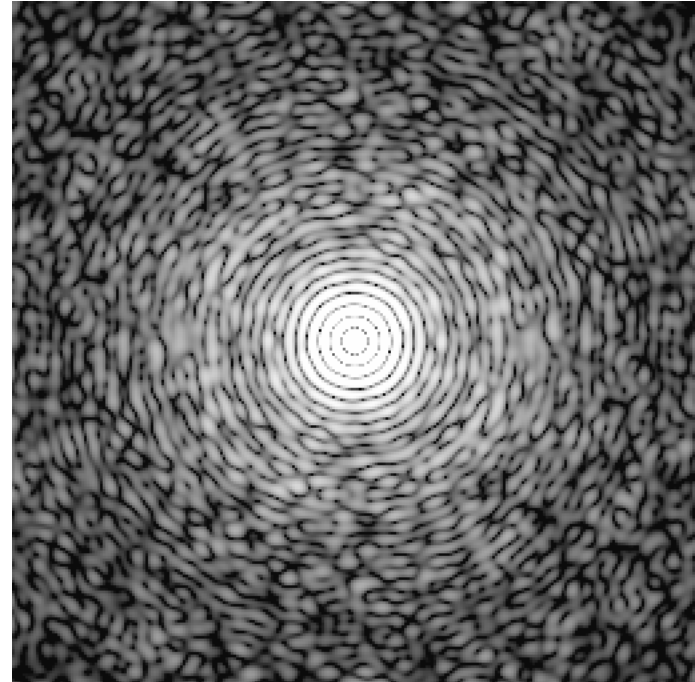
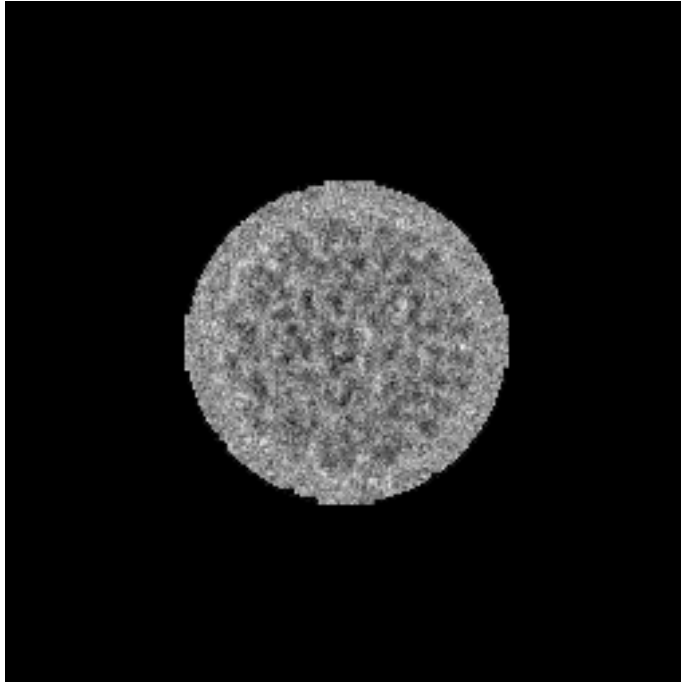
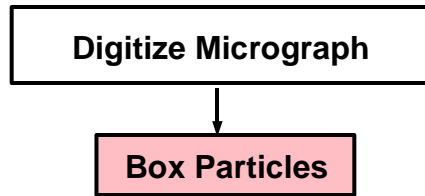
Apodized

Icosahedral Virus 3D Reconstruction Scheme



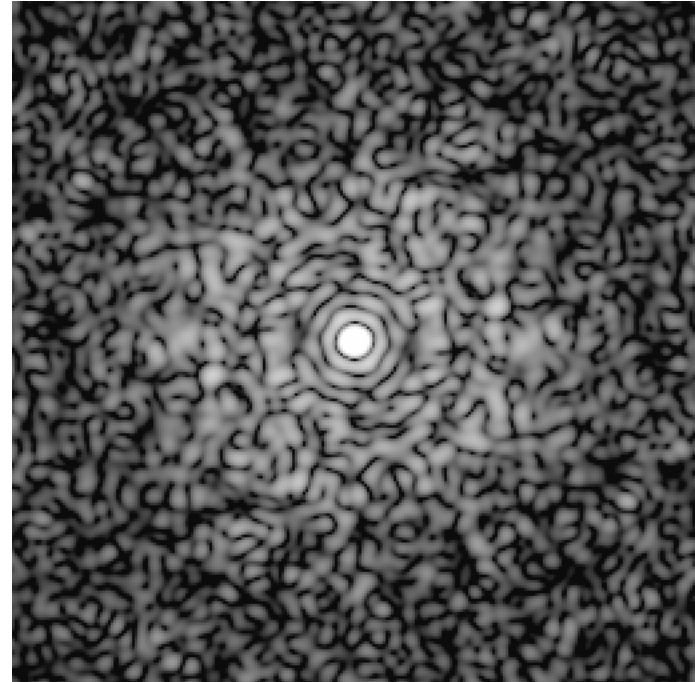
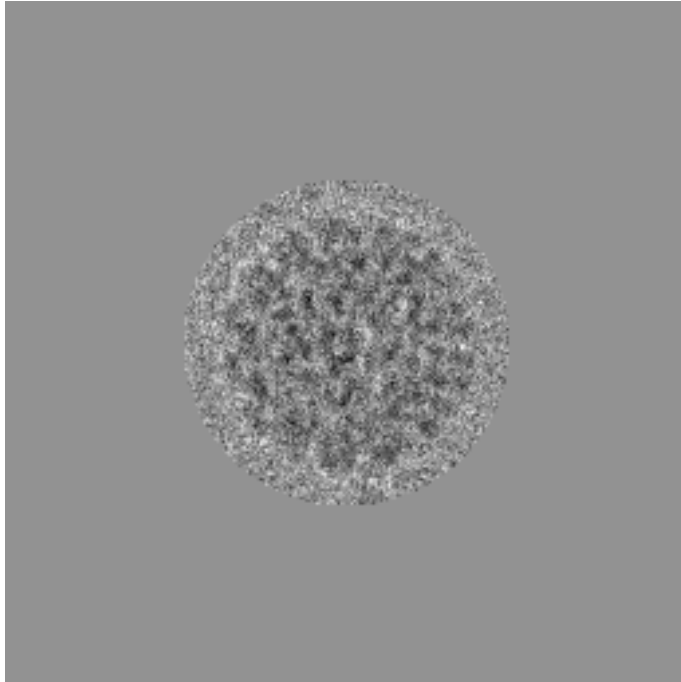
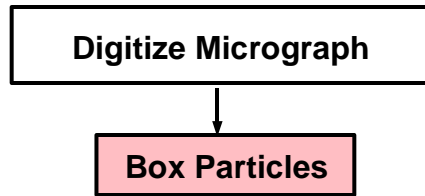
Square mask; unfloated

Icosahedral Virus 3D Reconstruction Scheme



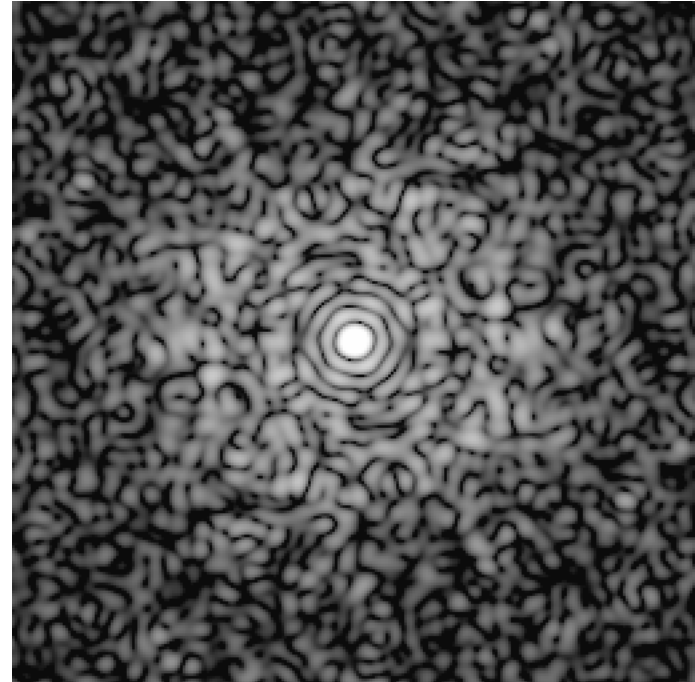
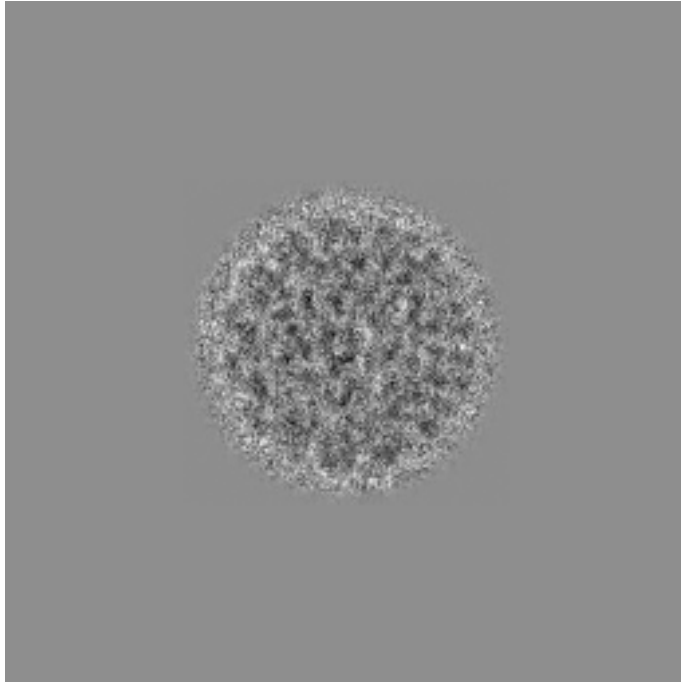
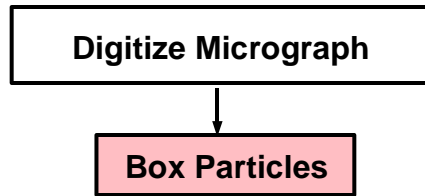
Circular mask; unfloated

Icosahedral Virus 3D Reconstruction Scheme



Circular mask; floated

Icosahedral Virus 3D Reconstruction Scheme



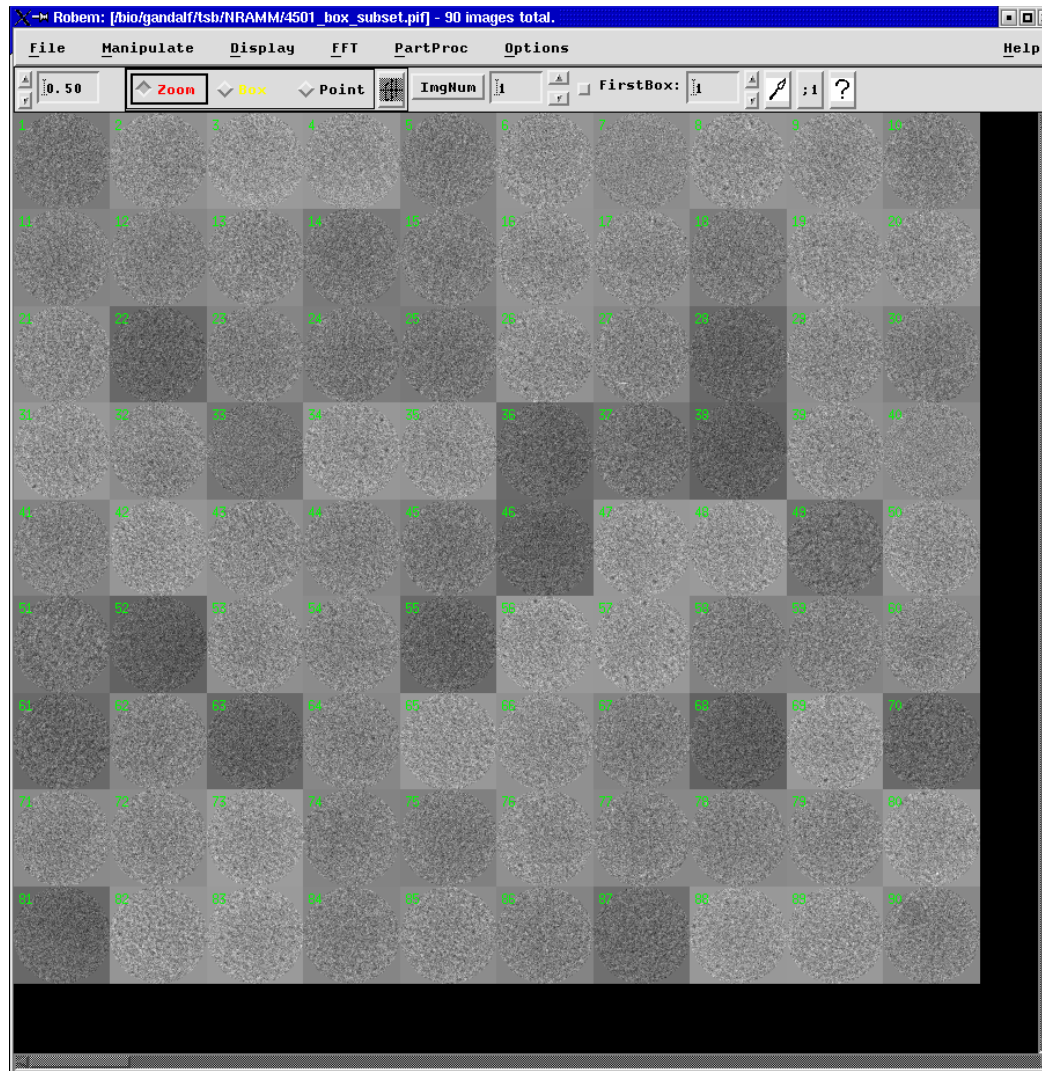
Circular mask; floated & apodized

Icosahedral Virus 3D Reconstruction Scheme

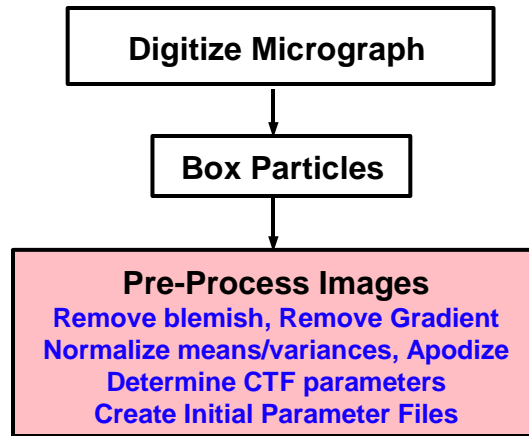
Digitize Micrograph



Box Particles



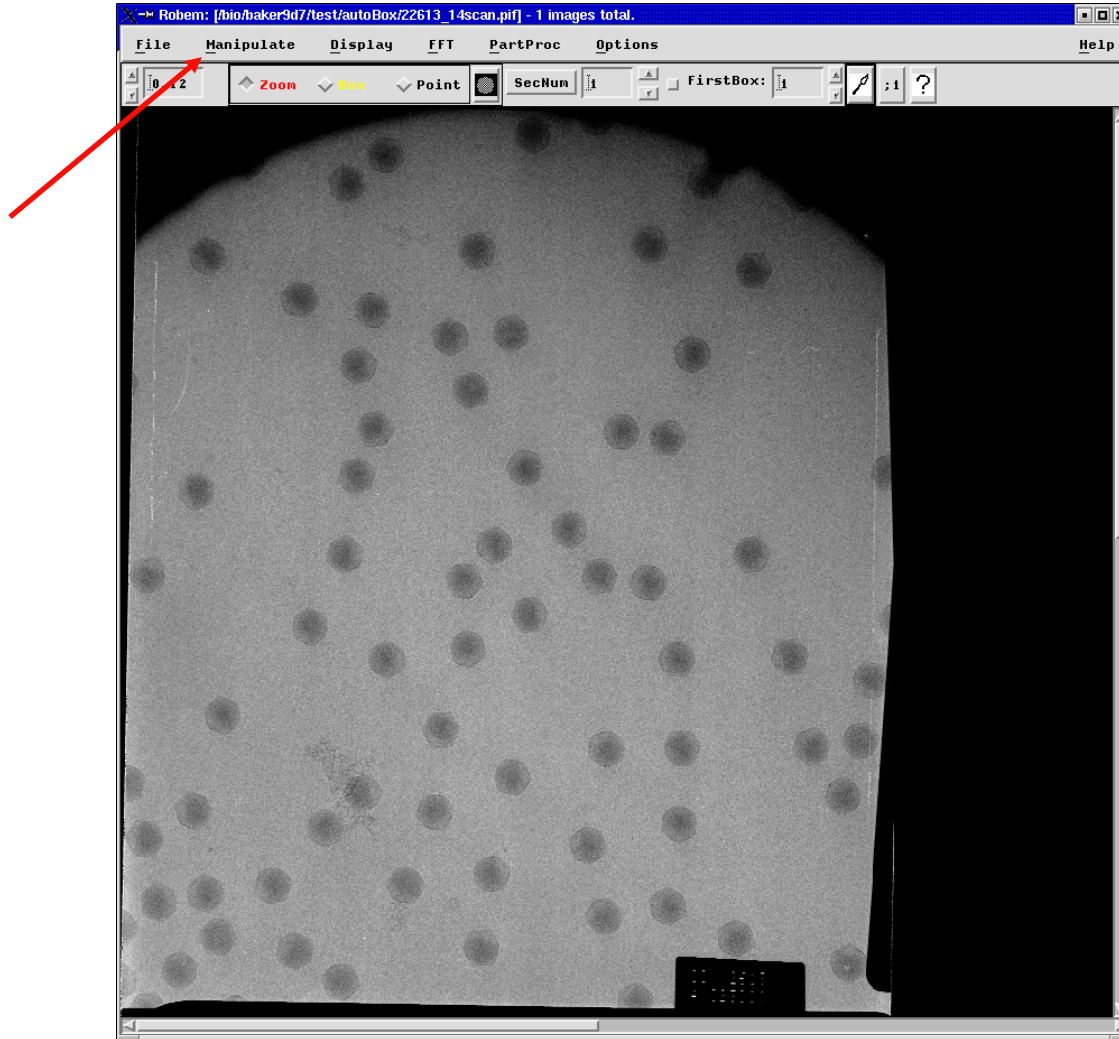
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



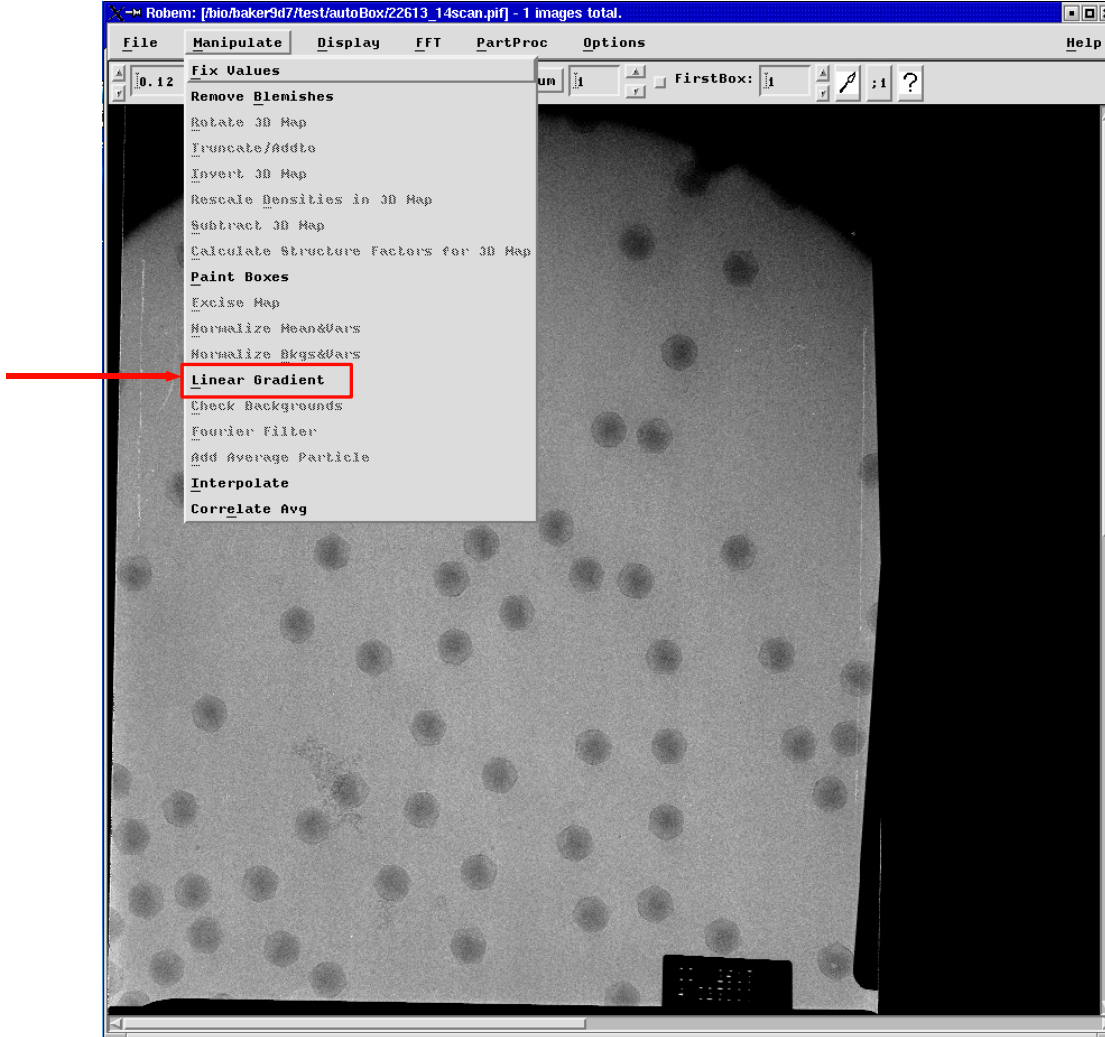
Pre-Process images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files



Icosahedral Virus 3D Reconstruction Scheme



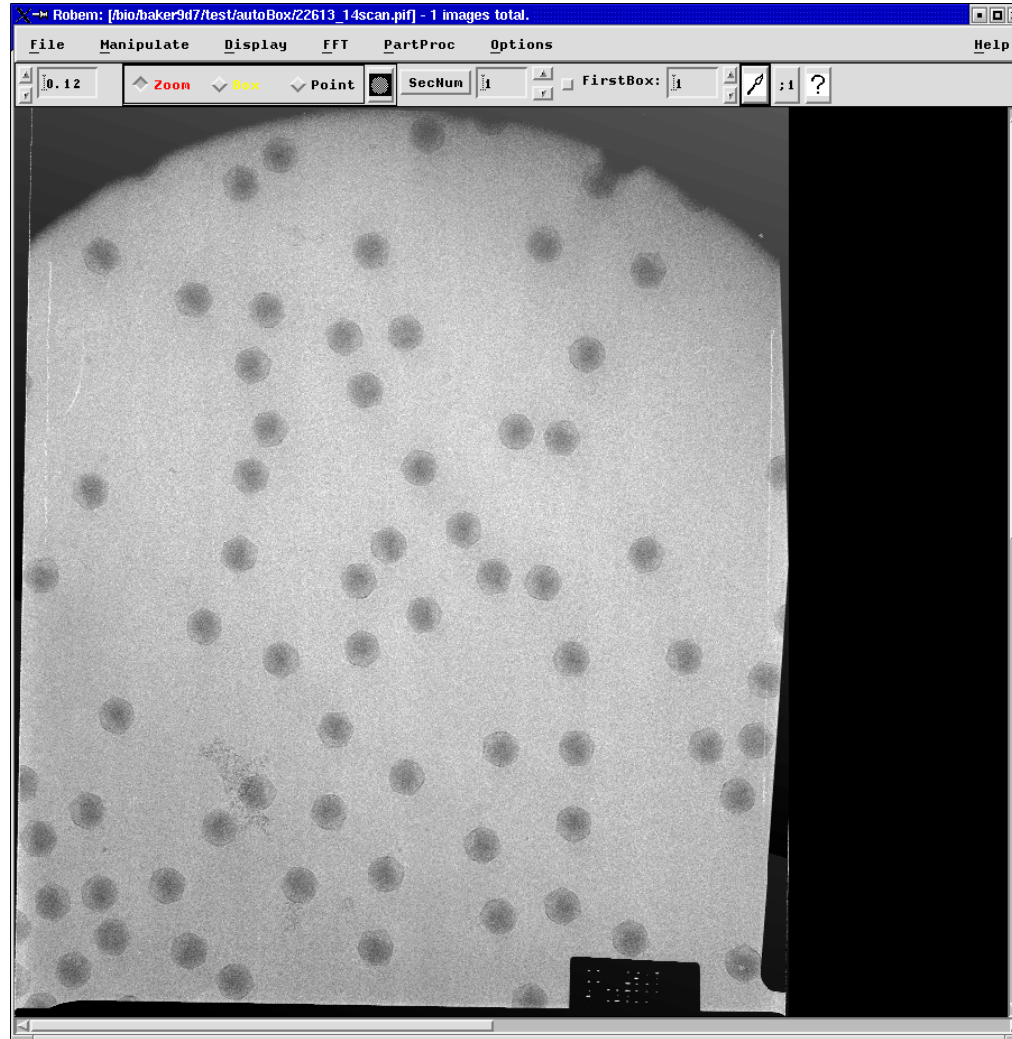
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Icosahedral Virus 3D Reconstruction Scheme



Pre-Process images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files

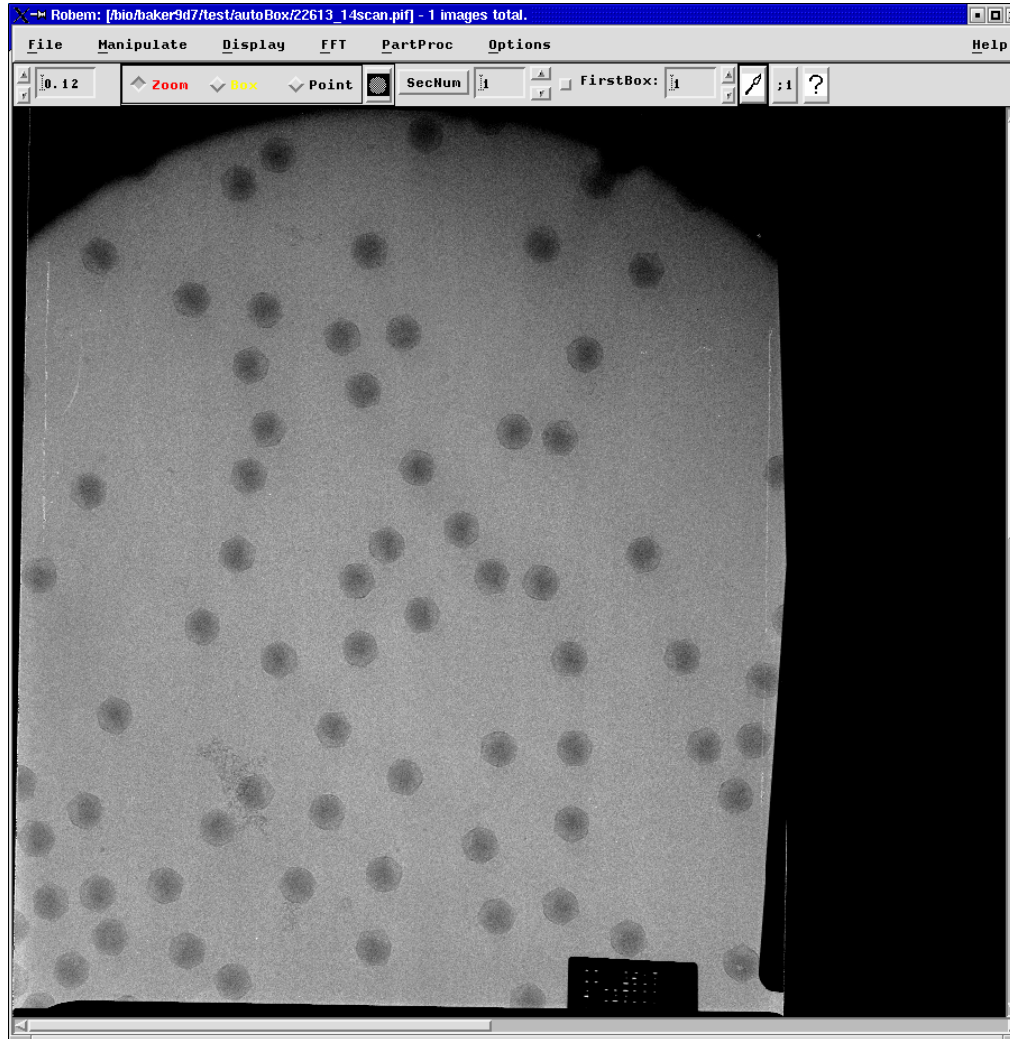


Gradient
removed

Icosahedral Virus 3D Reconstruction Scheme



Pre-Process images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files

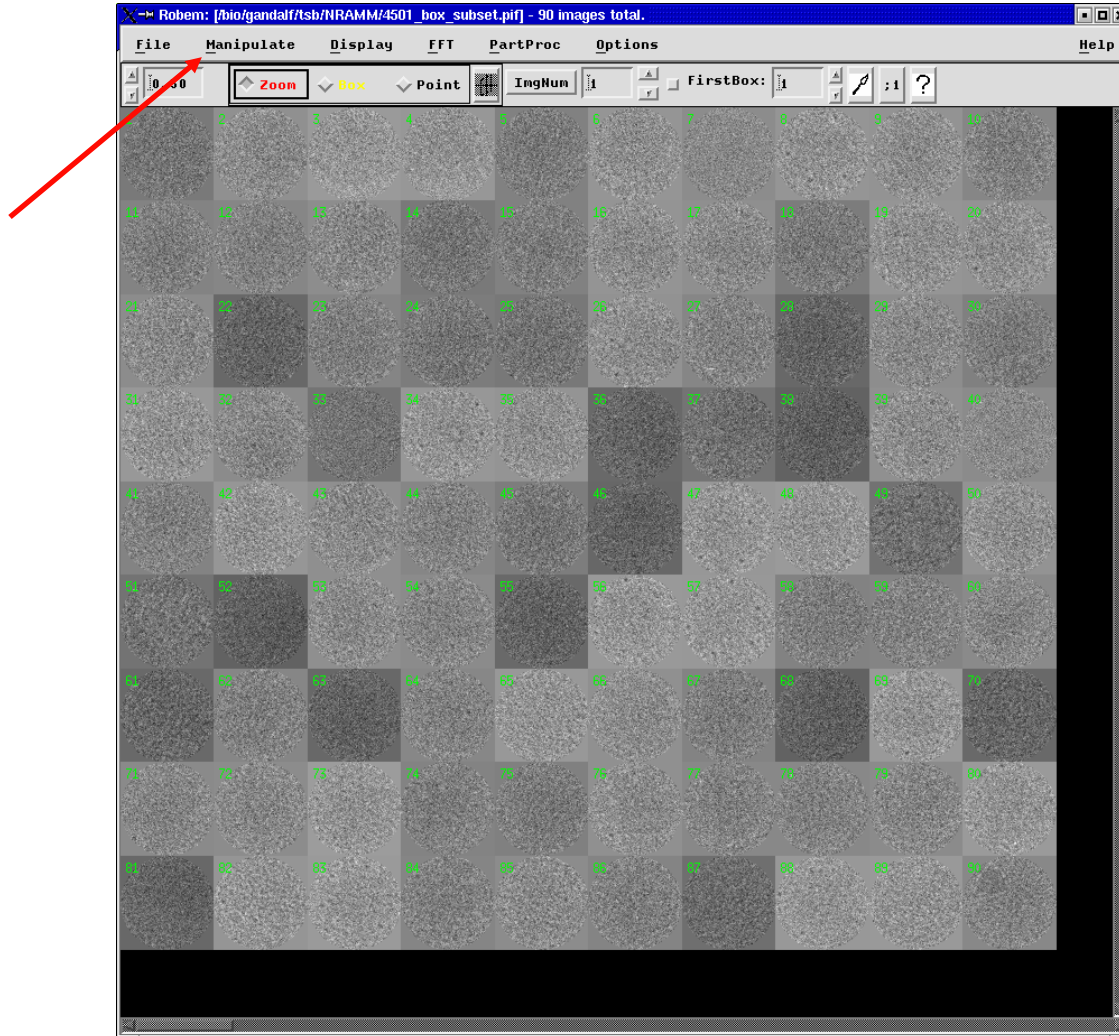


Gradient
not removed

Icosahedral Virus 3D Reconstruction Scheme



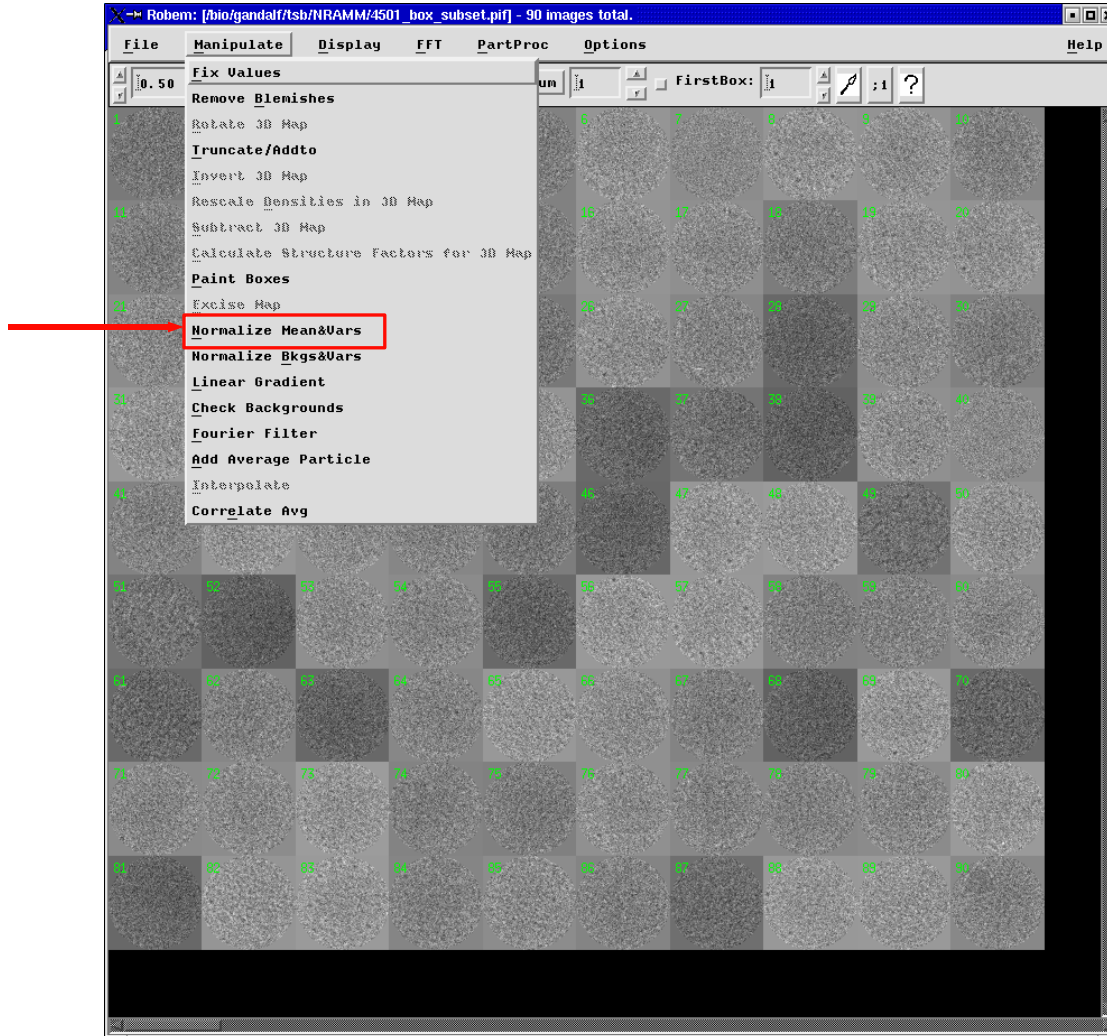
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Remove blemish, Remove Gradient
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Icosahedral Virus 3D Reconstruction Scheme



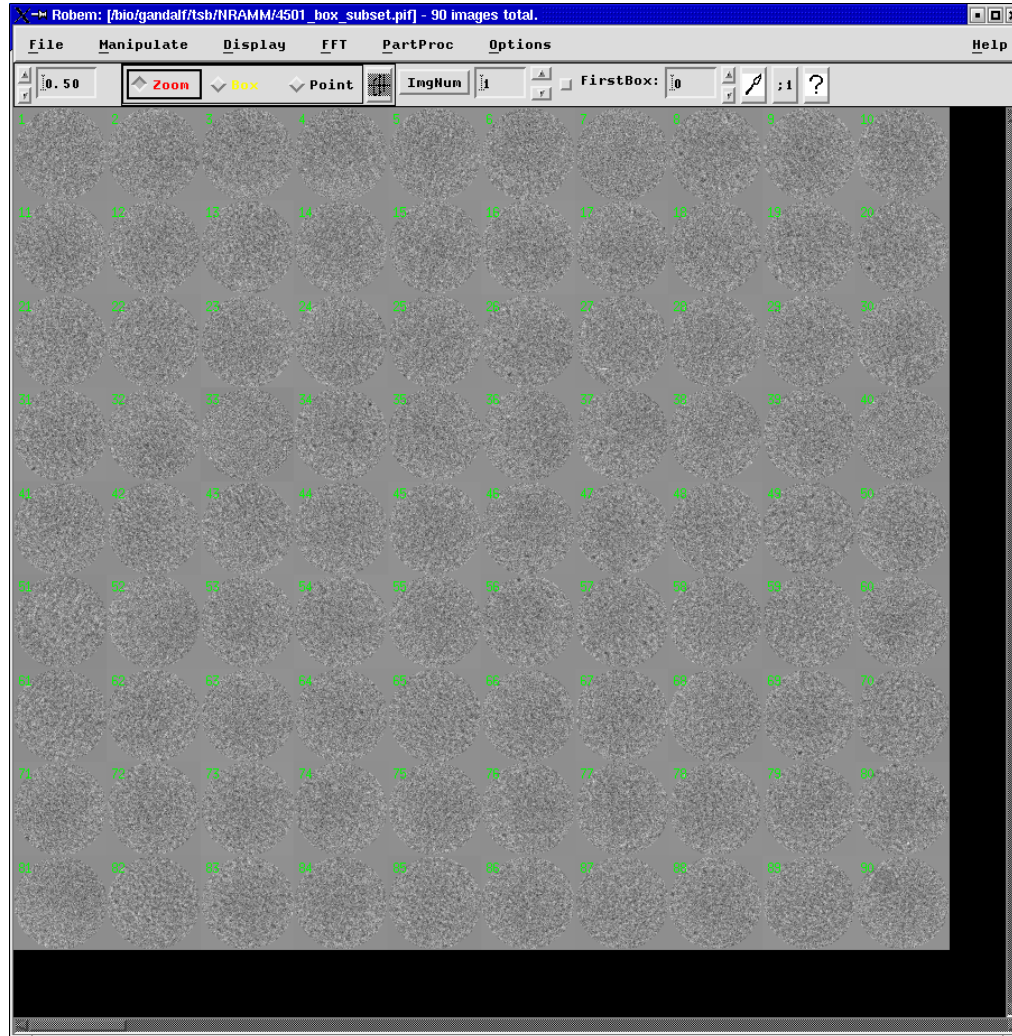
Pre-Process Images
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Icosahedral Virus 3D Reconstruction Scheme



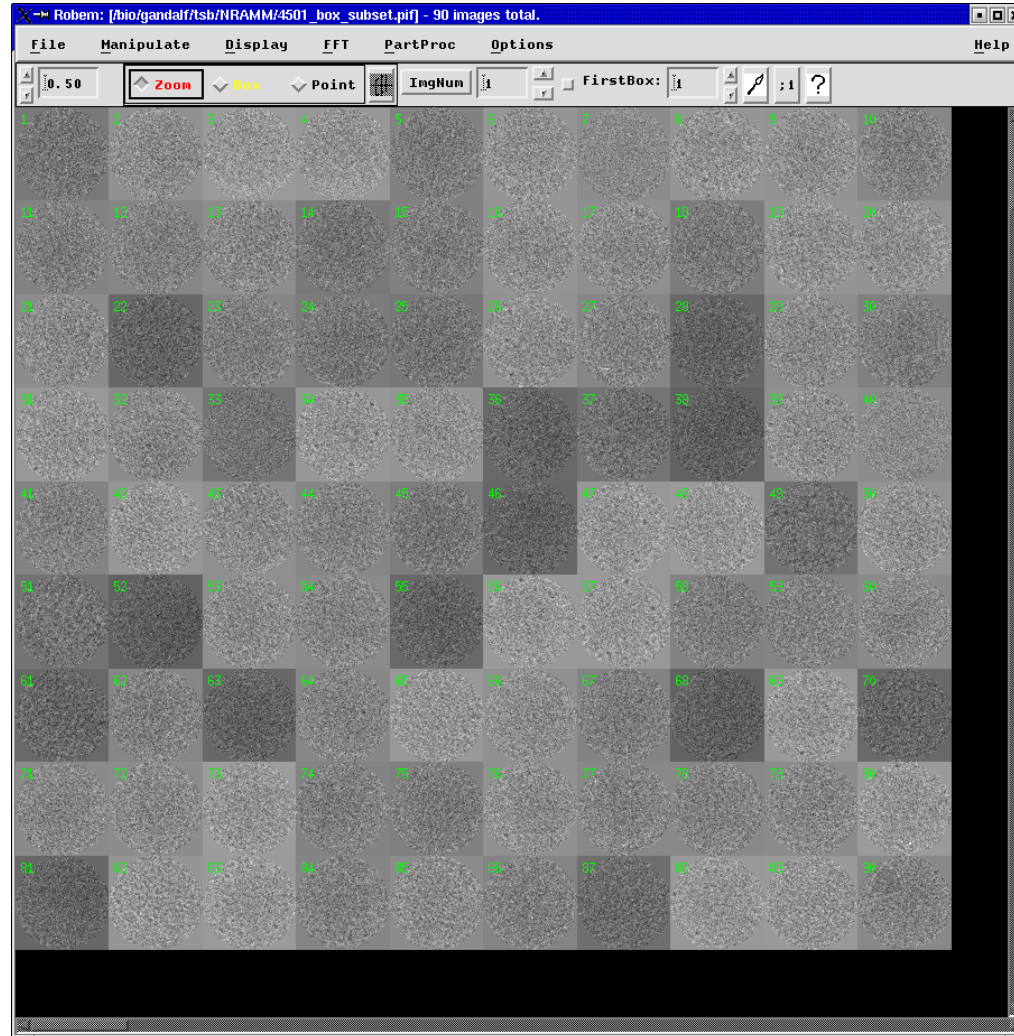
Pre-Process Images
Remove blemish, Remove Gradient
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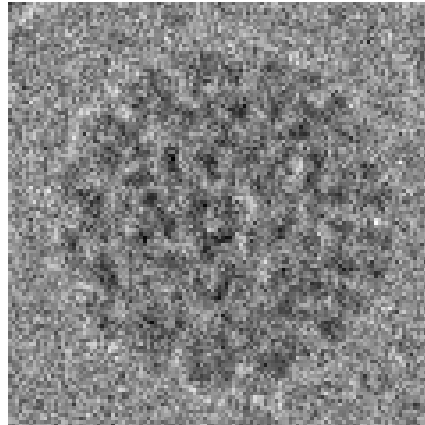
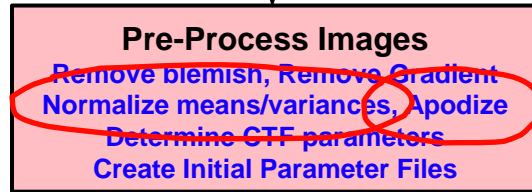
Icosahedral Virus 3D Reconstruction Scheme



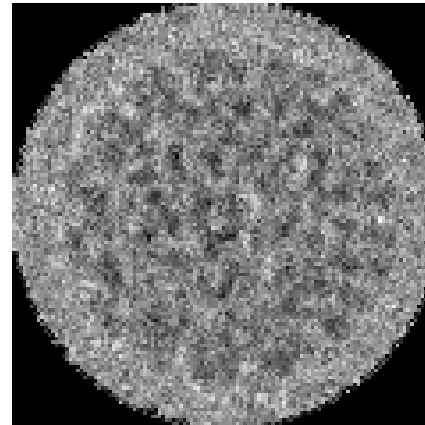
Pre-Process Images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files



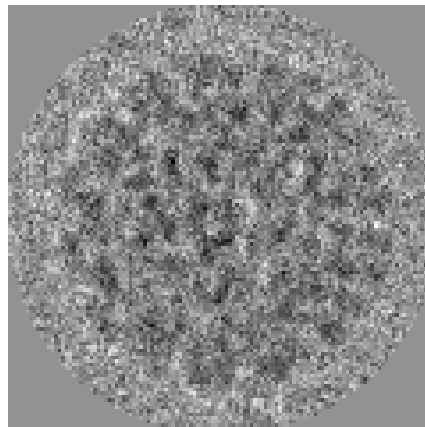
Icosahedral Virus 3D Reconstruction Scheme



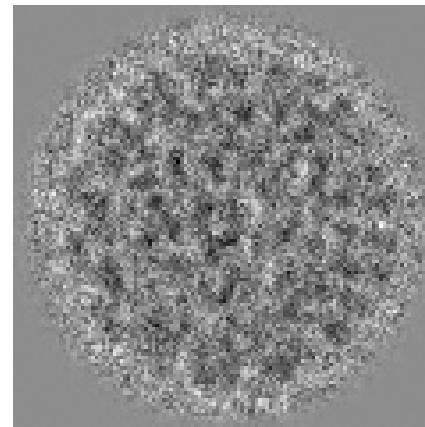
Extracted



Masked



Floated

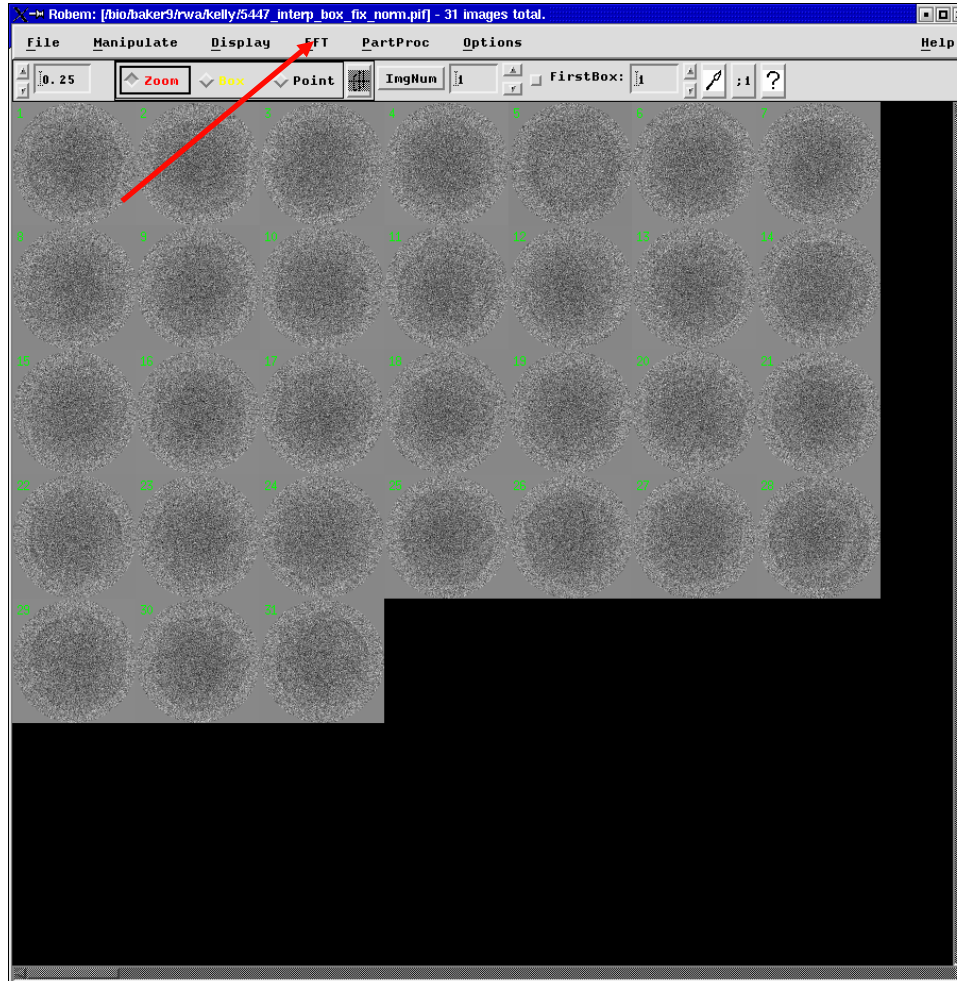


Apodized

Icosahedral Virus 3D Reconstruction Scheme



Pre-Process Images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files



Icosahedral Virus 3D Reconstruction Scheme



Pre-Process Images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files

FFT - CTF Estimation

Linear
 Log
 Non-Linear

1024 x 1024
512 x 512
256 x 256
128 x 128

Intensity
Recalc FFT

Lock Scrolls

Min: 7.61
Max: 8.52
TFac: 0
Range Min: 3.16 Max: 12.69

Back Transform
Pointer Detail

Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom
 Disp 1D CA
 Contour 1D CircAvg

Pick CTF Pts
Flicker Determine Image CTF

Ang Major
Focus Major
Focus Minor

CTF Node Num: 1

FFT Average
Average FFTs
Incoherent hvg

ScStp (um): 14 Mag: 33019
PxSiz (nm): 0.424 Wiener: 0.2
AmpCont: 0.07 AngMaj: 0.0
Volts (kV): 300 tFac: 0
Wave (nm): 0.001 Mode: 1
Cs (nm): 2.0 ctf: 1/ctf
FocMaj (um): 0.0 PixelSize in nanometers
FocMin (um): 0.0

Icosahedral Virus 3D Reconstruction Scheme

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Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files

FFT - CTF Estimation

Linear
Log
Non-Linear

1024 x 1024
512 x 512
256 x 256
128 x 128

Intensity
Recalc FFT

Lock Scrolls

Min 14.71
Max 17.42
TFac 0
Range Min: 14.71 Max: 25.16

Back Transform
Pointer Detail

Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom
Contour 1D CircAvg

Disp 1D CA

Pick CTF Pts Flicker Determine Image CTF

Ang Major
Focus Major
Focus Minor

CTF Node Num:
1

FFT Average
Aver Ing#
Average FFTs
Incoherent hvg

ScStp (um) 14 Mag 33019
PxSiz (nm) 0.424 Wiener 0.2
AmpCont 0.07 AngMaj 0.0
Volts (kV) 300 tFac 0
Wave (nm) 0.001 Mode 1
Cs (nm) 2.0 ctf 1/ctf
FocMaj (um) 0.0 PixelSize in nanometers
FocMin (um) 0.0

Icosahedral Virus 3D Reconstruction Scheme



Pre-Process Images
Remove blemish, Remove Gradient
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Range Min: 14.71 Max: 25.16

Back Transform
Pointer Detail

Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom
 Disp 1D CA
 Contour 1D CircAvg

Pick CTF Pts
Flicker Determine Image CTF

Ang Major: 359.4
Focus Major: 2.16
Focus Minor: 3.31

CTF Node Num: 1

FFT Average
Aver Ing#
Average FFTs
Incoherent hvj

ScStp (um): 14 Mag: 33019
PxSiz (nm): 0.424 Wiener: 0.2
AmpCont: 0.07 AngMaj: 359.4
Volts (kV): 300 tFac: 0
Wave (nm): 0.001 Mode: 1
Cs (nm): 2.0 ctf: 1/ctf
FocMaj (um): 2.16 PixelSize in nanometers
FocMin (um): 3.31

Icosahedral Virus 3D Reconstruction Scheme



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Min
Max
TFac
Range

Back Transfer
Pointer Detail

Overlay Intensity

Zoom
Disp 1D CA
Contour 1D CA

Pick CTF Pts
Flicker
Determine Image CTF

Ang Major: 359.4
Focus Major: 2.16
Focus Minor: 3.31

CTF Node Num: 1

FFT Average
Average FFTs
Incoherent hvg

Automatic Defocus Refinement

a0: 1
a1: 0
a2: 0
a3: 0
a4: 0

b0: 1
b1: 0
b2: 0
b3: 0
b4: 0

Highest Resolution (Angs): 8.480000

Estimated Defocus (um):
Chi squared:

Select different plots:
 Circular Average
 S
 S - B
 ctf^2
 D
 B

Zoom FFT image
Change Overlay Intensity to see CTF curves.

Estimate the Defocus *1
Update FFT screen

AmpCont: 0.07
Volts (kV): 300
Wave (nm): 0.001
Cs (nm): 2.0
FocMaj (um): 2.16
FocMin (um): 3.31

AngMaj: 359.4
tFac: 0
Mode: 1
ctf: 1/ctf
PixelSize in nanometers

Icosahedral Virus 3D Reconstruction Scheme



Pre-Process Images
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Log
Non-Linear

Min
Max
TFac
Range

Back Transfer
Pointer Detail

Overlay Intensity

Zoom
Disp 1D CA
Contour 1D CA

Pick CTF Pts
Flicker
Determine Image CTF

Ang Major: 359.4
Focus Major: 2.204
Focus Minor: 2.204

CTF Node Num: 1

FFT Average
Average Ing#
Average FFTs
Incoherent hvv

Automatic Defocus Refinement

a0: 1.739071E+00
a1: -6.500993E-0
a2: 3.357709E-05
a3: -1.871776E-0
a4: 3.767466E-10

b0: 2.172054E-01
b1: 2.077333E-03
b2: -4.360161E-0
b3: 2.043404E-07
b4: -3.037543E-1

Highest Resolution (Angs): 8.480000

Estimated Defocus (um): 2.204652
Chi squared: 0.111960

Select different plots:
 Circular Average
 S
 S - B
 ctf^2
 D
 B

Zoom FFT image
Change Overlay Intensity to see CTF curves.

Estimate the Defocus *1
Update FFT screen

AmpCont: 0.07
Volts (kV): 300
Wave (nm): 0.001
Cs (nm): 2.0
FocMaj (um): 2.20
FocMin (um): 2.20

AngMaj: 359.4
tFac: 0
Mode: 1
ctf: 1/ctf
PixelSize in nanometers

Icosahedral Virus 3D Reconstruction Scheme



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256 x 256
128 x 128

Intensity
Recalc FFT

Lock Scrolls

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Max: 17.42
TFac: 0
Range Min: 14.71 Max: 25.16

Back Transform
Pointer Detail

Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom
 Contour 1D CircAvg

Pick CTF Pts **Flicker** Determine Image CTF

Ang Major: 359.4
Focus Major: 2.204
Focus Minor: 2.204

CTF Node Num: 1

FFT Average
Aver Ing#
Average FFTs
Incoherent hvv

ScStp (um): 14 Mag: 33019
PxSiz (nm): 0.424 Wiener: 0.2
AmpCont: 0.07 AngMaj: 359.4
Volts (kV): 300 tFac: 0
Wave (nm): 0.001 Mode: 1
Cs (nm): 2.0 ctf: 1/ctf
FocMaj (um): 2.20 PixelSize in nanometers
FocMin (um): 2.20

Icosahedral Virus 3D Reconstruction Scheme



Pre-Process Images
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Max: 17.42
TFac: 0
Range Min: 14.71 Max: 25.16

Back Transform
Pointer Detail

Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom
 Contour 1D CircAvg

Pick CTF Pts **Flicker** Determine Image CTF

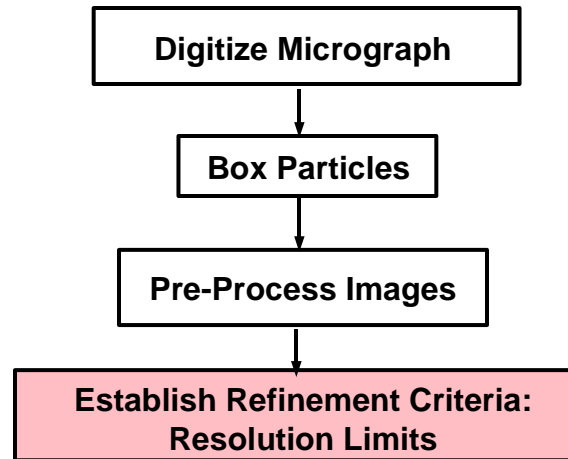
Ang Major: 359.4
Focus Major: 2.204
Focus Minor: 2.204

CTF Node Num: 1

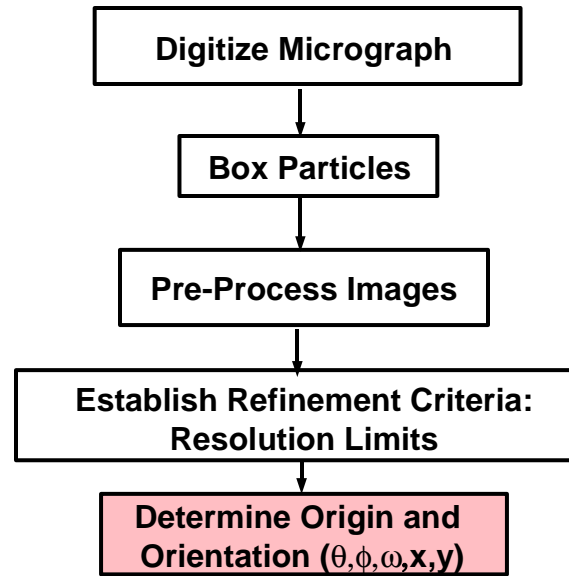
FFT Average
Aver Ing#
Average FFTs
Incoherent hvv

ScStp (um): 14 Mag: 33019
PxSiz (nm): 0.424 Wiener: 0.2
AmpCont: 0.07 AngMaj: 359.4
Volts (kV): 300 tFac: 0
Wave (nm): 0.001 Mode: 1
Cs (nm): 2.0 ctf: 1/ctf
FocMaj (um): 2.20 PixelSize in nanometers
FocMin (um): 2.20

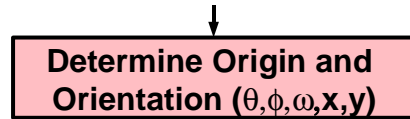
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



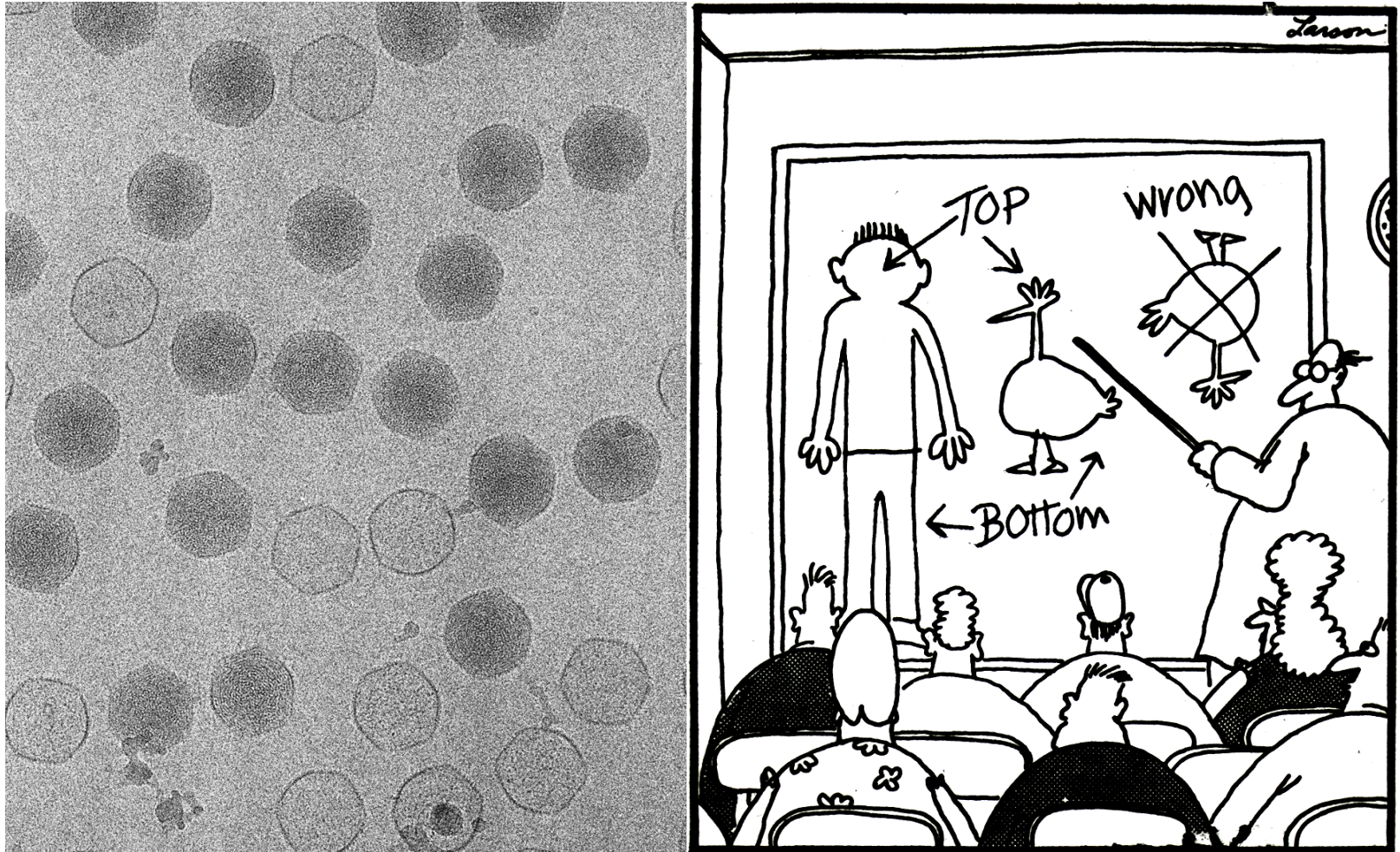
Goal: determine phase origin and view orientation for each boxed particle

MOST IMPORTANT STEP?

Garbage in -----> garbage out

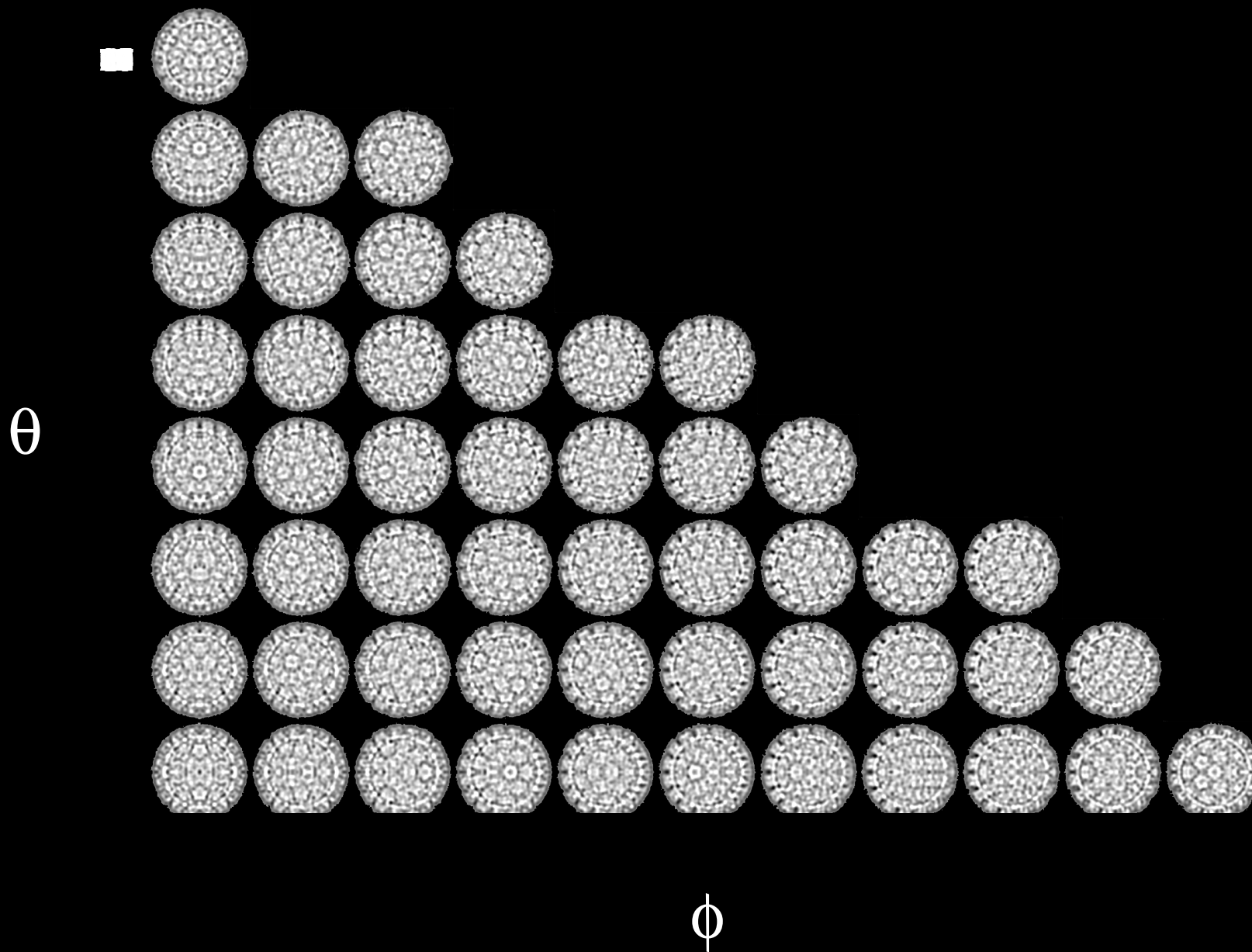
Icosahedral Virus 3D Reconstruction Scheme

↓
Determine Origin and
Orientation ($\theta, \phi, \omega, x, y$)

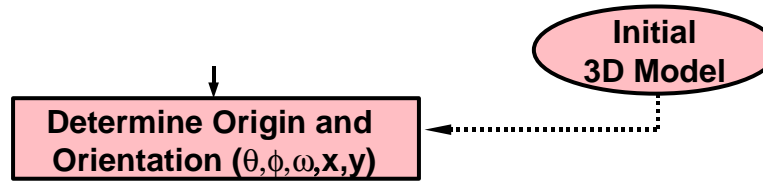


People who don't know which end is up

BPV Projections: 1/2 Icosahedral ASU



Icosahedral Virus 3D Reconstruction Scheme



How do we determine the ($\theta, \phi, \omega, x, y$) parameters?

Two methods:

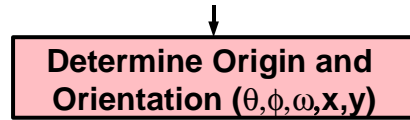
1. Common lines

New or unknown structure

2. Model-based (template) matching

General features of structure are known or a crude model can be generated

Icosahedral Virus 3D Reconstruction Scheme



How do we determine the ($\theta, \phi, \omega, x, y$) parameters?

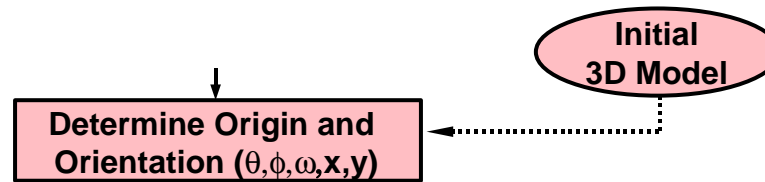
Two methods:

1. *Ab initio* (e.g. Common lines)

New or unknown structure

2. By guess and by golly

Icosahedral Virus 3D Reconstruction Scheme



How do we determine the ($\theta, \phi, \omega, x, y$) parameters?

Two methods:

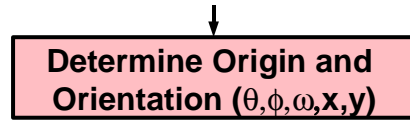
1. *Ab initio* (e.g. Common lines)

New or unknown structure

2. Model-based (template) matching

General features of structure are known or a crude model can be generated (...or, sometimes, even a lousy model will work)

Icosahedral Virus 3D Reconstruction Scheme



Common Lines

The 'gospel' according to Tony Crowther (*Phil. Trans. R. Soc. Lond. B.*(1971) **261:221-230**)

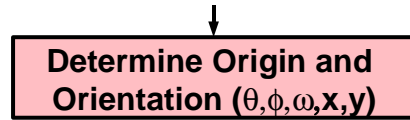
“[Common lines] arise as follows:”

*“An observed section of the transform intersects an identical symmetry-related section in a **line**, along which the transform must have the **same value in both sections**”*

“The common line lies in the original section.”

*“However, regarded as lying in the symmetry-related section it must have been generated by the symmetry operation from **some other line** in the original section.”*

Icosahedral Virus 3D Reconstruction Scheme



Common Lines

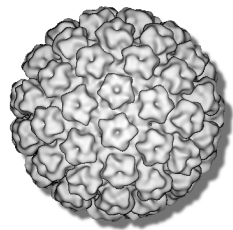
The 'gospel' continued:

“We therefore have a pair of lines in the original transform plane along which the transform must have identical values”

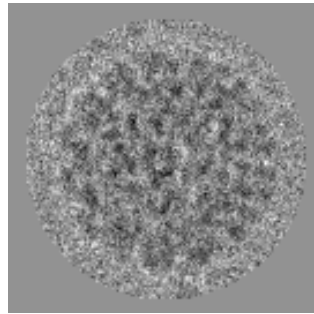
“A similar pair of lines will be generated by each possible choice of pairs of symmetry operations”

“The angular positions of these lines are dependent on the orientation of the particle.”

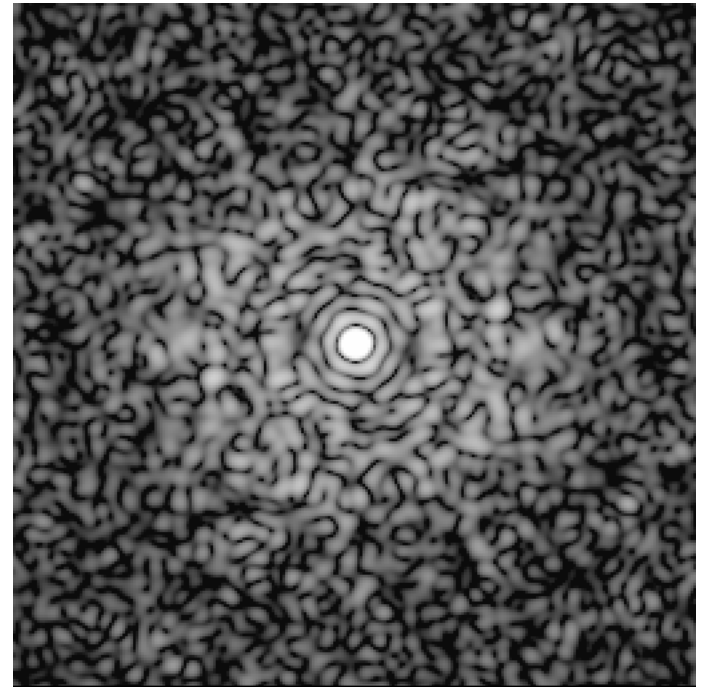
Orientation Determination by Common Lines



3D Object



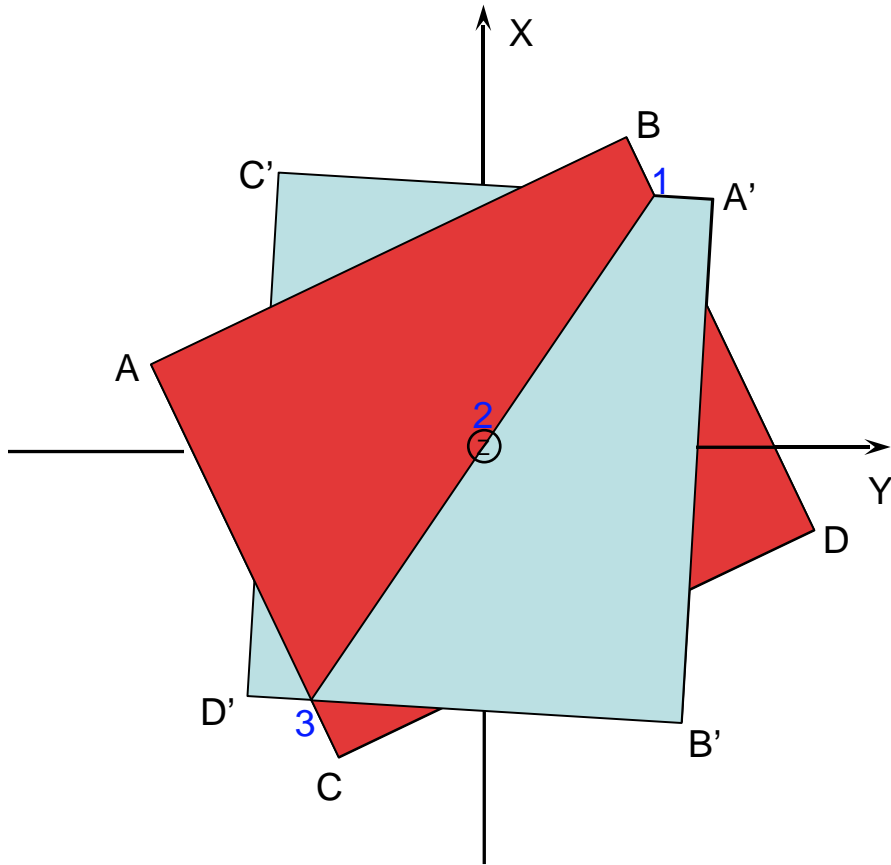
2D Projection
(θ, ϕ, ω)



2D Fourier Transform

Orientation Determination by Common Lines

Simple example: object with single three-fold axis of symmetry



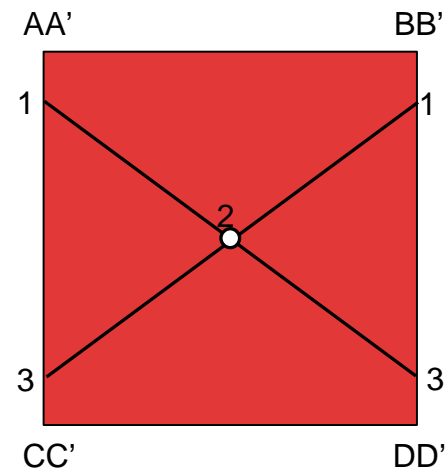
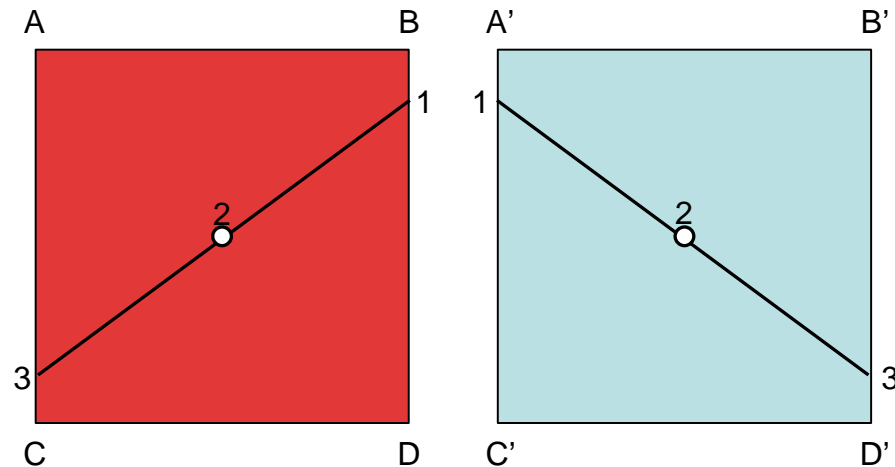
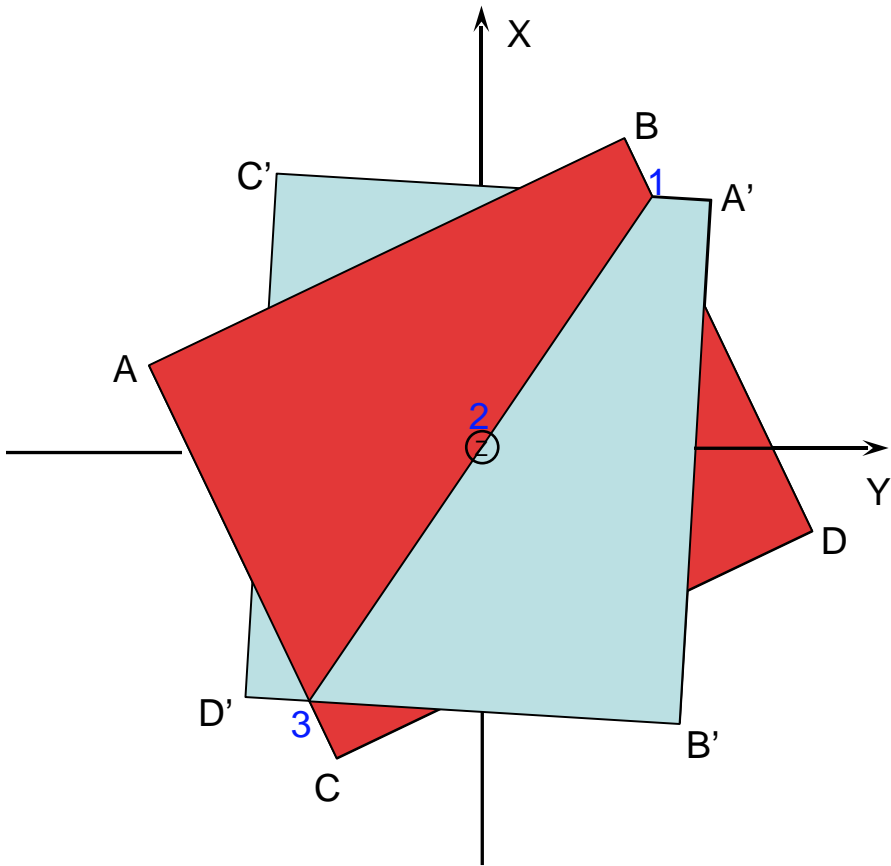
ABCD = 2D transform of image from particle **not** viewed along an axis of symmetry

Let Z-direction coincide with **3-fold** axis of symmetry

3-fold operation generates **two** additional FT sections (only A'B'C'D' is shown)

Both planes have **common values** along the **line** (1,2,3) of their intersection

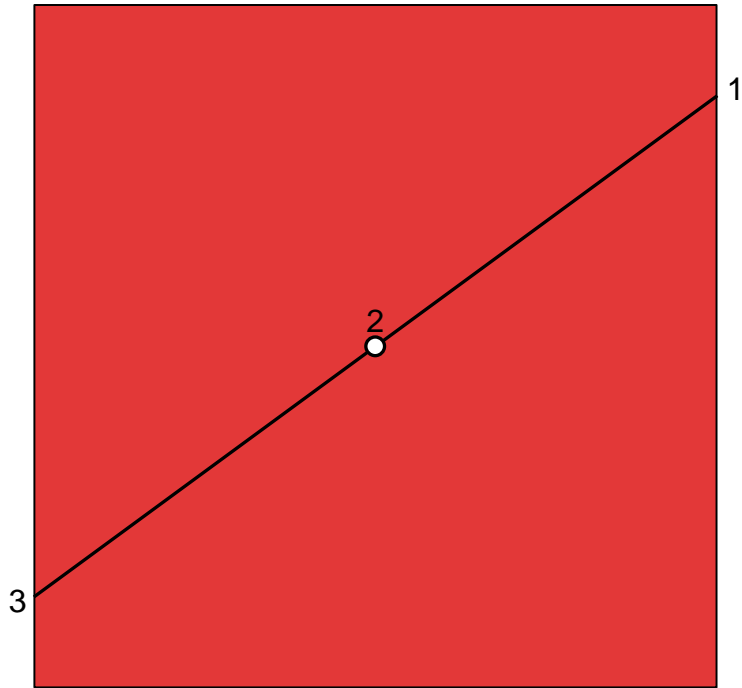
Orientation Determination by Common Lines



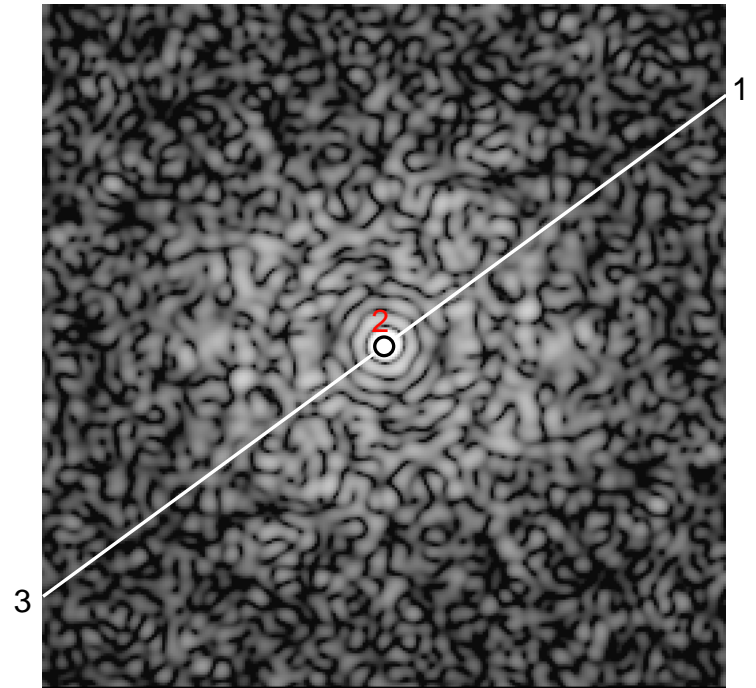
Adapted from Moody (1990) Fig. 7.68,
p.245

Adapted from Moody (1990) Fig. 7.69, p.246

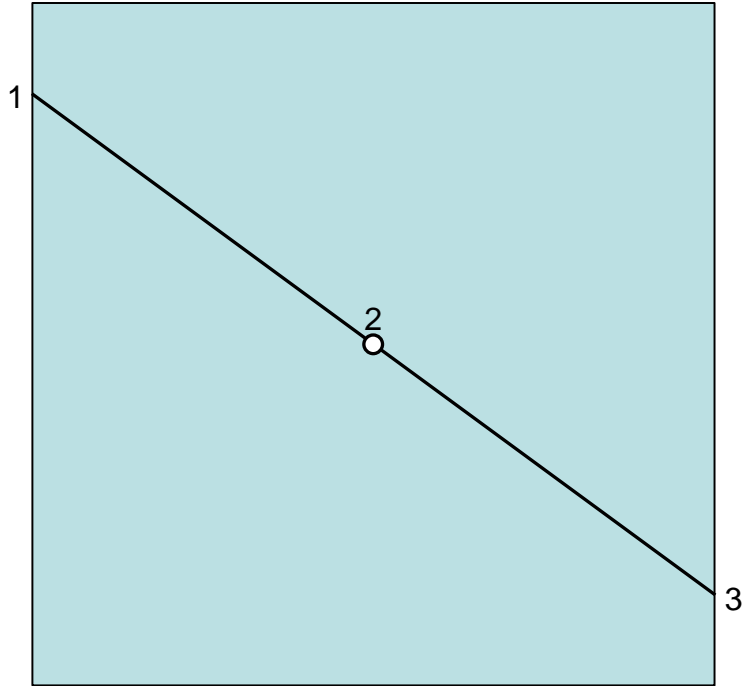
Orientation Determination by Common Lines



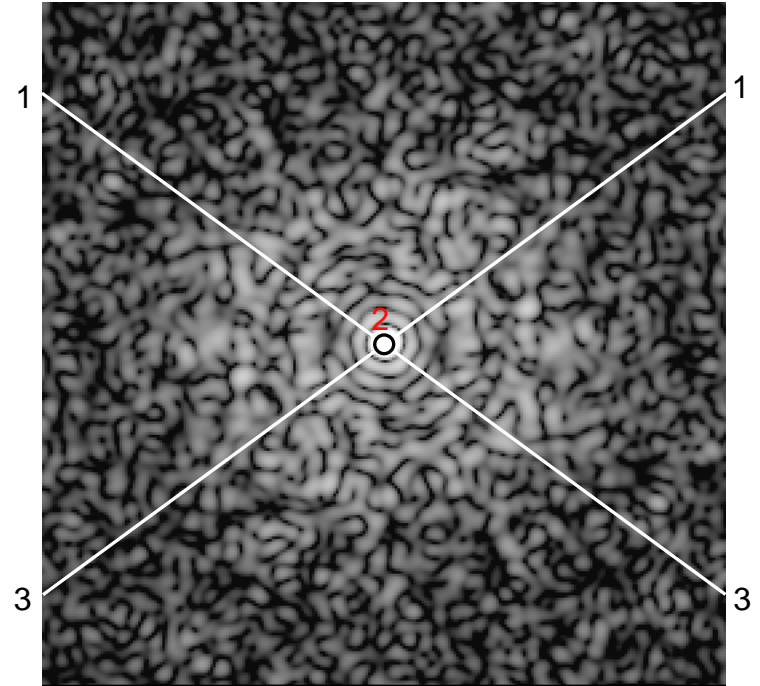
Original Transform Plane



Orientation Determination by Common Lines



Symmetry-Related
Transform Plane



Orientation Determination by Common Lines

Ok, that's easy (simple object with single 3-fold axis)

What about an object with 532 symmetry?

For a **general view**, icosahedral symmetry generates:

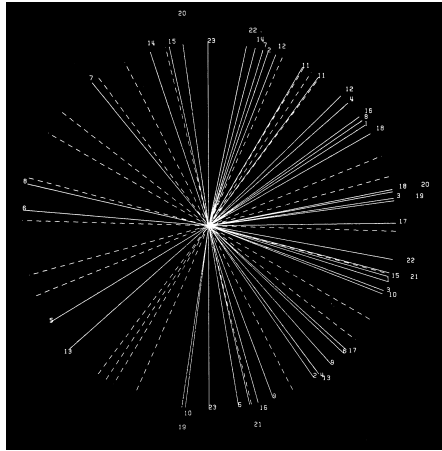
$$\text{5-folds: } \frac{12}{2} \times 2 = 12 \text{ pairs}$$

$$\text{3-folds: } \frac{20}{2} \times 1 = 10 \text{ pairs}$$

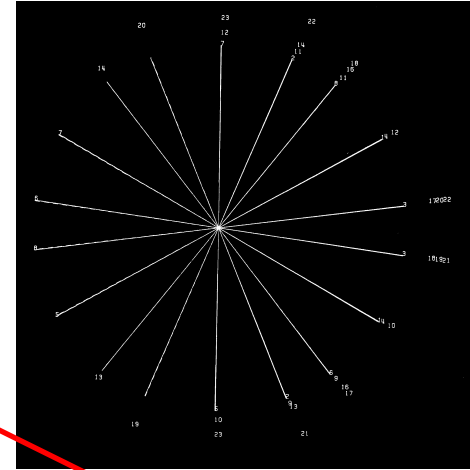
$$\text{2-folds: } \frac{30}{2} \times 1 = \underline{15} \text{ real lines}$$

37 common lines

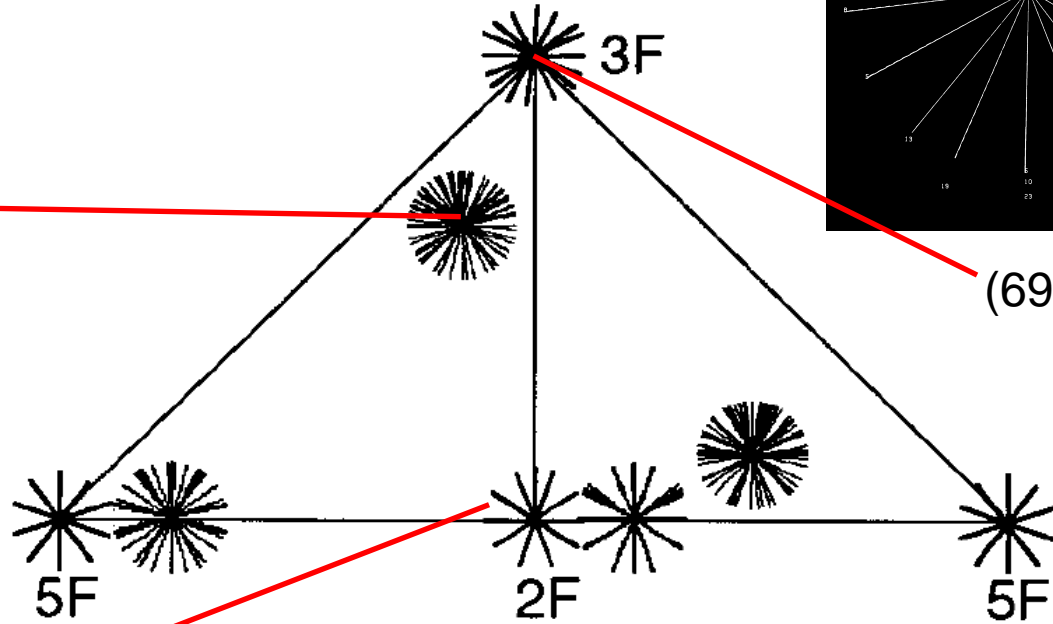
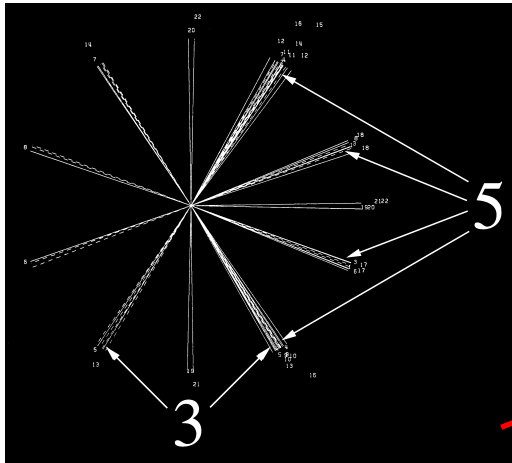
Orientation Determination by Common Lines



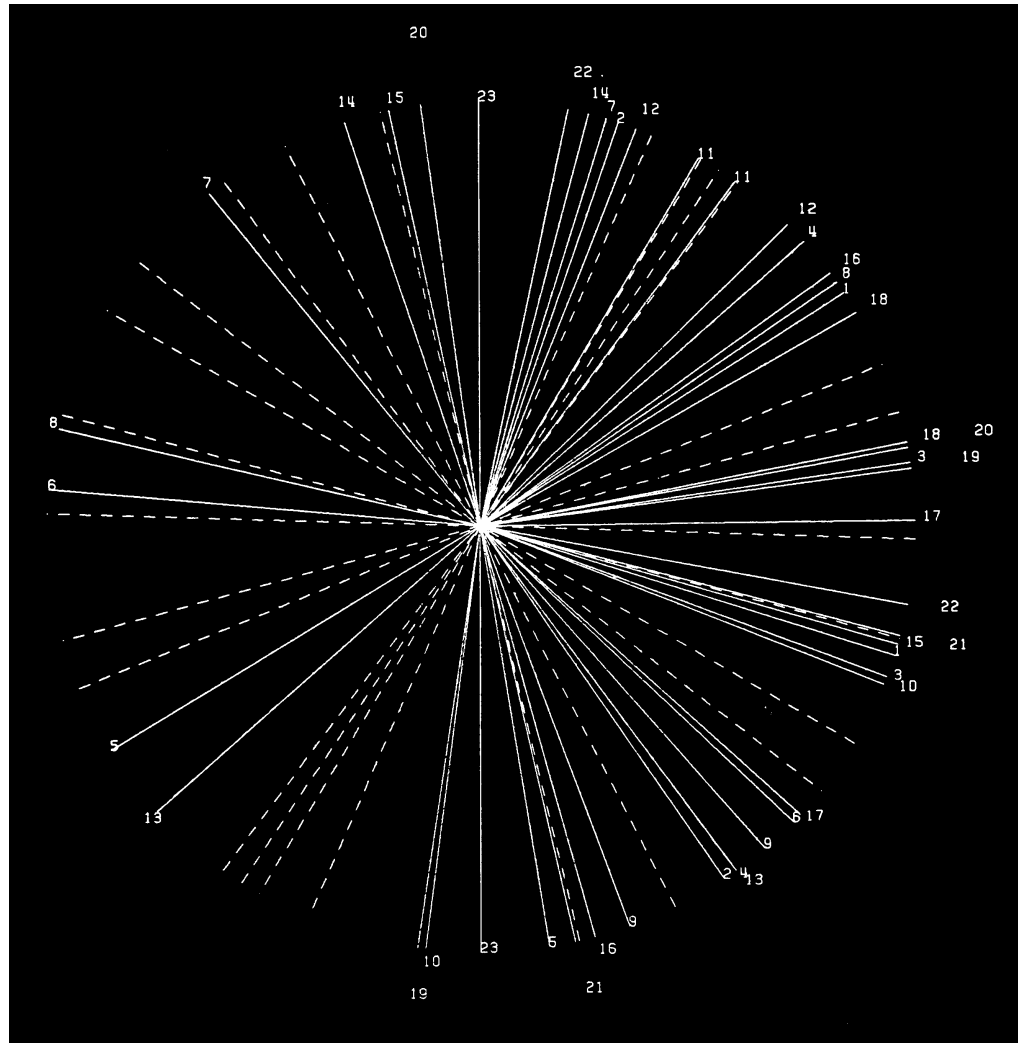
(80,11)



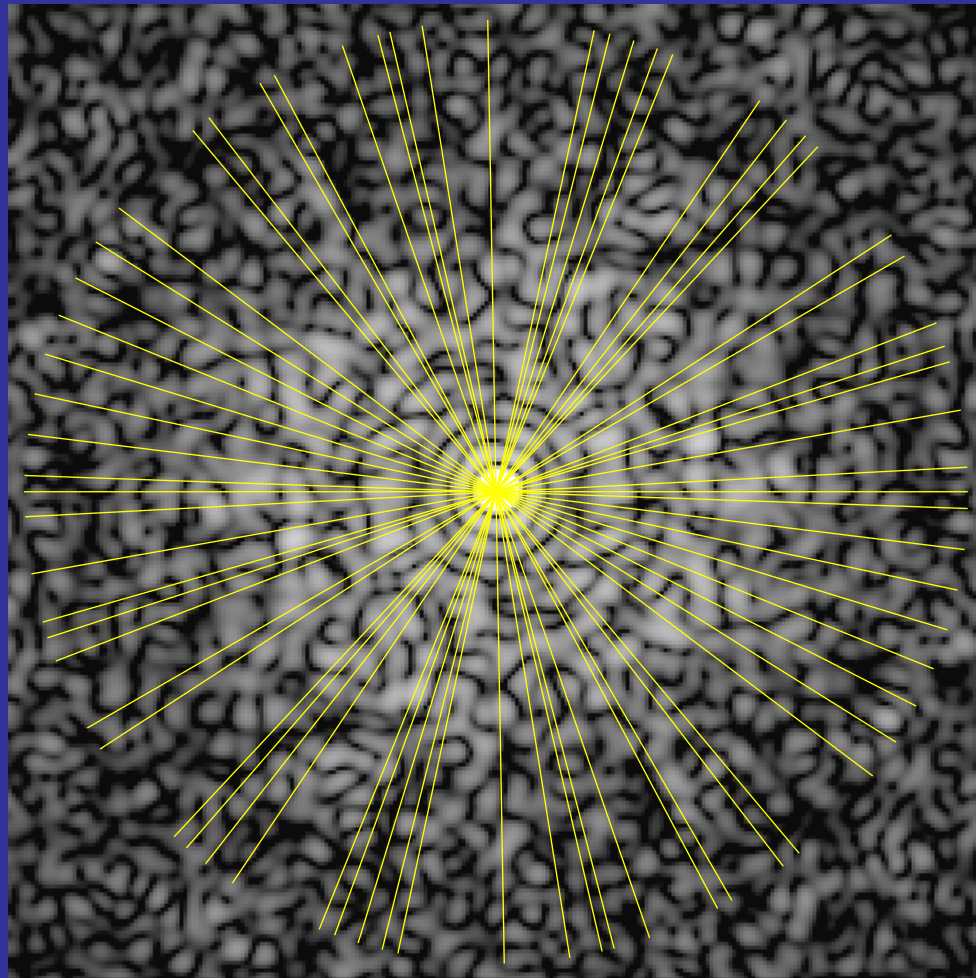
(69,0)



Orientation Determination by Common Lines



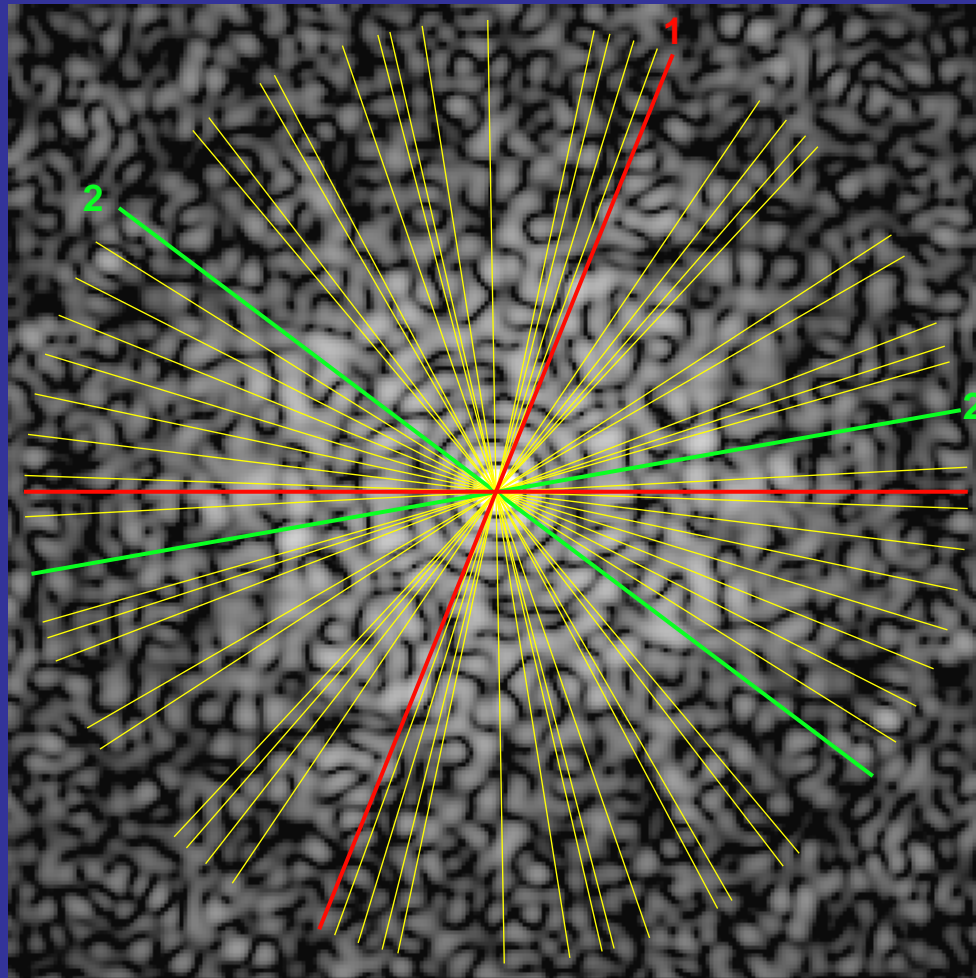
Orientation Determination by Common Lines



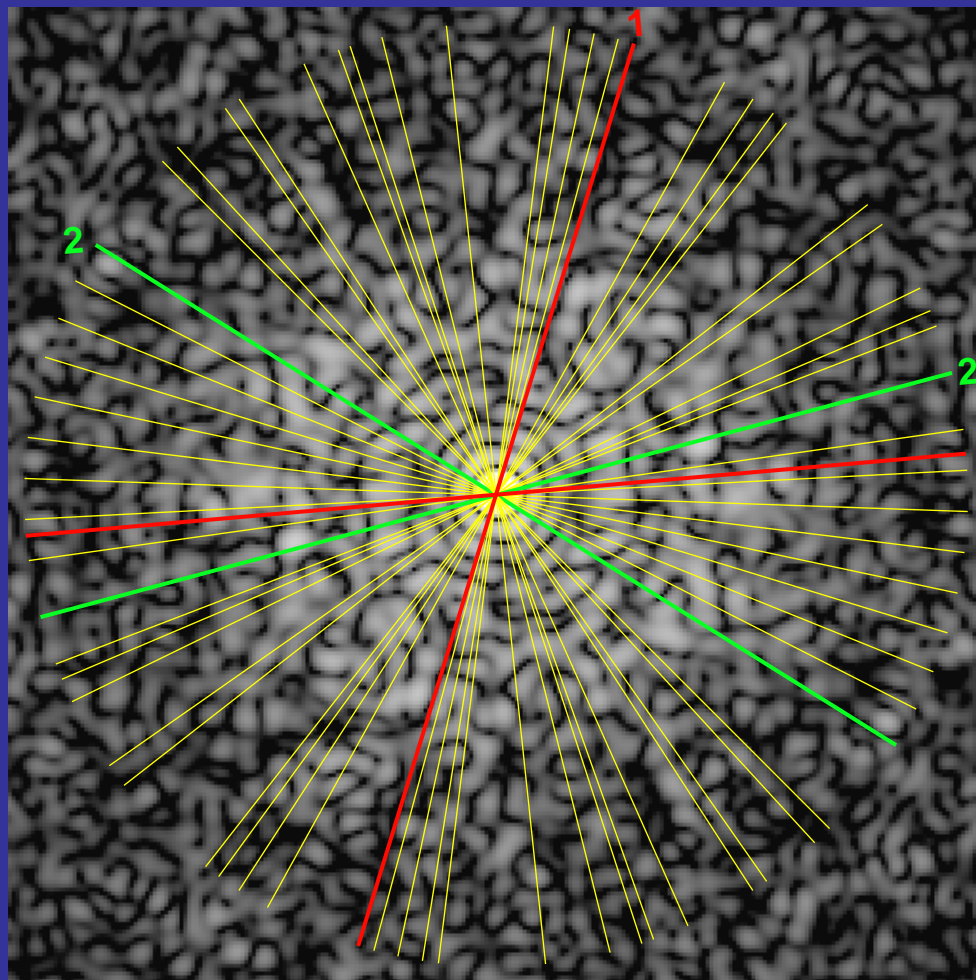
(80,11)

What is (θ, ϕ, ω) for this particle?

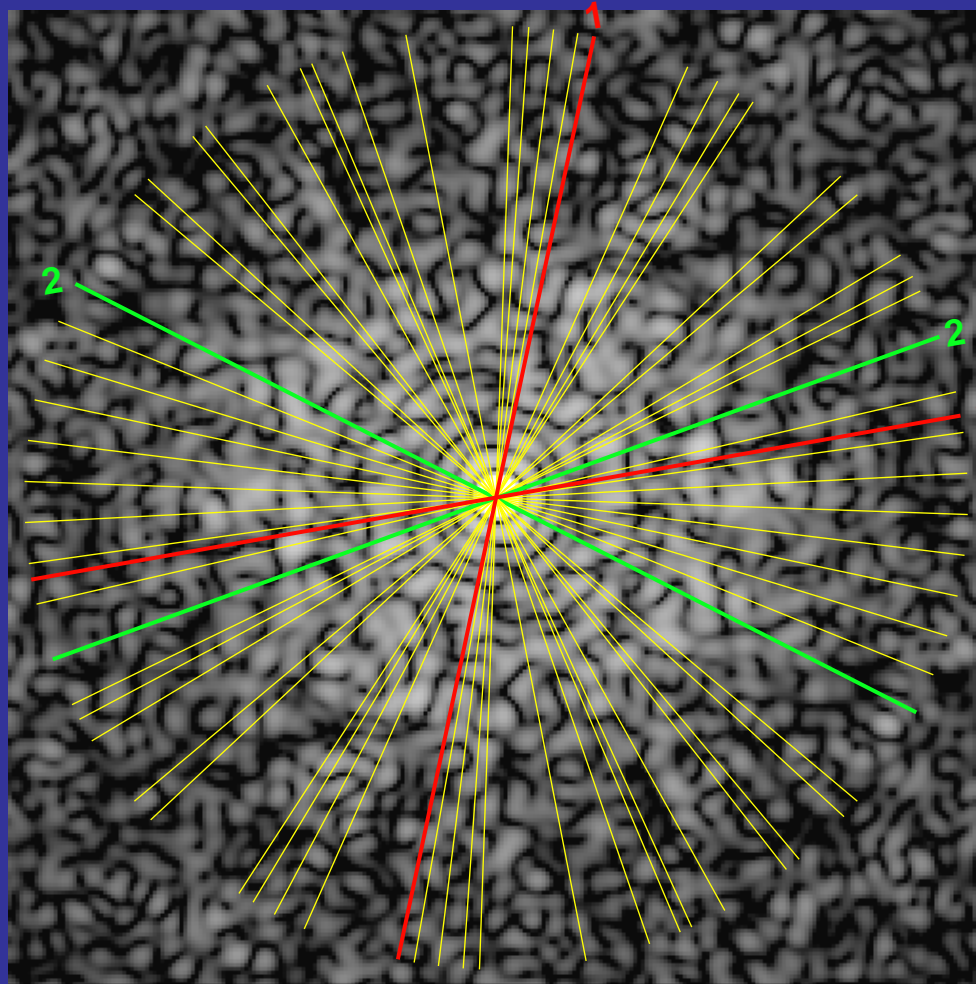
Orientation Determination by Common Lines



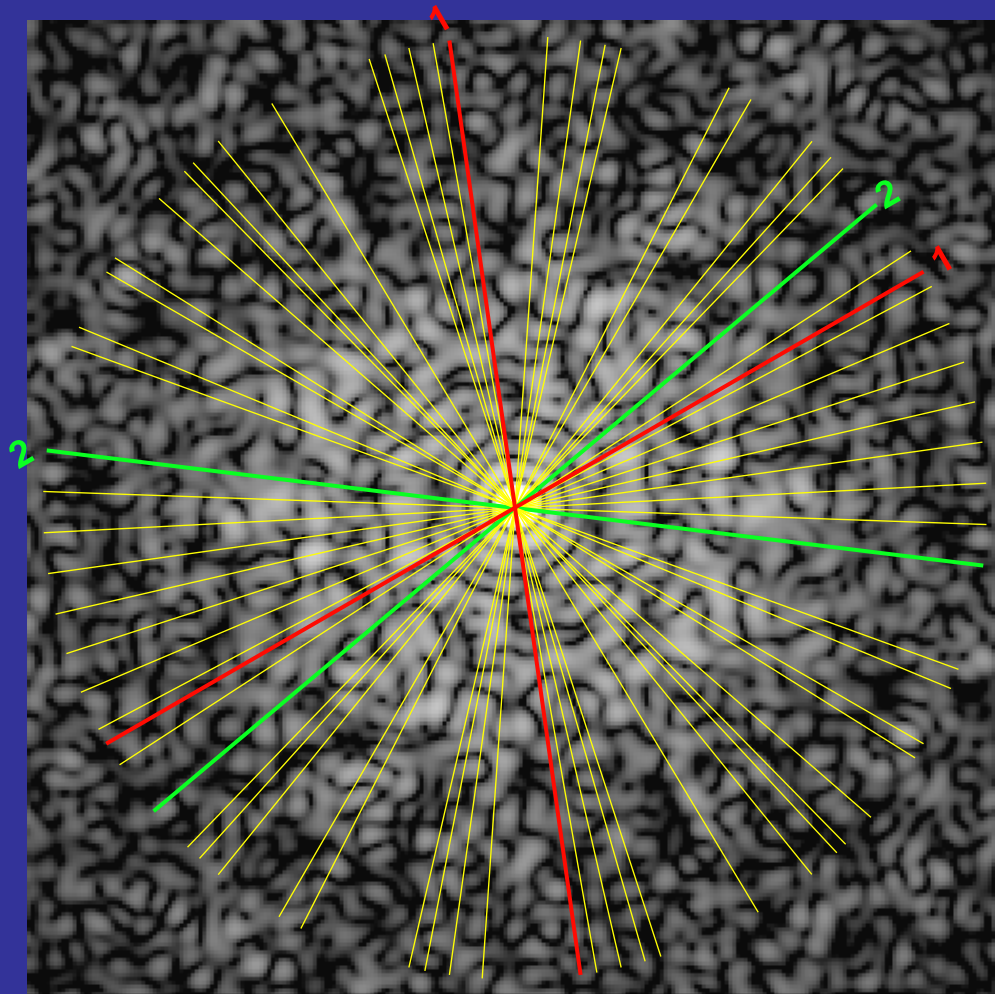
Orientation Determination by Common Lines



Orientation Determination by Common Lines

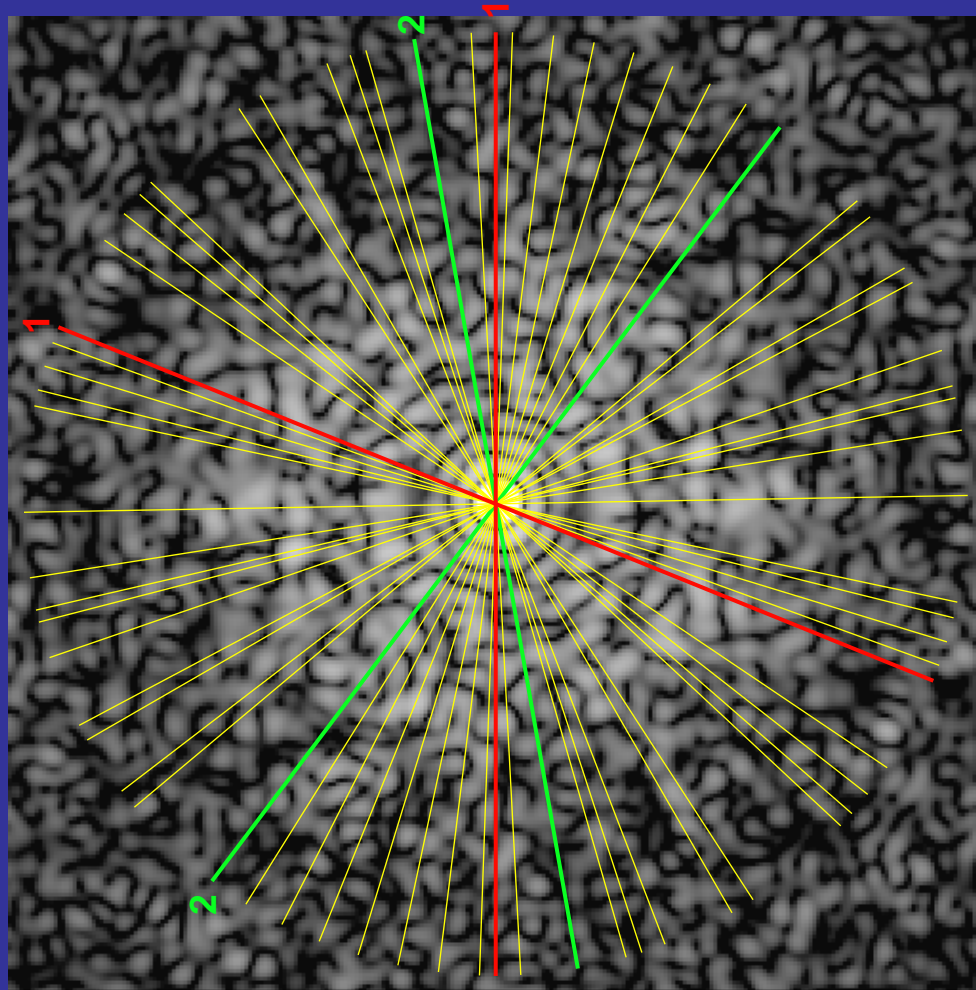


Orientation Determination by Common Lines

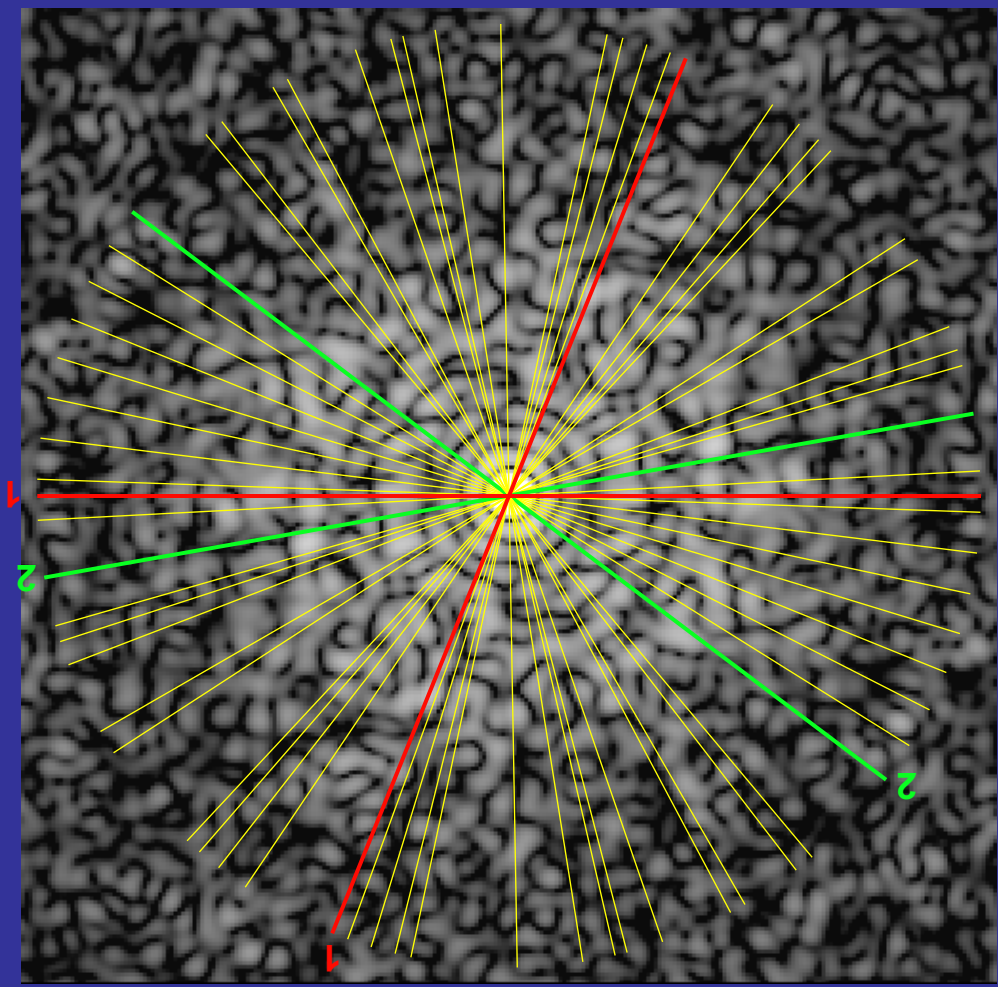


ω
↓
(80, 11, 30)

Orientation Determination by Common Lines

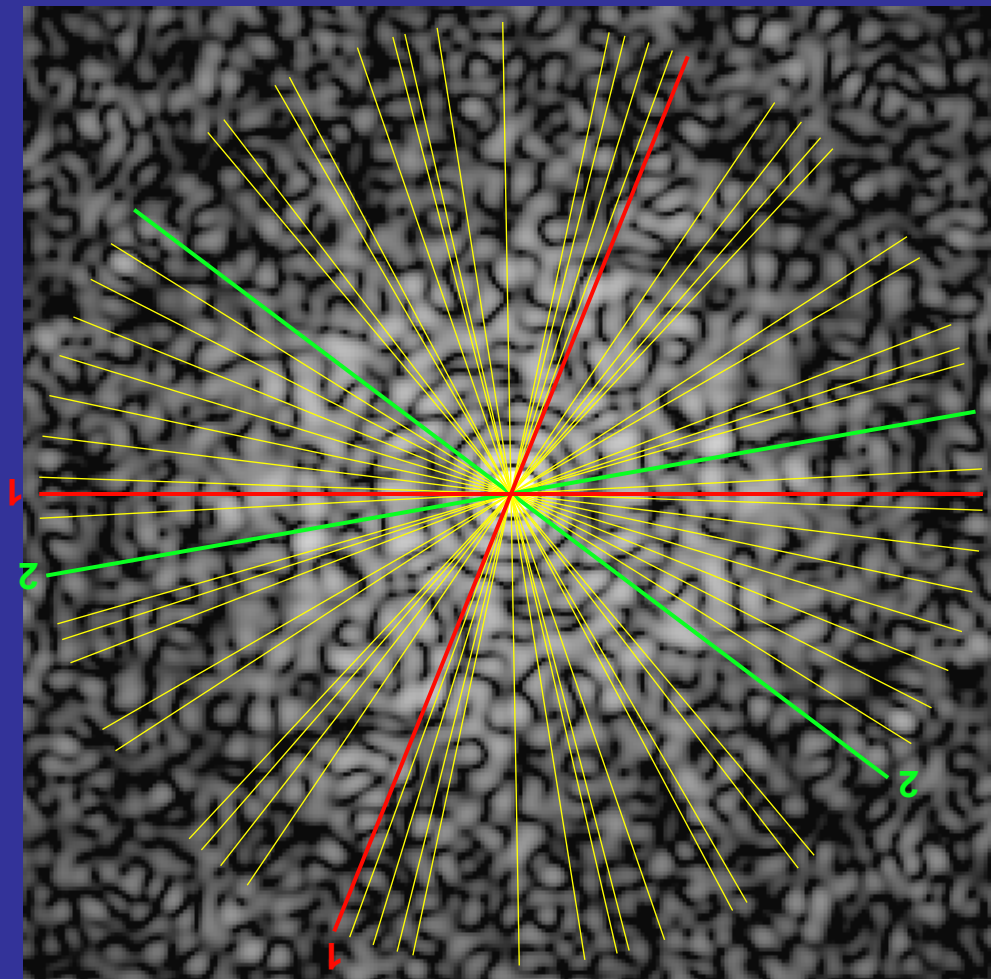


Orientation Determination by Common Lines



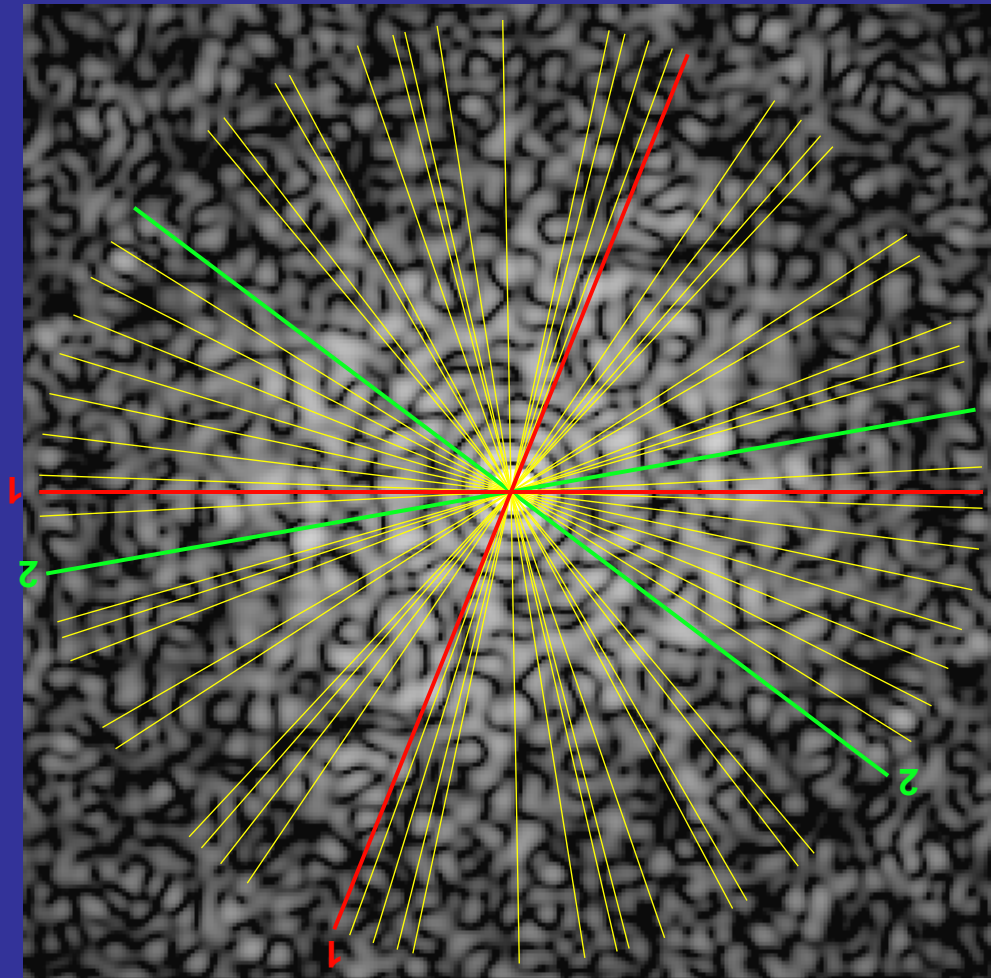
ω
↓
(80, 11, 180)

Orientation Determination by Common Lines



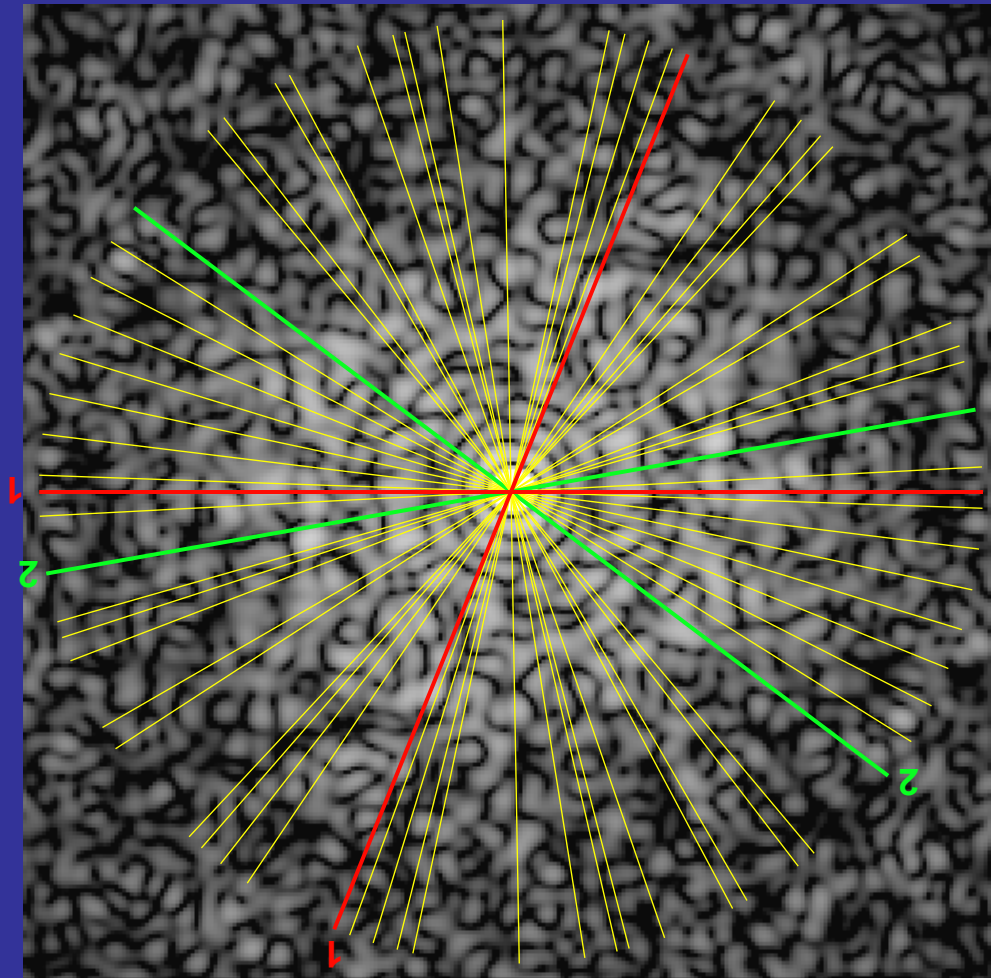
Metric: Identify ω that gives lowest phase residual

Orientation Determination by Common Lines



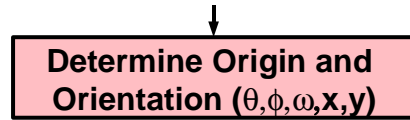
Repeat process for all possible (θ, ϕ, ω) combinations

Orientation Determination by Common Lines



> 250,000 combinations for 1° angular search intervals

Icosahedral Virus 3D Reconstruction Scheme



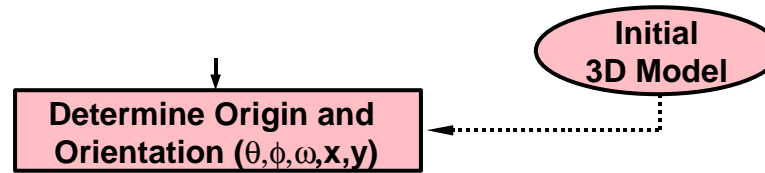
Common Lines

The (θ, ϕ, ω) that results in the lowest phase residual is selected as the best estimate for the particle view orientation

The 'common lines' procedure is similarly used to determine the particle phase origin (x, y)

Not to worry.....I'll spare you the details!!!

Icosahedral Virus 3D Reconstruction Scheme



Recall: two methods to determine $(\theta, \phi, \omega, x, y)$:

1. Common lines
2. Model-based (template) matching

Bulk of structures now solved this way

Details discussed in practical session

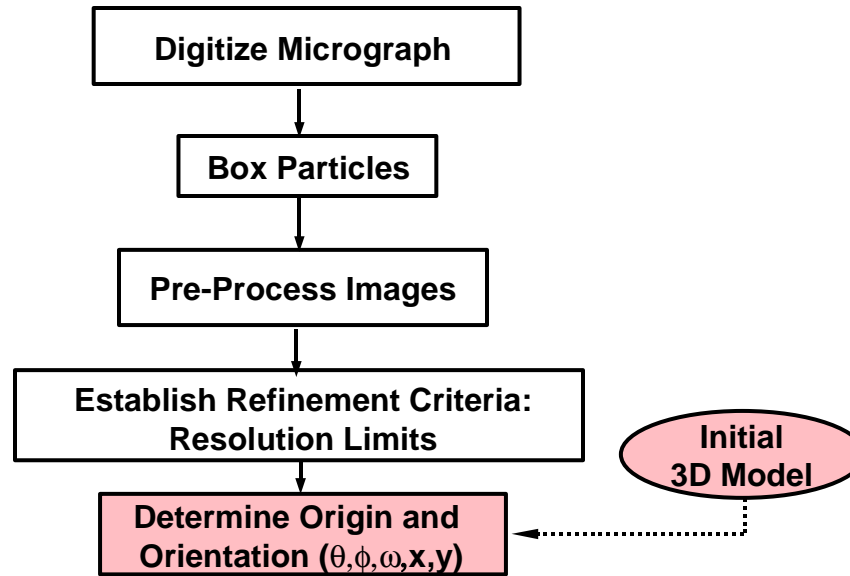
Icosahedral Virus 3D Reconstruction Scheme

Initial
3D Model

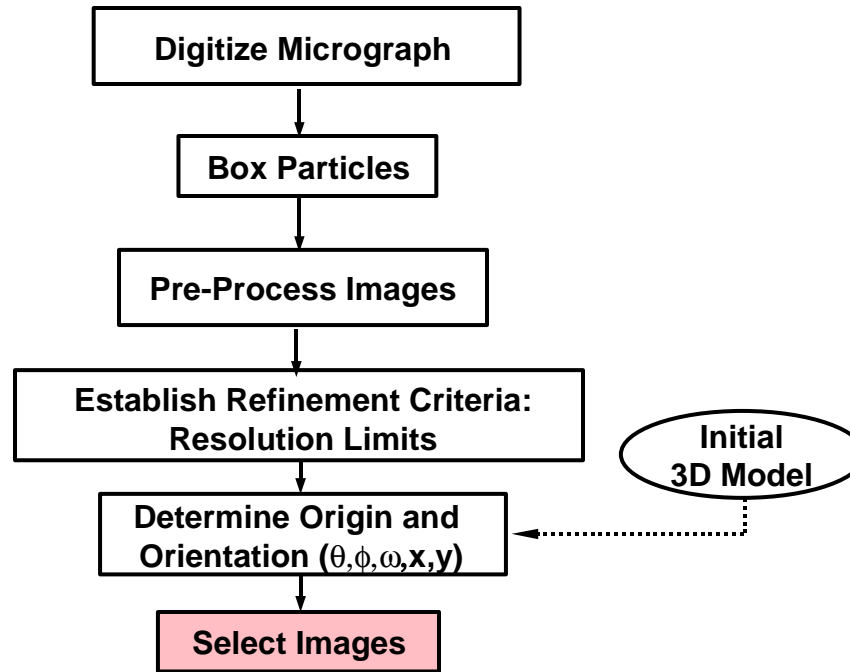
Determine Origin and
Orientation ($\theta, \phi, \omega, x, y$)

ID	θ	ϕ	ω	x	y				
1,	80,000,	-13,201,	313,594,	48,751,	48,849,	1,015,	0,492,	0,713,	0,557
2,	85,000,	23,088,	49,922,	49,387,	48,429,	1,010,	0,598,	0,725,	0,609
3,	77,000,	-5,132,	334,688,	49,051,	48,364,	1,005,	0,728,	0,749,	0,616
4,	81,000,	5,062,	15,469,	49,215,	49,138,	1,000,	0,718,	0,778,	0,656
5,	81,000,	0,000,	148,359,	49,681,	48,118,	1,005,	0,725,	0,712,	0,646
6,	79,000,	-2,037,	338,203,	49,320,	48,202,	1,005,	0,746,	0,738,	0,647
7,	83,000,	1,008,	175,781,	49,223,	48,802,	1,010,	0,687,	0,703,	0,620
8,	83,000,	-3,023,	32,344,	49,147,	48,901,	1,005,	0,658,	0,762,	0,574
9,	88,000,	1,001,	15,469,	49,843,	49,042,	1,005,	0,732,	0,796,	0,662
10,	75,000,	-7,247,	158,203,	47,869,	49,199,	1,000,	0,744,	0,784,	0,622
11,	87,000,	-7,010,	222,891,	48,247,	48,675,	1,000,	0,623,	0,765,	0,577
12,	86,000,	-1,002,	19,688,	49,127,	48,761,	0,995,	0,784,	0,783,	0,607
13,	80,000,	-5,077,	68,906,	48,997,	48,623,	1,000,	0,720,	0,780,	0,600
14,	84,000,	5,028,	279,141,	49,449,	48,400,	1,000,	0,707,	0,761,	0,662
15,	86,000,	9,022,	175,781,	49,635,	49,787,	1,000,	0,625,	0,760,	0,589
16,	87,000,	11,015,	2,813,	49,797,	49,182,	1,000,	0,567,	0,761,	0,609
17,	89,000,	30,005,	89,297,	49,754,	48,938,	1,000,	0,730,	0,785,	0,632
18,	85,000,	1,004,	170,156,	49,710,	49,029,	1,000,	0,767,	0,780,	0,642
19,	86,000,	0,000,	126,563,	49,944,	48,914,	1,000,	0,775,	0,808,	0,668
20,	89,000,	5,001,	290,391,	49,925,	48,704,	1,000,	0,699,	0,765,	0,642
21,	78,000,	7,156,	108,281,	49,310,	49,342,	1,000,	0,732,	0,792,	0,657
22,	81,000,	2,025,	355,078,	49,317,	48,172,	1,000,	0,729,	0,766,	0,632
23,	80,000,	-3,046,	180,000,	49,873,	48,313,	1,005,	0,756,	0,738,	0,578
24,	86,000,	-9,022,	351,563,	49,649,	48,755,	1,005,	0,608,	0,760,	0,573
25,	84,000,	10,055,	168,047,	49,792,	49,281,	1,000,	0,634,	0,755,	0,586
26,	84,000,	15,083,	5,625,	49,824,	48,378,	1,000,	0,548,	0,737,	0,621
27,	83,000,	4,030,	180,703,	48,852,	48,378,	0,995,	0,726,	0,770,	0,654

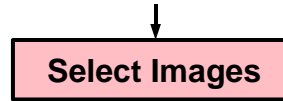
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Goal: weed out 'bad' particle images before computing 3D reconstruction

Icosahedral Virus 3D Reconstruction Scheme

Select Images

ID	θ	ϕ	ω	x	y				
1,	80,000,	-13,201,	313,594,	48,751,	48,849,	1,015,	0,492,	0,713,	0,557
2,	85,000,	23,088,	49,922,	49,387,	48,429,	1,010,	0,598,	0,725,	0,609
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6,	79,000,	-2,037,	338,203,	49,320,	48,202,	1,005,	0,746,	0,738,	0,647
7,	83,000,	1,008,	175,781,	49,223,	48,802,	1,010,	0,687,	0,703,	0,620
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9,	88,000,	1,001,	15,469,	49,843,	49,042,	1,005,	0,732,	0,796,	0,662
10,	75,000,	-7,247,	158,203,	47,869,	49,199,	1,000,	0,744,	0,784,	0,622
11,	87,000,	-7,010,	222,891,	48,247,	48,675,	1,000,	0,623,	0,765,	0,577
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13,	80,000,	-5,077,	68,906,	48,997,	48,623,	1,000,	0,720,	0,780,	0,600
14,	84,000,	5,028,	279,141,	49,449,	48,400,	1,000,	0,707,	0,761,	0,662
15,	86,000,	9,022,	175,781,	49,635,	49,787,	1,000,	0,625,	0,760,	0,589
16,	87,000,	11,015,	2,813,	49,797,	49,182,	1,000,	0,567,	0,761,	0,609
17,	89,000,	30,005,	89,297,	49,754,	48,938,	1,000,	0,730,	0,785,	0,632
18,	85,000,	1,004,	170,156,	49,710,	49,029,	1,000,	0,767,	0,780,	0,642
19,	86,000,	0,000,	126,563,	49,944,	48,914,	1,000,	0,775,	0,808,	0,668
20,	89,000,	5,001,	290,391,	49,925,	48,704,	1,000,	0,699,	0,765,	0,642
21,	78,000,	7,156,	108,281,	49,310,	49,342,	1,000,	0,732,	0,792,	0,657
22,	81,000,	2,025,	355,078,	49,317,	48,172,	1,000,	0,729,	0,766,	0,632
23,	80,000,	-3,046,	180,000,	49,873,	48,313,	1,005,	0,756,	0,738,	0,578
24,	86,000,	-9,022,	351,563,	49,649,	48,755,	1,005,	0,608,	0,760,	0,573
25,	84,000,	10,055,	168,047,	49,792,	49,281,	1,000,	0,634,	0,755,	0,586
26,	84,000,	15,083,	5,625,	49,824,	48,378,	1,000,	0,548,	0,737,	0,621
27,	83,000,	4,030,	180,703,	48,852,	48,378,	0,995,	0,726,	0,770,	0,654

--More-- (46%)

Icosahedral Virus 3D Reconstruction Scheme

Select Images

PRJ CC

PFT CC

CMP CC

1,	80,000,	-13,201,	313,594,	48,751,	48,849,	1,015,	0,492,	0,713,	0,557
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7,	83,000,	1,008,	175,781,	49,223,	48,802,	1,010,	0,687,	0,703,	0,620
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10,	75,000,	-7,247,	158,203,	47,869,	49,199,	1,000,	0,744,	0,784,	0,622
11,	87,000,	-7,010,	222,891,	48,247,	48,675,	1,000,	0,623,	0,765,	0,577
12,	86,000,	-1,002,	19,688,	49,127,	48,761,	0,995,	0,784,	0,783,	0,607
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14,	84,000,	5,028,	279,141,	49,449,	48,400,	1,000,	0,707,	0,761,	0,662
15,	86,000,	9,022,	175,781,	49,635,	49,787,	1,000,	0,625,	0,760,	0,589
16,	87,000,	11,015,	2,813,	49,797,	49,182,	1,000,	0,567,	0,761,	0,609
17,	89,000,	30,005,	89,297,	49,754,	48,938,	1,000,	0,730,	0,785,	0,632
18,	85,000,	1,004,	170,156,	49,710,	49,029,	1,000,	0,767,	0,780,	0,642
19,	86,000,	0,000,	126,563,	49,944,	48,914,	1,000,	0,775,	0,808,	0,668
20,	89,000,	5,001,	290,391,	49,925,	48,704,	1,000,	0,699,	0,765,	0,642
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23,	80,000,	-3,046,	180,000,	49,873,	48,313,	1,005,	0,756,	0,738,	0,578
24,	86,000,	-9,022,	351,563,	49,649,	48,755,	1,005,	0,608,	0,760,	0,573
25,	84,000,	10,055,	168,047,	49,792,	49,281,	1,000,	0,634,	0,755,	0,586
26,	84,000,	15,083,	5,625,	49,824,	48,378,	1,000,	0,548,	0,737,	0,621
27,	83,000,	4,030,	180,703,	48,852,	48,378,	0,995,	0,726,	0,770,	0,654

0.784

0.567 ?

0.775

0.548 ?

Icosahedral Virus 3D Reconstruction Scheme

Select Images

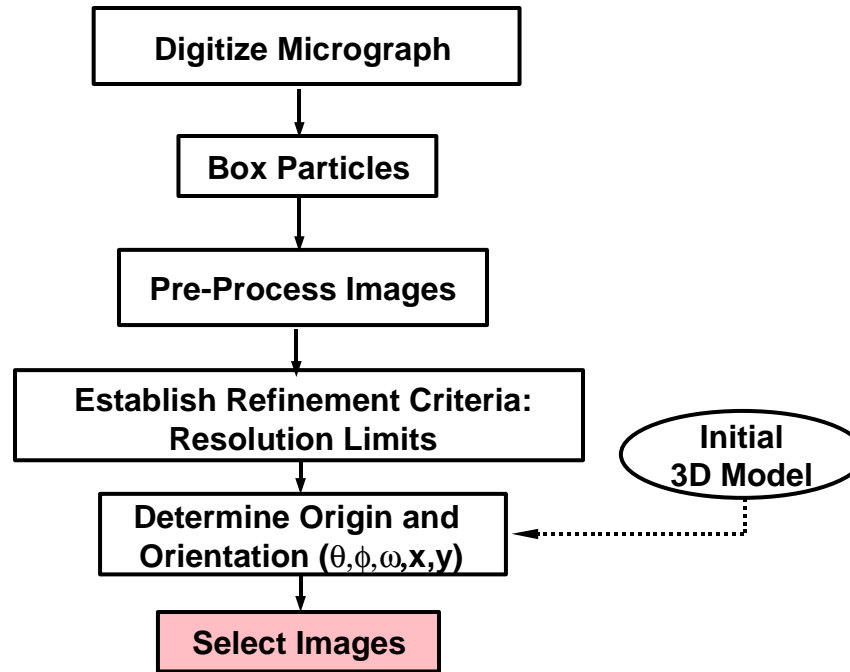
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19, 86.000, 0.000, 126.563, 49.944, 48.914, 1.000, 0.775, 0.808, 0.668
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22, 81.000, 2.025, 355.078, 49.317, 48.172, 1.000, 0.729, 0.766, 0.632
23, 80.000, -3.046, 180.000, 49.873, 48.313, 1.005, 0.756, 0.738, 0.578
24, 86.000, -9.022, 351.563, 49.649, 48.755, 1.005, 0.608, 0.760, 0.573
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26, 84.000, 15.083, 5.625, 49.824, 48.378, 1.000, 0.548, 0.737, 0.621
27, 83.000, 4.030, 180.703, 48.852, 48.378, 0.995, 0.726, 0.770, 0.654
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 55, 88.000, 21.013, 162.422, 48.765, 49.285, 0.995, 0.557, 0.749, 0.580
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 57, 80.000, -2.031, 213.047, 47.831, 48.686, 0.995, 0.722, 0.744, 0.590
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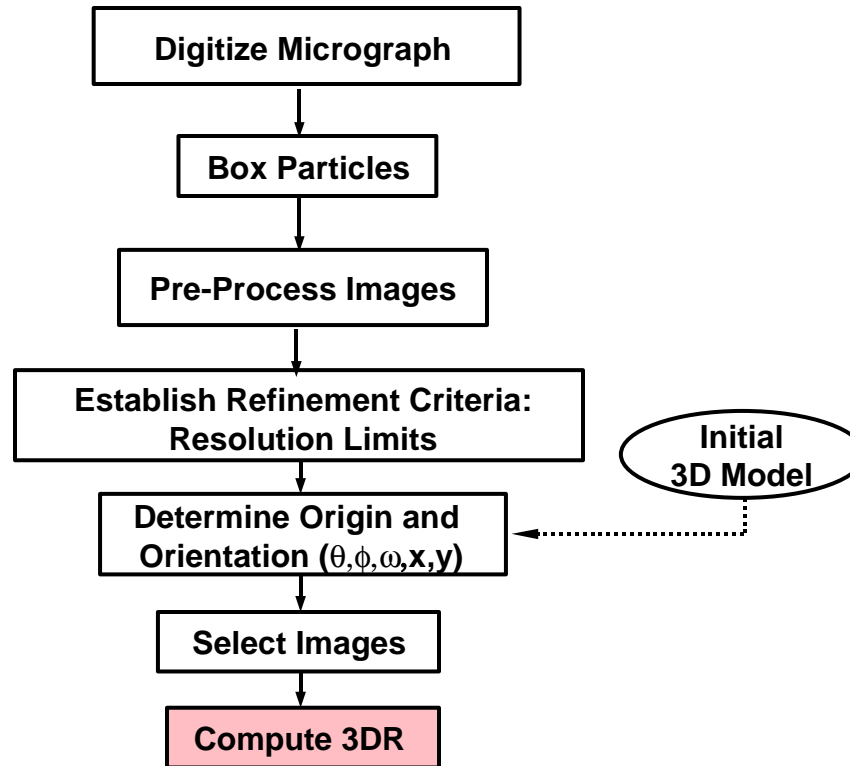
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Compute average MAG factor and coefficients, N = 120
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                          Average Std. Dev.
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MAG Factor                1.000    0.005
PFT Coefficient            0.679    0.075
PRJ Coefficient            0.756    0.023
CMP Coefficient            0.609    0.038
PFT finished.
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gandalf #
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PFT Coefficient 0.679 ± 0.075

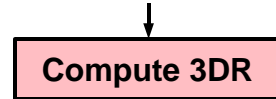
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

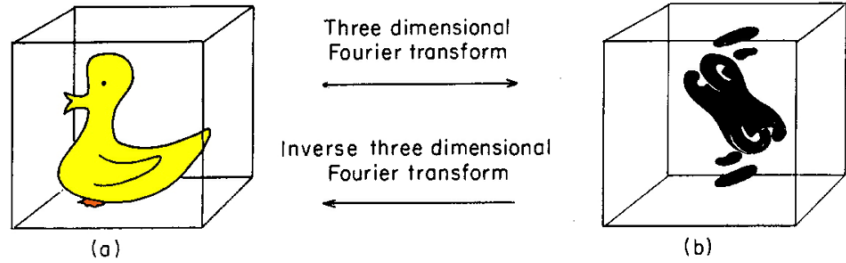


Goal: combine only “good” particle images
to compute a 3D density map

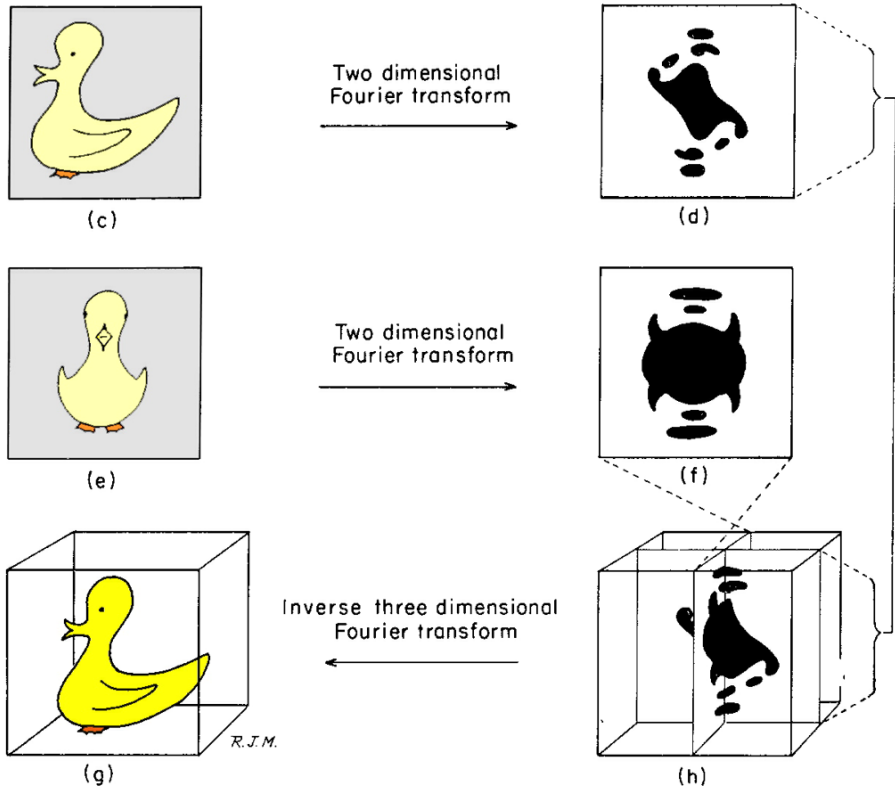
Icosahedral Virus 3D Reconstruction Scheme

↓
Compute 3DR

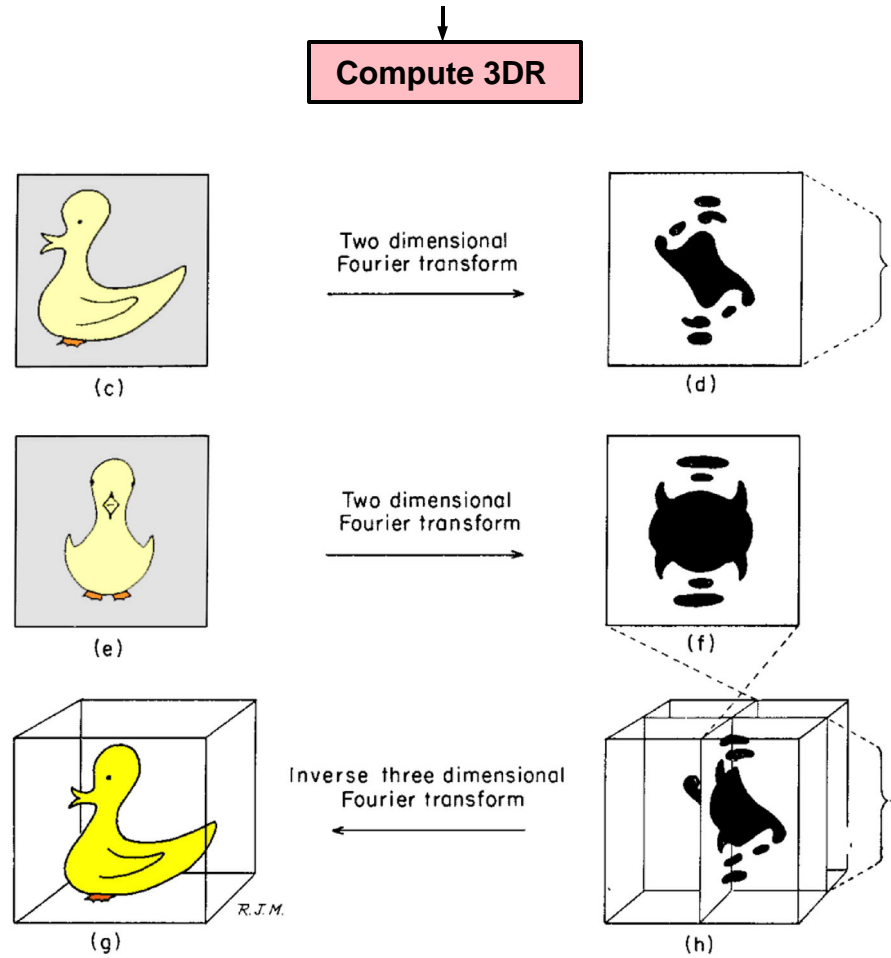
In theory



In practice



Icosahedral Virus 3D Reconstruction Scheme



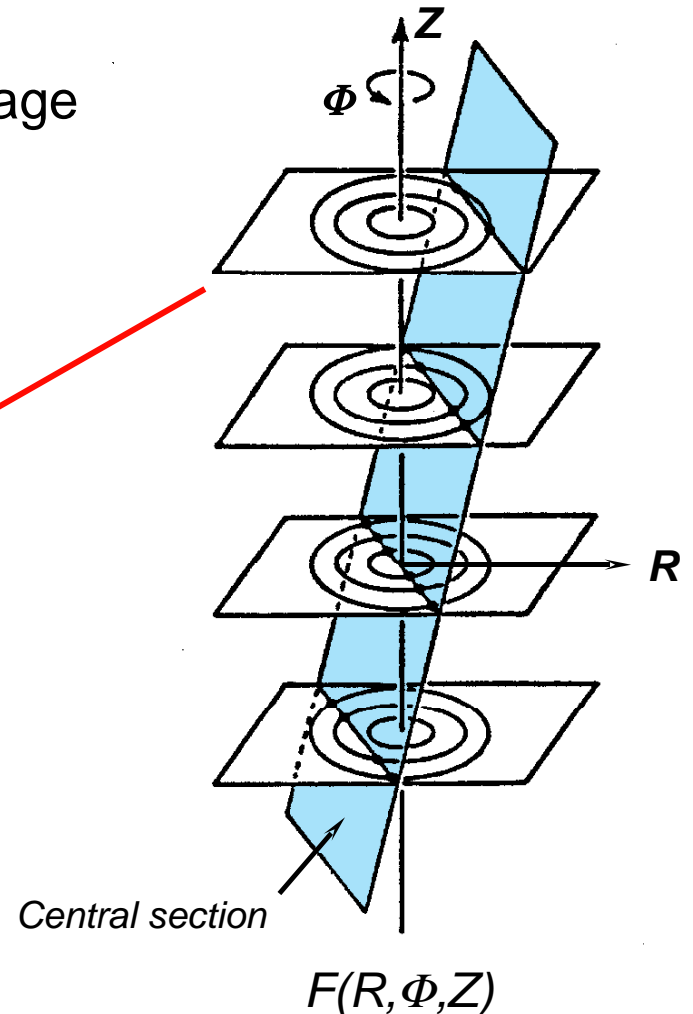
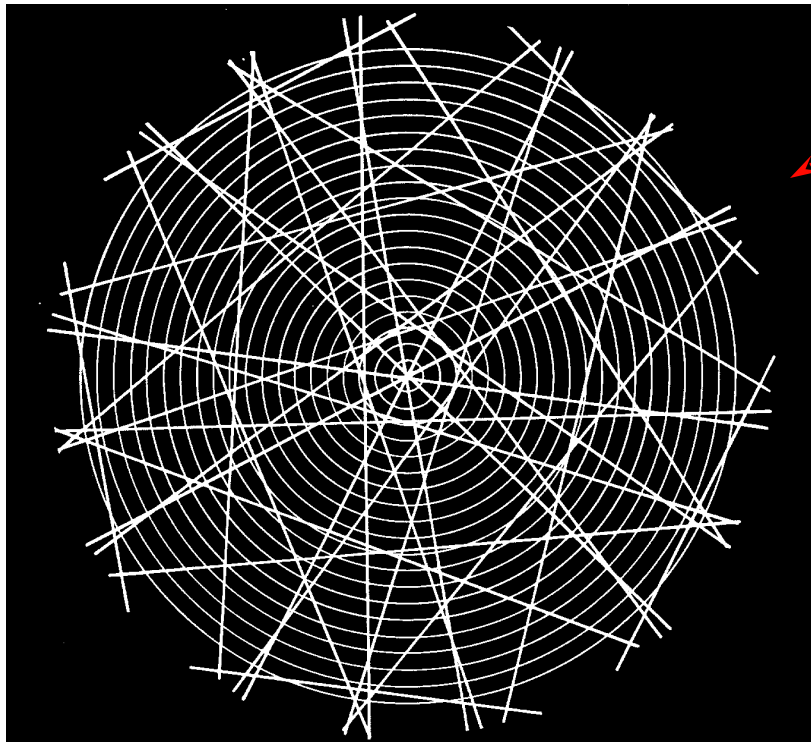
Overall scheme: $\rho \leftarrow g \leftarrow G \leftarrow F$

Icosahedral Virus 3D Reconstruction Scheme

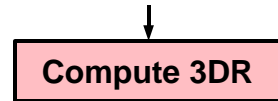
↓
Compute 3DR

Steps:

1. Compute 2D FFT of each particle image
2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform



Icosahedral Virus 3D Reconstruction Scheme

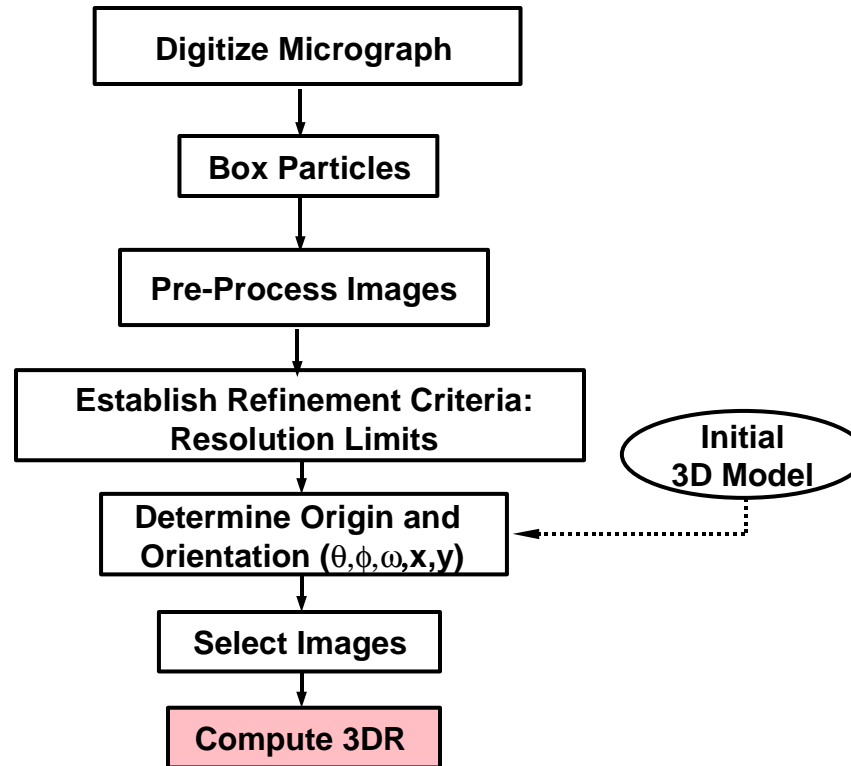


$$\rho \leftarrow g \leftarrow G \leftarrow F$$

Steps:

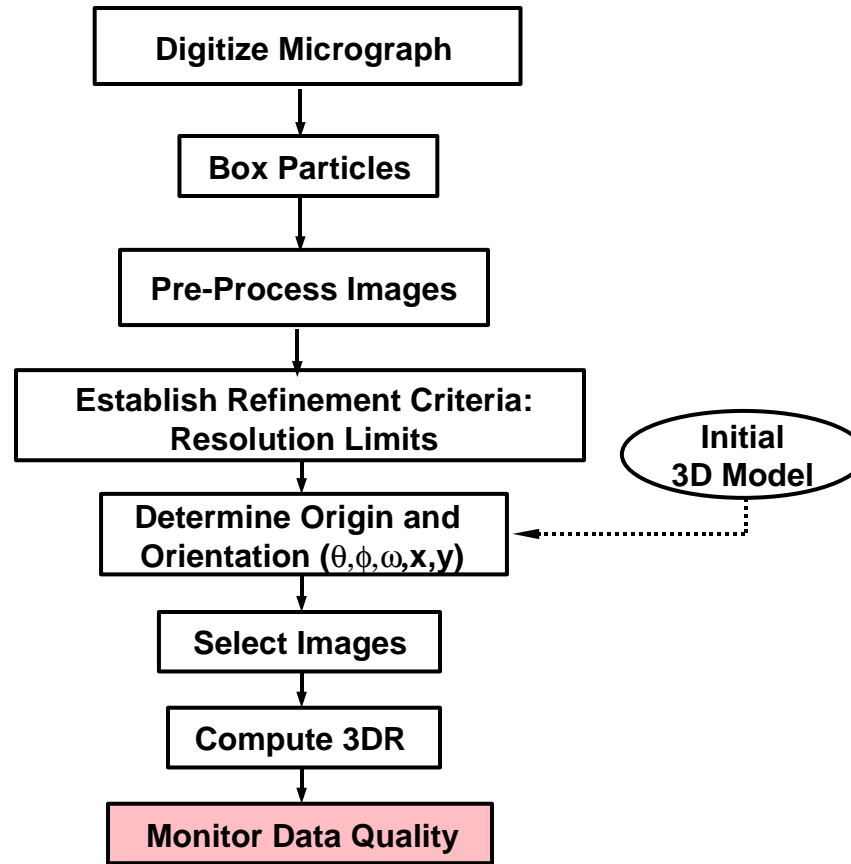
1. Compute 2D FFT of each particle image
2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform
3. Compute G_n 's on each annulus $G = (B B)^{-1} B F$
4. Compute g_n 's from G_n 's (Fourier-Bessel transform)
5. Compute polar density map ($\rho(r, \phi, z)$) from g_n 's
6. Convert from polar to Cartesian map ($\rho(r, \phi, z) \rightarrow \rho(x, y, z)$)

Icosahedral Virus 3D Reconstruction Scheme



Option: correct for CTF effects in particle FFTs before FFTs are merged to form the 3D FFT

Icosahedral Virus 3D Reconstruction Scheme



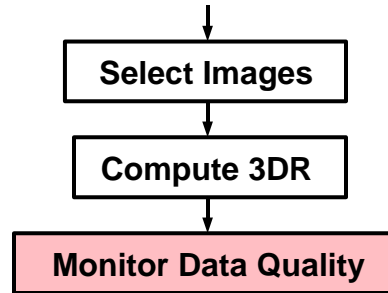
Icosahedral Virus 3D Reconstruction Scheme



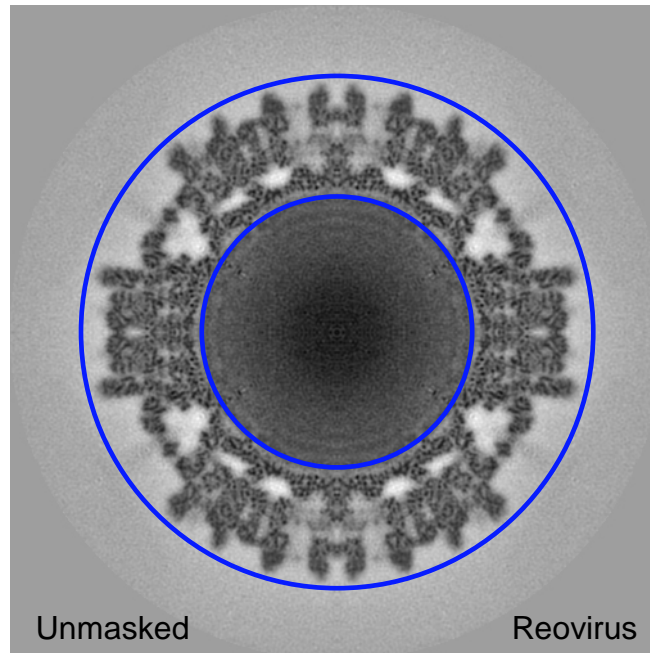
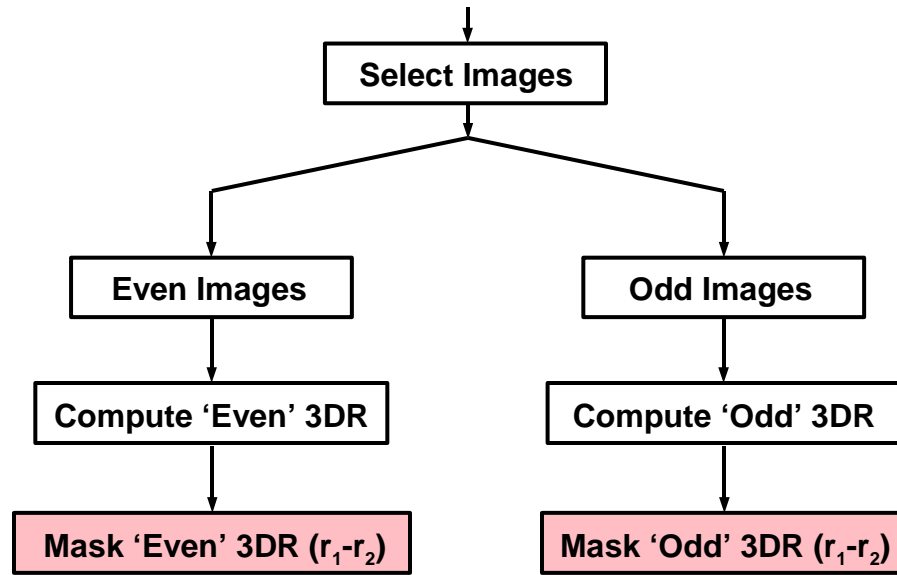
Monitor Data Quality

Goal: assess resolution of 3D density map
to determine what to do next

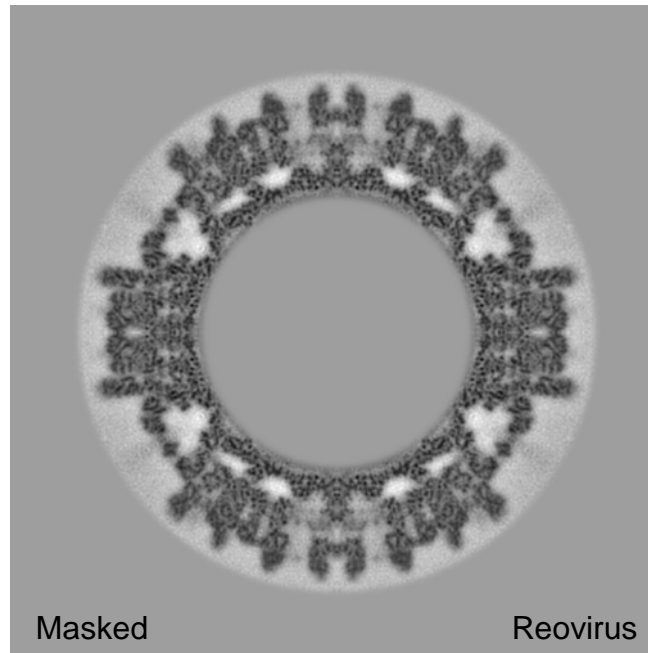
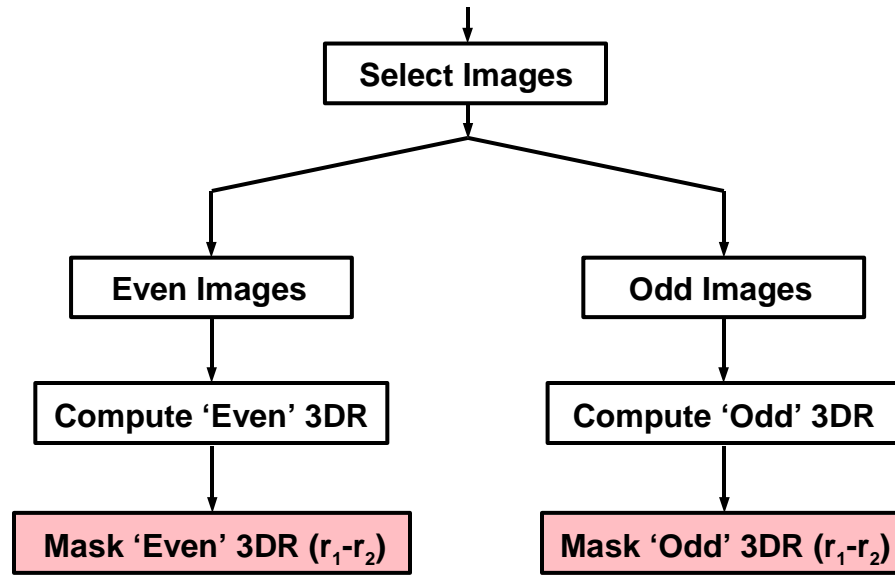
Icosahedral Virus 3D Reconstruction Scheme



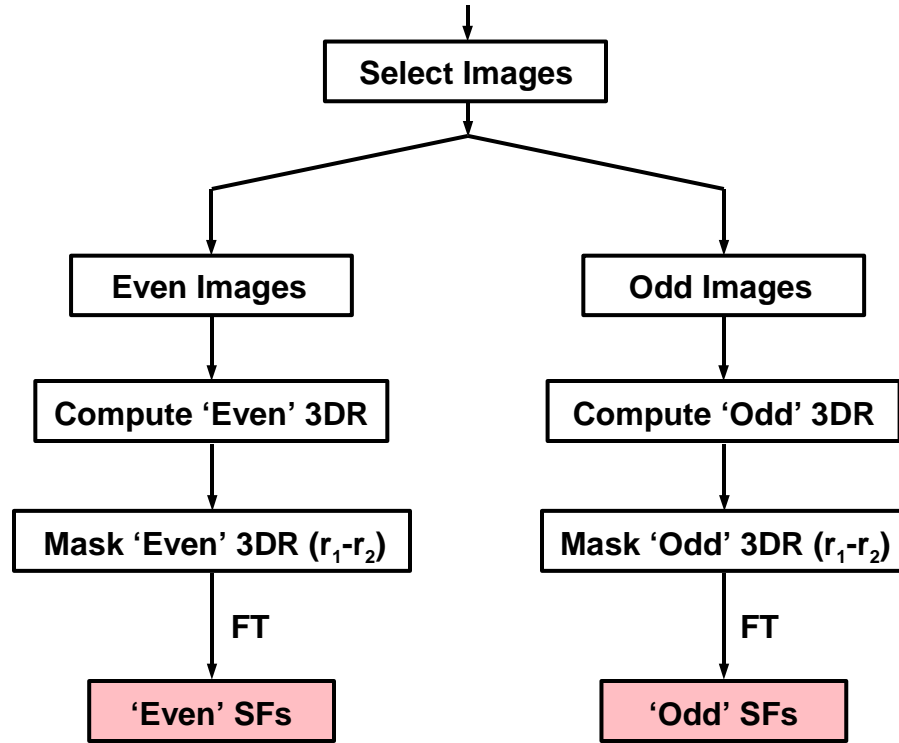
Icosahedral Virus 3D Reconstruction Scheme



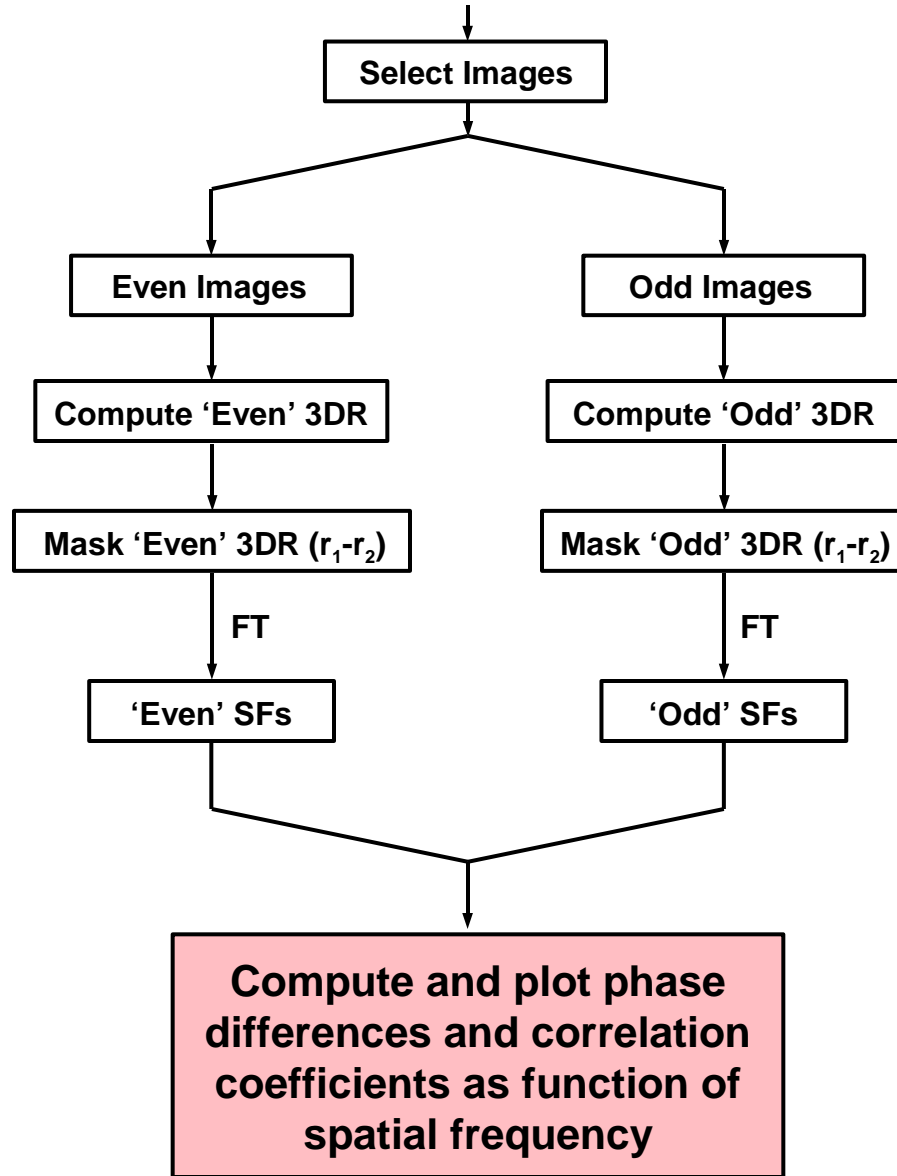
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

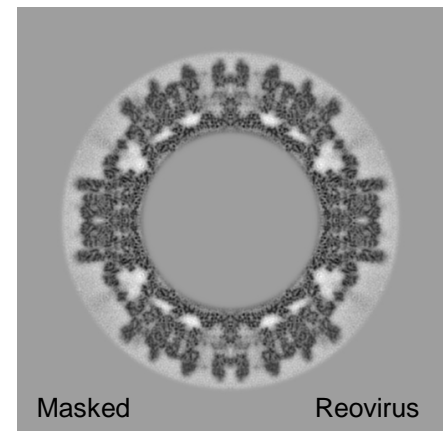
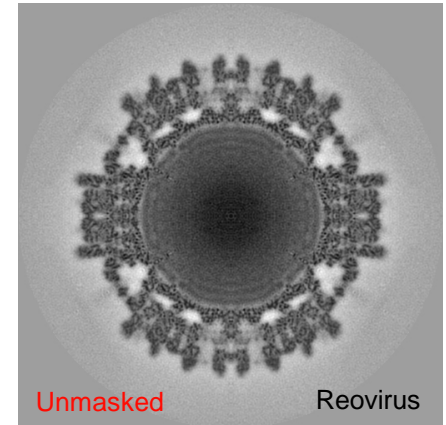
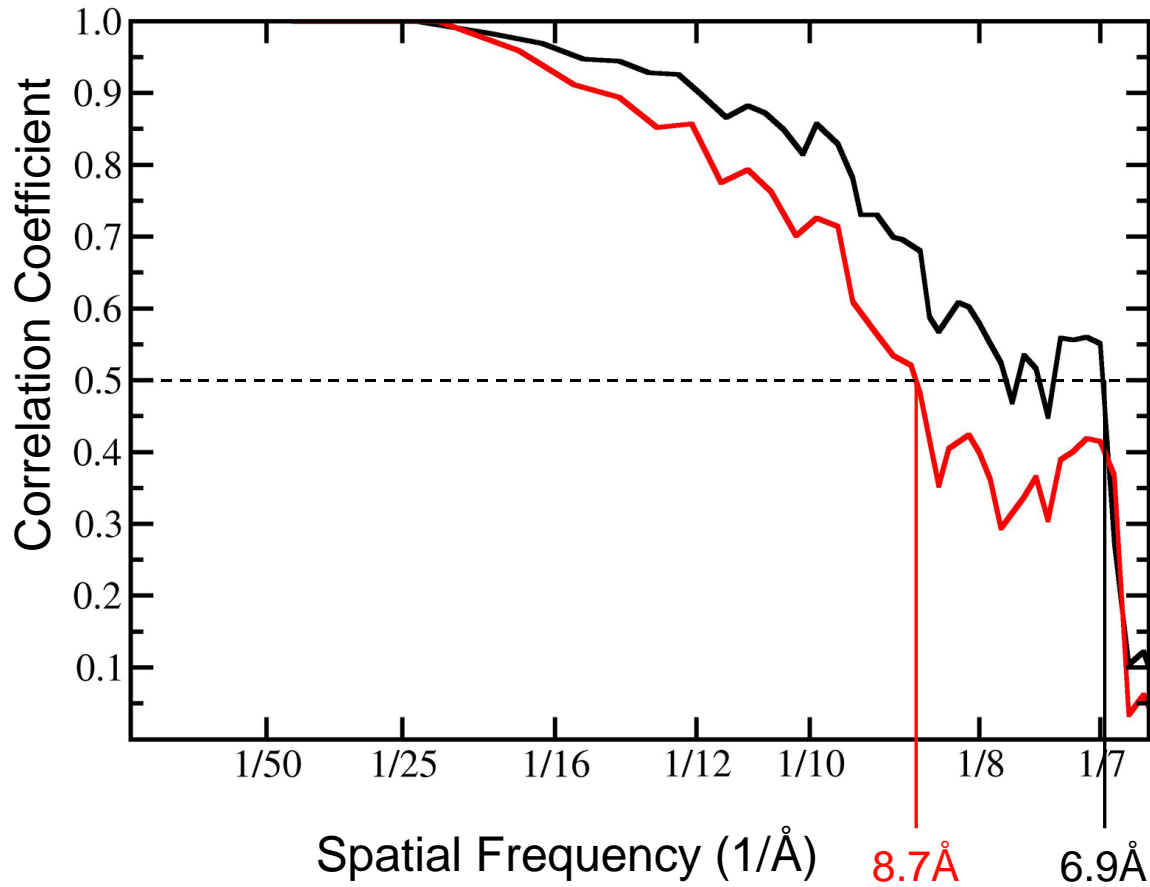


Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

↓
Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme

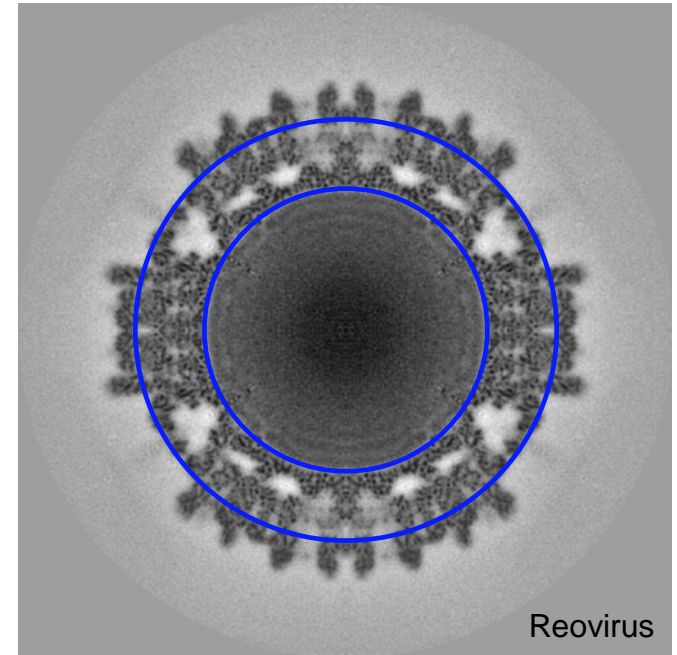
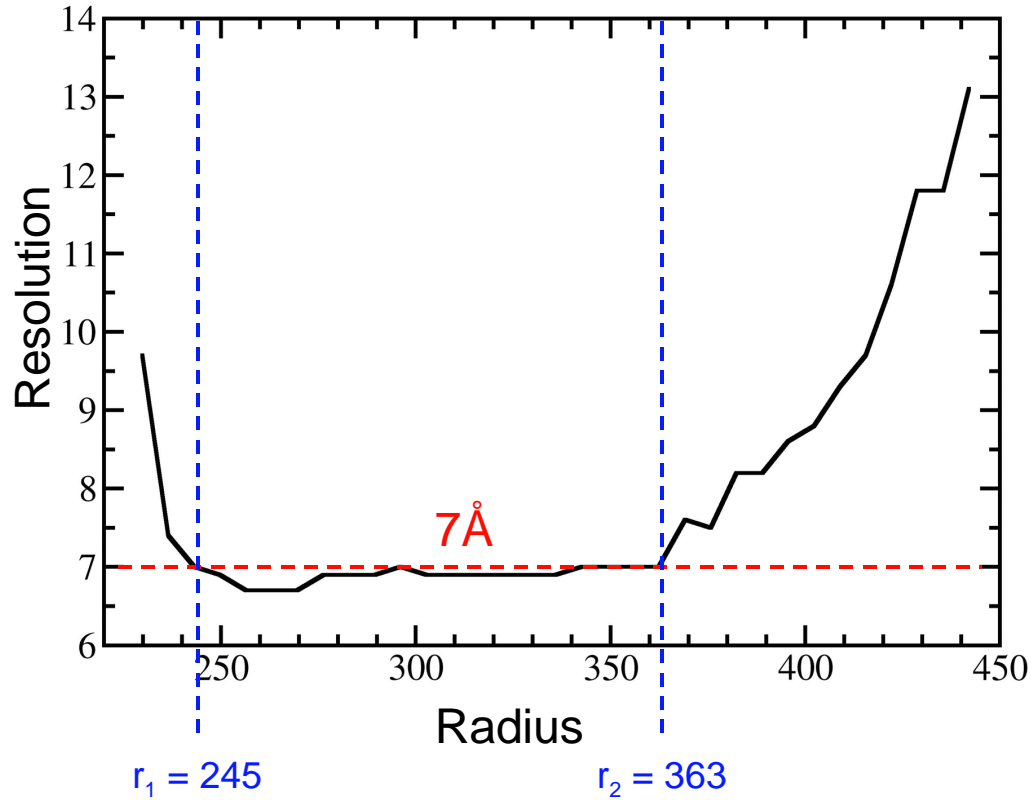


Monitor Data Quality

Note: quality of 3D density map is not identical everywhere in the map

Icosahedral Virus 3D Reconstruction Scheme

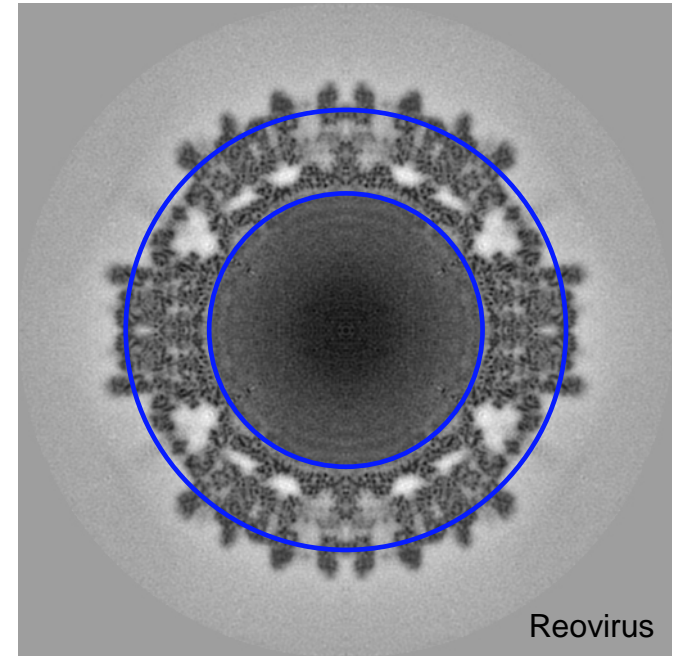
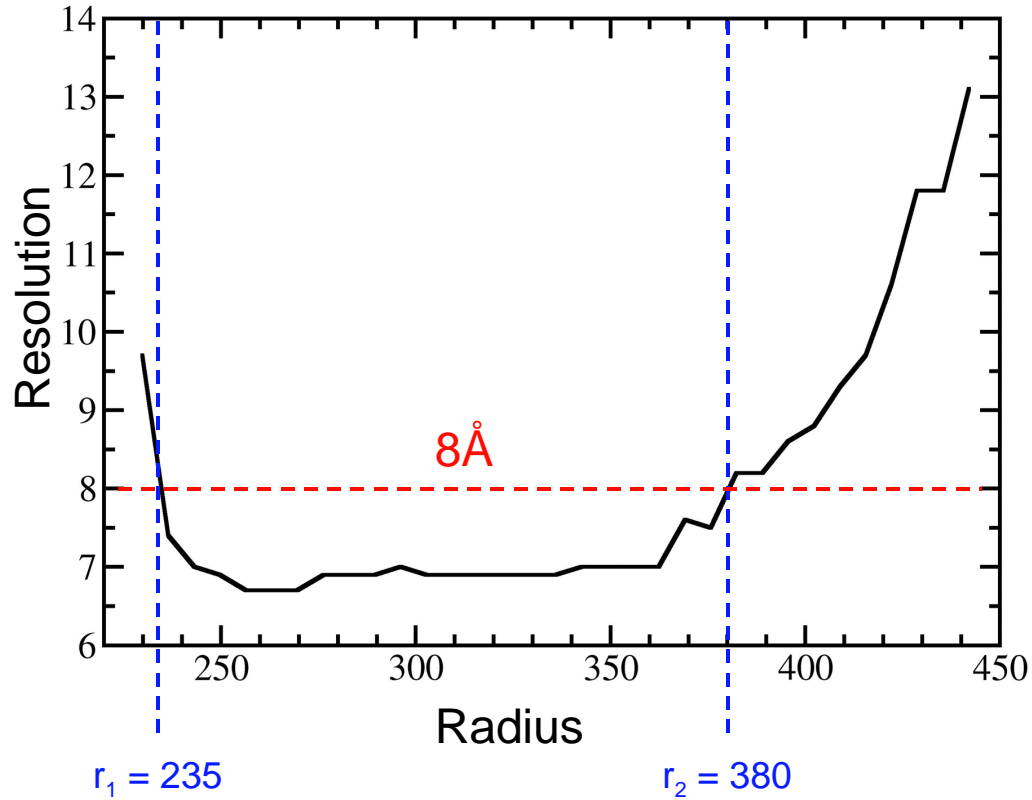
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Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme

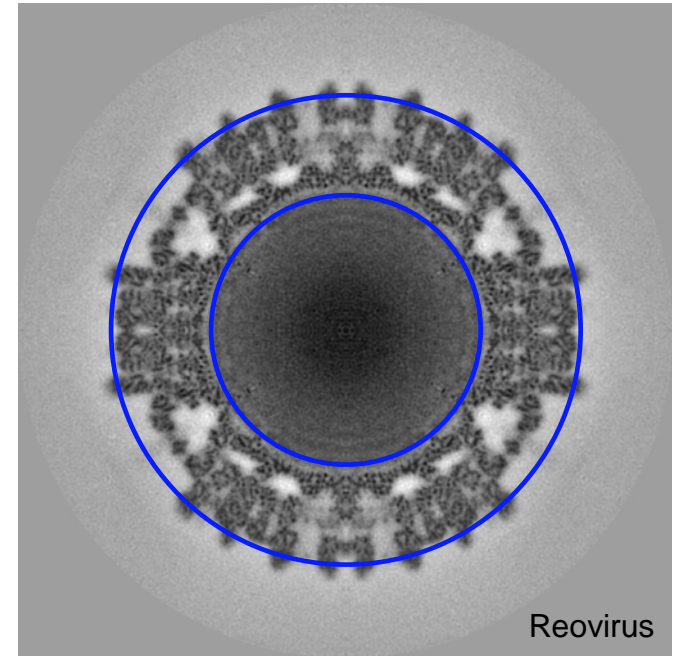
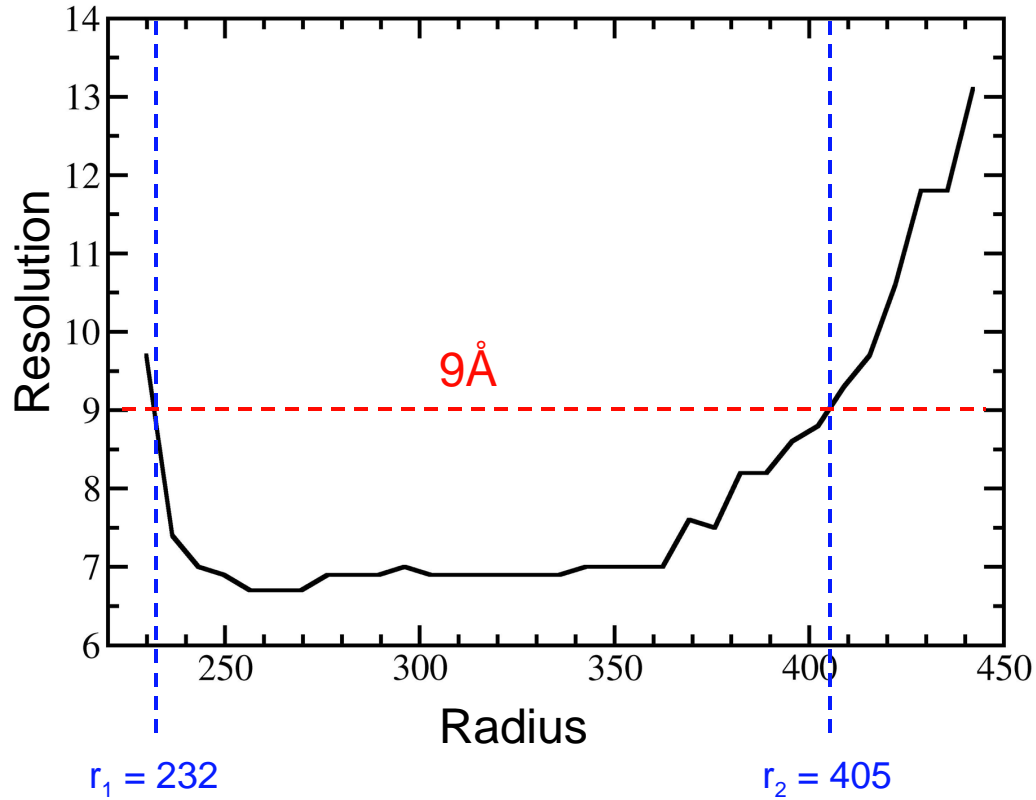


Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme

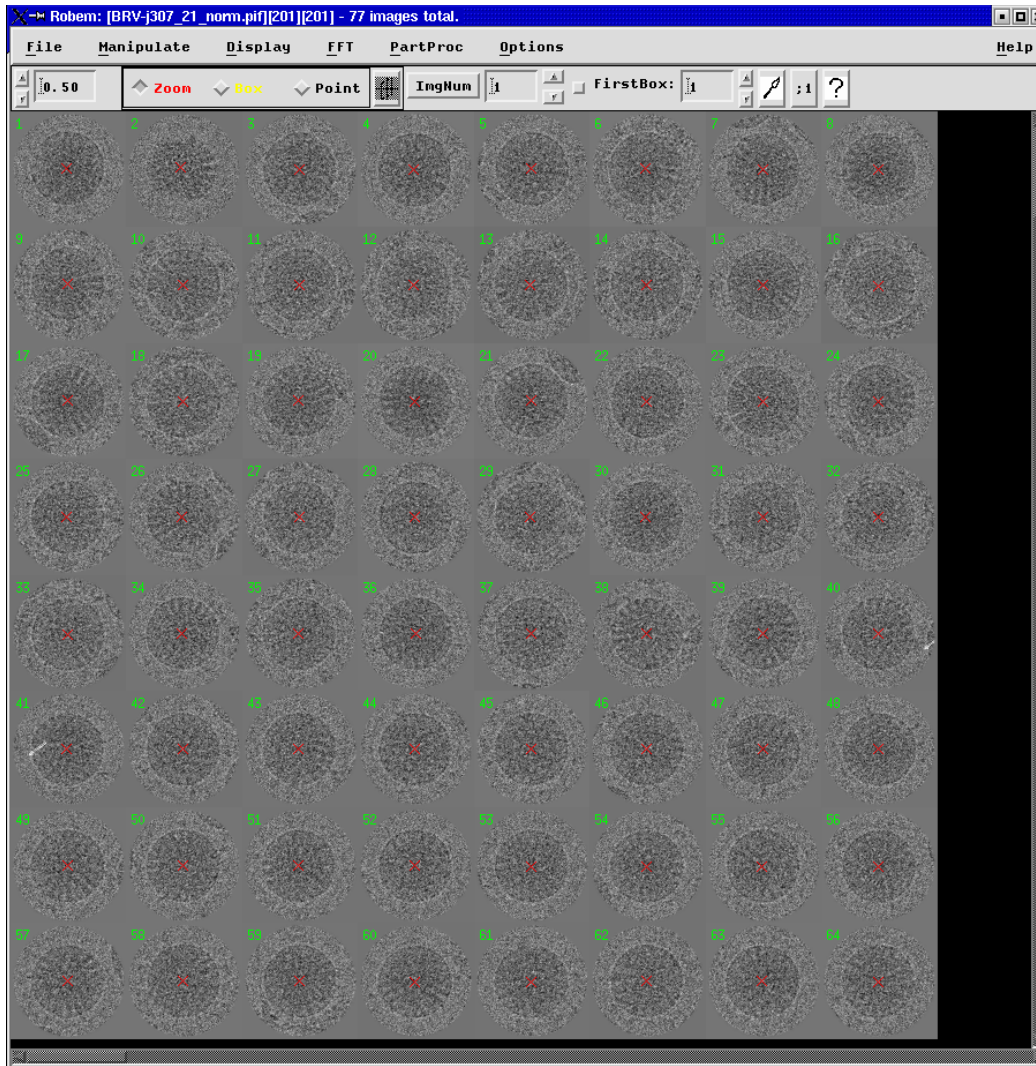
Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme



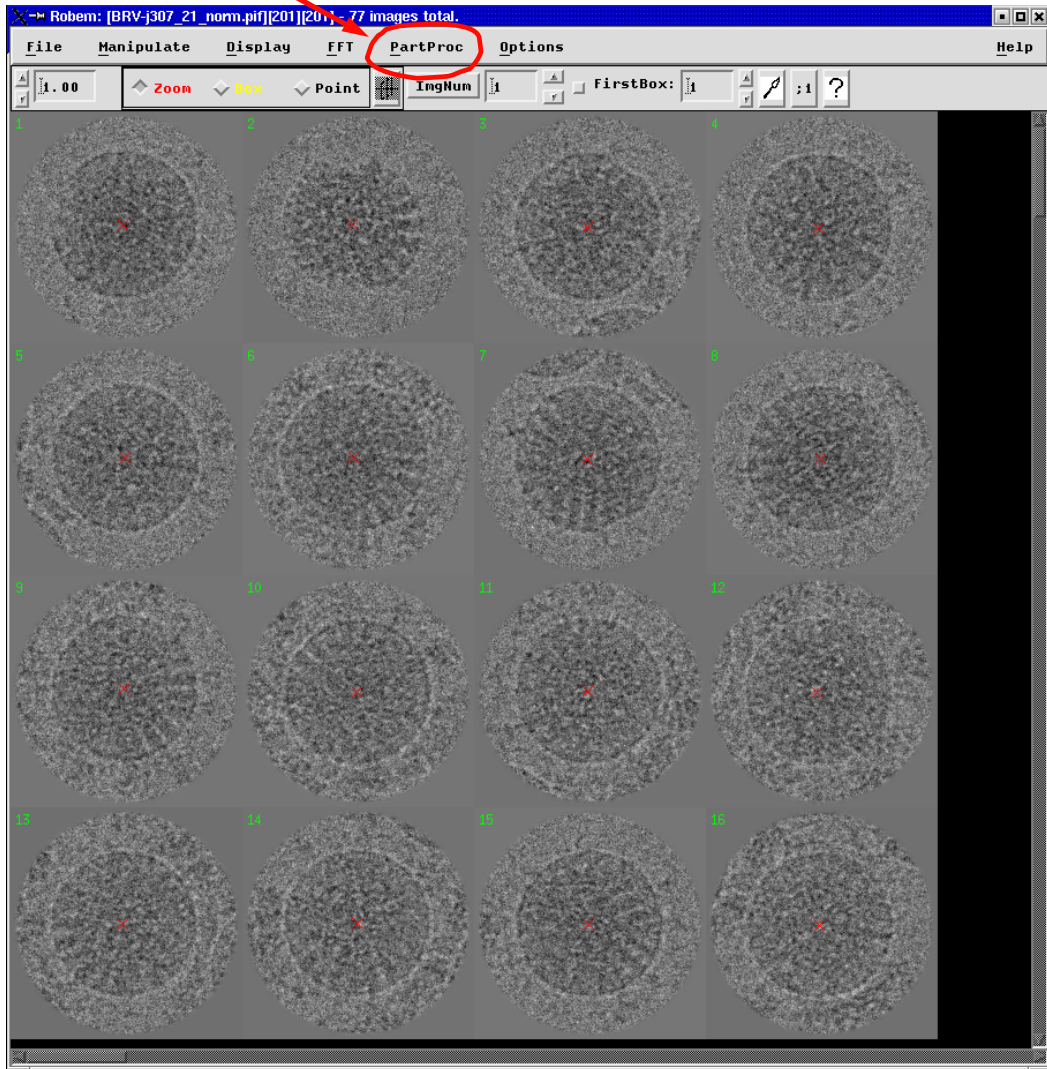
Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality

The screenshot displays the PartProc software interface. The main window shows a grid of particle images. The central control panel includes the following parameters and buttons:

- Parameter File: `/bio/gandalf/tsb/NRAHM/B`
- Map file: `/bio/gandalf/tsb/NRAHM/B`
- Scan File:
- Display centers
- Pick new particle center
- MAP Projections Projection Contour
- PixSize: `6.0000` Units: `1`
- Voltage: `100000.0`
- Ampl Factor: `0.0700`
- Δf major: `1.6910`
- Δf minor: `1.6910`
- Ang major: `0.0000`
- Cs Coef: `2.00`
- Buttons: Compute Circular Avg, Add delta omega, Manipulate Particle Params, Add delta X,Y to centers, Rebox ALL Particles, Rebox THIS Particle, Write New Parm File

The bottom-right window shows a triangular grid of red 'x' markers representing particle positions. The axes are labeled with values -31, 0, and 31. A red arrow points to a specific 'x' marker in the grid.

Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

Robem: [BRV-j307_21_norm.pif][201][201] - 77 images total.

File Manipulate Display FFT PartProc Options Help

1.00 Zoom Box Point IngNum 44 FirstBox: 1

Partproc

Parameter File: /bio/gandalf/tsb/NRAHM/B Browse
Map File: /bio/gandalf/tsb/NRAHM/B Browse
Scan File: Browse

Display centers
Pick new particle center
MAP Projections Projection Contour

PixSize 6.0000 Units 1
Voltage 100000.0
Amp Factor 0.0700
 Δf major 1.6910
 Δf minor 1.6910
Ang major 0.0000
Cs Coef 2.00

Compute Circular Avg
Add delta omega Manipulate Particle Params
Add delta X,Y to centers
Rebox ALL Particles
Rebox THIS Particle
Write New Parn File

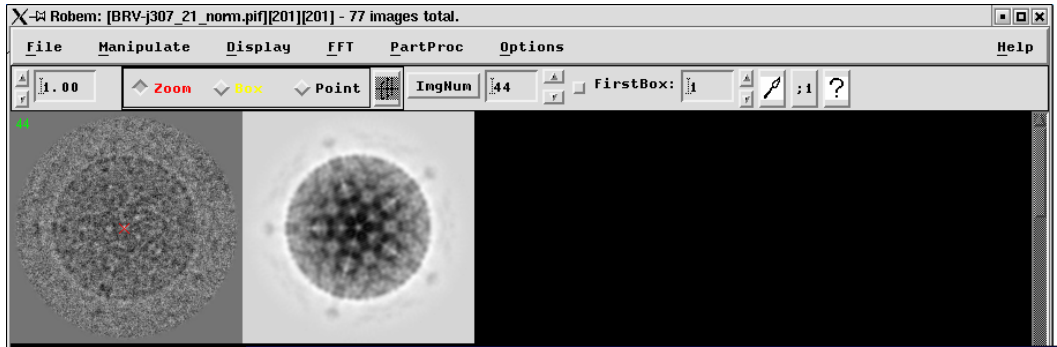
Particle[44] Theta [90.000] Phi[31.000] Omega[55.141]
X[98.694] Y[99.617] Mag[1.000]
pft_cc[0.536] prj_cc[0.959] cmp_cc[0.567]
deltaX[-1.306] deltaY[-0.383] distance[1.361]
state[ON] rank[44]

90 31

Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality



Partproc

Parameter File:

Map File:

Scan File:

Display centers

Pick new particle center

MAP Projections Projection Contour

PixSize	<input type="text" value="6.0000"/>	Units	<input type="text" value="1"/>
Voltage	<input type="text" value="100000.0"/>		
Amp factor	<input type="text" value="0.0700"/>		
Δf major	<input type="text" value="1.6910"/>		
Δf minor	<input type="text" value="1.6910"/>		
Ang major	<input type="text" value="0.0000"/>		
Cs Coef	<input type="text" value="2.00"/>		

Particle[44	Theta [90.000]	Phi[31.000]	Omega[55.141]
X[98.694]	Y[99.617]	Mag[1.000]		
pft_cc[0.536]	prj_cc[0.959]	cmp_cc[0.967]		
deltaK[-1.306]	deltaY[-0.383]	distance[1.361]		
state[ON]	rank[44]					

Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

The screenshot displays a software interface for 3D reconstruction, divided into two main windows: the main application window and the 'Partproc' (Particle Processor) window.

Main Application Window:

- Title bar: Robem: [BRV-j307_21_norm.tif][201][201] - 77 images total.
- Menu bar: File, Manipulate, Display, FFT, PartProc, Options, Help.
- Toolbar: Includes buttons for Zoom, Box, Point, and a numeric input field for 'IngNum' set to 26. A 'FirstBox' field is also present.
- Viewports: Two circular grayscale images showing particle distributions. The left image is a noisy, low-resolution view, while the right image is a clearer, higher-resolution view of a circular particle arrangement.

Partproc Window:

- Parameter File: `/bio/gandalf/tsb/NRAHM/B`
- Map File: `/bio/gandalf/tsb/NRAHM/B`
- Scan File:
- Buttons: Browse, Batch Job, Calc, Symmetry, Display.
- Display centers: (unchecked)
- Pick new particle center: (unchecked)
- MAP Projections: (unchecked)
- Projection Contour:
- Parameters table:

Particle[26	Theta [76.644]	Phi [11.927]	Omega [92.277]
X [97.863]	Y [99.927]	Mag [1.000]		
pft_cc [0.542]	prj_cc [0.605]	cmp_cc [0.578]		
deltaK [-2.137]	deltaY [-0.073]	distance [2.138]		
state [ON]	rank [26]				
- Input fields:

PixSize	6.0000	Units	1
Voltage	100000.0		
Amp factor	0.0700		
Δf major	1.6910		
Δf minor	1.6910		
Ang major	0.0000		
Cs Coef	2.00		
- Buttons: Compute Circular Avg, Add delta omega, Manipulate Particle Params, Add delta X,Y to centers, Rebox ALL Particles, Rebox THIS Particle, Write New Parm File.
- Plot: A triangular plot with axes ranging from -31 to 31. It contains a grid of red 'x' marks representing particles. A blue dashed triangle is overlaid on the plot. A red arrow points to a specific particle at coordinates (76.7, 12.0). Two icosahedral virus models are shown in the top right corner of the plot area: one in blue and one in red.

Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

The screenshot shows a software interface for 3D reconstruction. The main window displays two images: a noisy 2D projection on the left and a clearer 2D projection on the right. The 'Partproc' window is open, showing various parameters and a plot of particle positions.

Partproc Parameters:

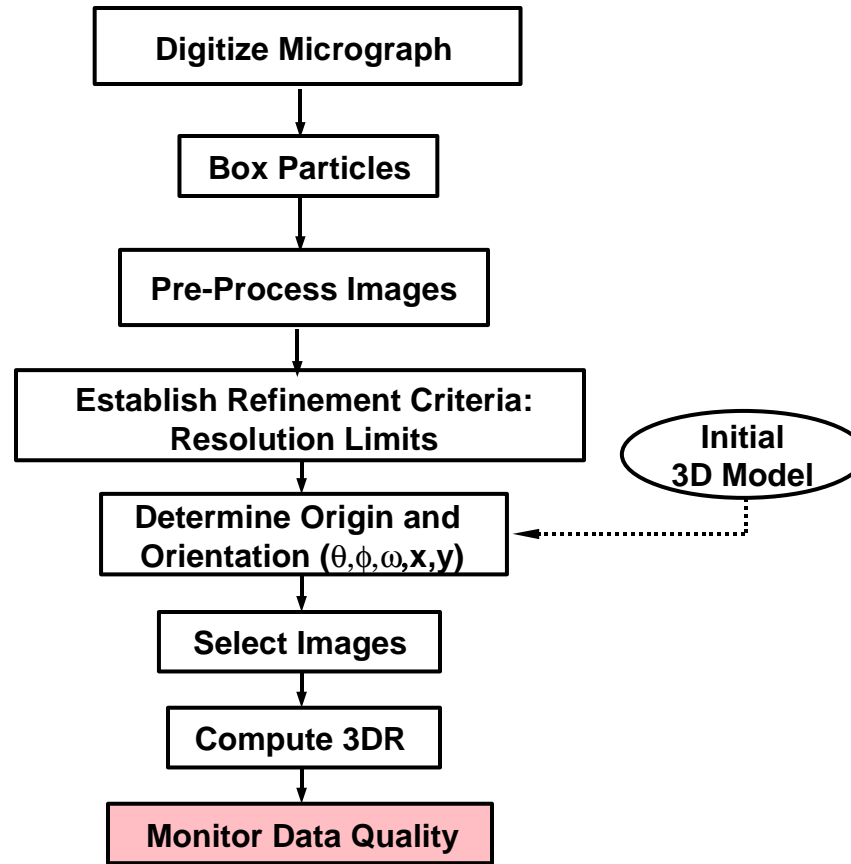
- Parameter File: `/bio/gandalf/tsb/NRAHM/B`
- Map File: `/bio/gandalf/tsb/NRAHM/B`
- Scan File:
- Display centers:
- Pick new particle center:
- MAP Projections: Projection Contour:
- PixSize: `6.0000` Units: `1`
- Voltage: `100000.0`
- Amp Factor: `0.0700`
- Δf major: `1.6910`
- Δf minor: `1.6910`
- Ang major: `0.0000`
- Cs Coef: `2.00`

Particle Parameters Table:

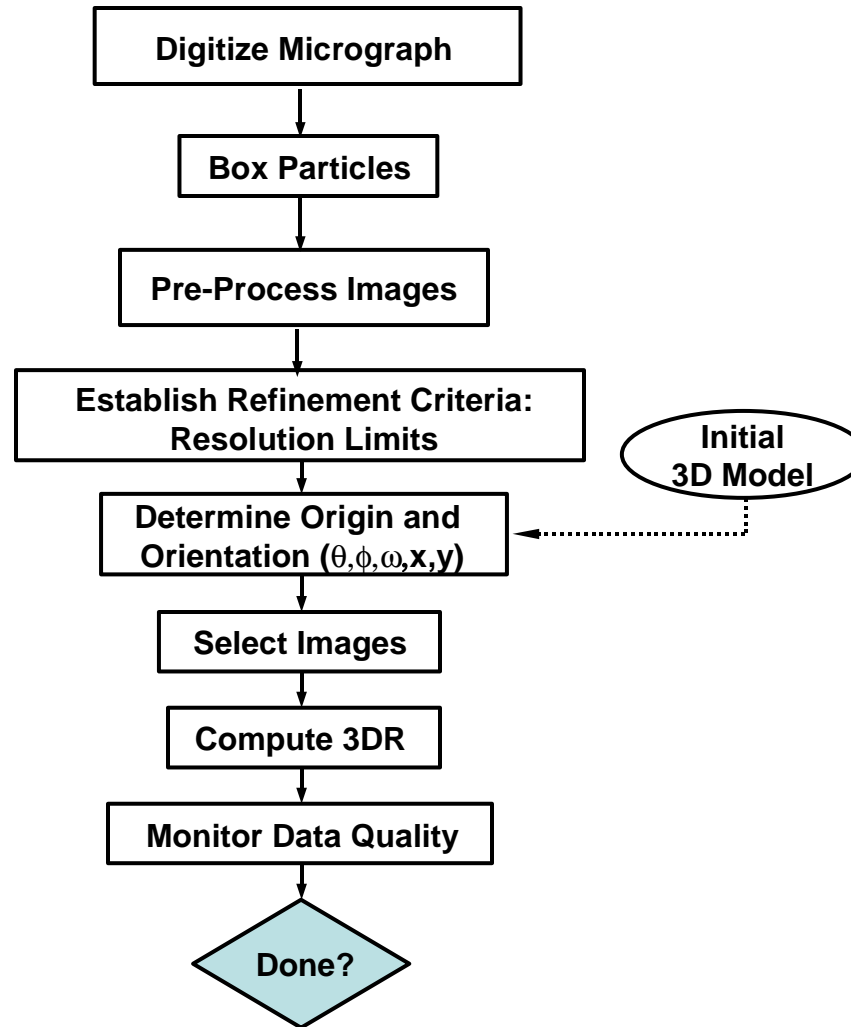
Particle	20	Theta	70,000	Phi	-1,064	Omega	181,813
X	99,732	Y	99,592	Mag	1,000		
pft_cc	0,560	prj_cc	0,623	cmp_cc	0,577		
deltaK	-0,268	deltaY	-0,408	distance	0,488		
state	[ON]	rank	[20]				

Plot: A triangular plot showing particle positions (red 'x' marks) and a selected particle (blue 'x' mark) with coordinates `70,0,-1,0`. The plot includes two icosahedral virus models (blue and red) and axes labeled `-31`, `0`, and `31`.

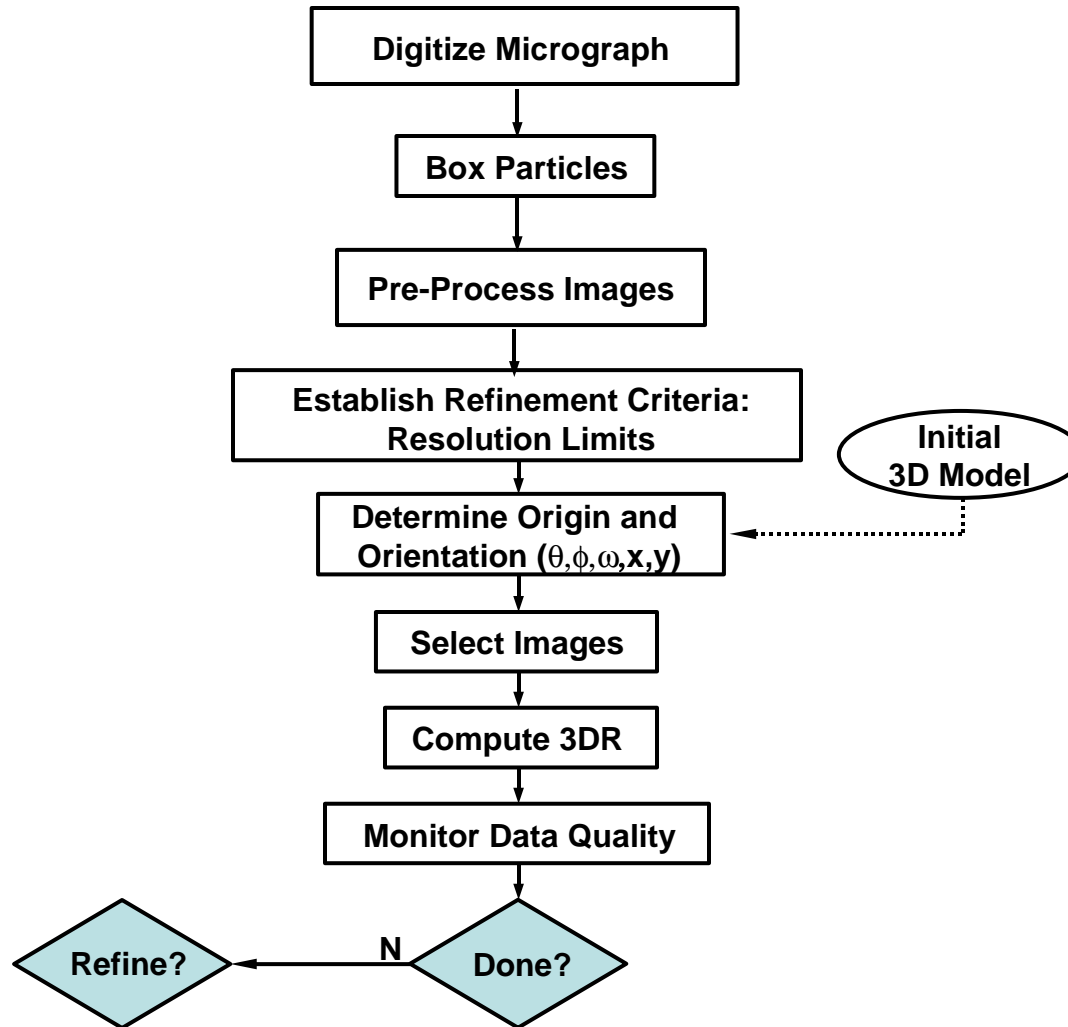
Icosahedral Virus 3D Reconstruction Scheme



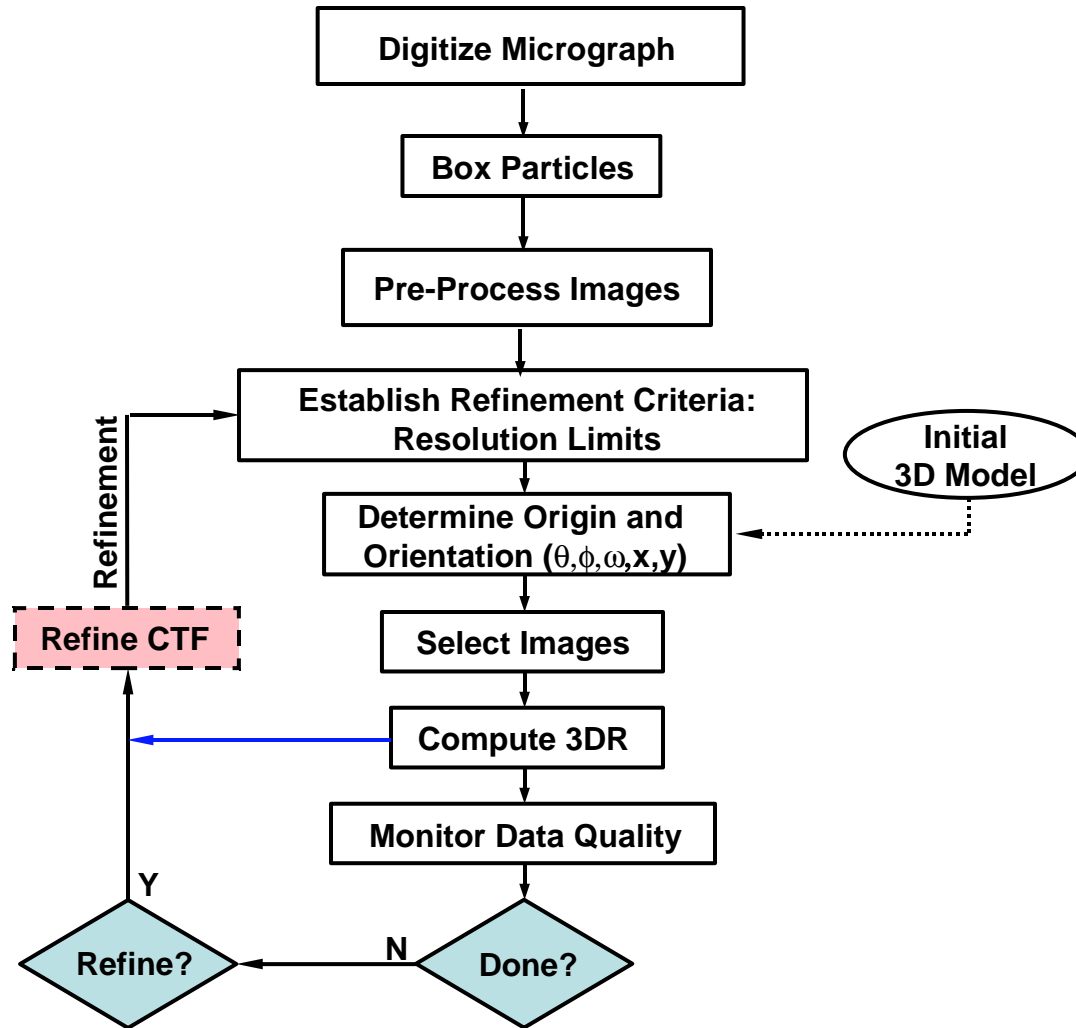
Icosahedral Virus 3D Reconstruction Scheme



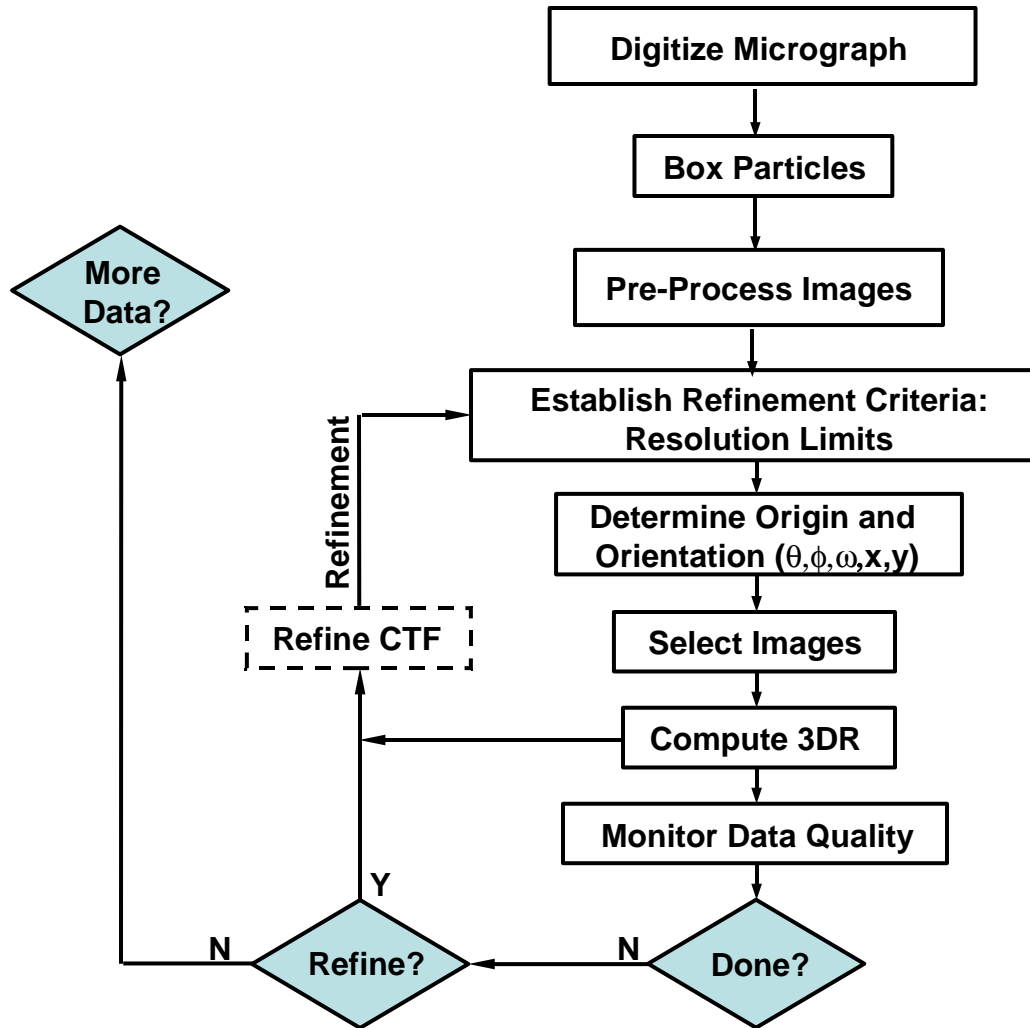
Icosahedral Virus 3D Reconstruction Scheme



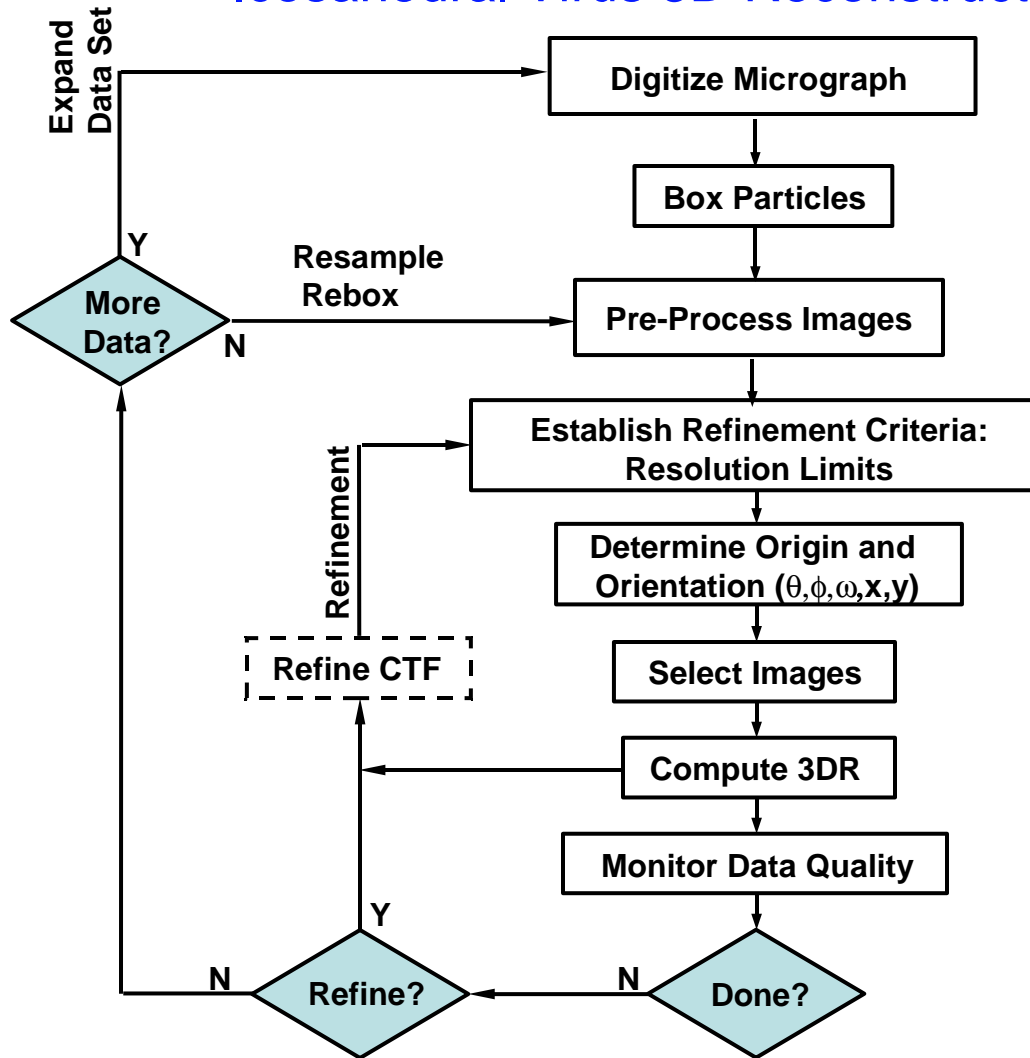
Icosahedral Virus 3D Reconstruction Scheme



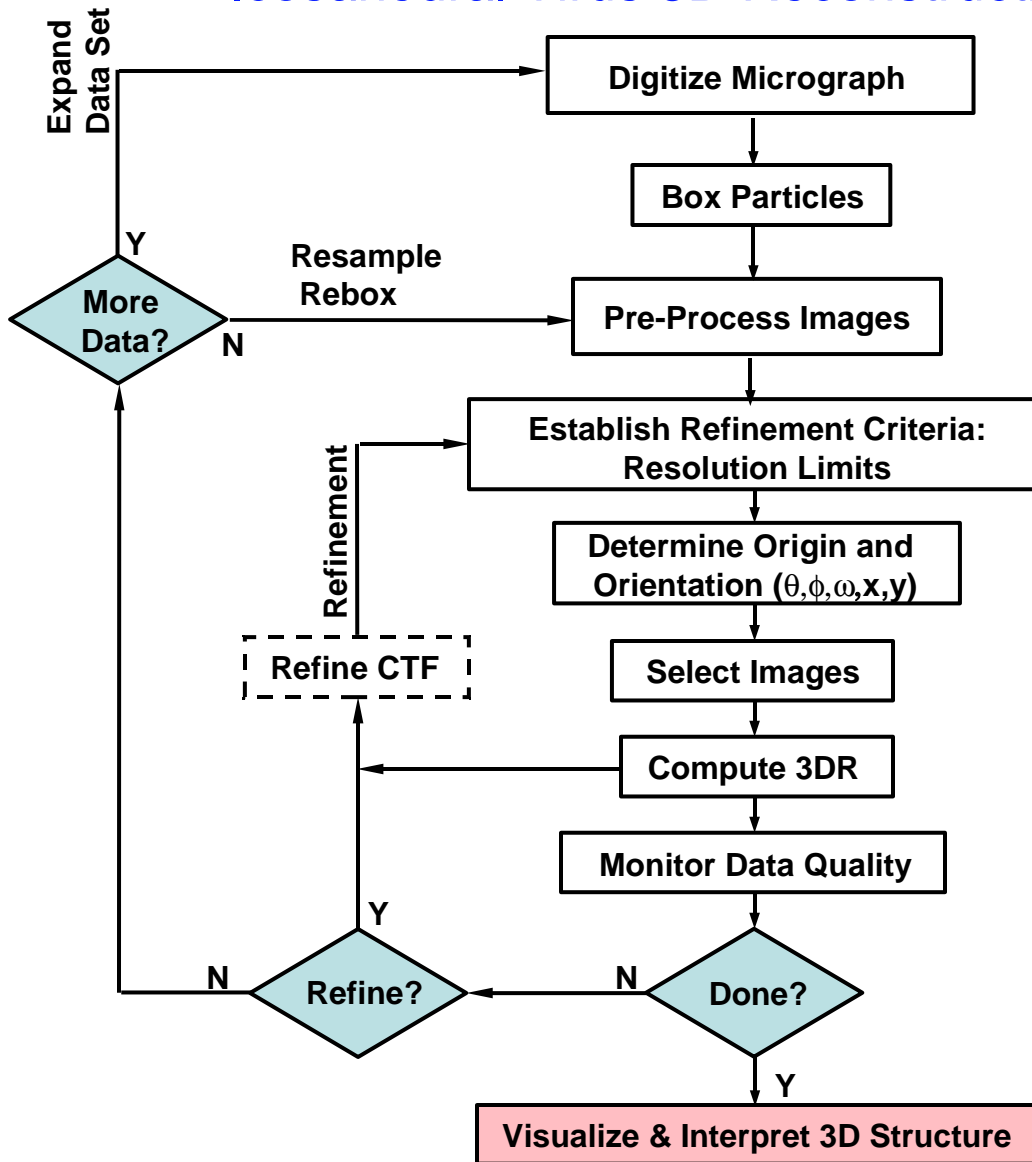
Icosahedral Virus 3D Reconstruction Scheme



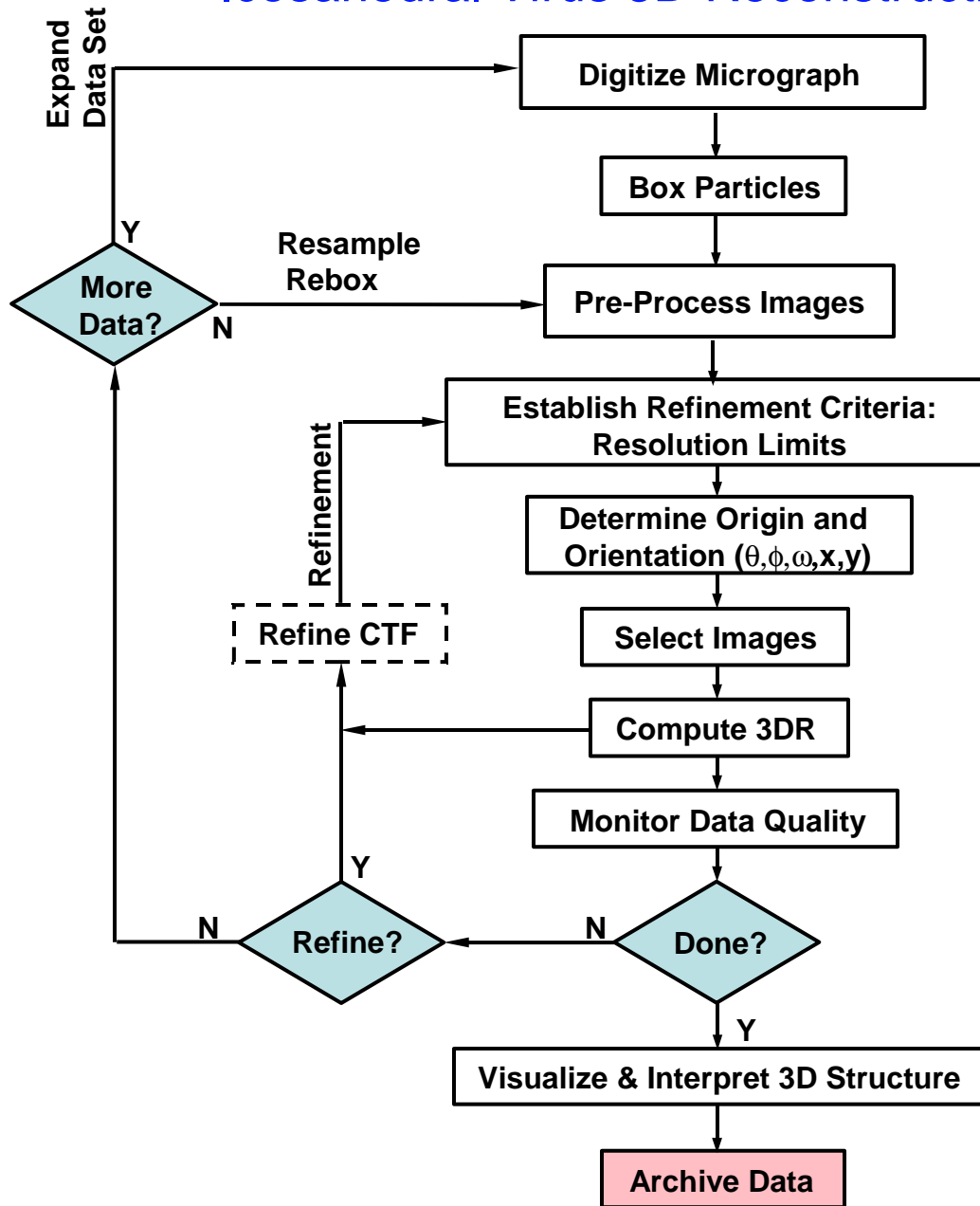
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



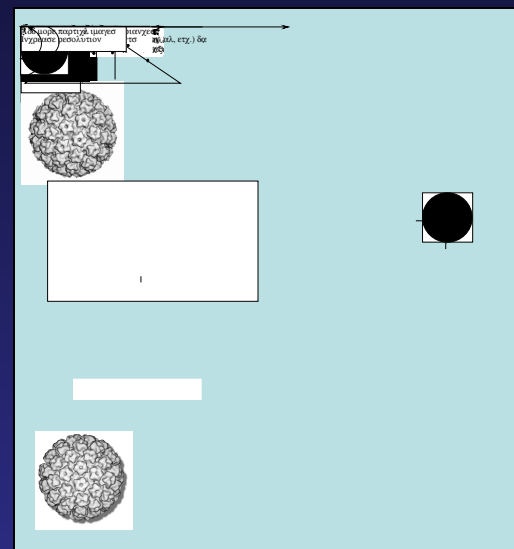
Icosahedral Virus 3D Reconstruction Scheme



3D Reconstruction of Icosahedral Particles

Outline

- Background
 - References; examples; etc.
- Symmetry
 - Icosahedral (532) point group symmetry
 - Triangulation symmetry
- “Typical” procedure (flow chart)
 - Digitization and boxing
 - Image preprocessing / CTF estimation
 - Initial particle orientation/origin search
 - Orientation/origin refinement
 - 3D reconstruction with CTF corrections
 - Validation (resolution assessment)
- Current and future strategies



3D Reconstruction of Icosahedral Particles

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3D Reconstruction of Icosahedral Particles

Current and Future Strategies

- Parallelization and new algorithms

- “Parallel” versions of EM3DR, PFTSEARCH, OOR
- EM3DR ---> P3DR
- OOR -----> PO²R

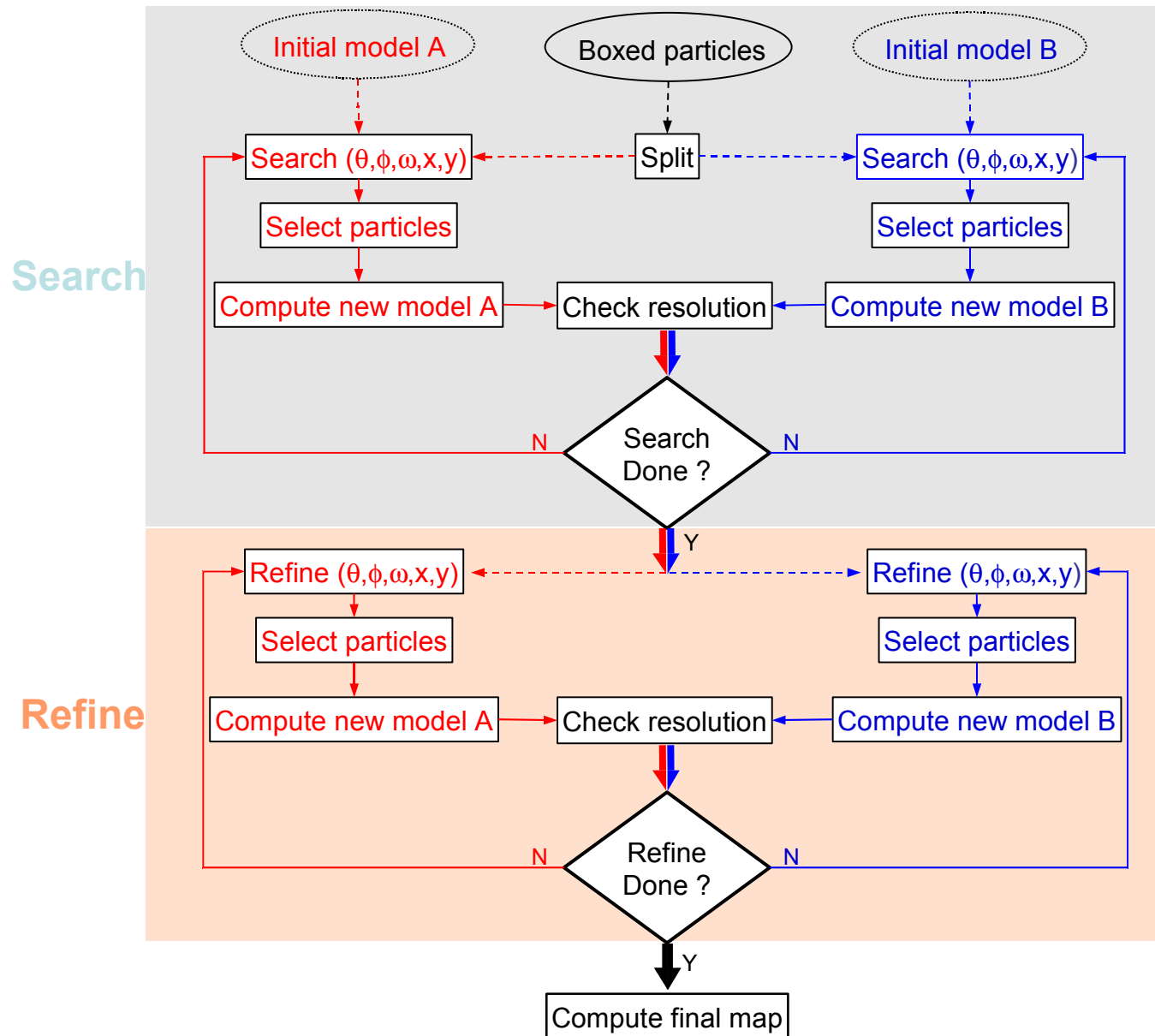
- Automation

- Semi-auto boxing (RobEM)
- Automated origin/orientation refinement (AUTO3DEM)

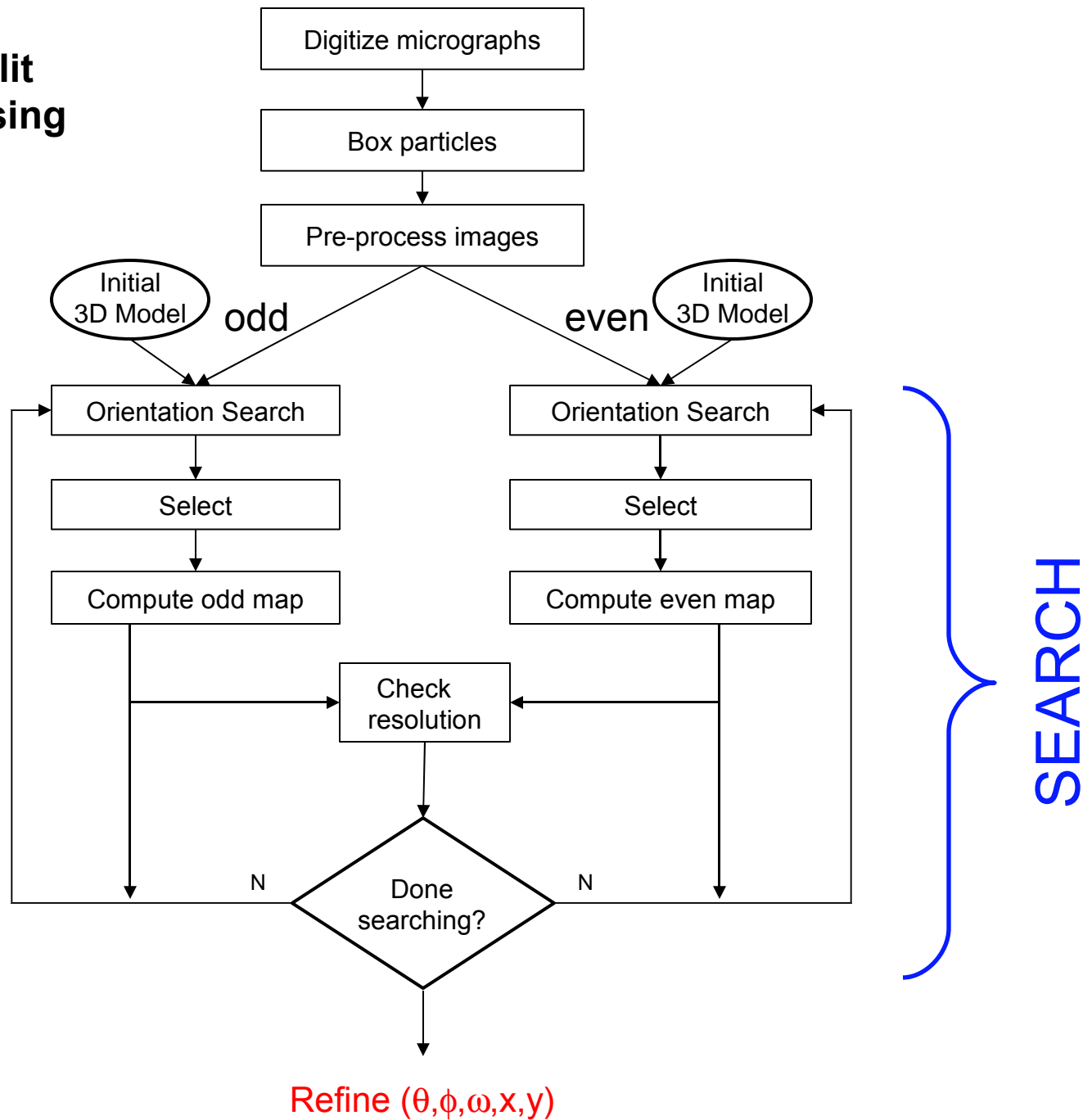
- Split data set processing

- Divide image data at very beginning and refine ‘even’ and ‘odd’ data independently.
- Minimizes (eliminates ?) bias in resolution assessment
- Combine independent reconstructions to obtain ‘final’ 3DR with highest S/N

Structure Determination Flow Chart



Data Flow for Split Data Set Processing



3D Reconstruction of Icosahedral Particles

Current and Future Strategies

- Data selection

Trying to improve resolution by substantially increasing the number of images averaged *ad infinitum* may prove less beneficial than simply applying more rigorous quality control measures to weed out 'bad' data.

Borgnia, M. J., D. Shi, P. Zhang and J. L. Milne (2004) Visualization of α -helical features in a density map constructed using 9 molecular images of the 1.8 MDa icosahedral core of pyruvate dehydrogenase. *J. Struct. Biol.* **147**:136-145.

