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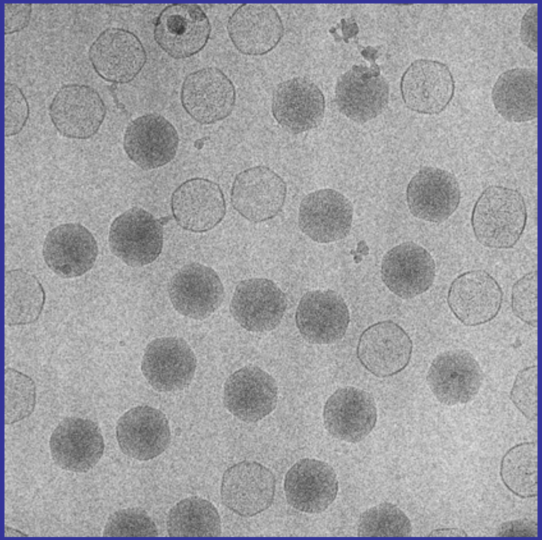
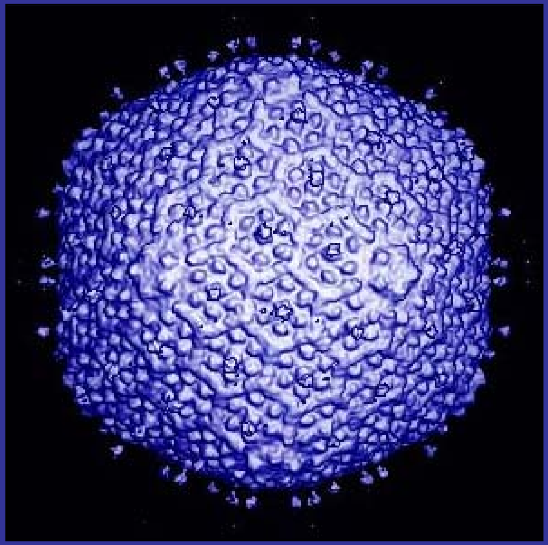
3D Reconstruction of Icosahedral Particles



2D

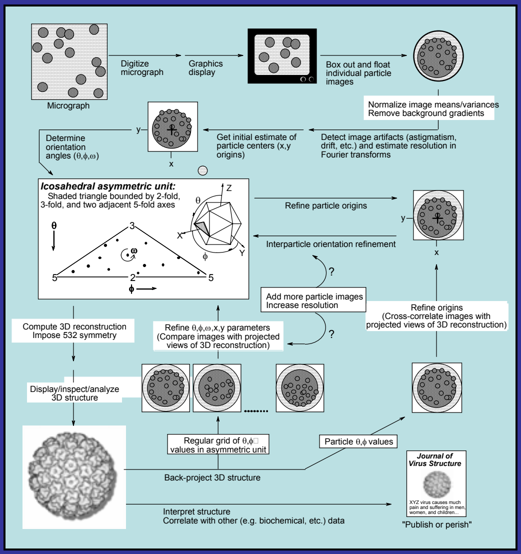


3D

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

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

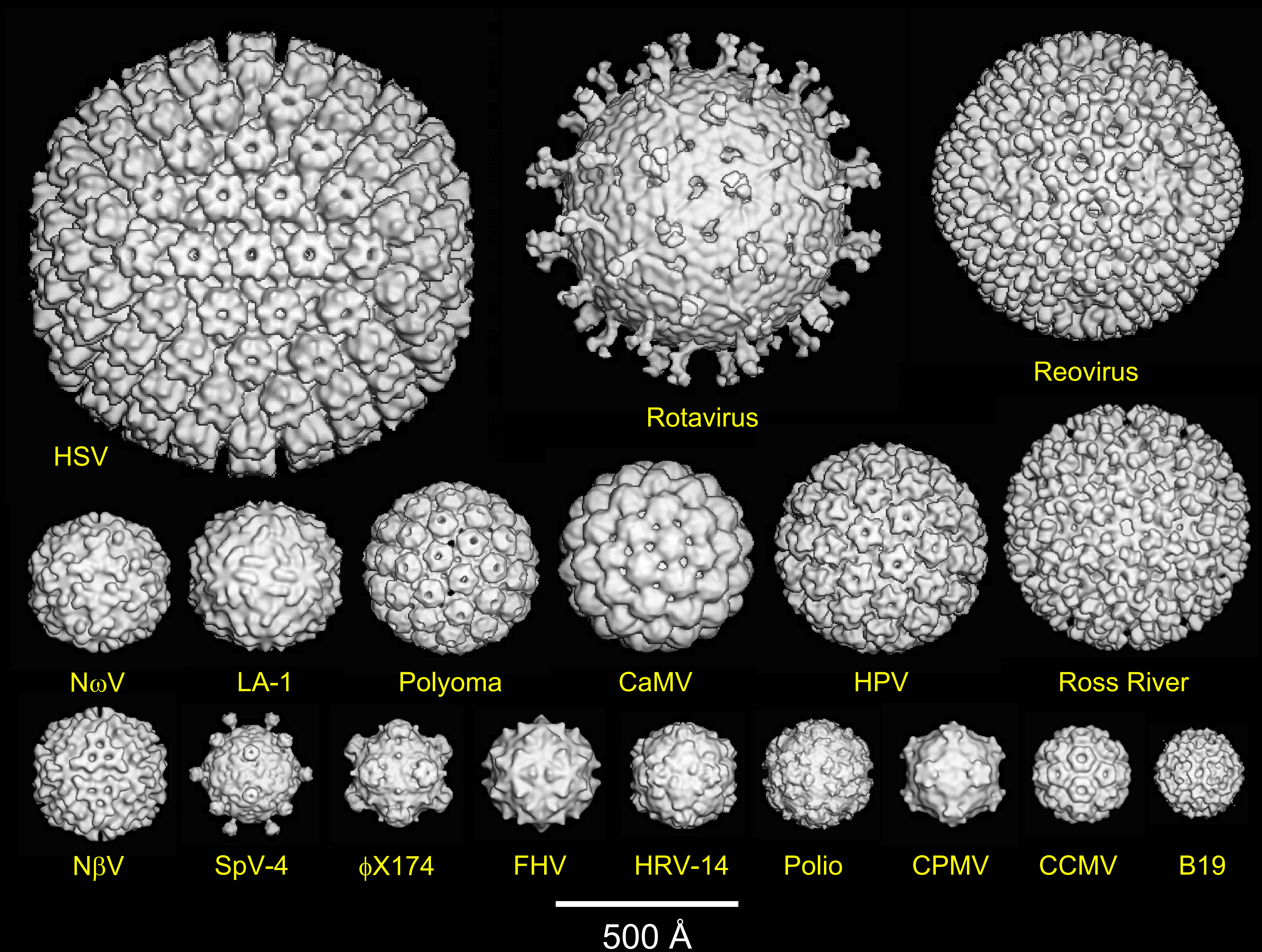
3D Reconstruction of Icosahedral Particles

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
Ross River
NωV
LA-1
Polyoma
CaMV
HPV
NβV
SpV-4
φX174
FHV
HRV-14
Polio
CPMV
CCMV
B19
500 Å

3D Reconstruction of Icosahedral Particles

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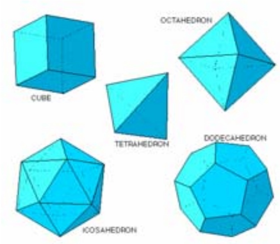
3D Reconstruction of Icosahedral Particles

Symmetry

1. Icosahedral (532) point group symmetry

2. Triangulation symmetry

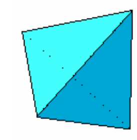


Regular Polyhedra

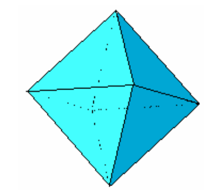
(Platonic Solids)

There are just five platonic solids:

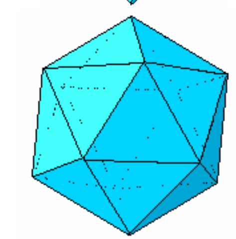
From **equilateral triangles** you can make:
with 3 faces at each vertex, a **tetrahedron**



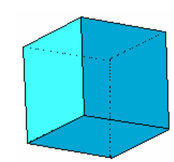
with 4 faces at each vertex, an **octahedron**



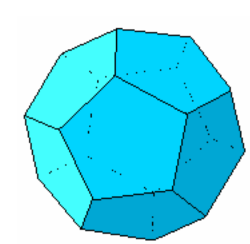
with 5 faces at each vertex, an **icosahedron**



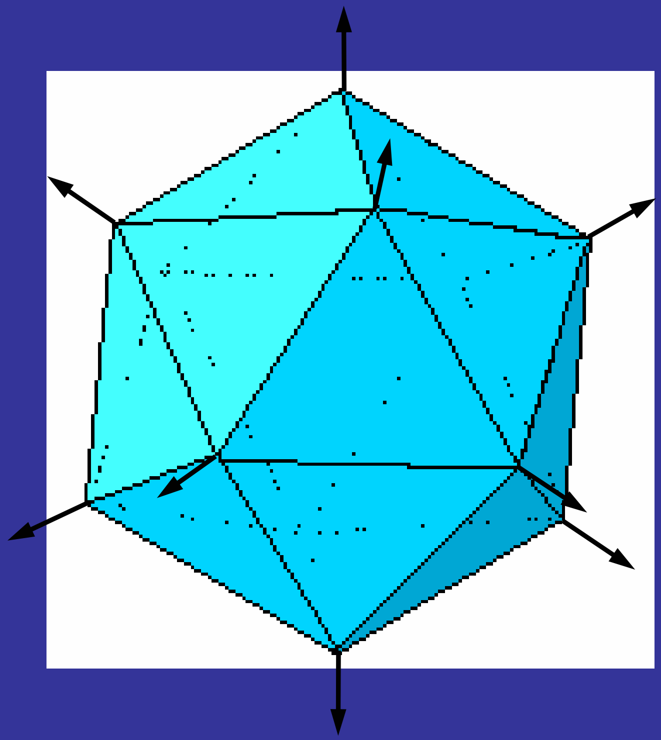
From **squares** you can make:
with 3 faces at each vertex, a **cube**



From **pentagons** you can make:
with 3 faces at each vertex, a **dodecahedron**

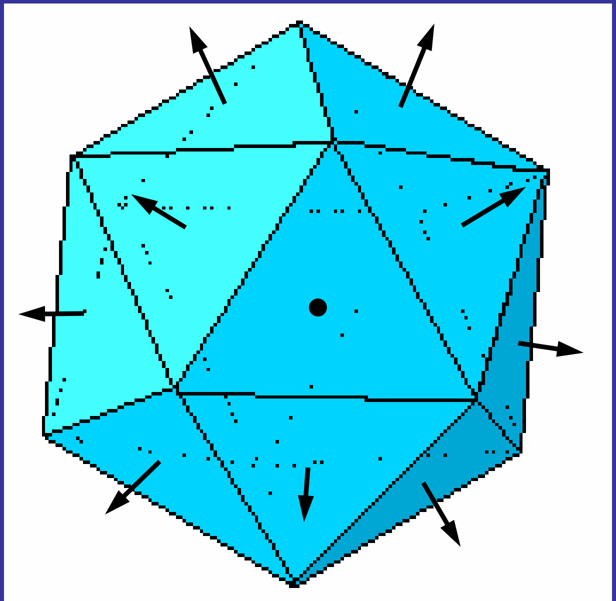


Icosahedral (532) Point Group Symmetry



12 vertices (5-fold)

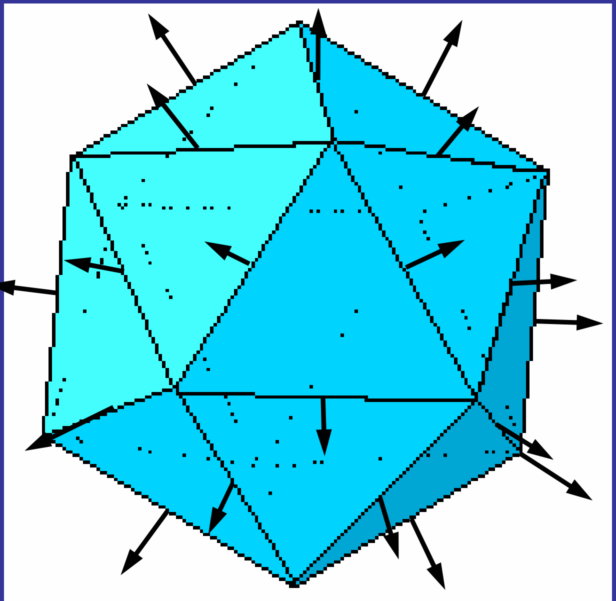
Icosahedral (532) Point Group Symmetry



12 vertices (5-fold)

20 faces (3-fold)

Icosahedral (532) Point Group Symmetry



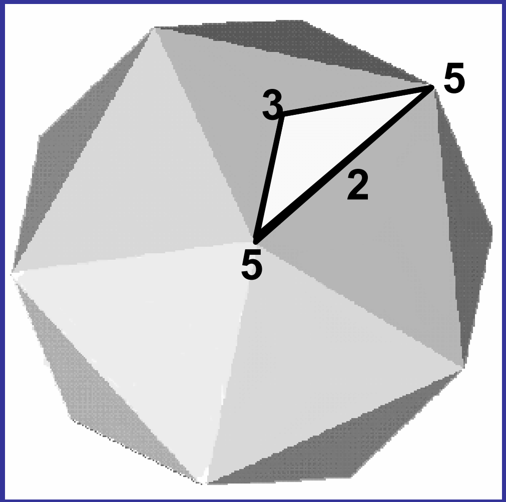
12 vertices (5-fold)

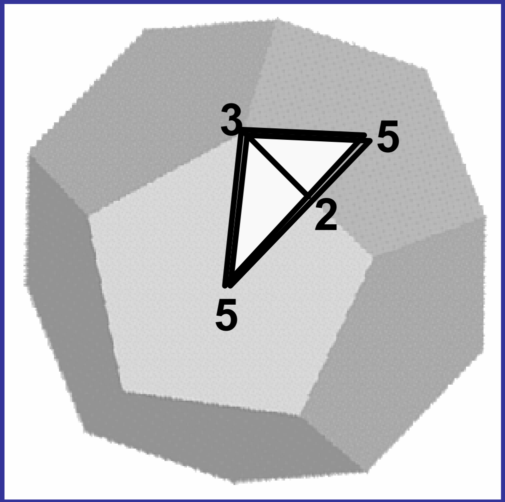
20 faces (3-fold)

30 edges (2-fold)

Icosahedron

Dodecahedron





Different shapes, but both have 532 symmetry

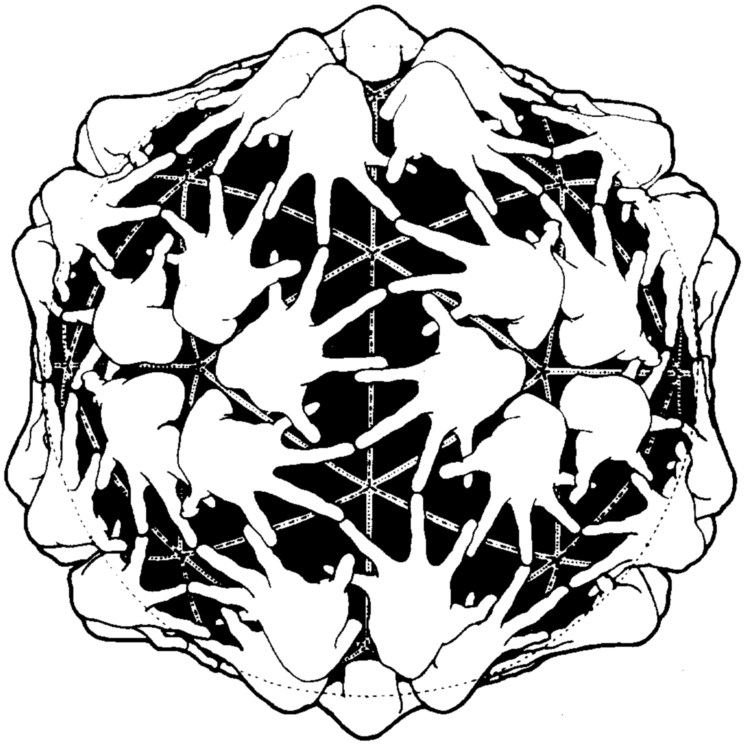
12 vertices, 20 faces, 30 edges
(6 5-folds, 10 3-folds, 15 2-folds)

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

Asymmetric unit is 1/60th of whole object

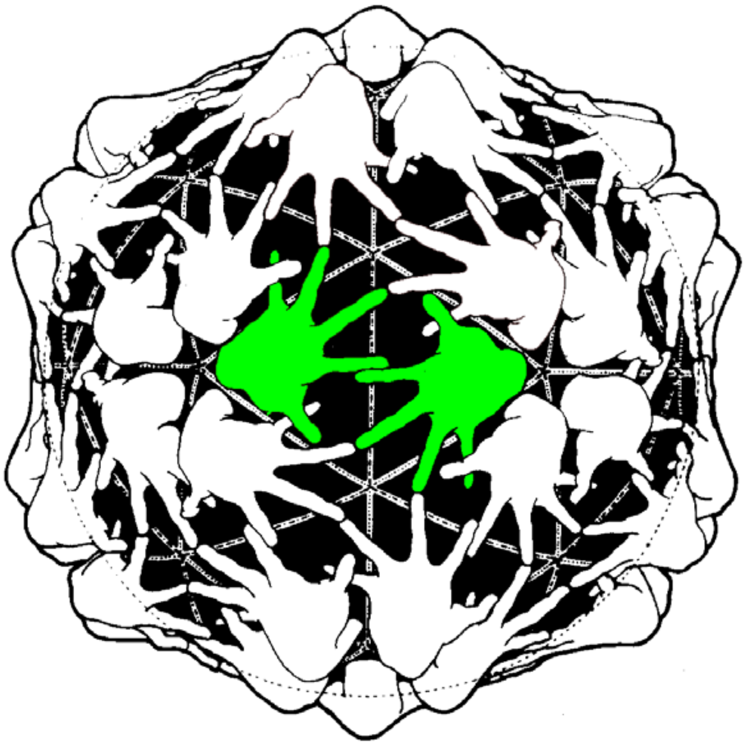
Object consists of 60 identical ‘subunits’ arranged with icosahedral symmetry

Icosahedral (532) Point Group Symmetry



From Eisenberg & Crothers, Table 16-3, p.767

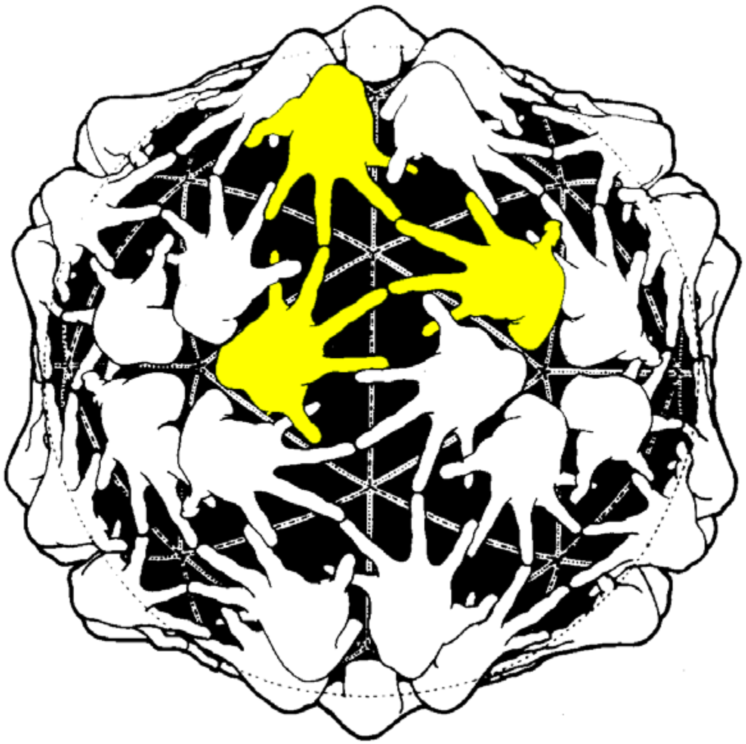
Icosahedral (532) Point Group Symmetry



30 dimers

From Eisenberg & Crothers, Table 16-3, p.767

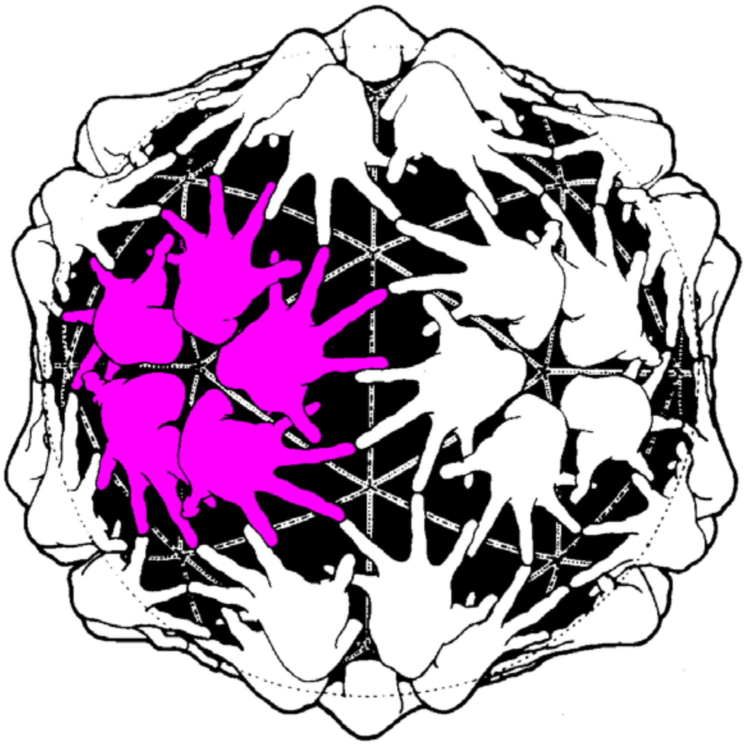
Icosahedral (532) Point Group Symmetry



20 trimers

From Eisenberg & Crothers, Table 16-3, p.767

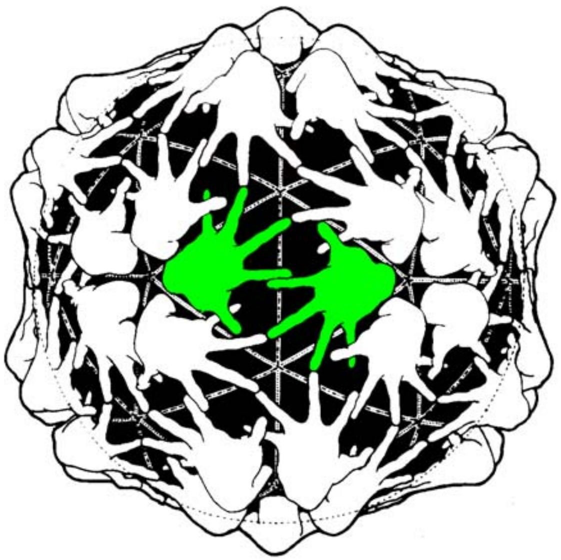
Icosahedral (532) Point Group Symmetry



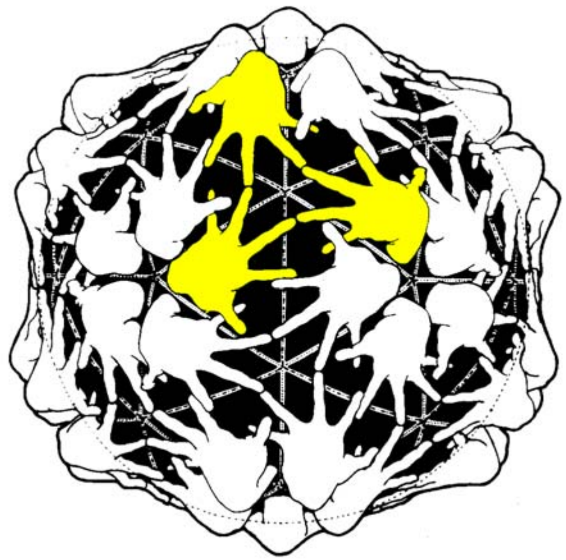
12 pentamers

From Eisenberg & Crothers, Table 16-3, p.767

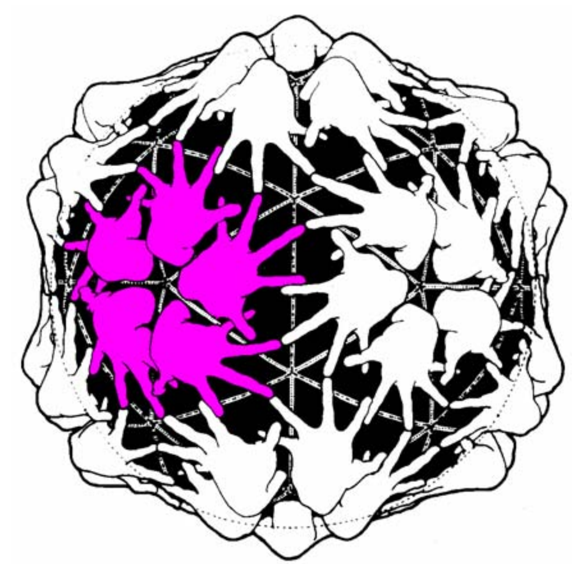
Icosahedral (532) Point Group Symmetry



30 dimers



20 trimers



12 pentamers

From Eisenberg & Crothers, Table 16-3, p.767

3D Reconstruction of Icosahedral Particles

Symmetry

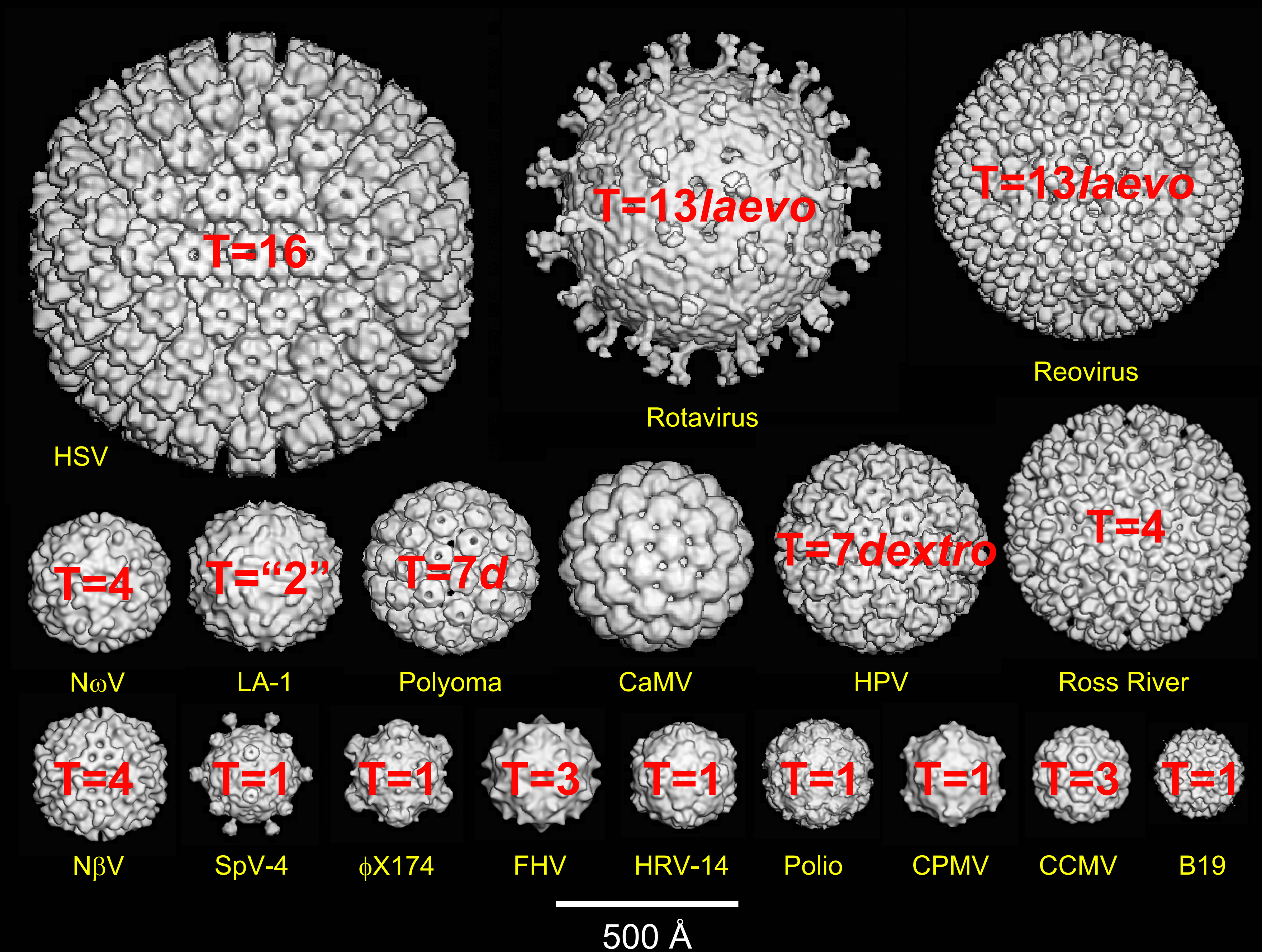
1. Icosahedral (532) point group symmetry

➡ 2. Triangulation symmetry

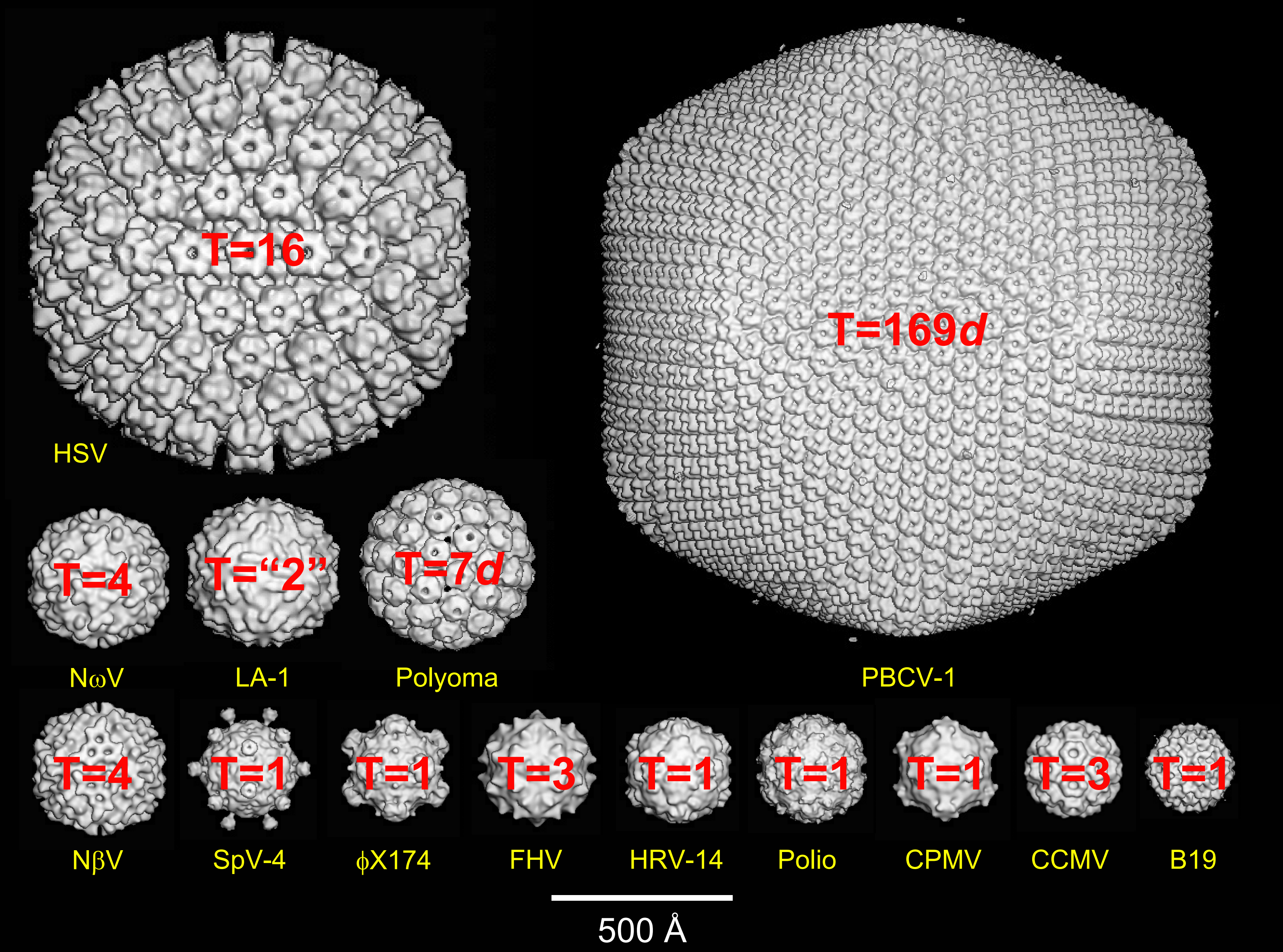
Purely mathematical concept (concerns lattices)

Real objects (*e.g.* viruses) with 532 symmetry often consists of multiples of 60 ‘subunits’

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



T=16
HSV
T=13laevo
Rotavirus
T=13laevo
Reovirus
T=4
NωV
T="2"
LA-1
T=7d
Polyoma
CaMV
T=7dextro
HPV
T=4
Ross River
T=4
NβV
T=1
SpV-4
T=1
φX174
T=3
FHV
T=1
HRV-14
T=1
Polio
T=1
CPMV
T=3
CCMV
T=1
B19
500 Å


T=16
HSV
T=169*d*
T=4
T="2"
T=7*d*
NωV
LA-1
Polyoma
PBCV-1
T=4
T=1
T=1
T=3
T=1
T=1
T=1
T=3
T=1
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

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

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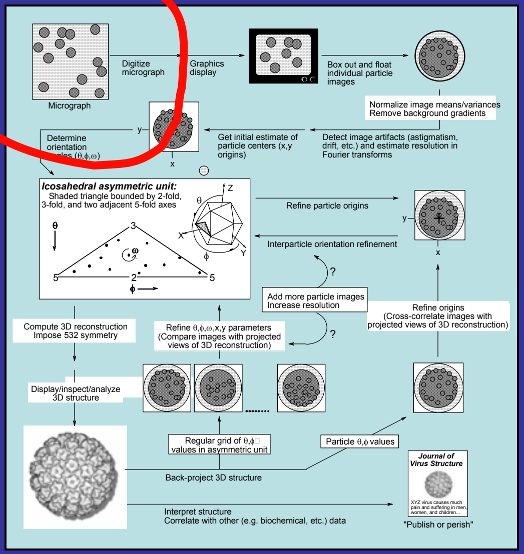


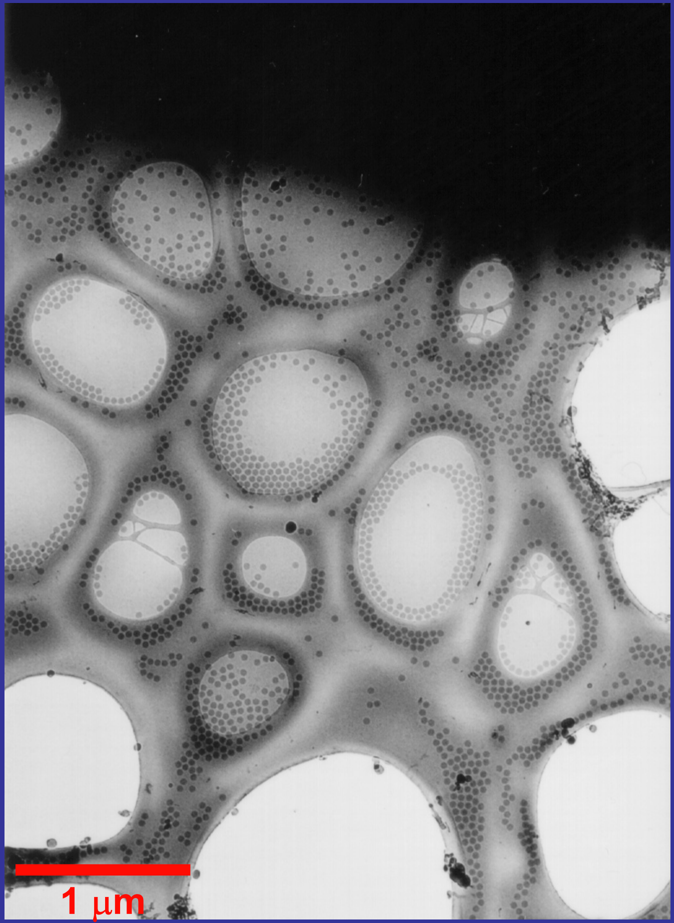
3D Reconstruction of Icosahedral Particles

Protocol

Electron Cryo-Microscopy

Sample : ~2-3 μl at 1-5 mg/ml

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



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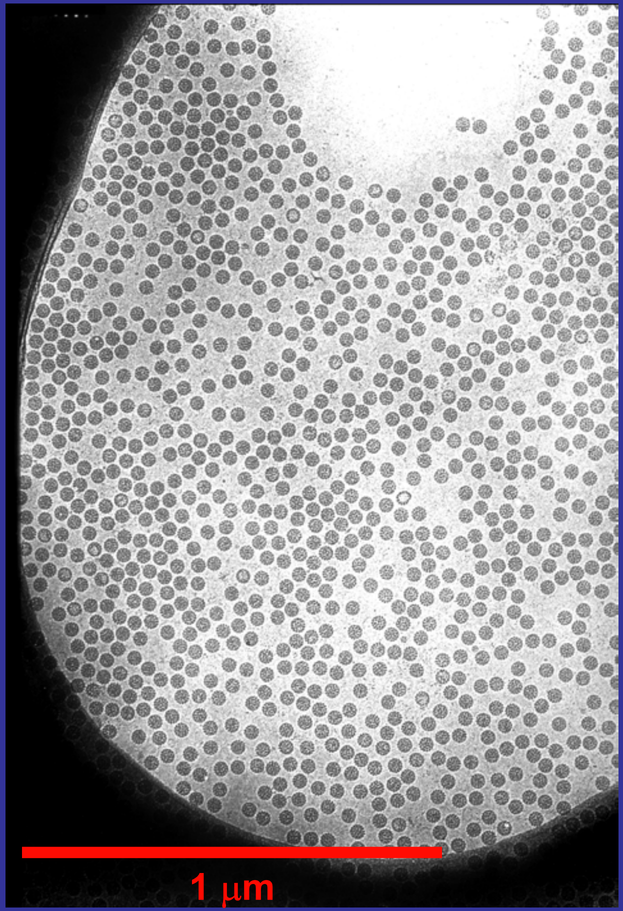

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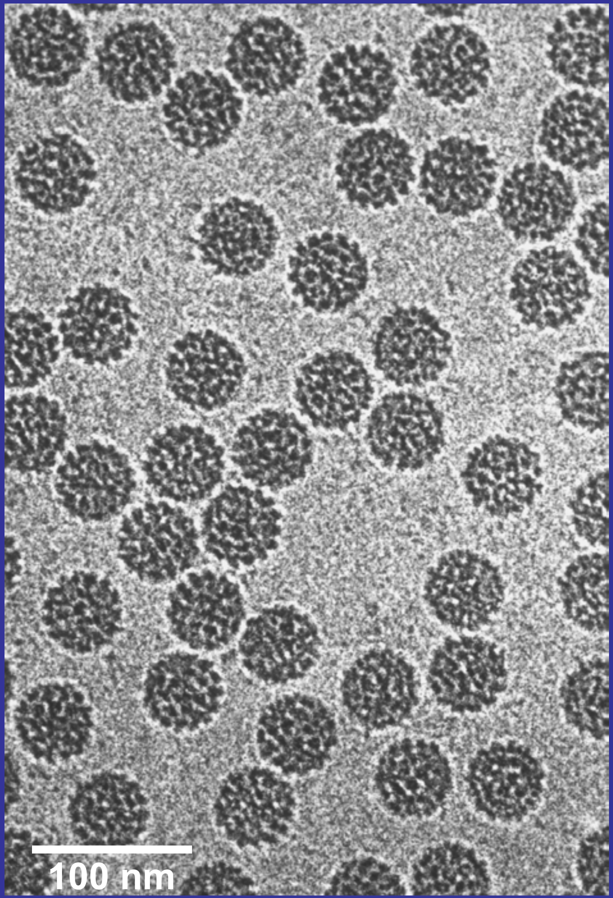


3D Reconstruction of Icosahedral Particles

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

Defocus range: 1-3 μm underfocus

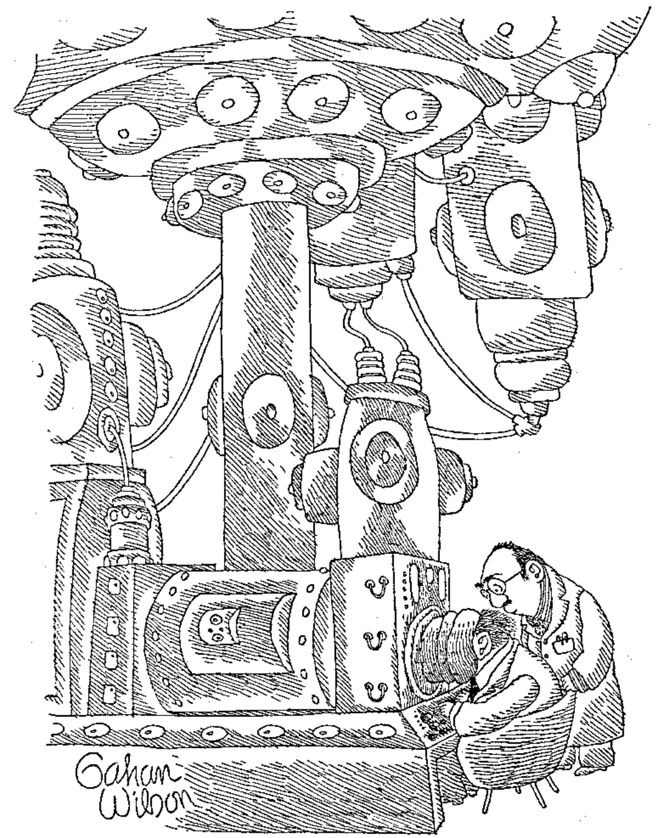
Dose: 10-20 $e^-/Å^2$

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

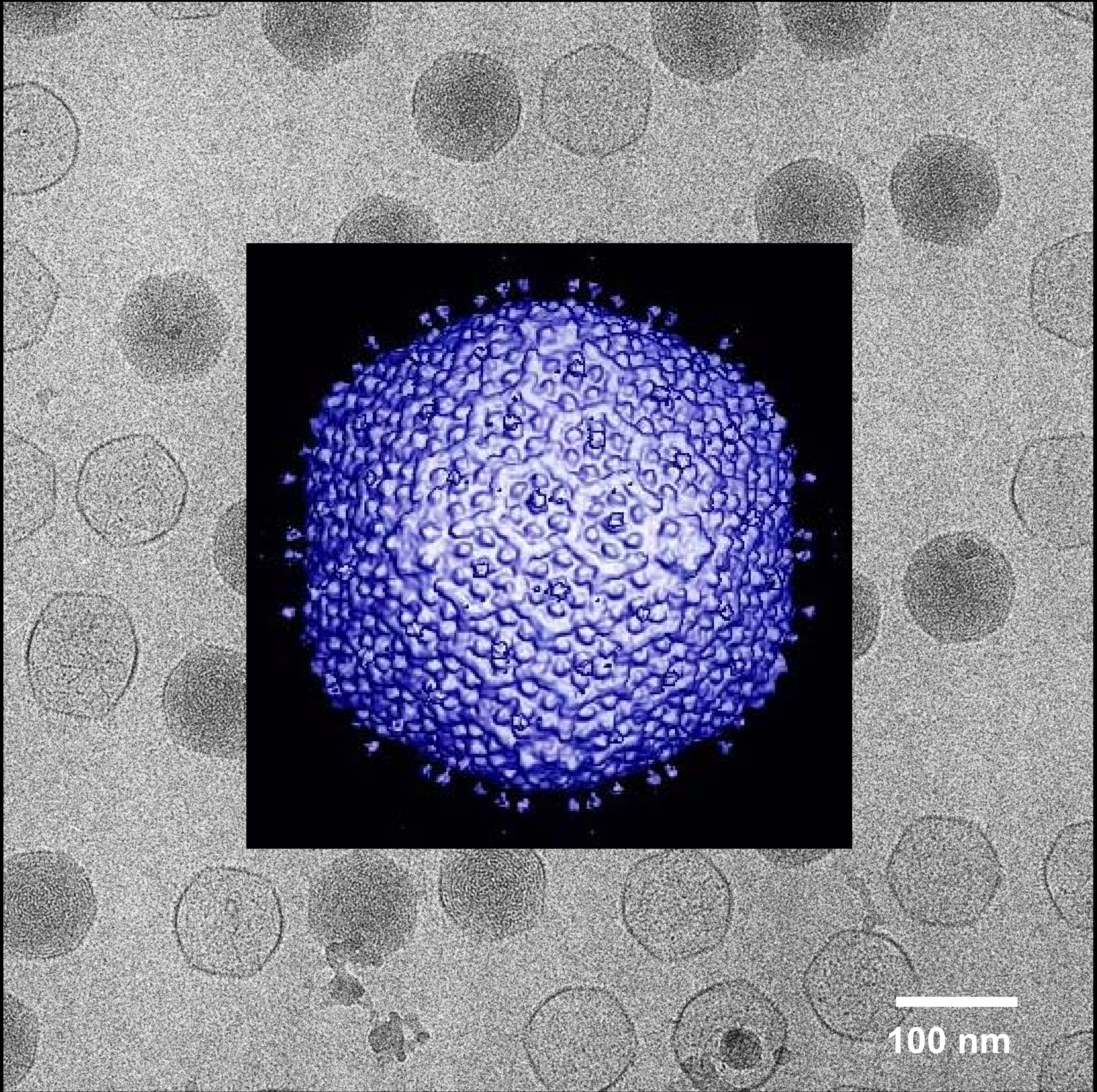
Micrographs: 25-100

Particles: 10^3-10^4

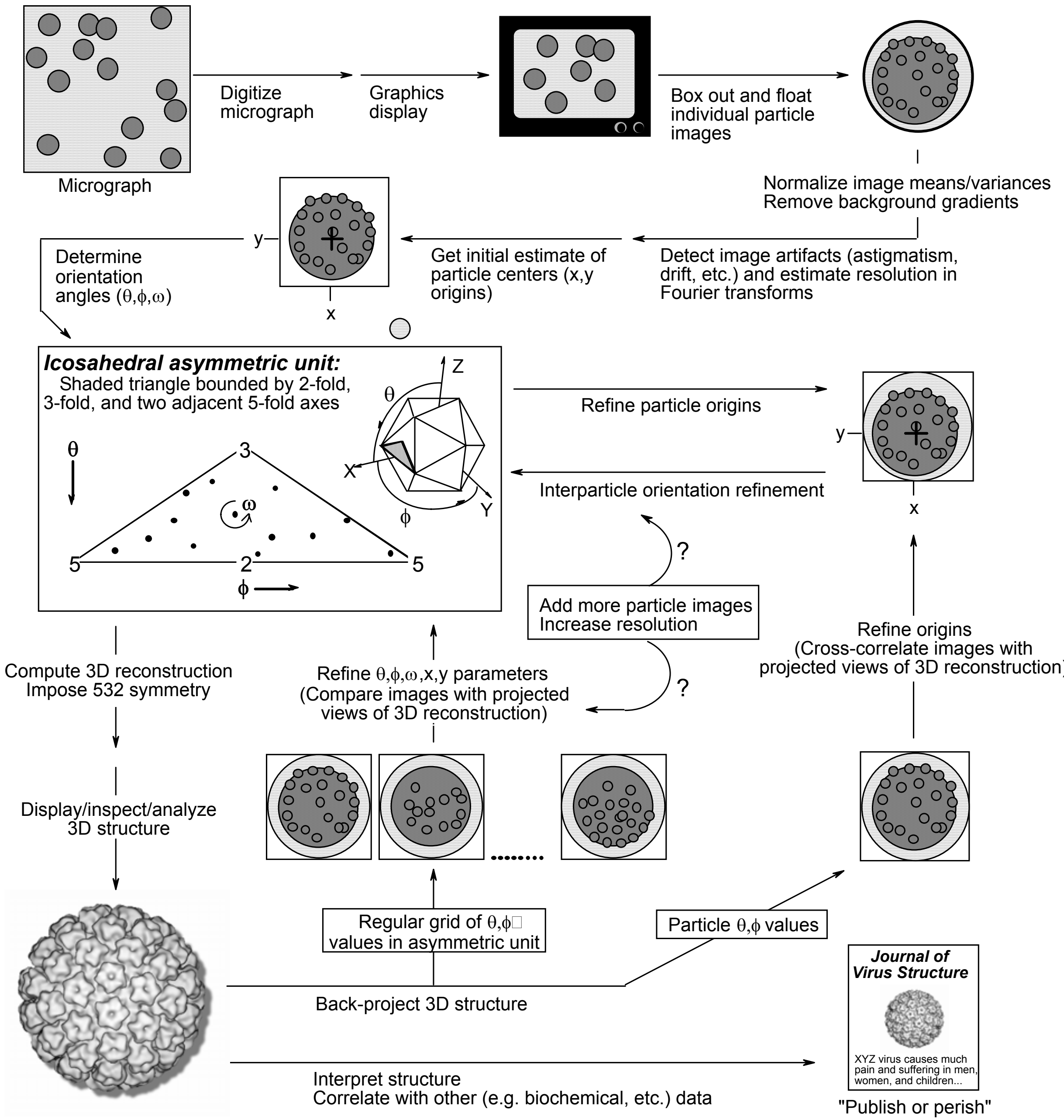
Target resolution: 12 - 6 Å



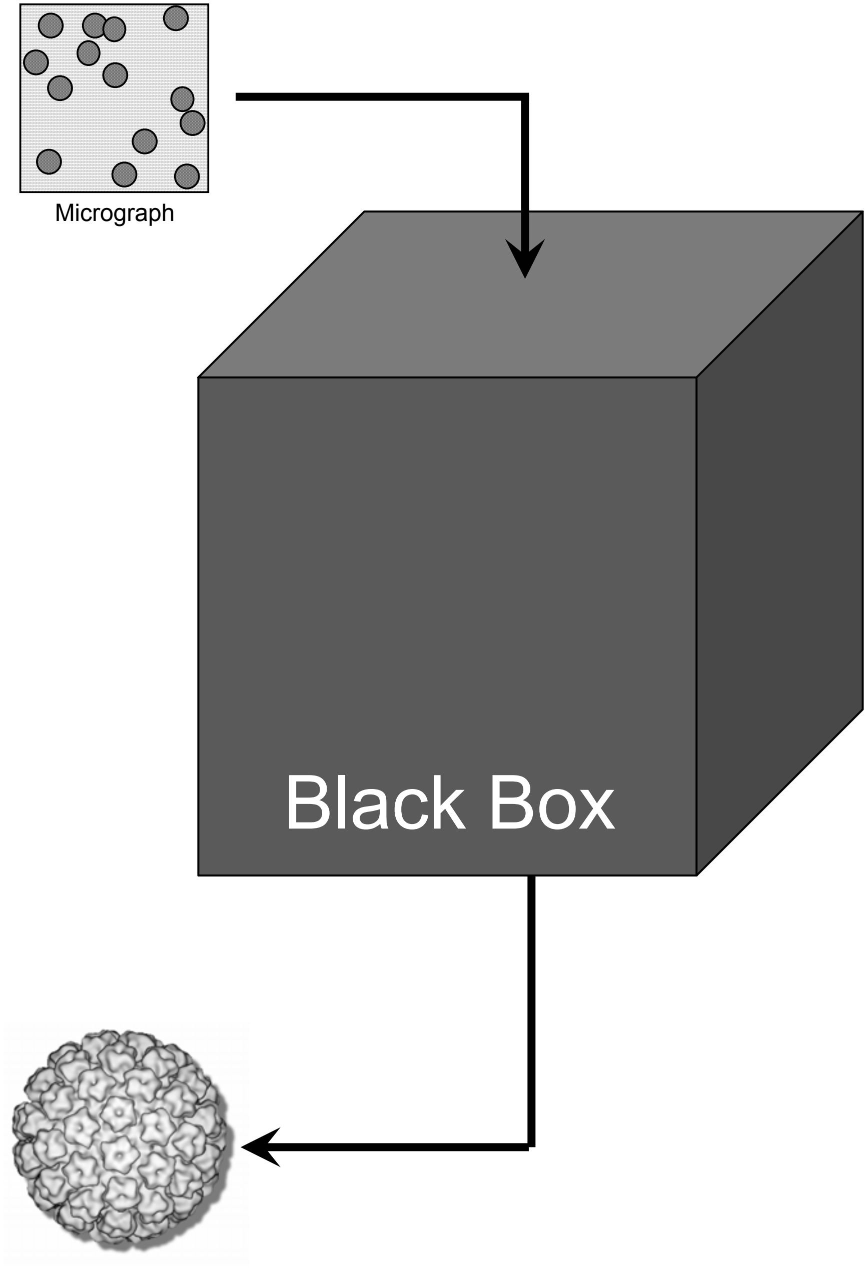
"I think I may have spotted something!"


100 nm

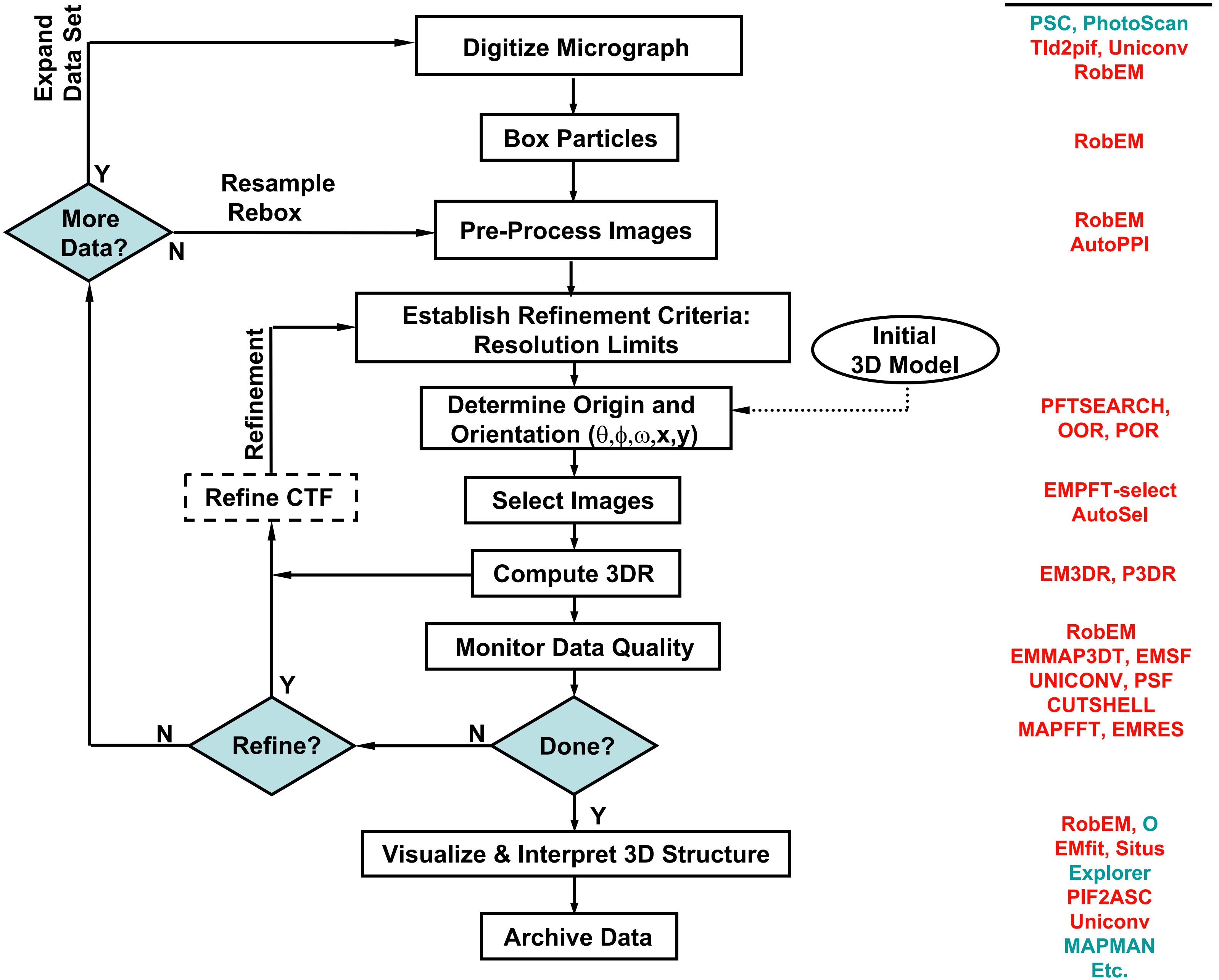
Icosahedral Particle Image Reconstruction Scheme



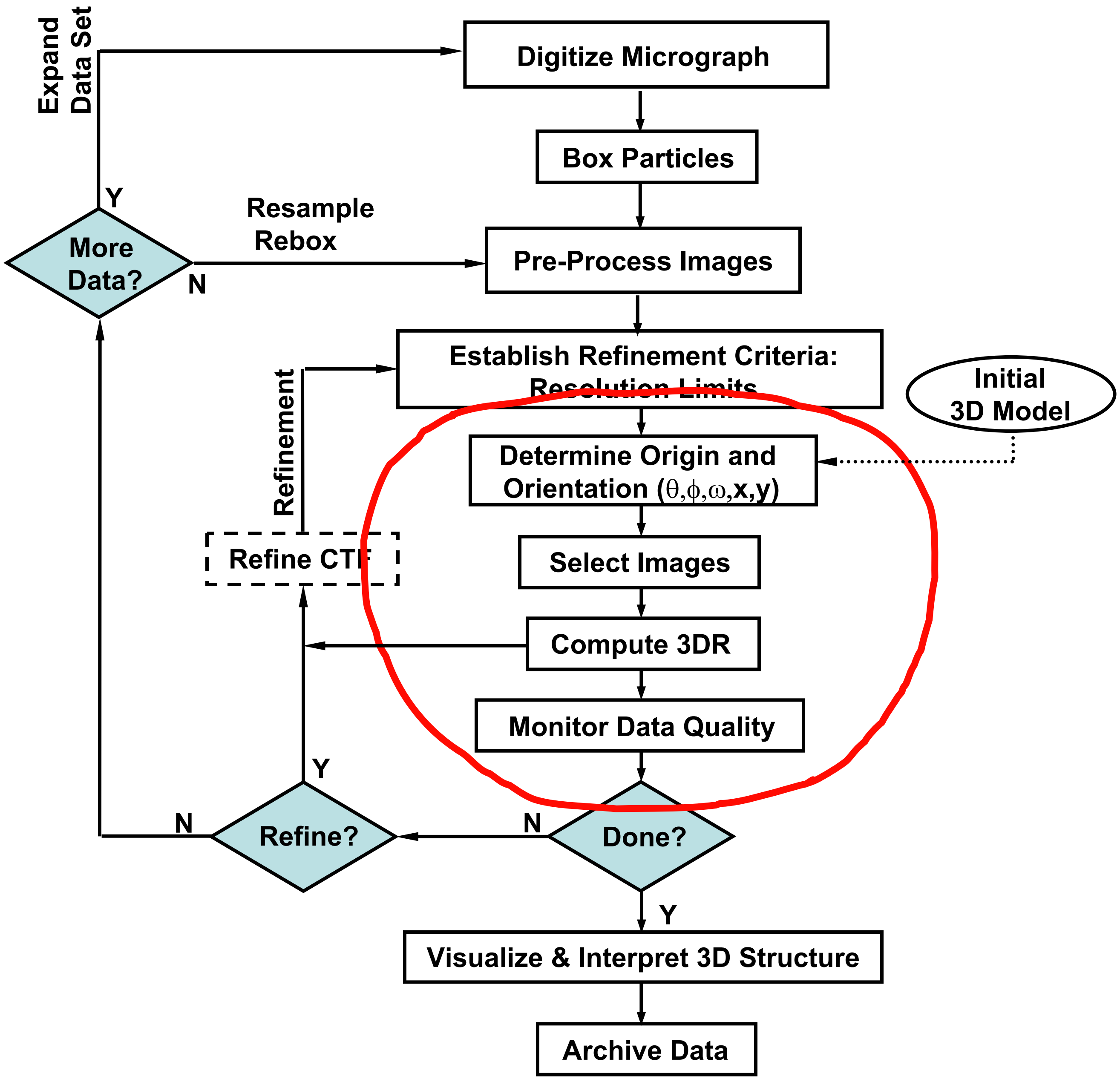
Icosahedral Particle Image Reconstruction Scheme



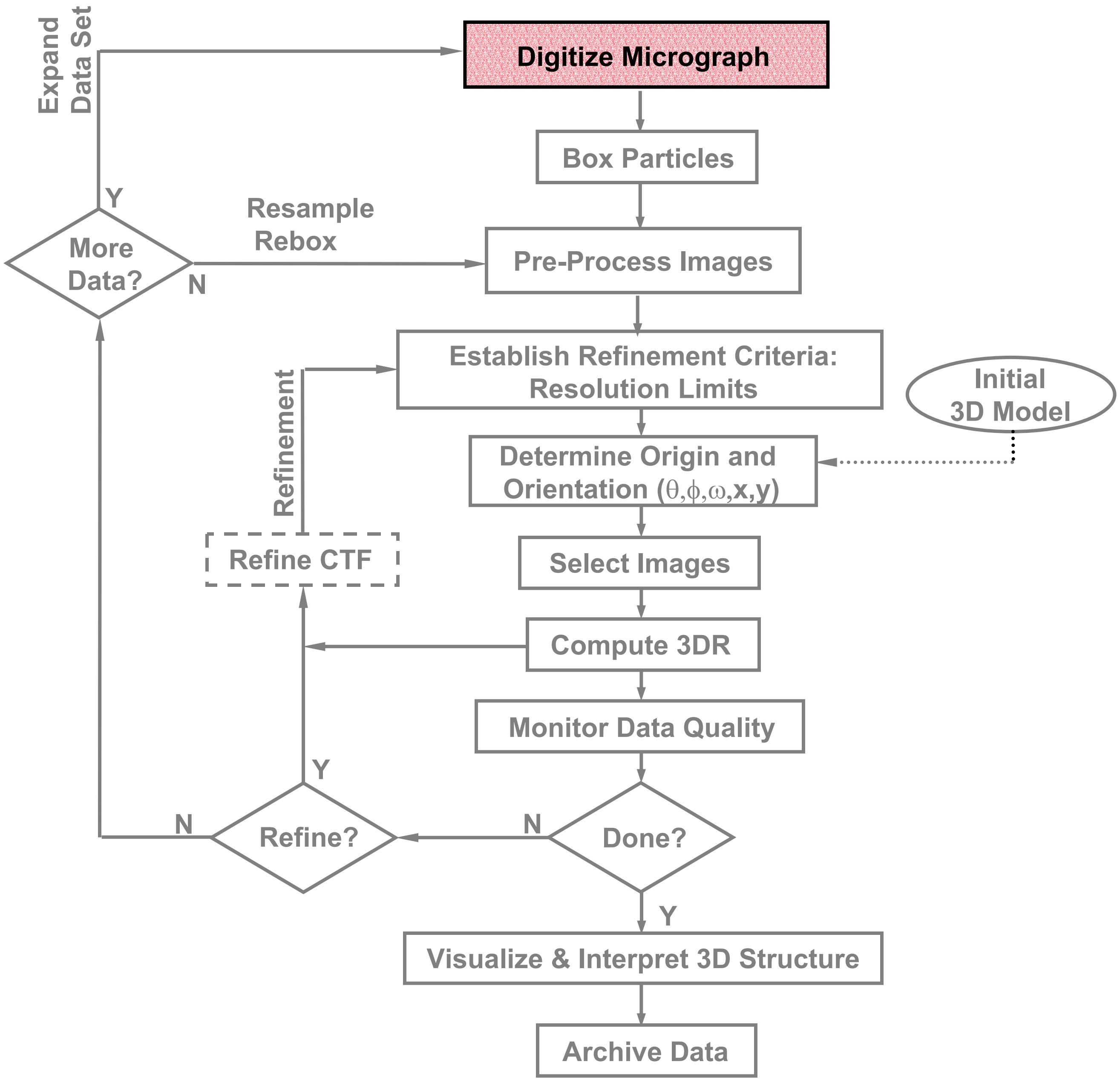
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

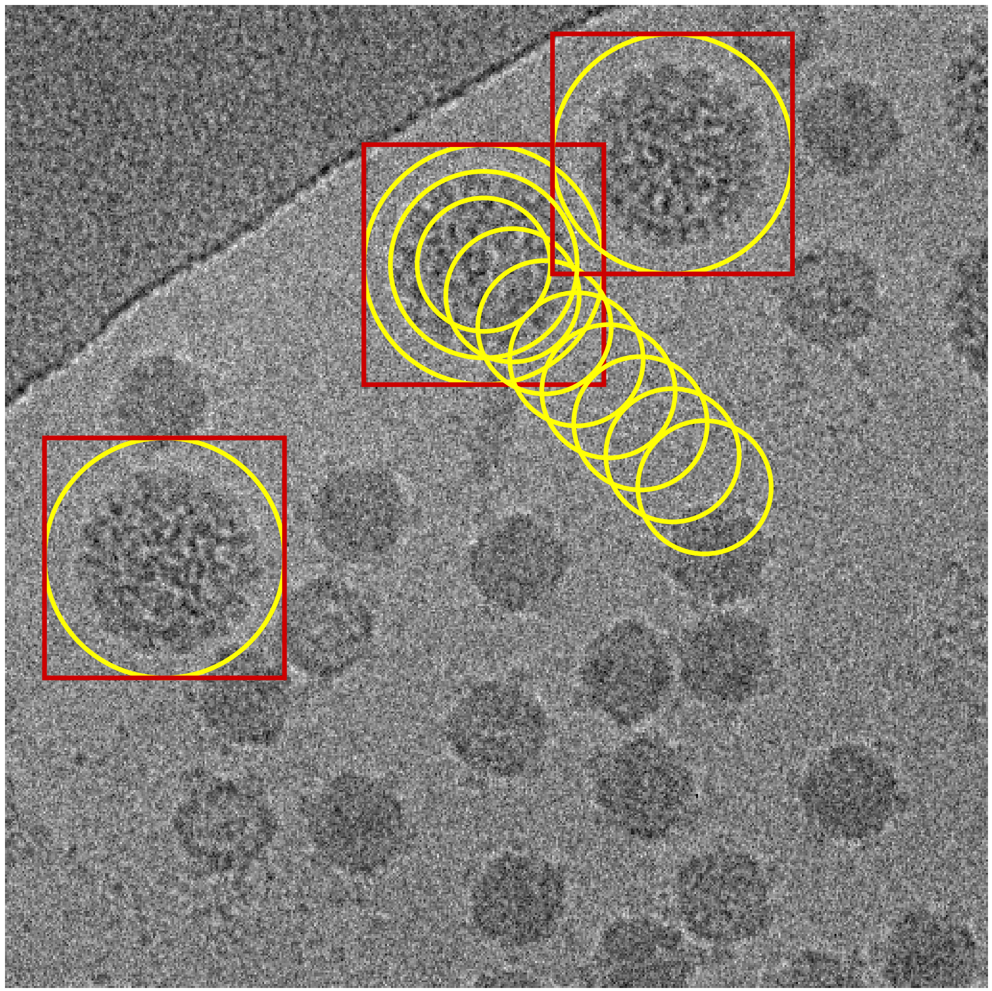
Digitize Micrograph

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles

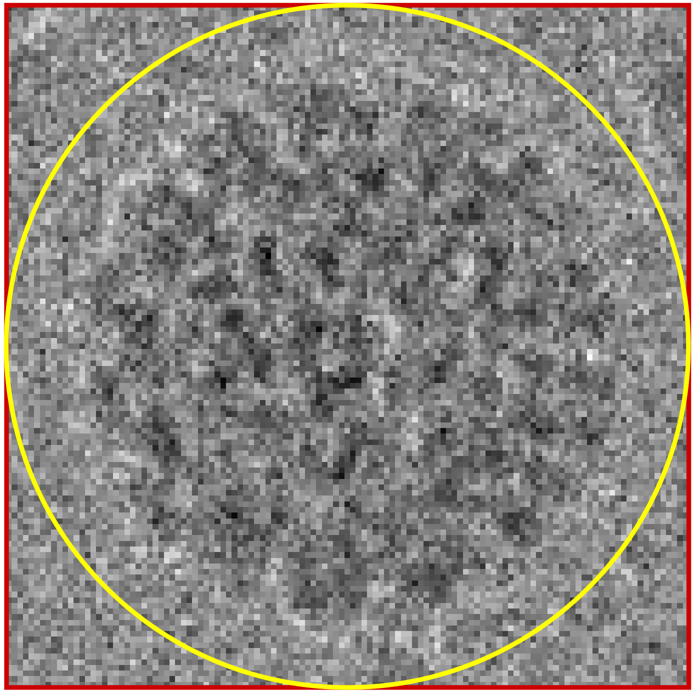


Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles



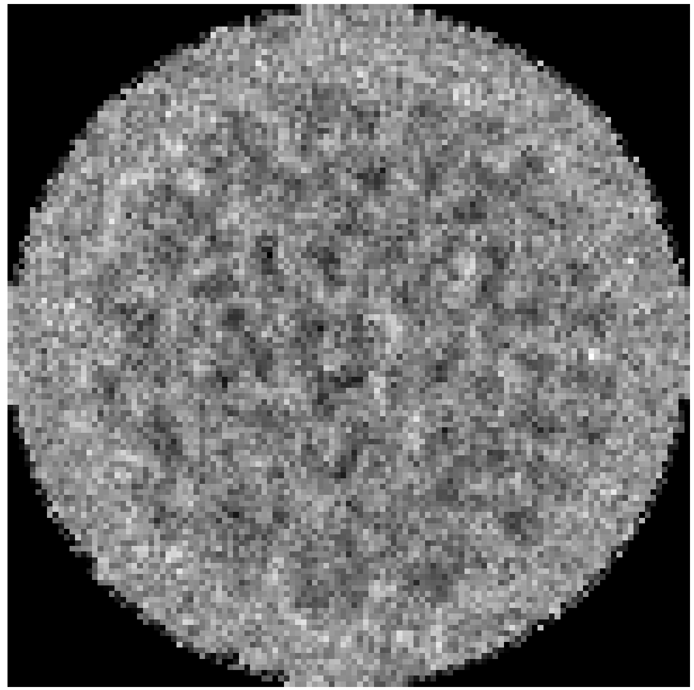
Extracted

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

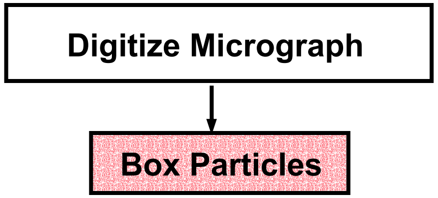
↓

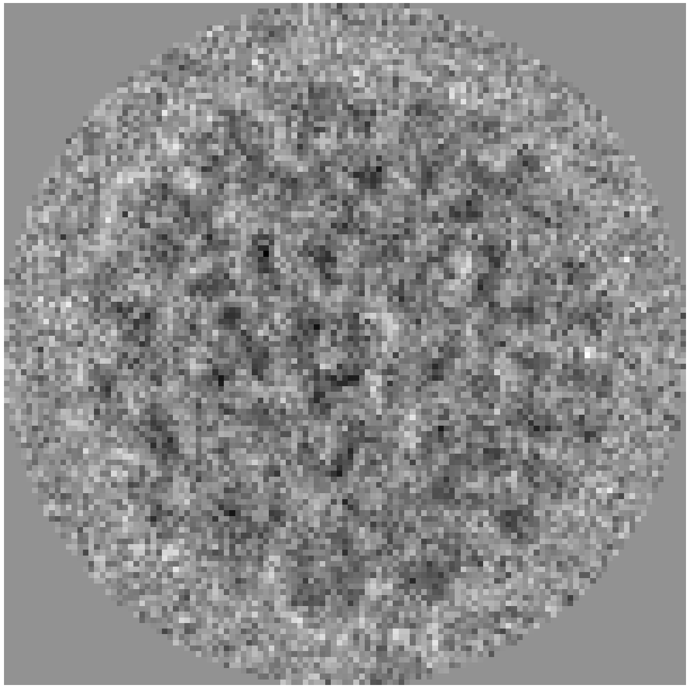
Box Particles



Masked

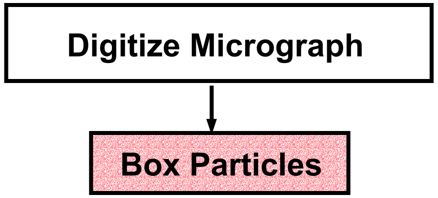
Icosahedral Virus 3D Reconstruction Scheme

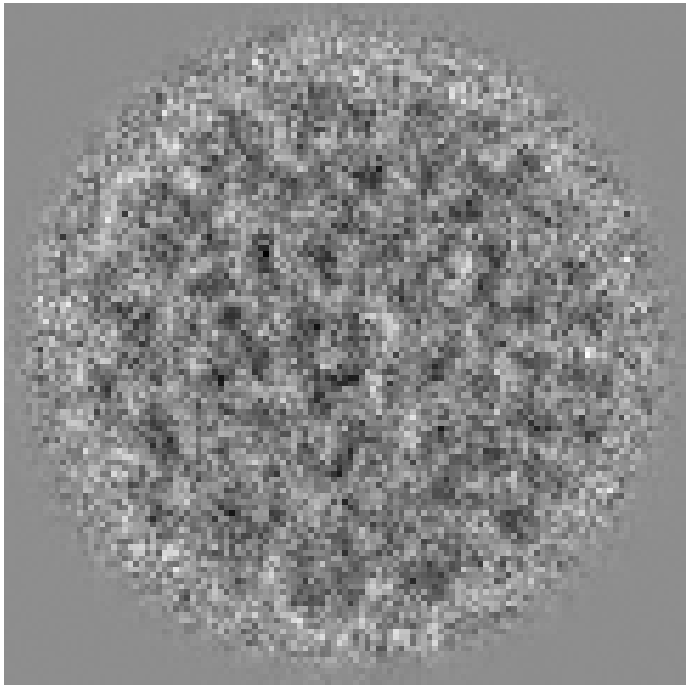




Floated

Icosahedral Virus 3D Reconstruction Scheme





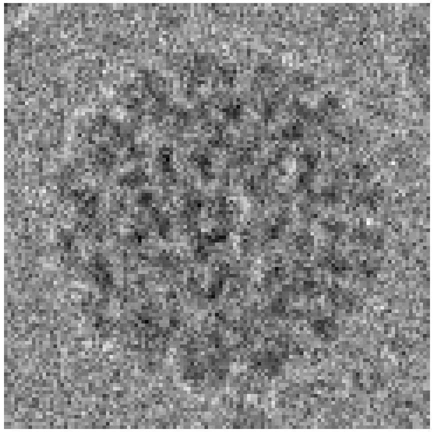
Apodized

Icosahedral Virus 3D Reconstruction Scheme

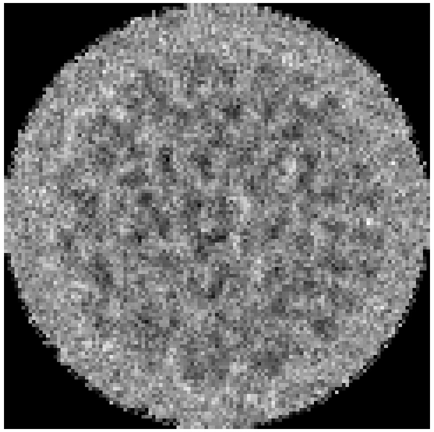
Digitize Micrograph

↓

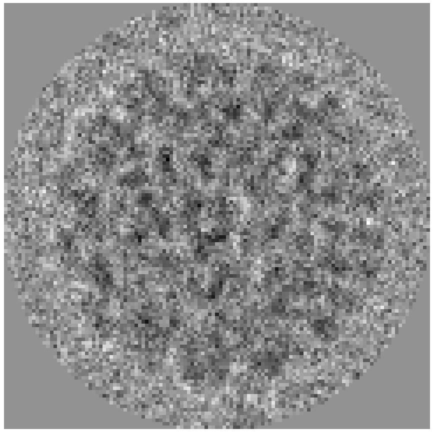
Box Particles



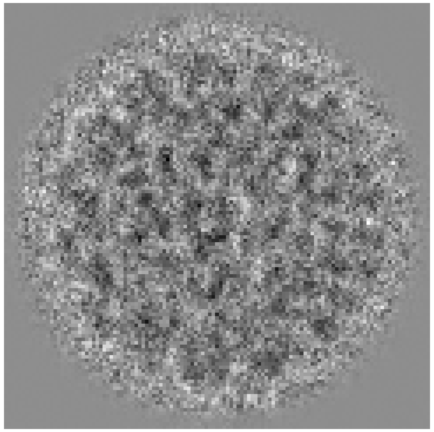
Extracted



Masked



Floated



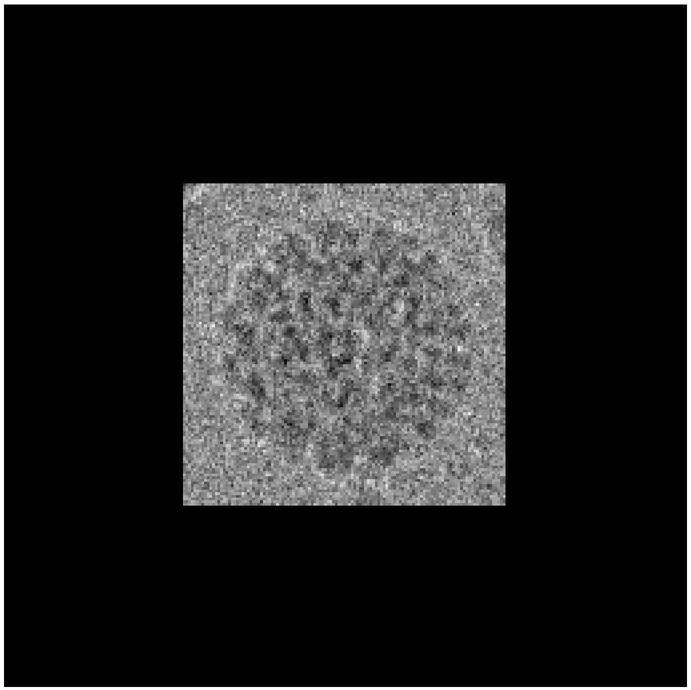
Apodized

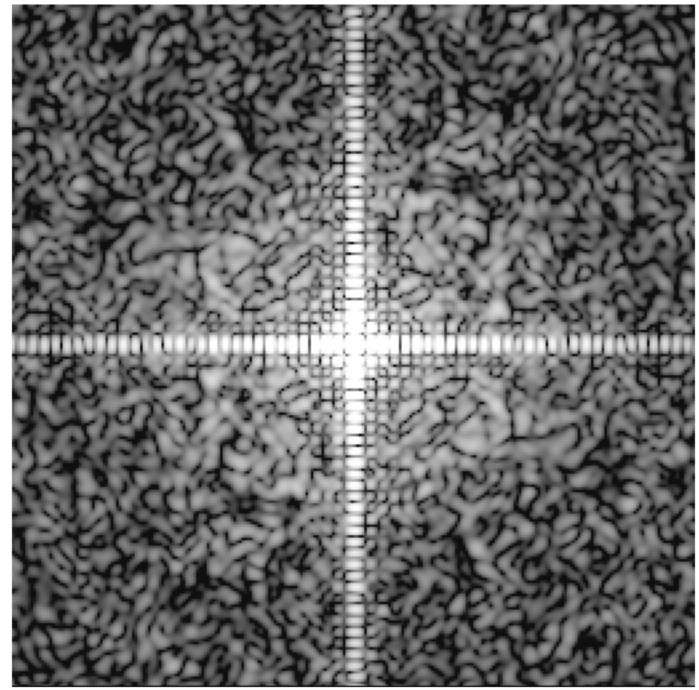
Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles





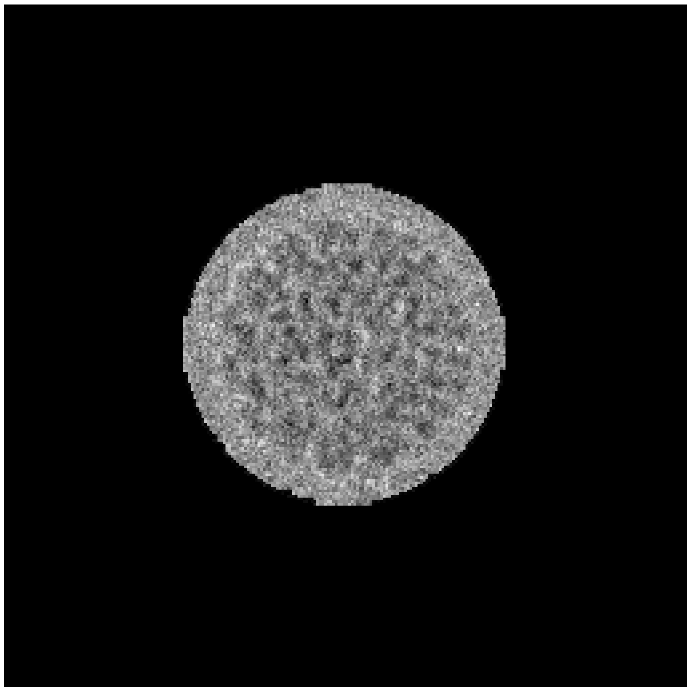
Square mask; **un**floated

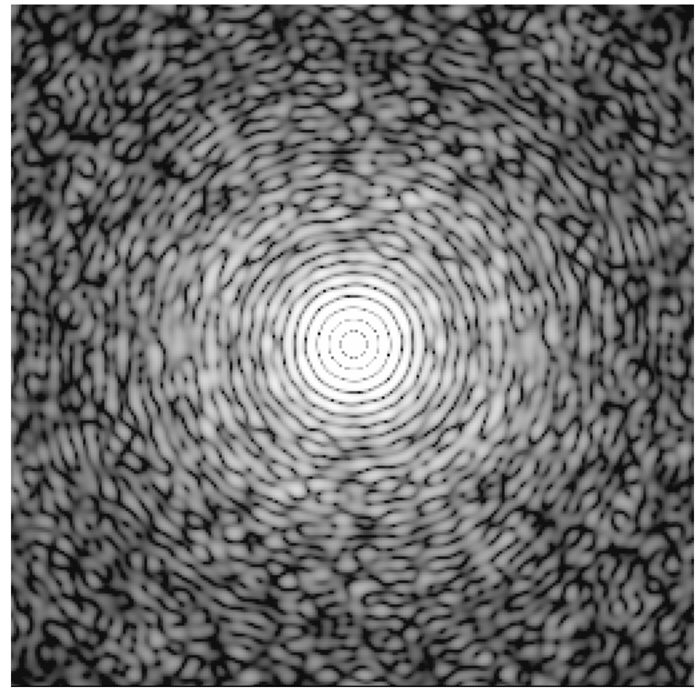
Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

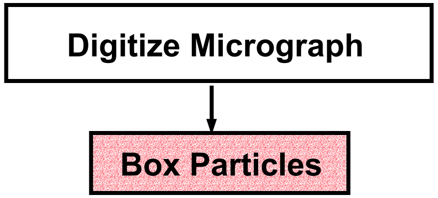
Box Particles

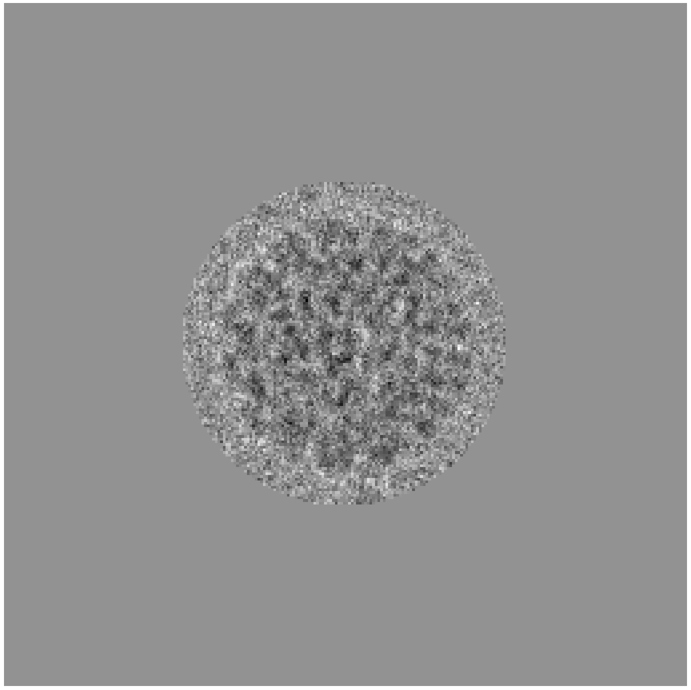


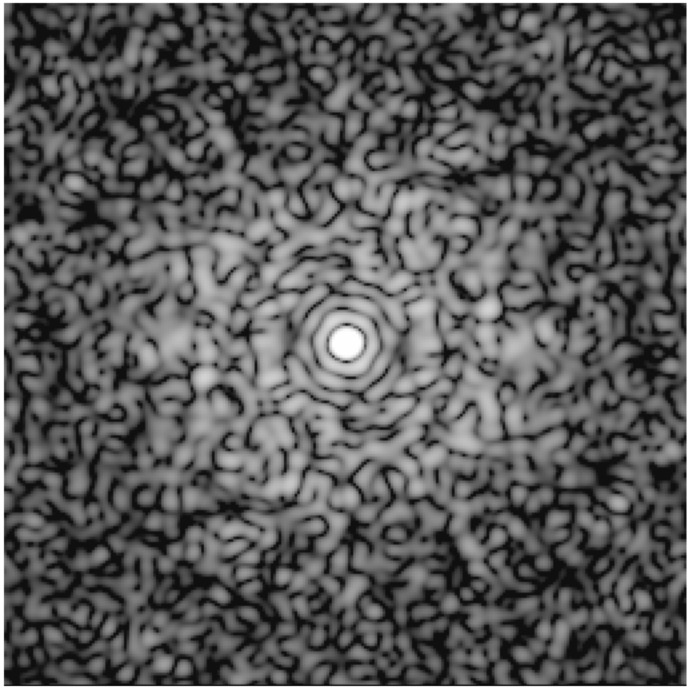


Circular mask; **<u>un</u>**floated

Icosahedral Virus 3D Reconstruction Scheme







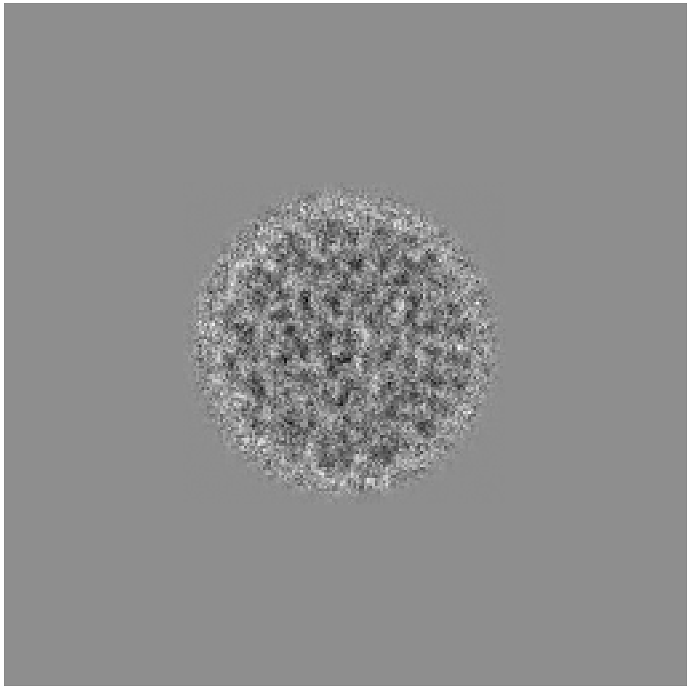
Circular mask; floated

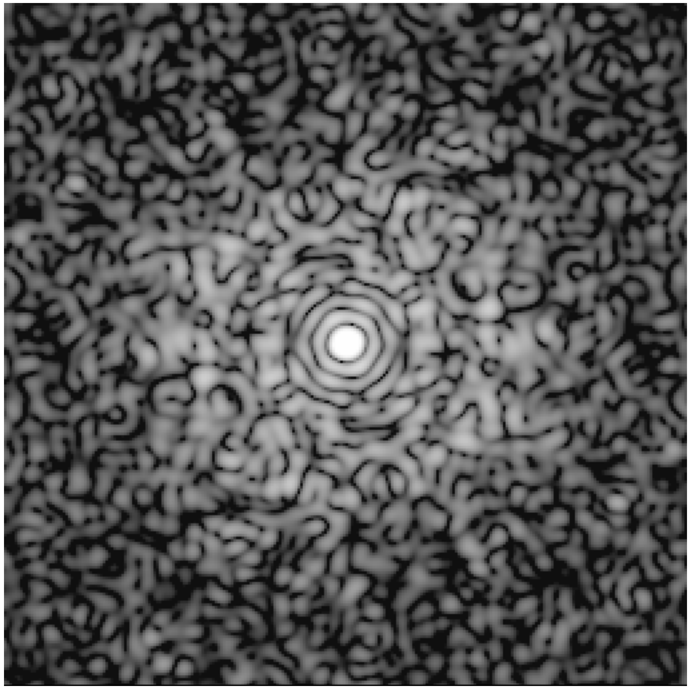
Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles





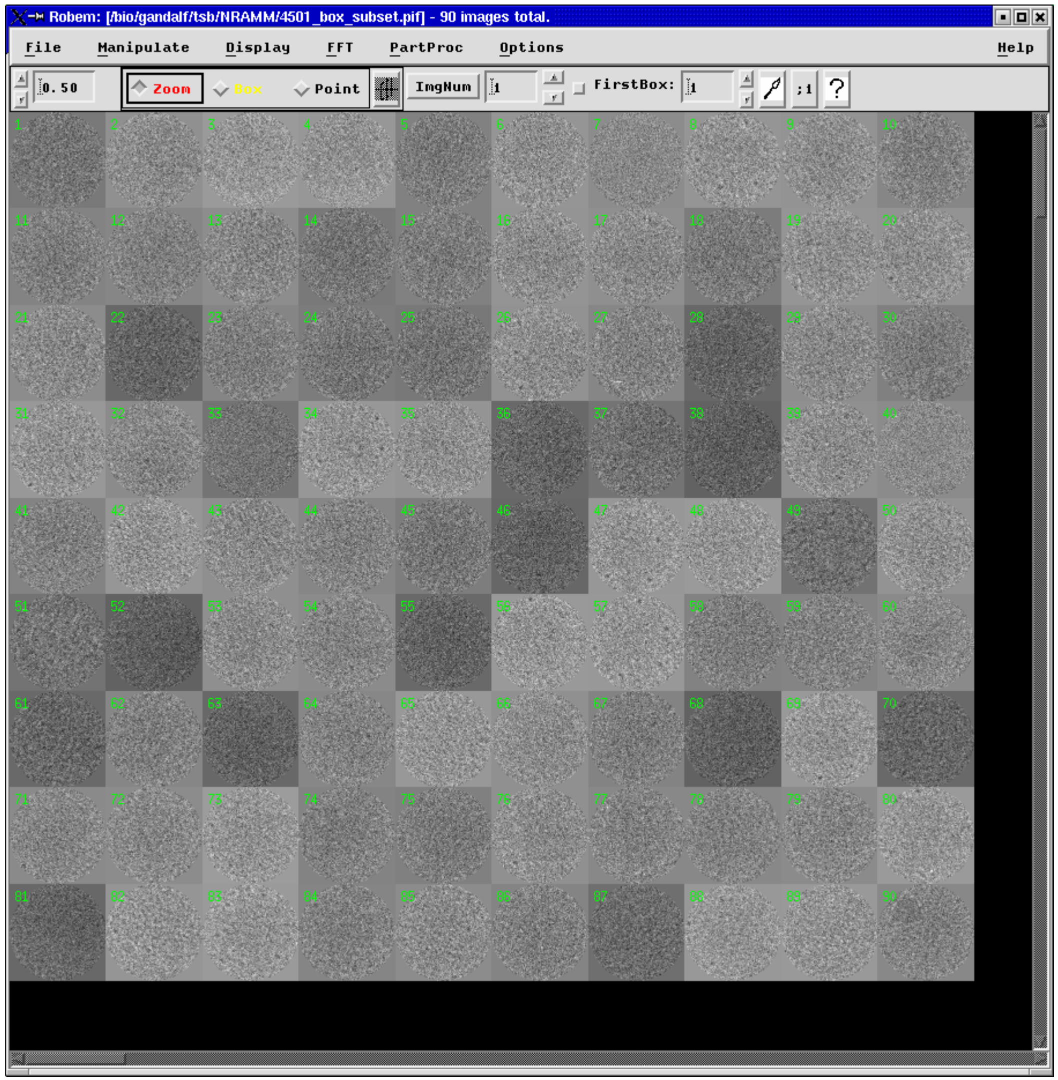
Circular mask; floated & apodized

Icosahedral Virus 3D Reconstruction Scheme

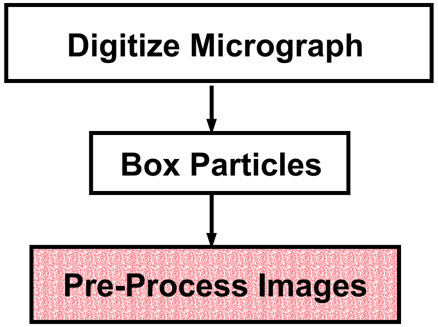
Digitize Micrograph

↓

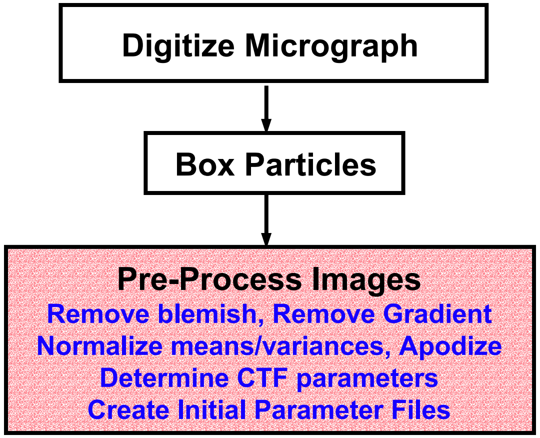
Box Particles



Icosahedral Virus 3D Reconstruction Scheme



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

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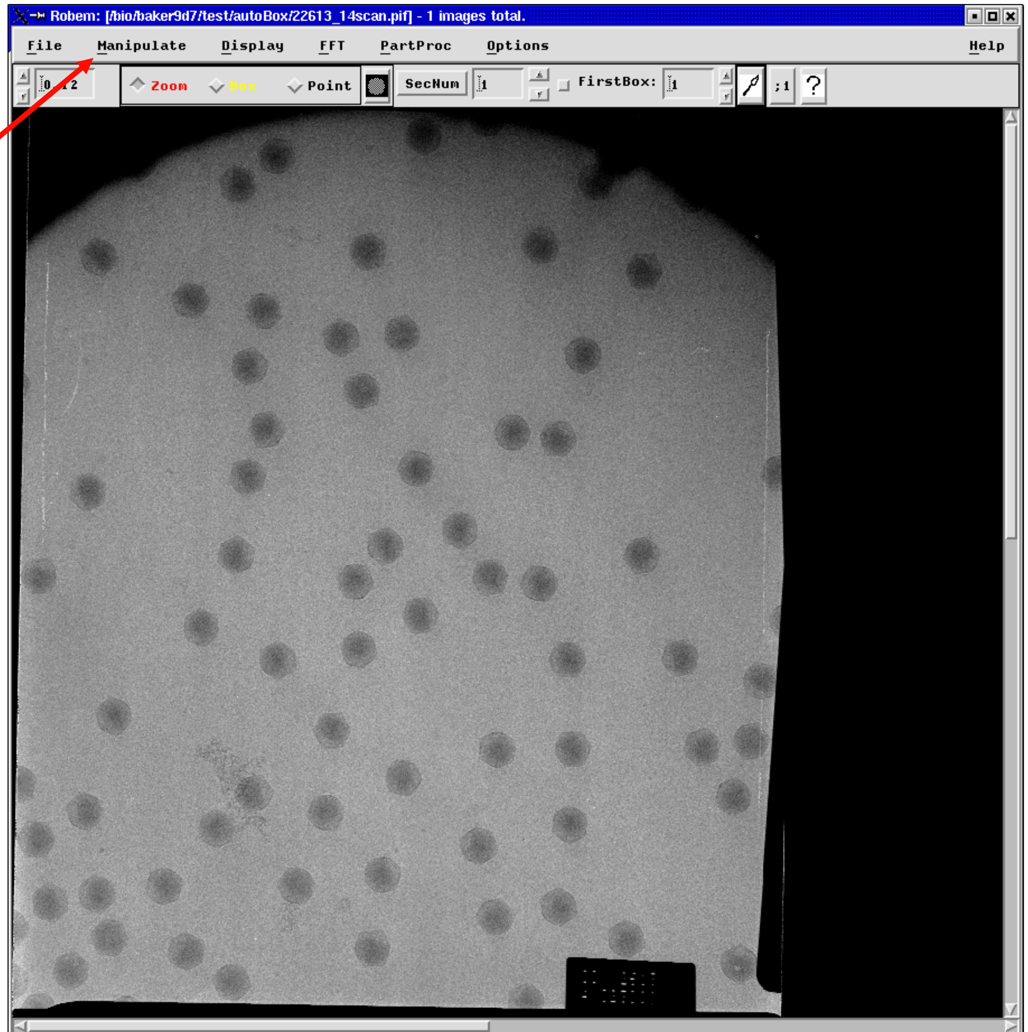

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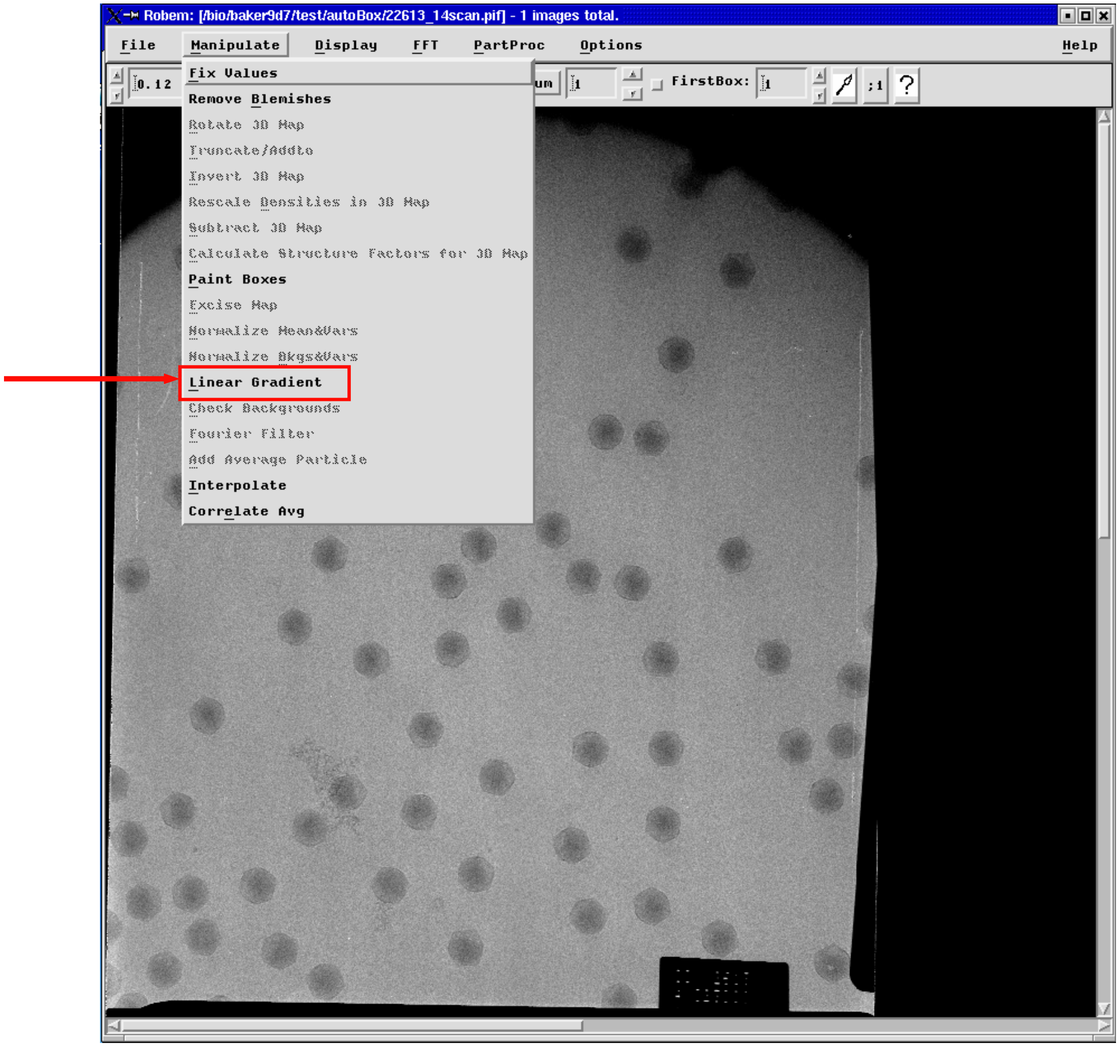

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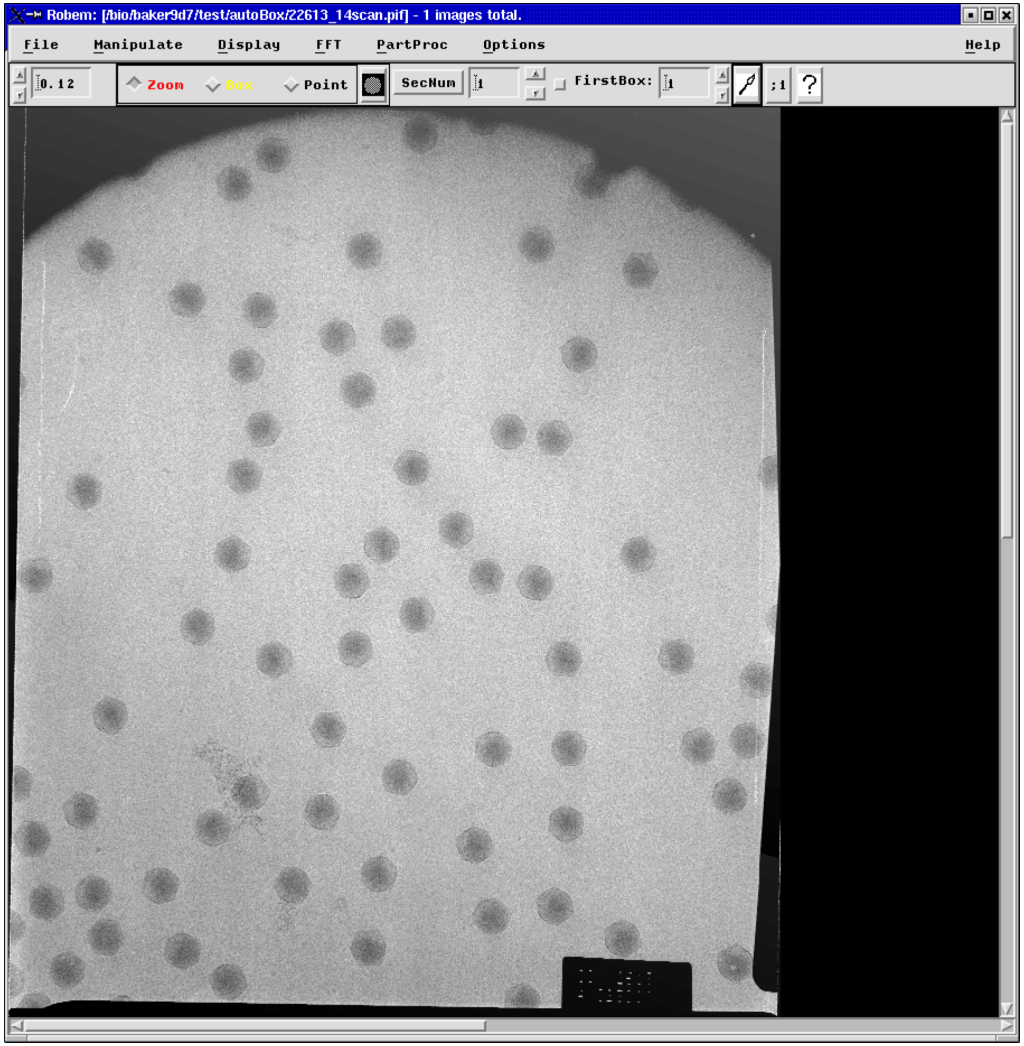

Gradient removed

Icosahedral Virus 3D Reconstruction Scheme

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Remove blemish, Remove Gradient
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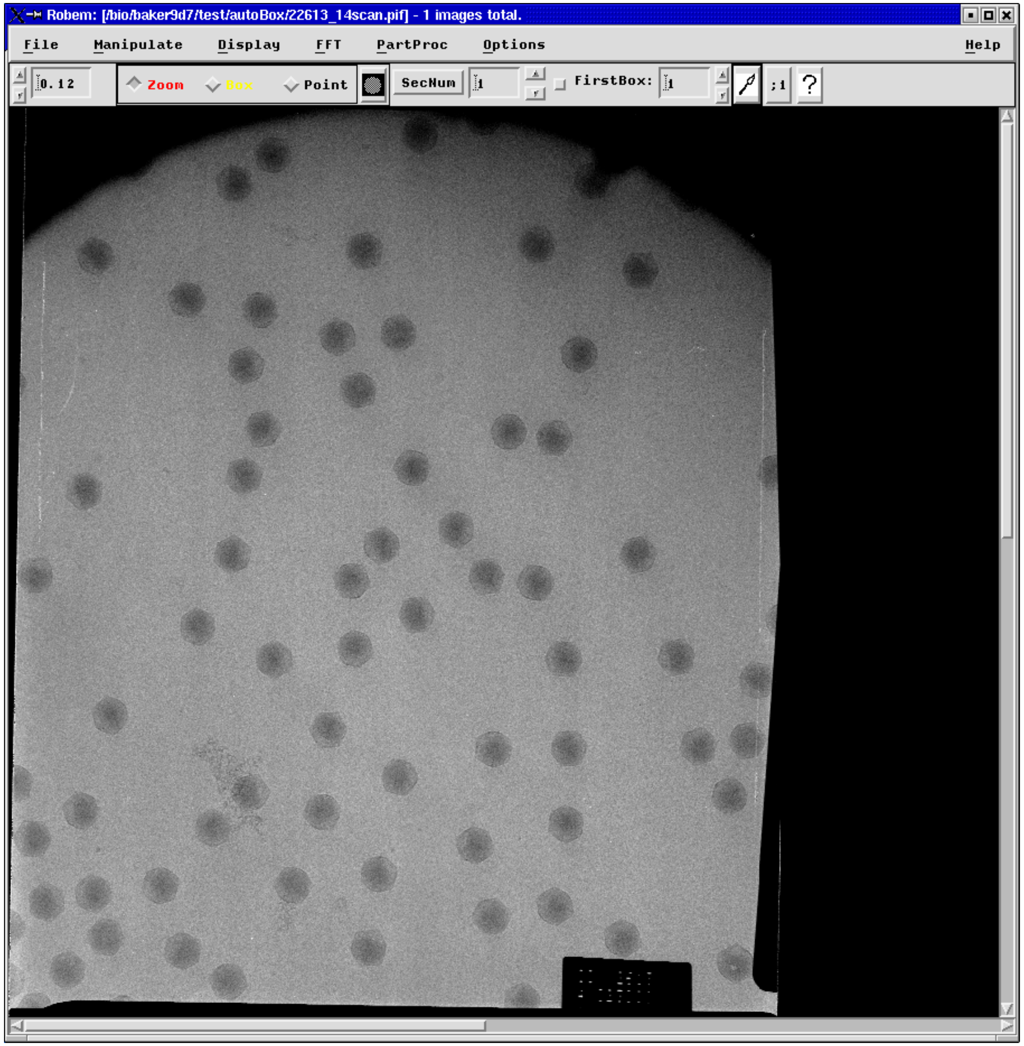

Gradient
not removed

Icosahedral Virus 3D Reconstruction Scheme

Pre-Process images

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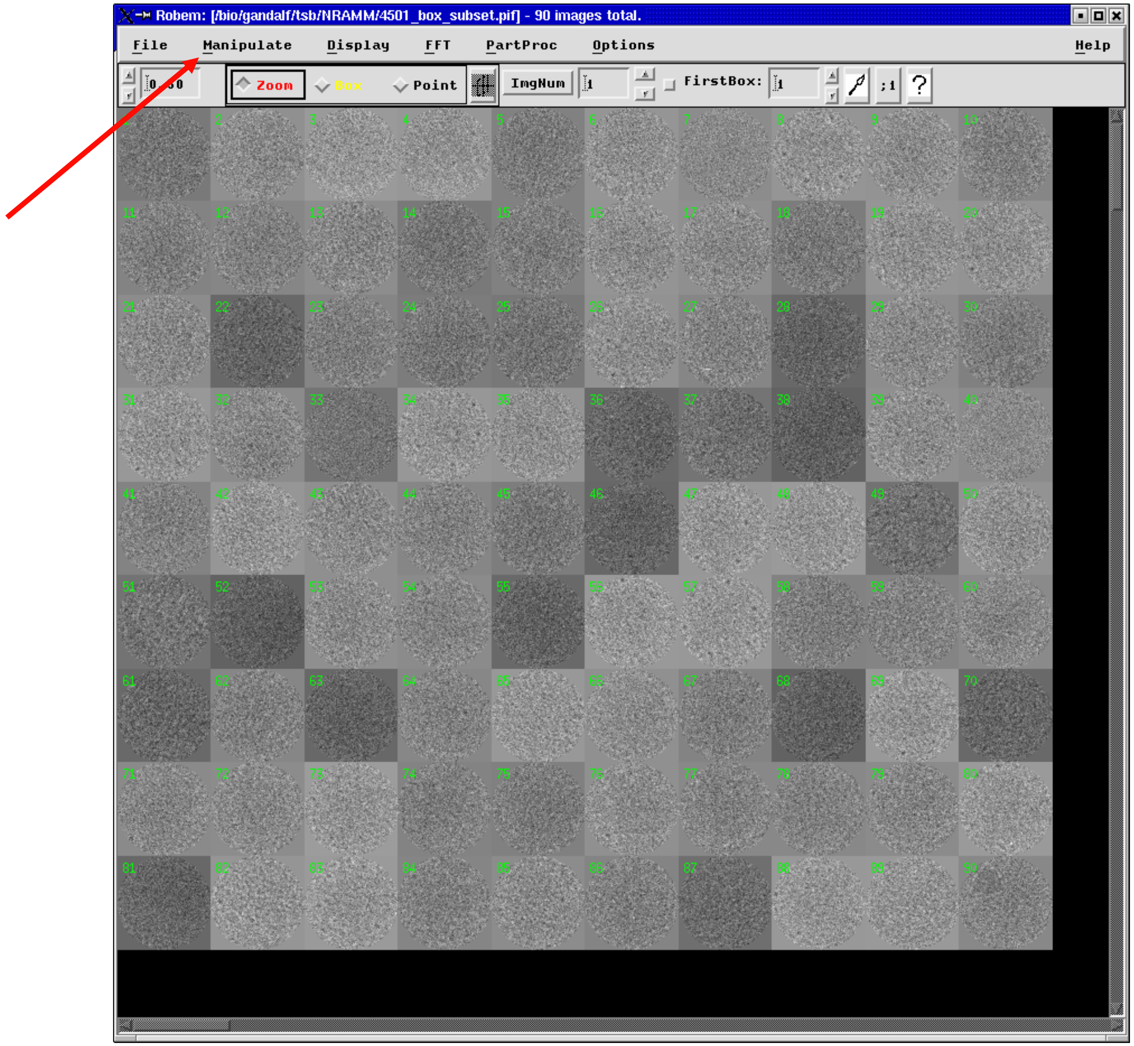

Icosahedral Virus 3D Reconstruction Scheme

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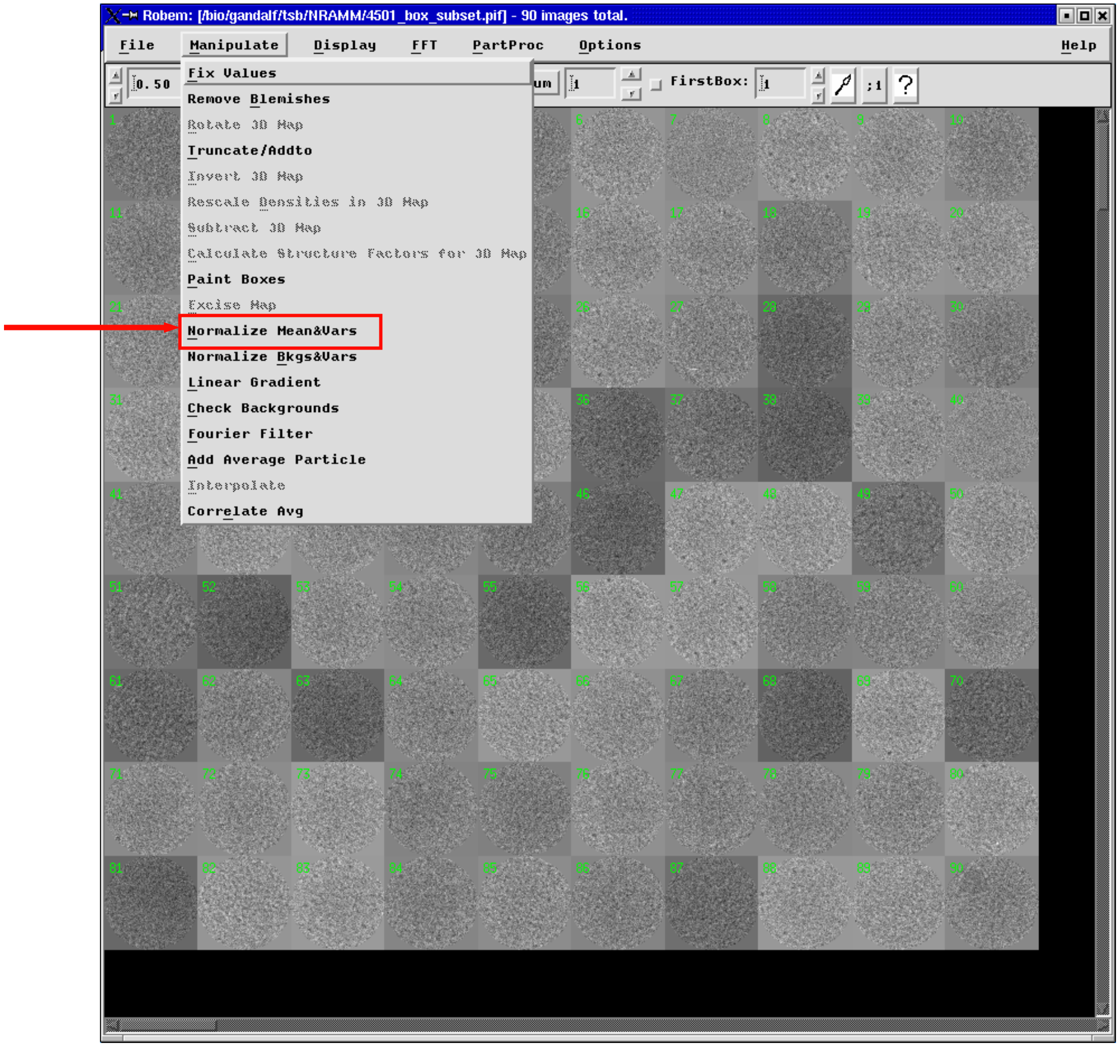

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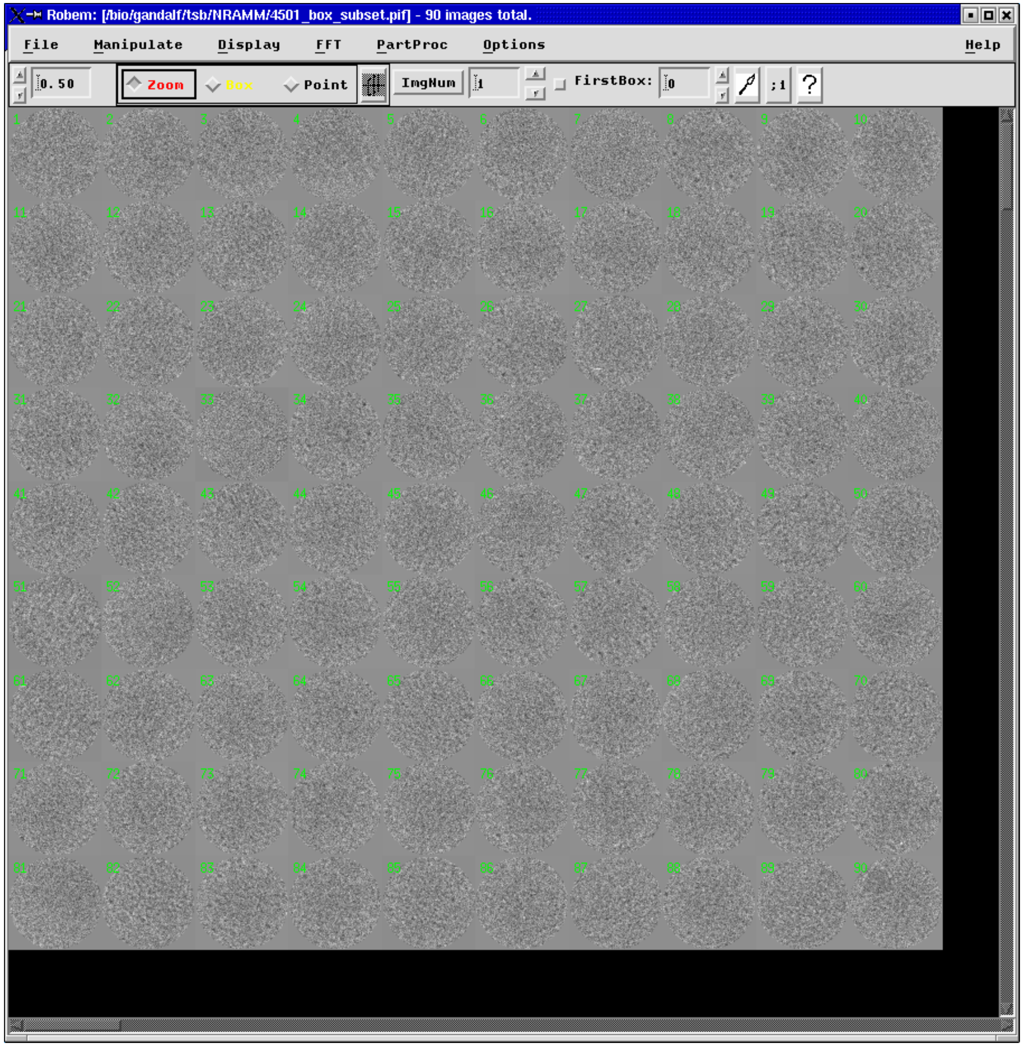

Icosahedral Virus 3D Reconstruction Scheme

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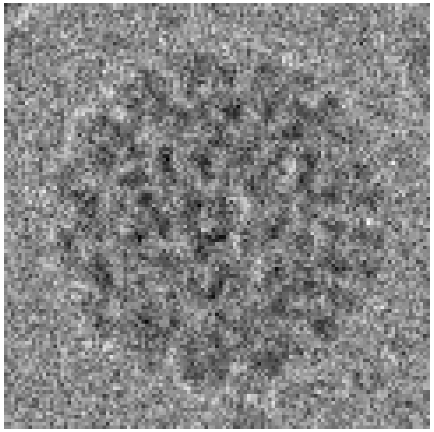
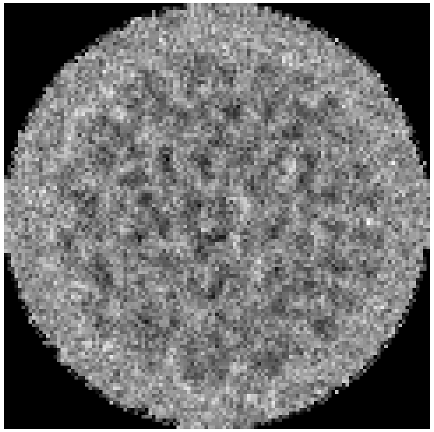
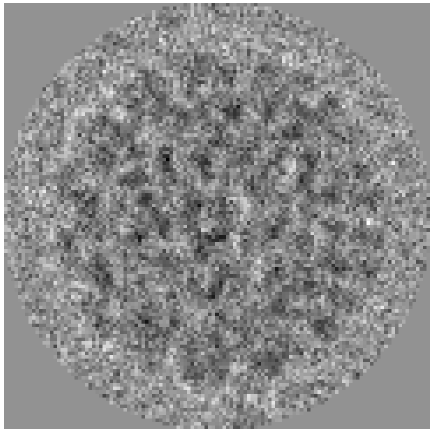


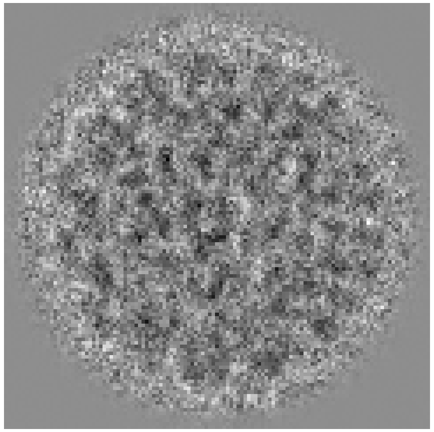
Extracted



Masked

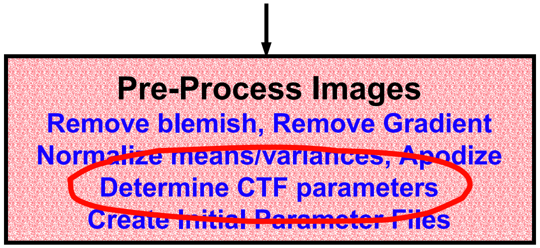


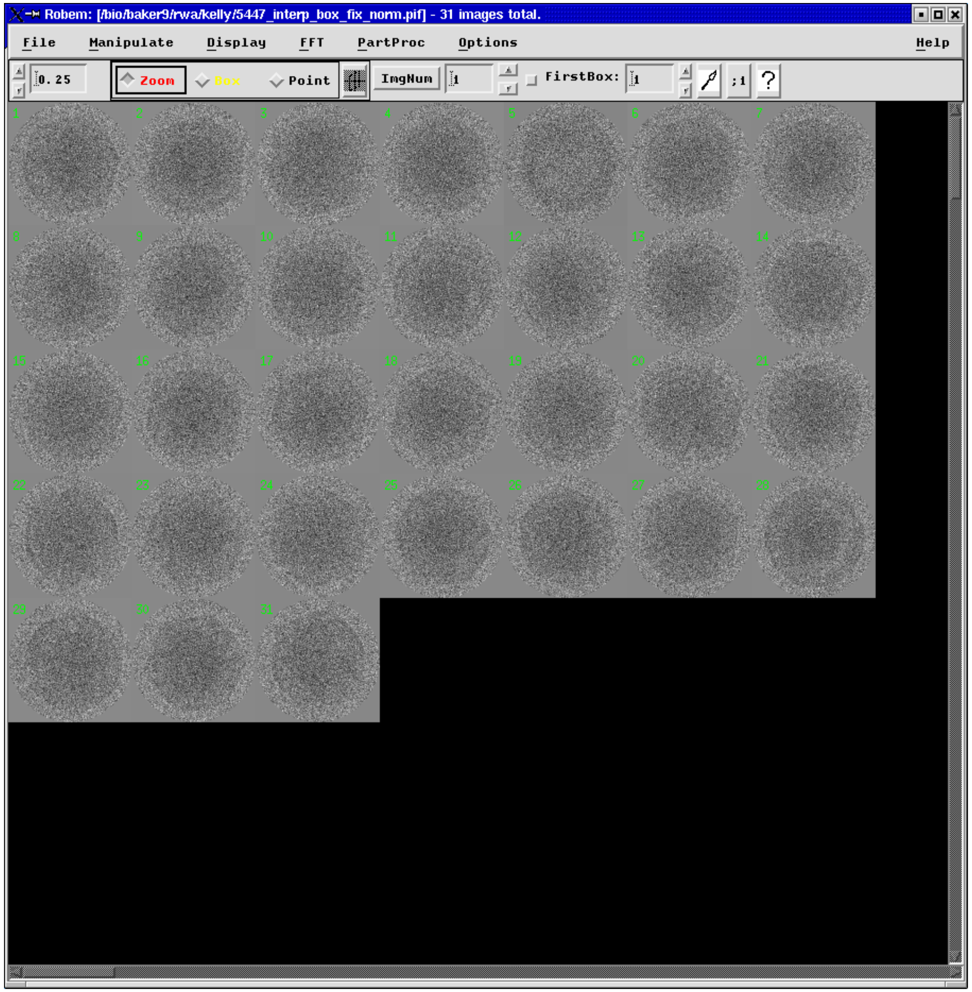
Floated



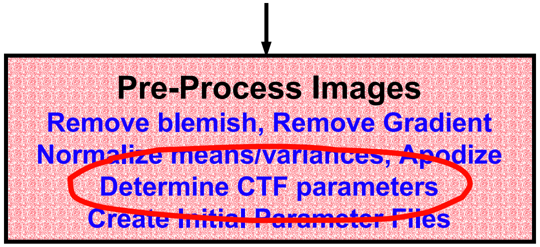
Apodized

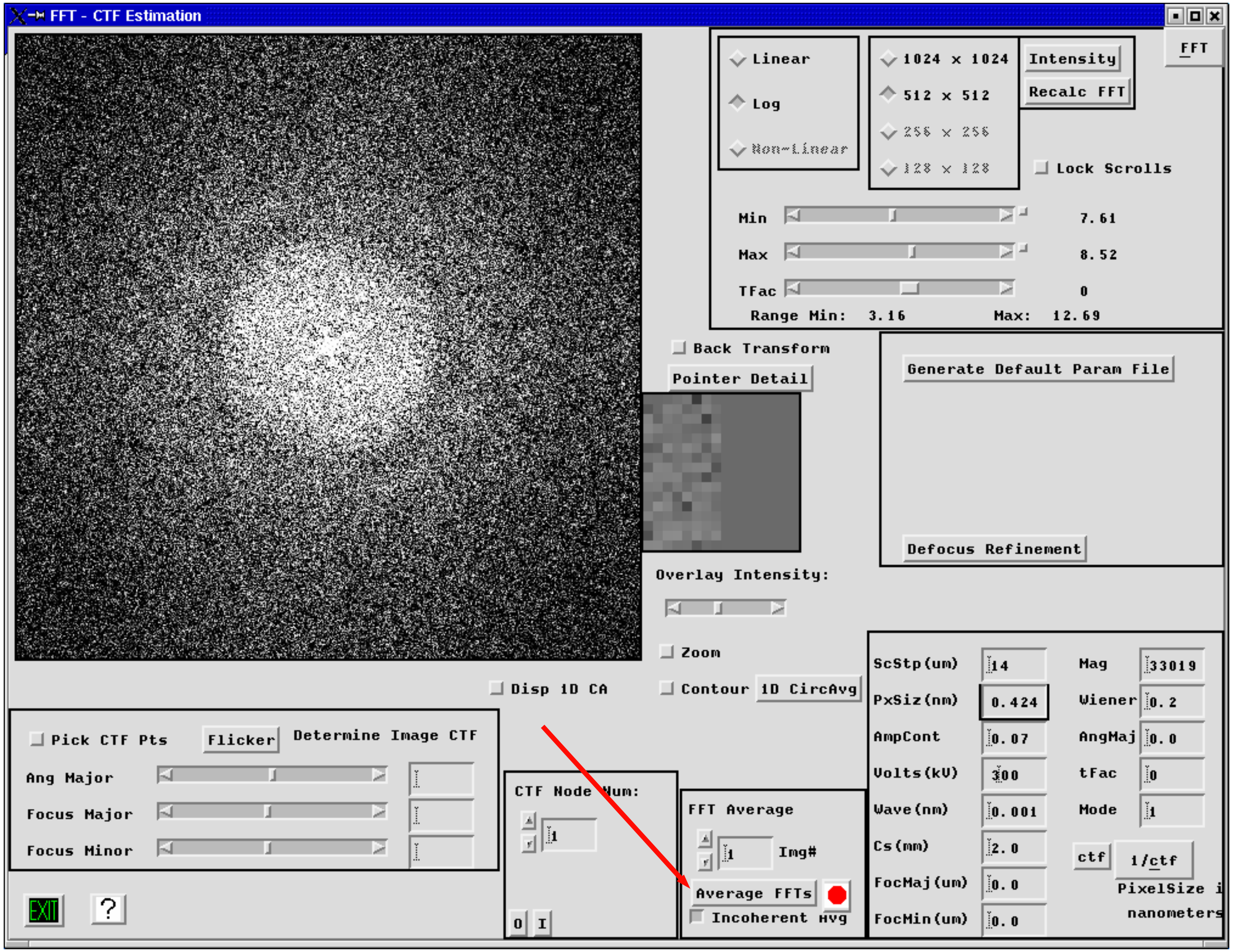
Icosahedral Virus 3D Reconstruction Scheme





Icosahedral Virus 3D Reconstruction Scheme





Icosahedral Virus 3D Reconstruction Scheme

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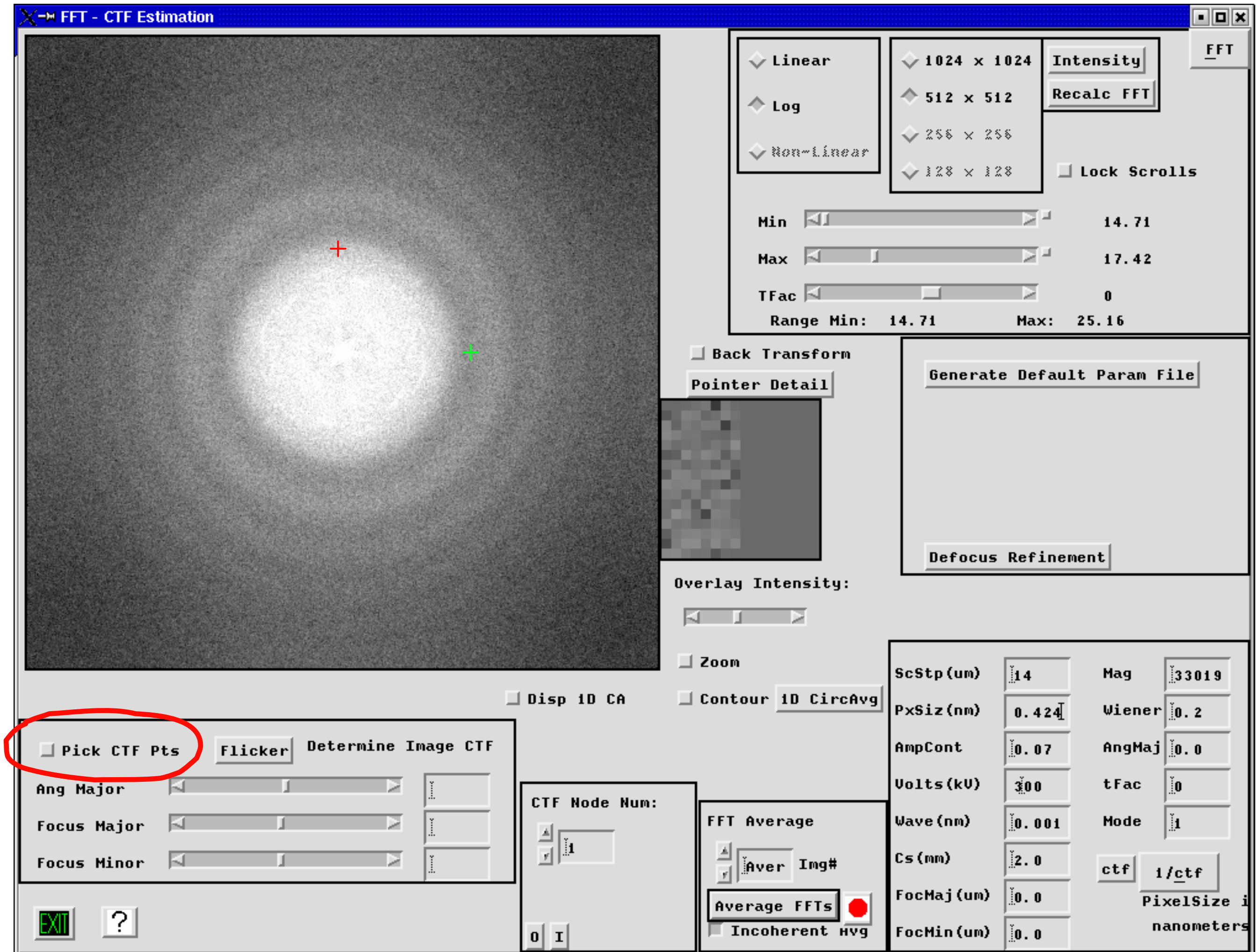

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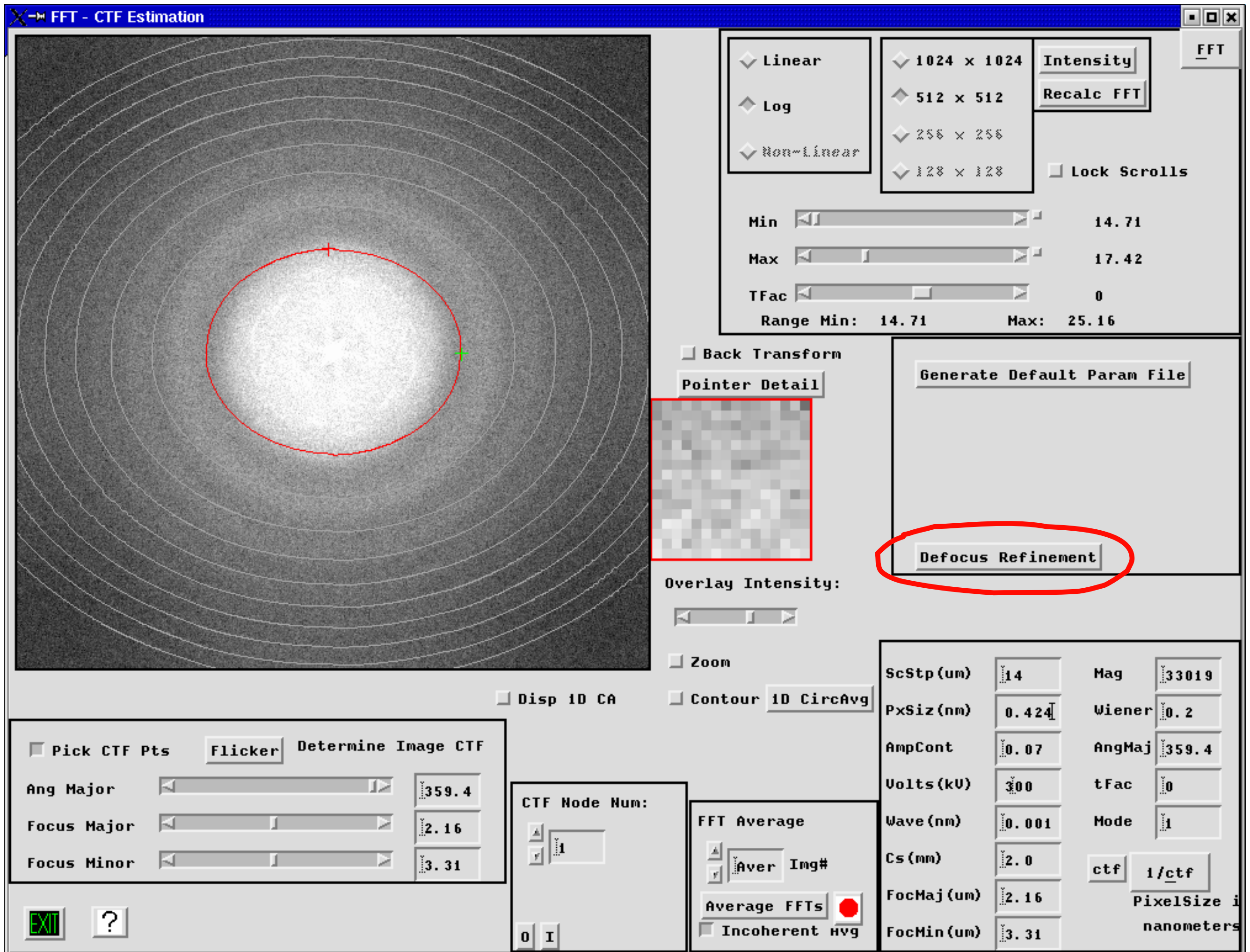

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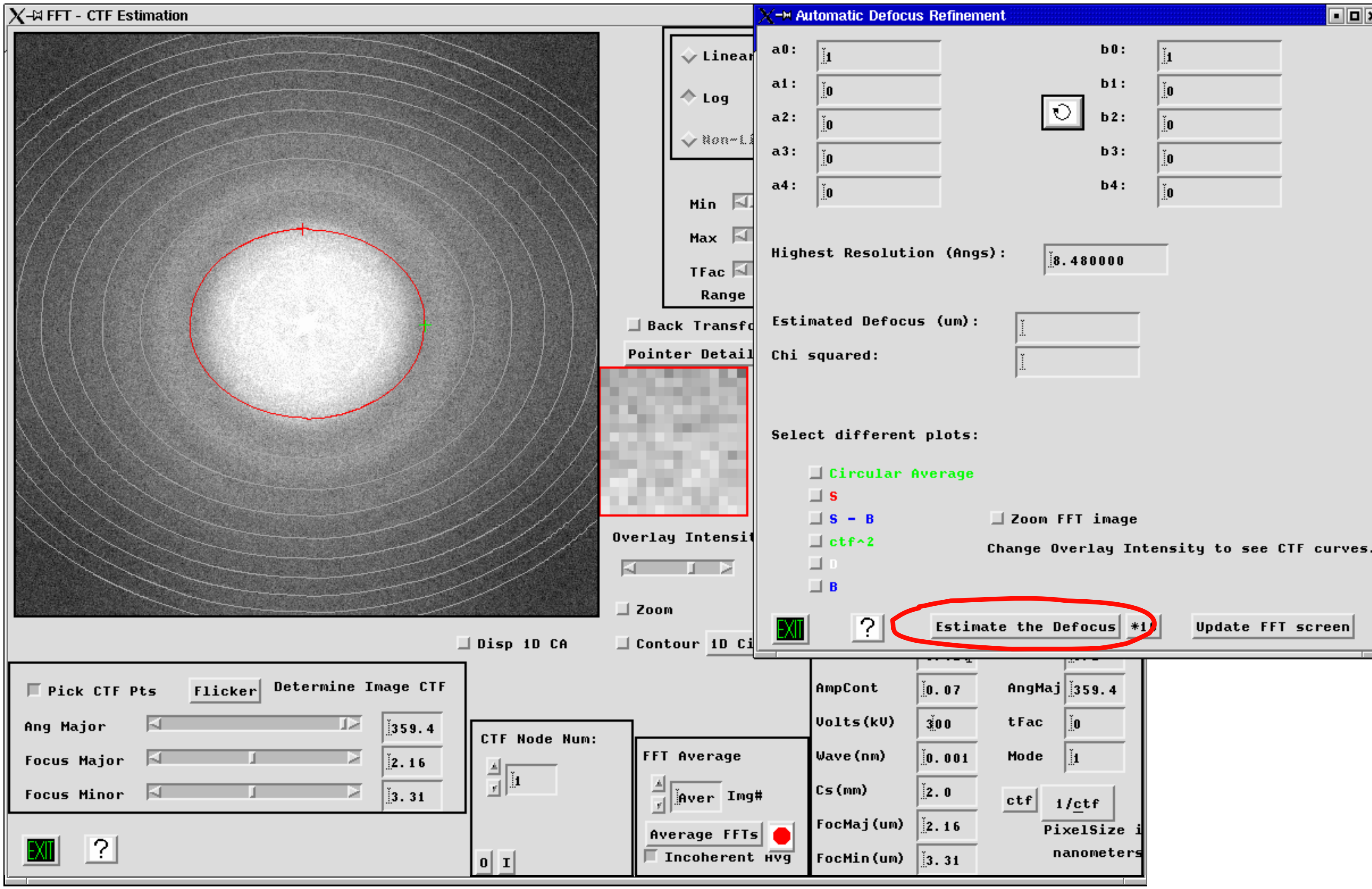

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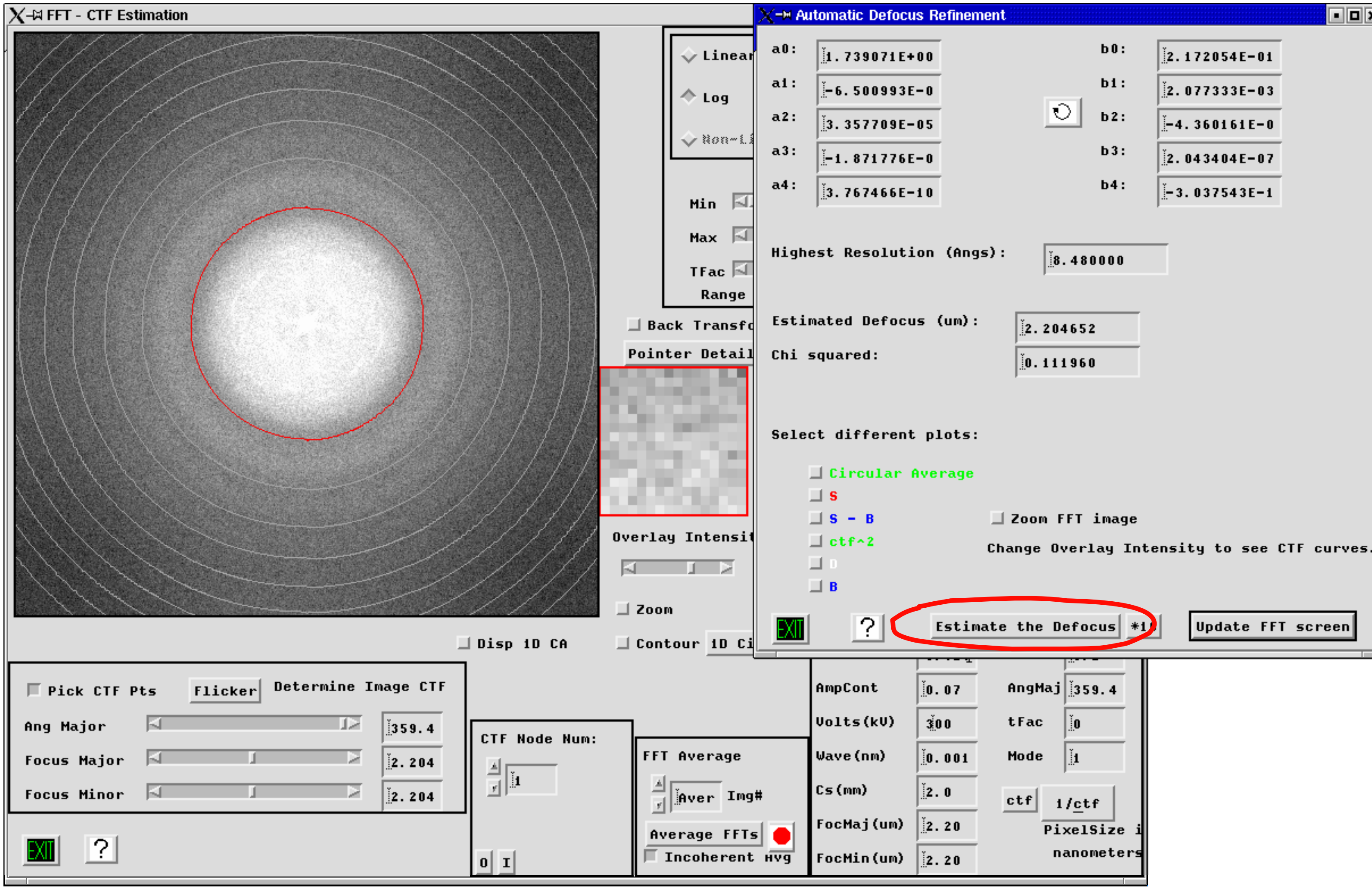

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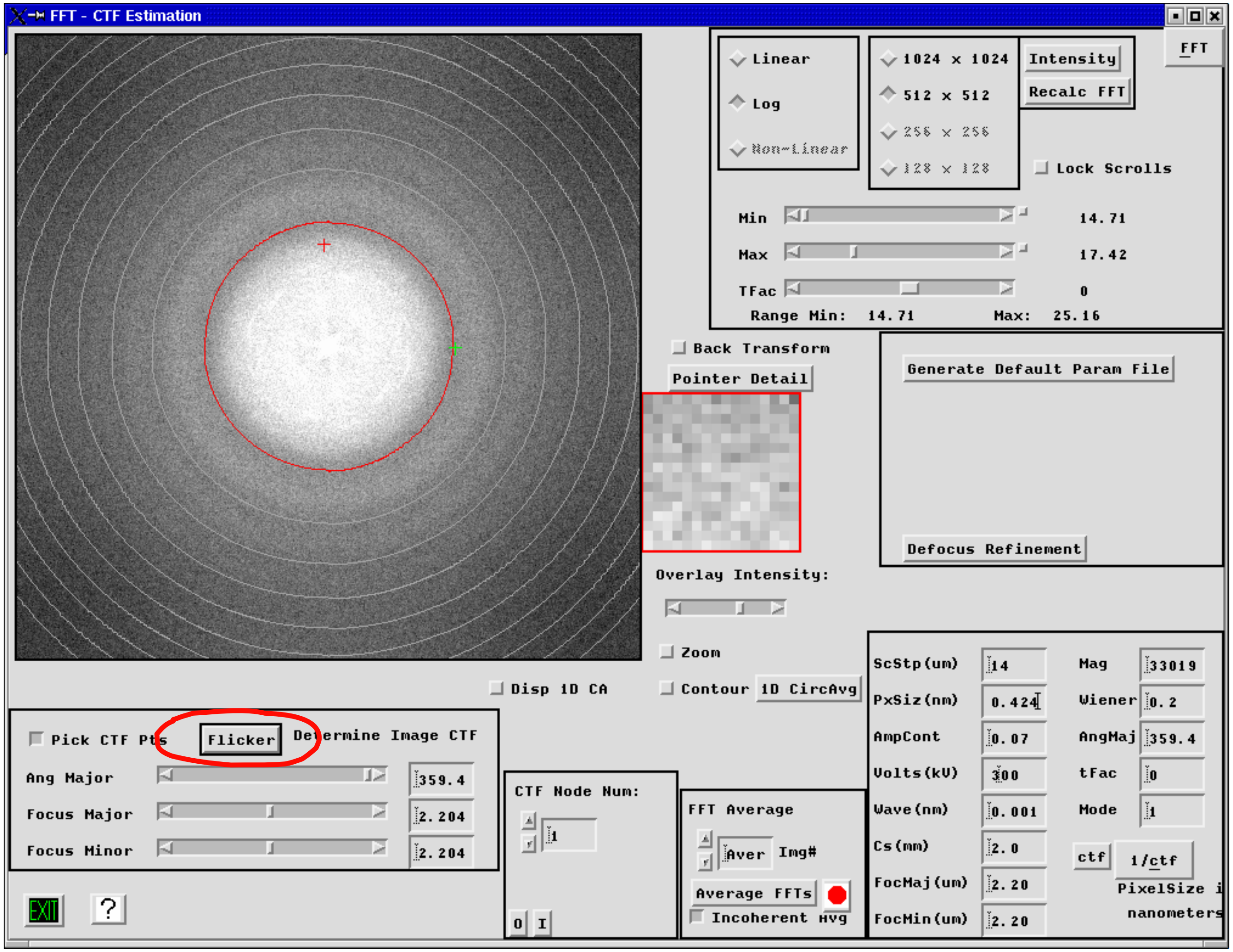

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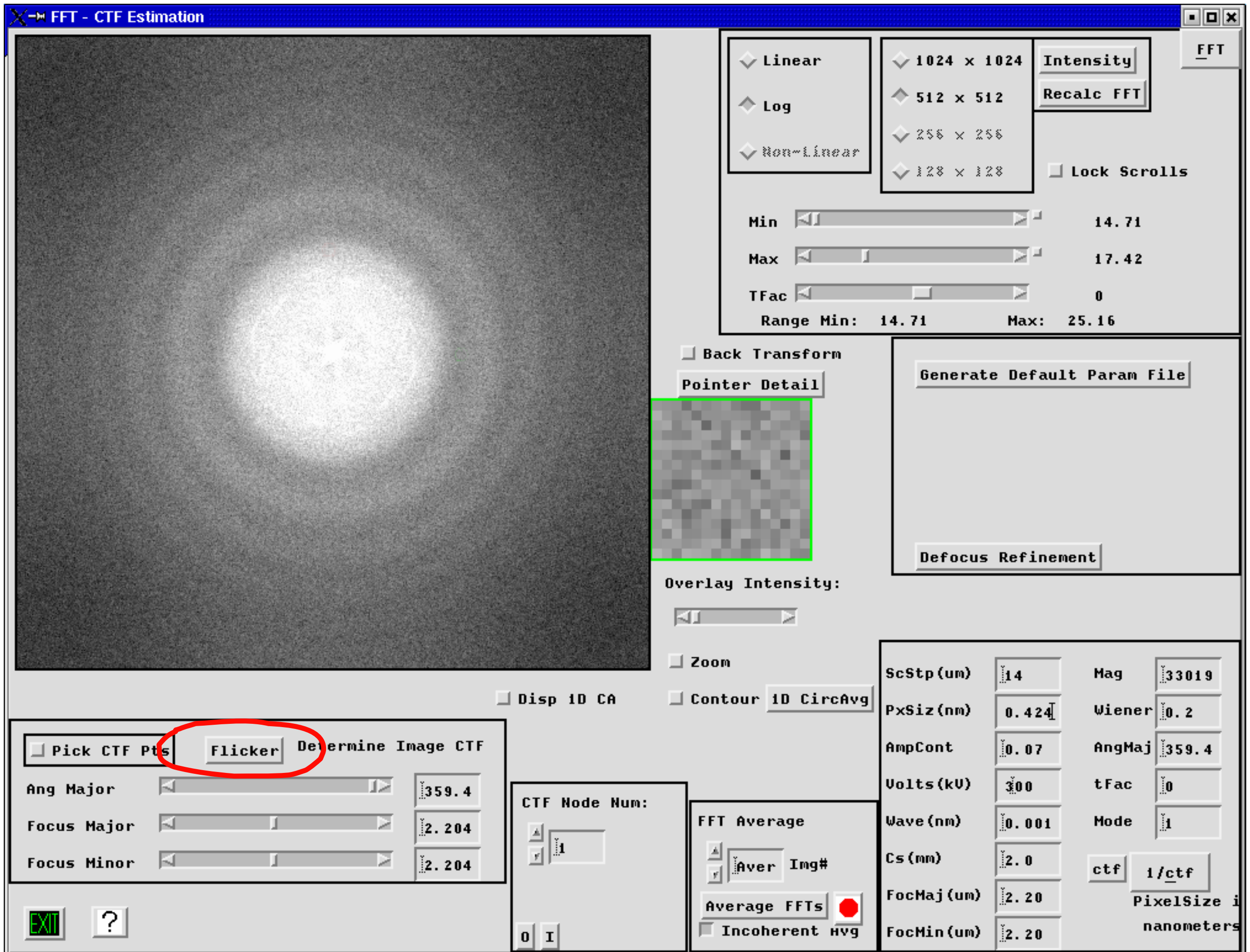

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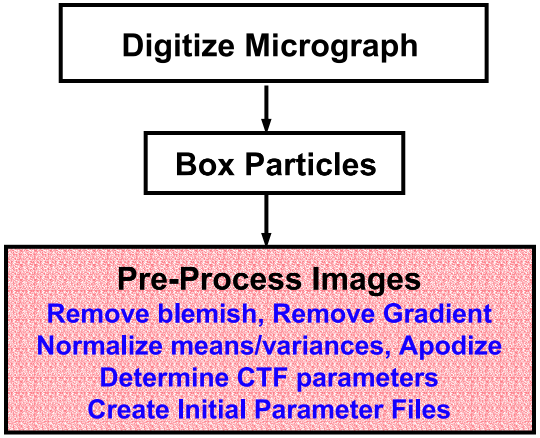
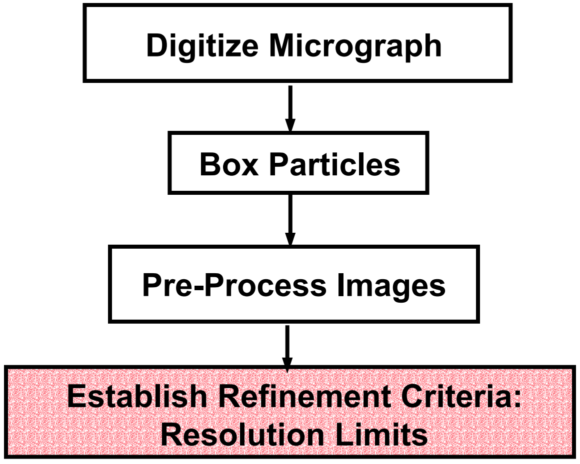
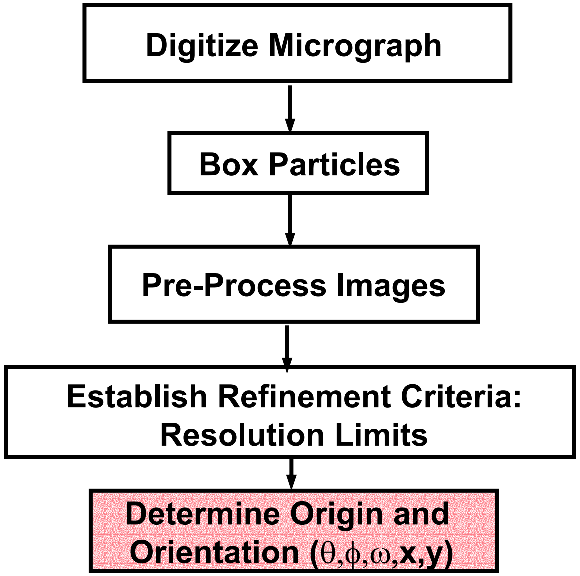


Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

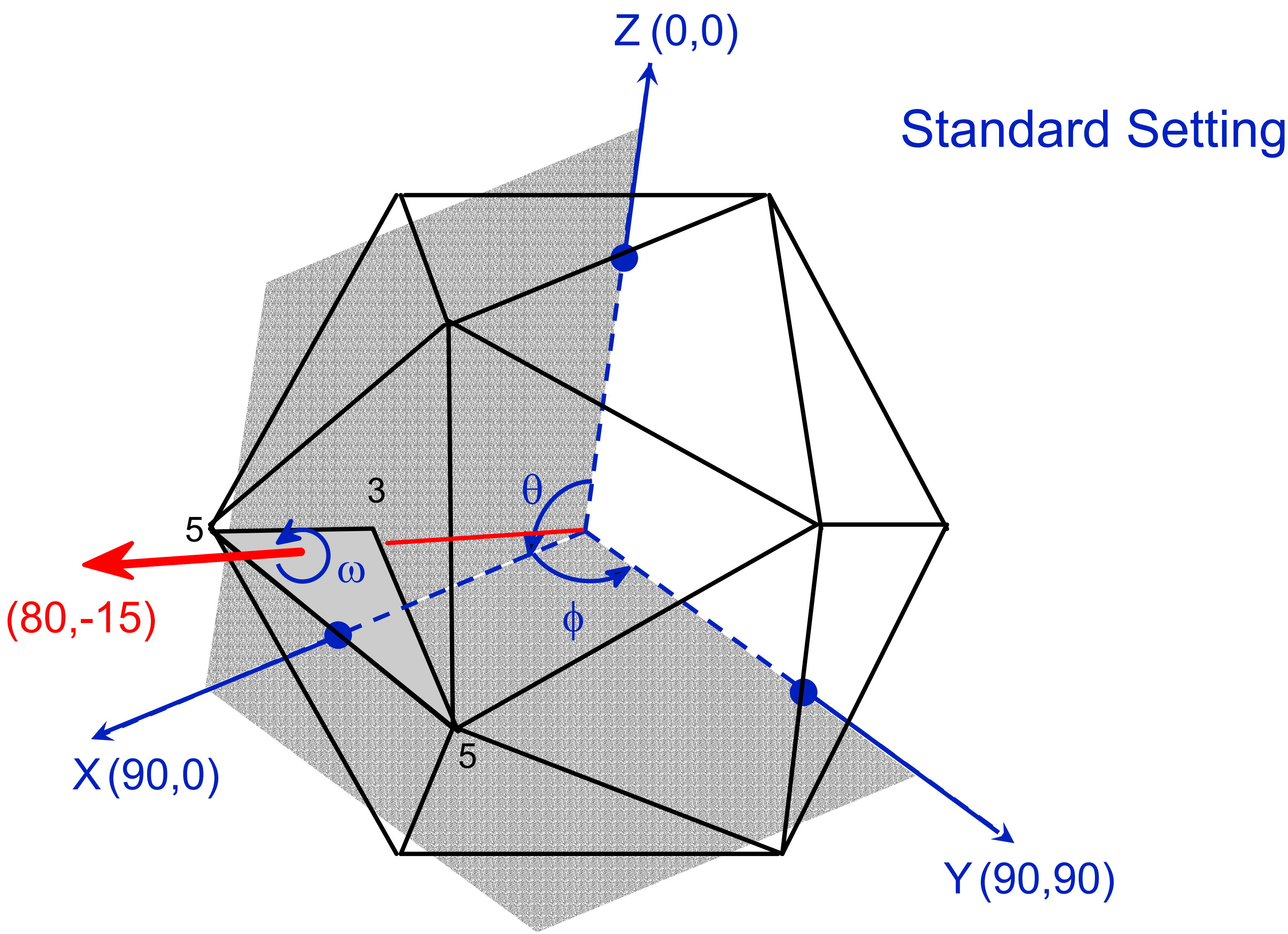
Determine Origin and Orientation (θ,ϕ,ω,x,y)

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

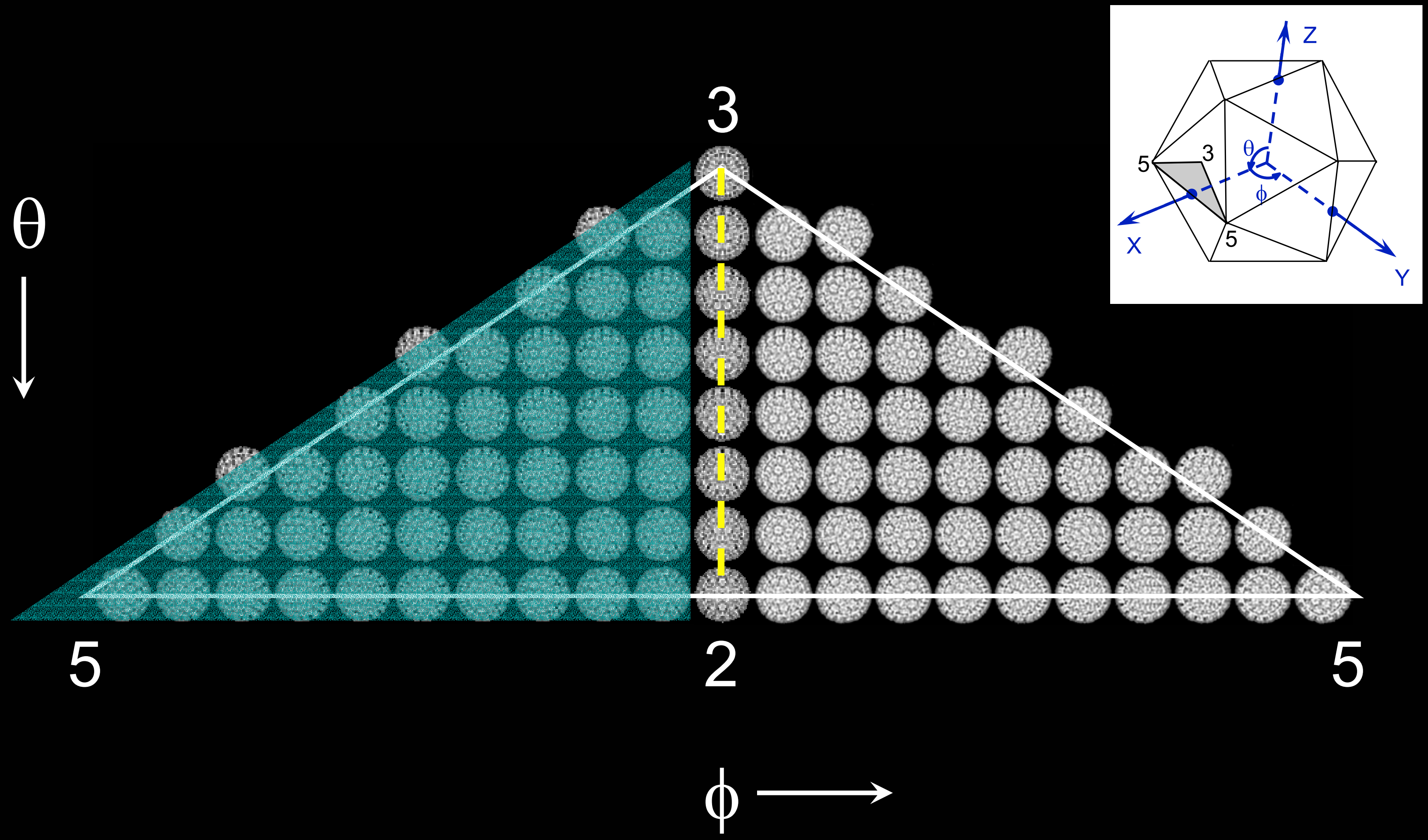
MOST IMPORTANT STEP?

Garbage in ------> garbage out

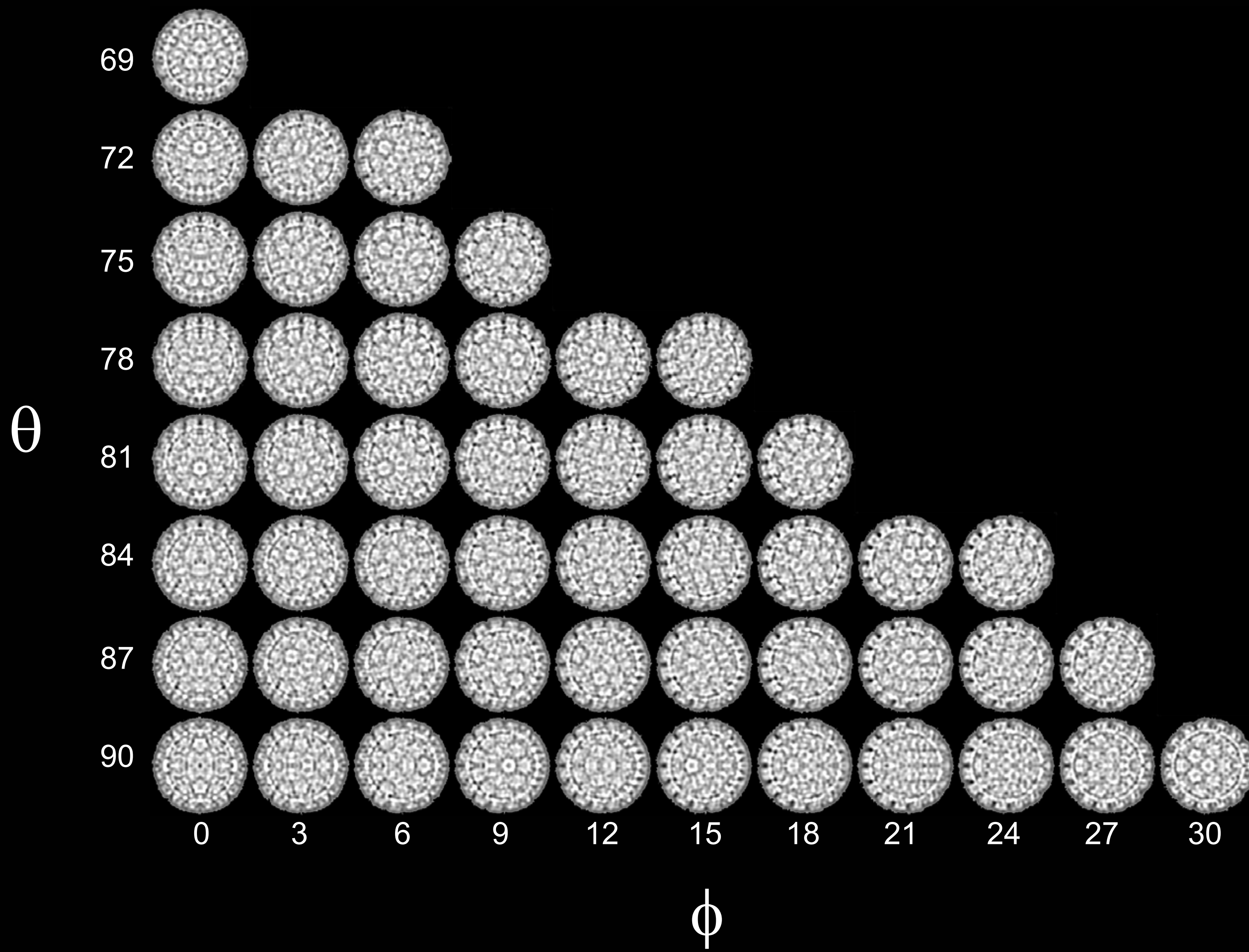
Specifying Direction of View: (θ,ϕ,ω) Orientation



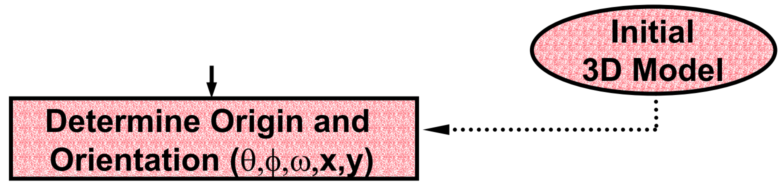
BPV Projections: Icosahedral ASU



BPV Projections: 1/2 Icosahedral ASU



Icosahedral Virus 3D Reconstruction Scheme



How do we determine the (θ, ϕ, ω, 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

Determine Origin and Orientation (θ,ϕ,ω,x,y)

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.”

Determine Origin and Orientation (θ,φ,ω,x,y)

Common Lines

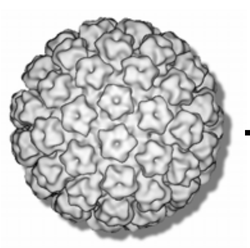
The ‘gospel’ continued:

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

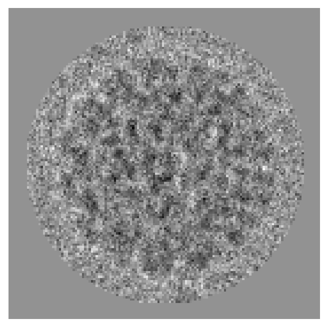
“A similar pair of lines will be generated by each possible choice of pairs of symmetry operations”

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

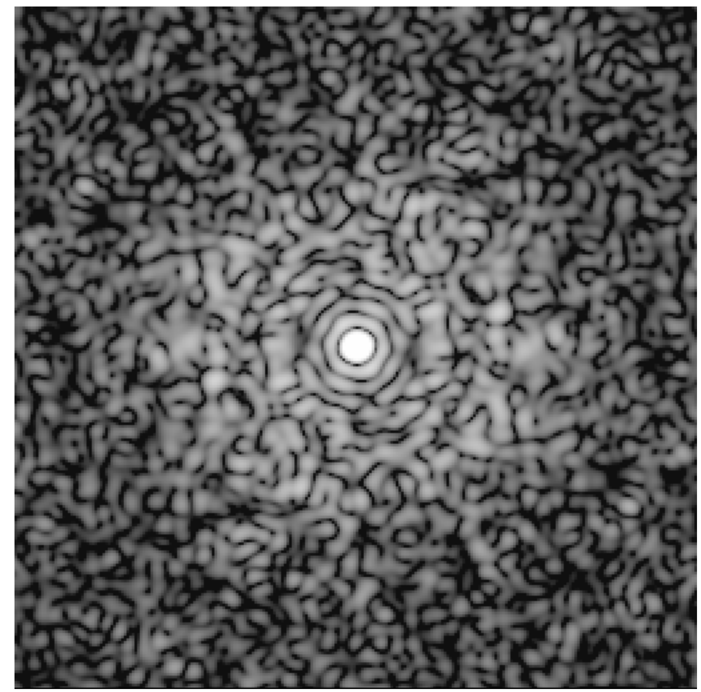
Orientation Determination by Common Lines



3D Object



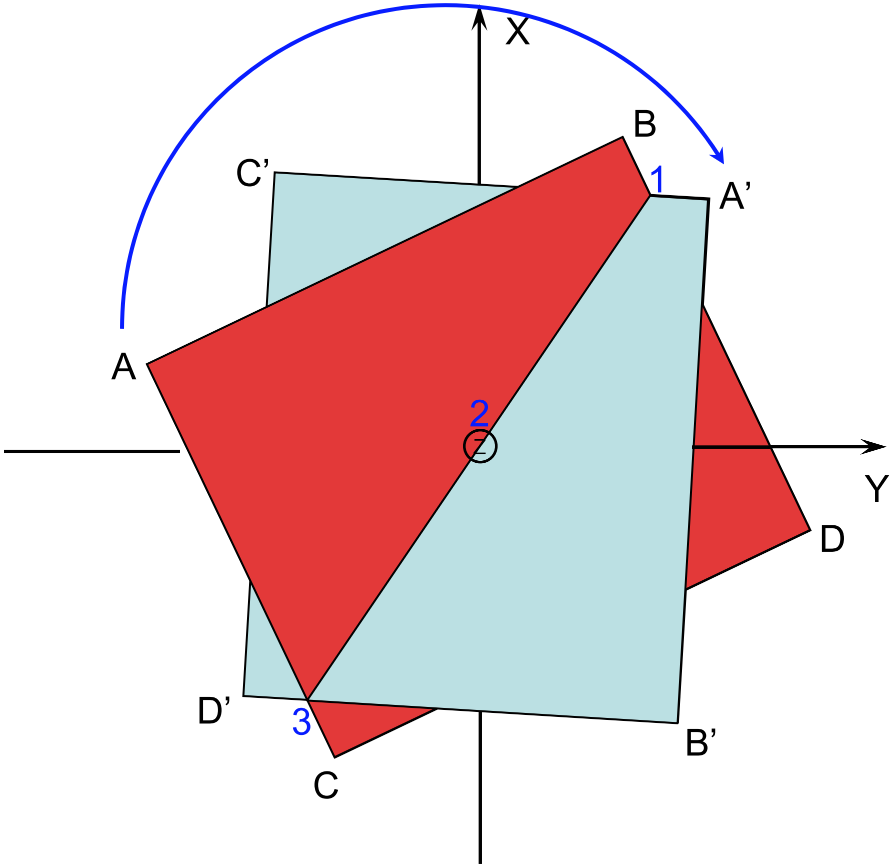
2D Projection
(θ,ϕ,ω)



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

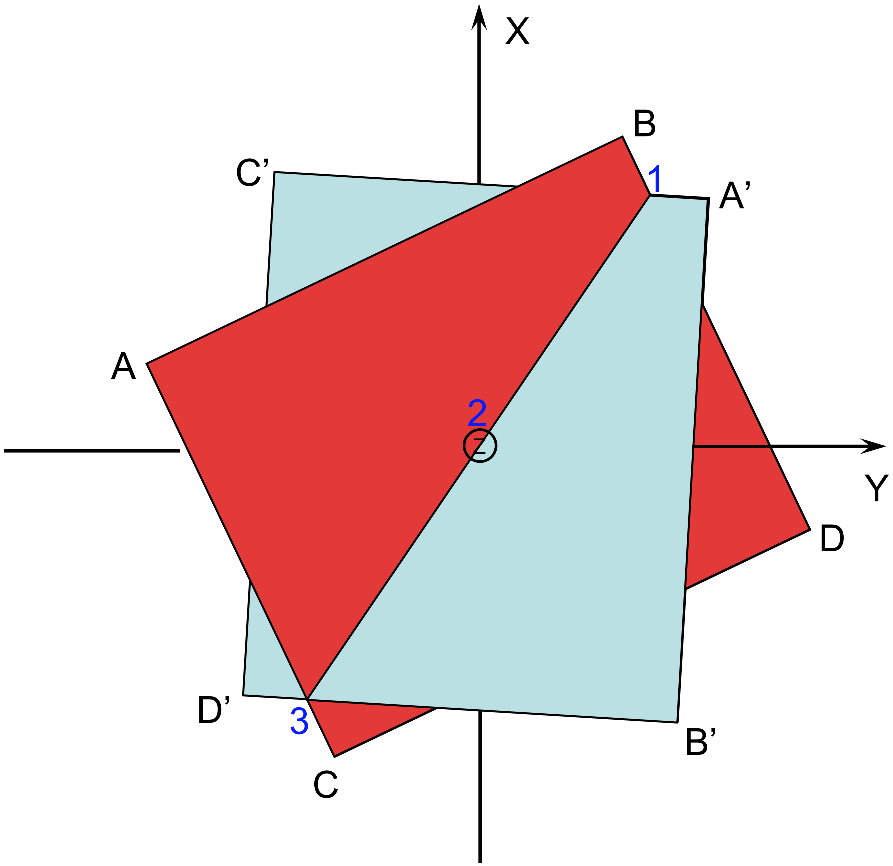
Let z-direction coincide with **3-fold** axis of symmetry

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

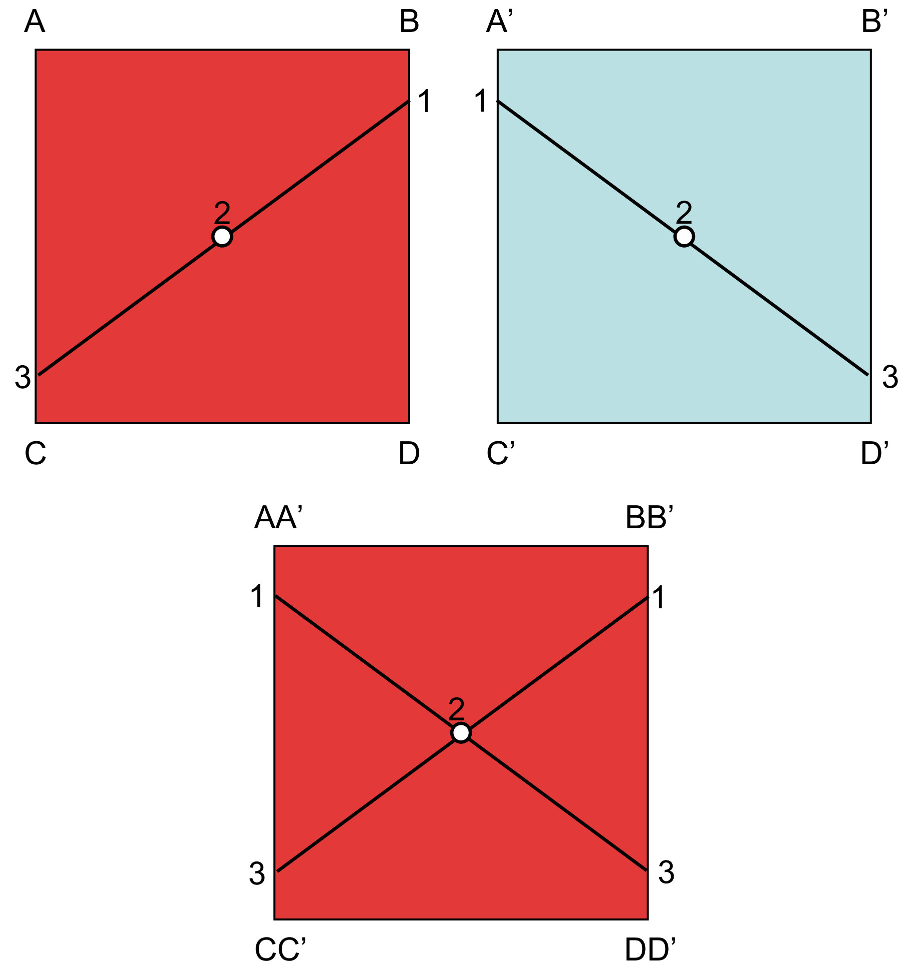
Both planes have **common values** along the **line** (1,2,3) of their intersection

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

Orientation Determination by Common Lines

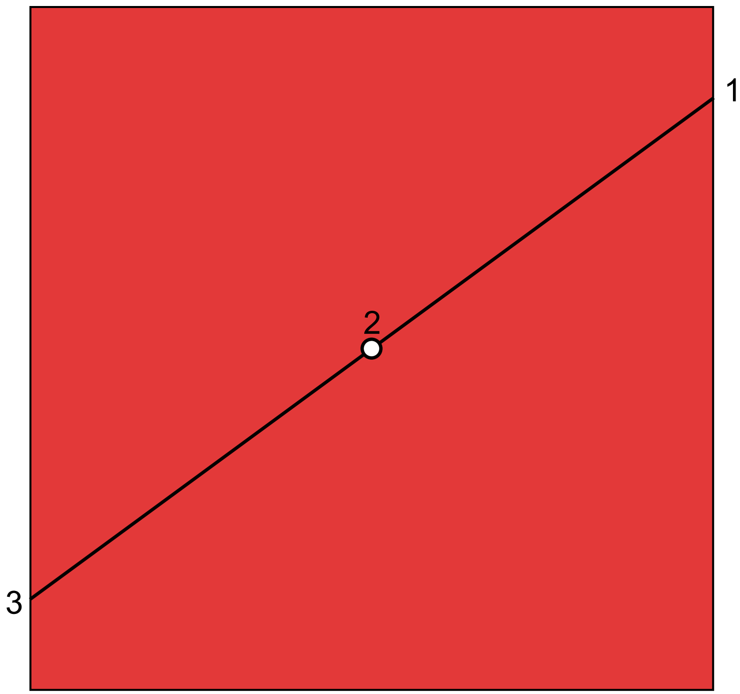


Adapted from Moody (1990) Fig. 7.68, p.245

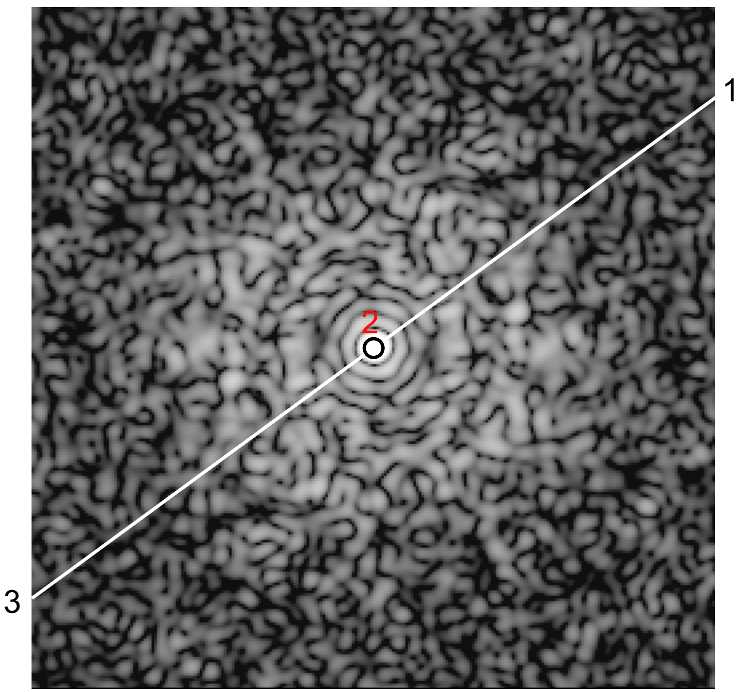


Adapted from Moody (1990) Fig. 7.69, p.246

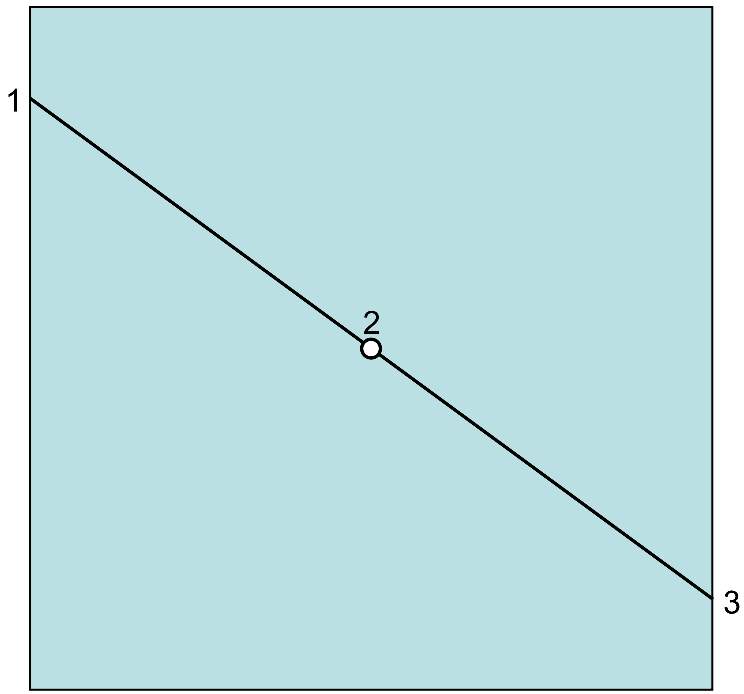
Orientation Determination by Common Lines



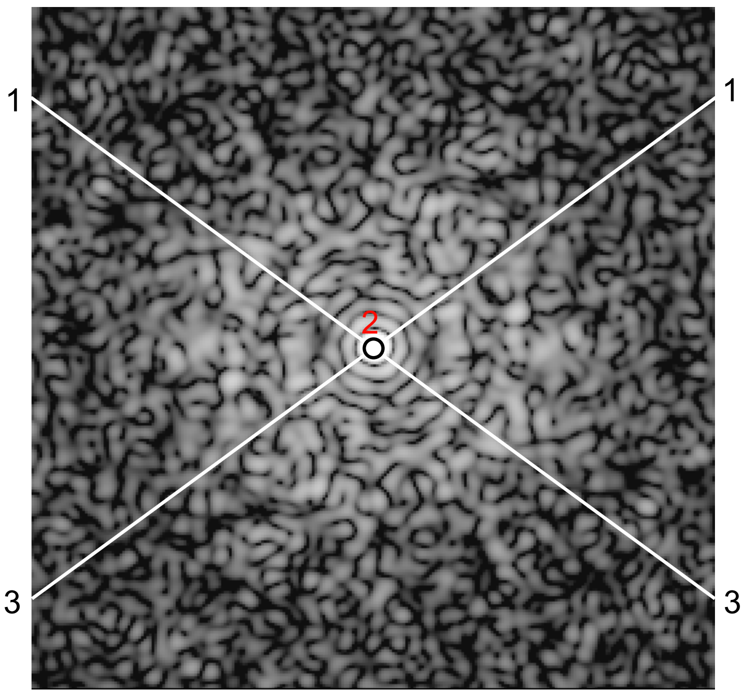
Original Transform Plane



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:

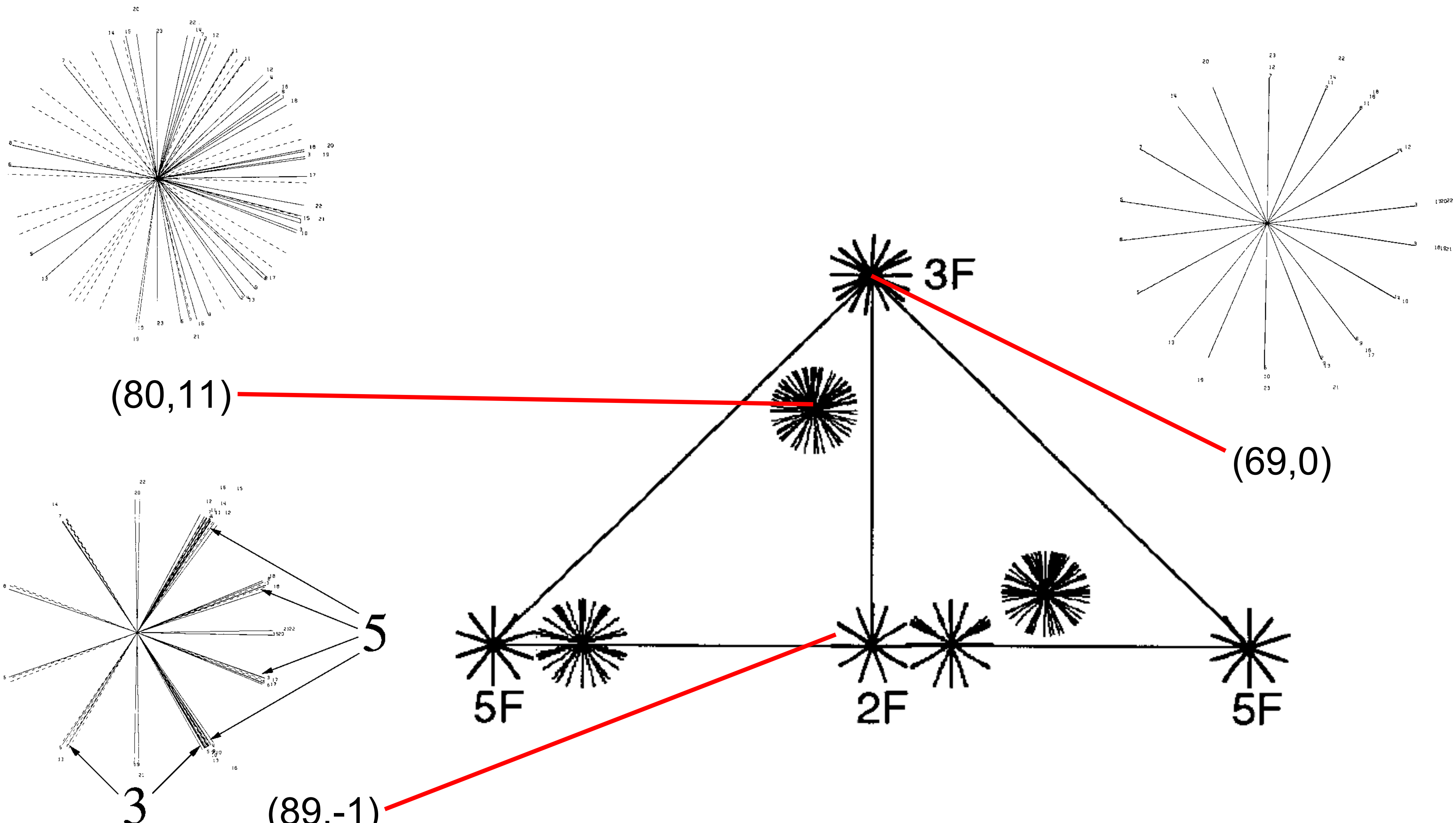
5-folds: $\frac{12}{2}$ x 2 = 12 pairs

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

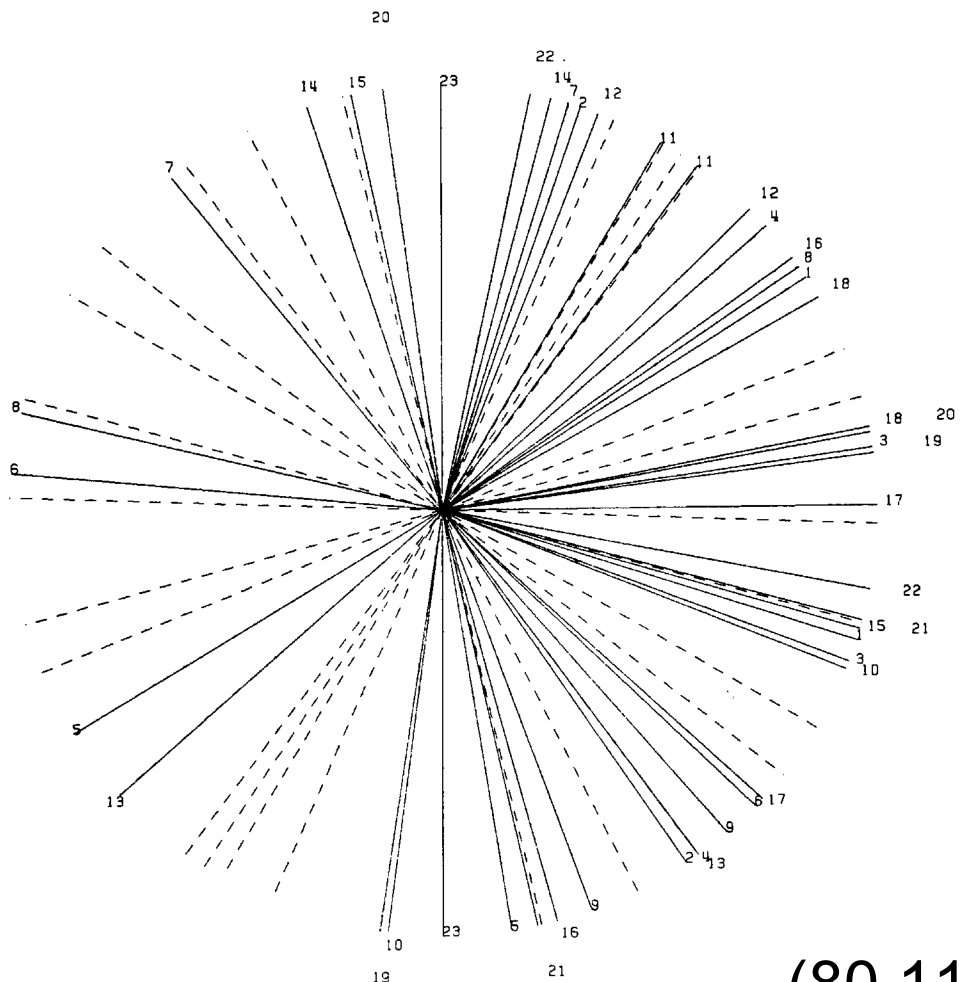
2-folds: $\frac{30}{2}$ x 1 = 15 real lines

37 common lines

Orientation Determination by Common Lines

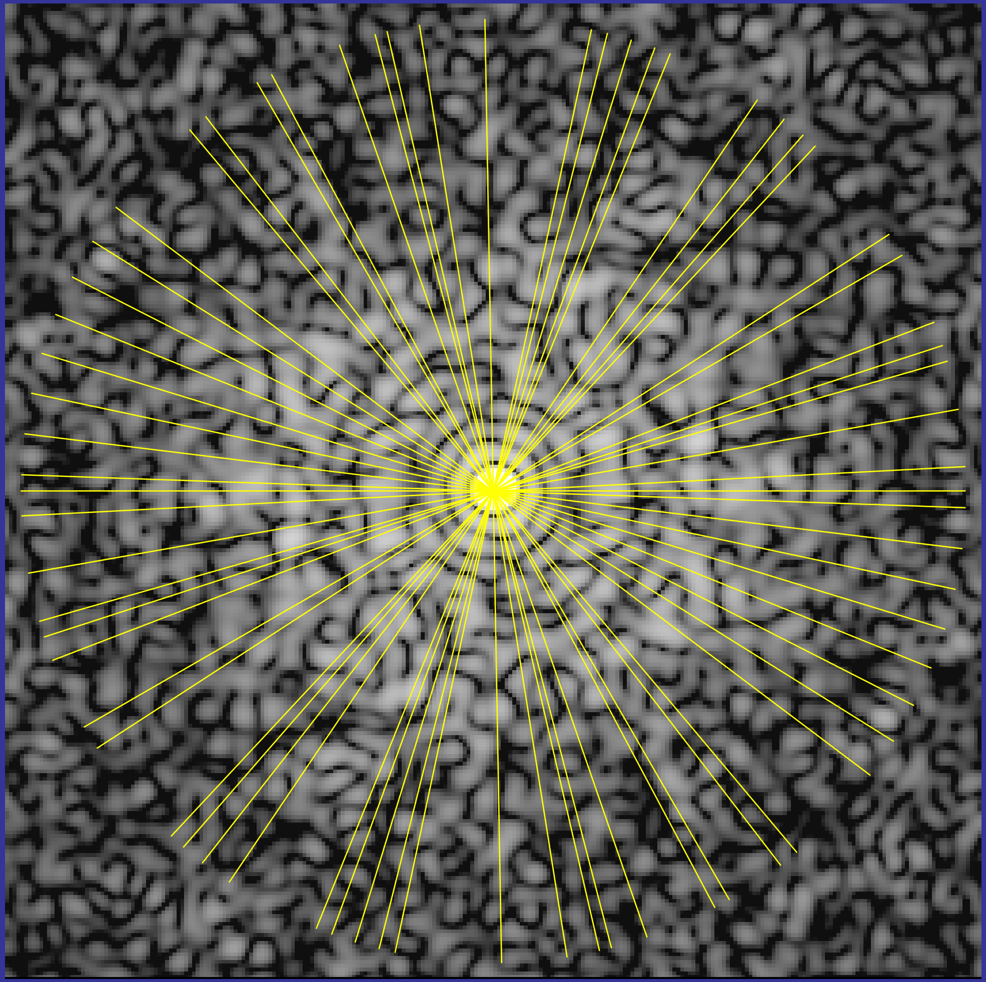


Orientation Determination by Common Lines



(80,11)

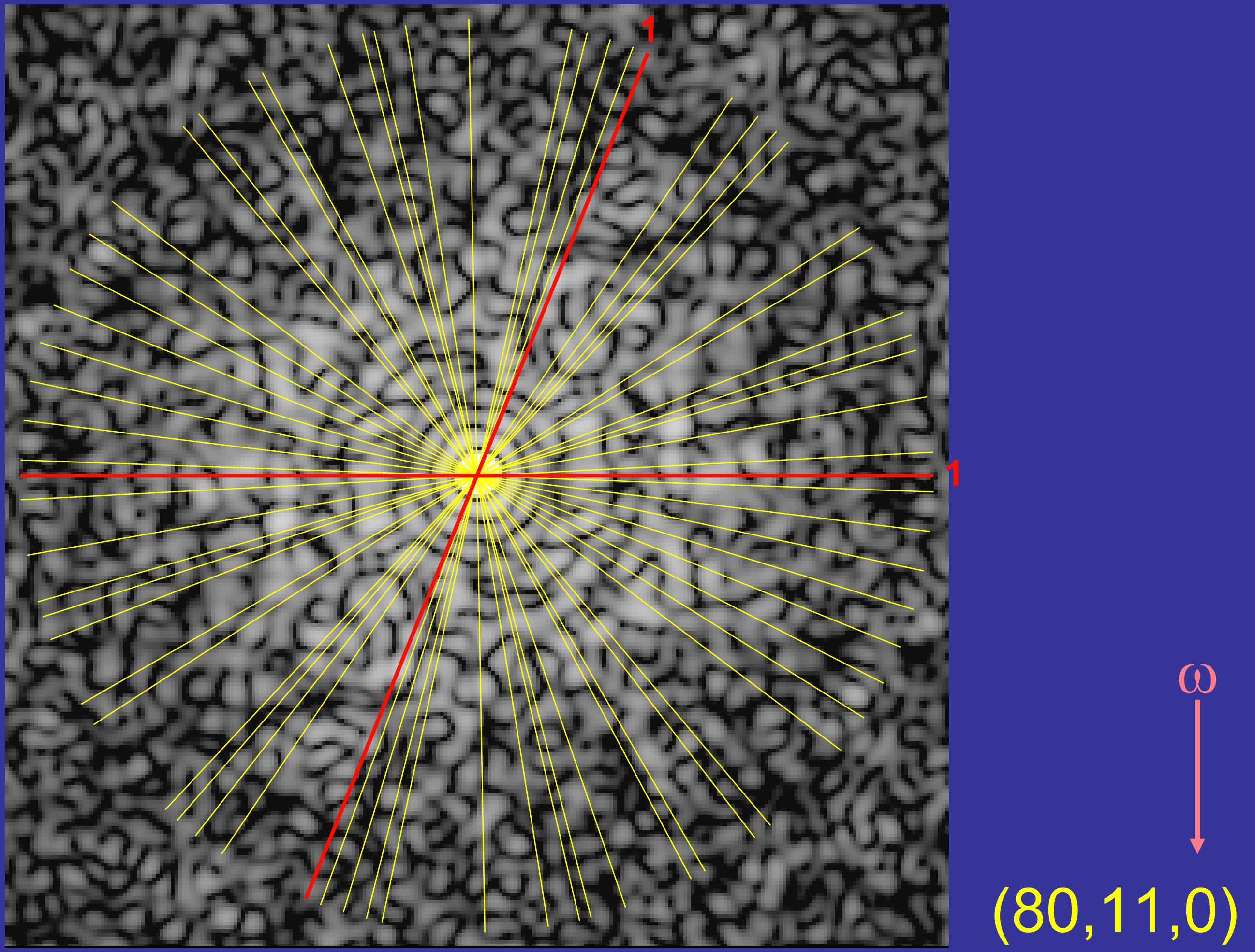
Orientation Determination by Common Lines



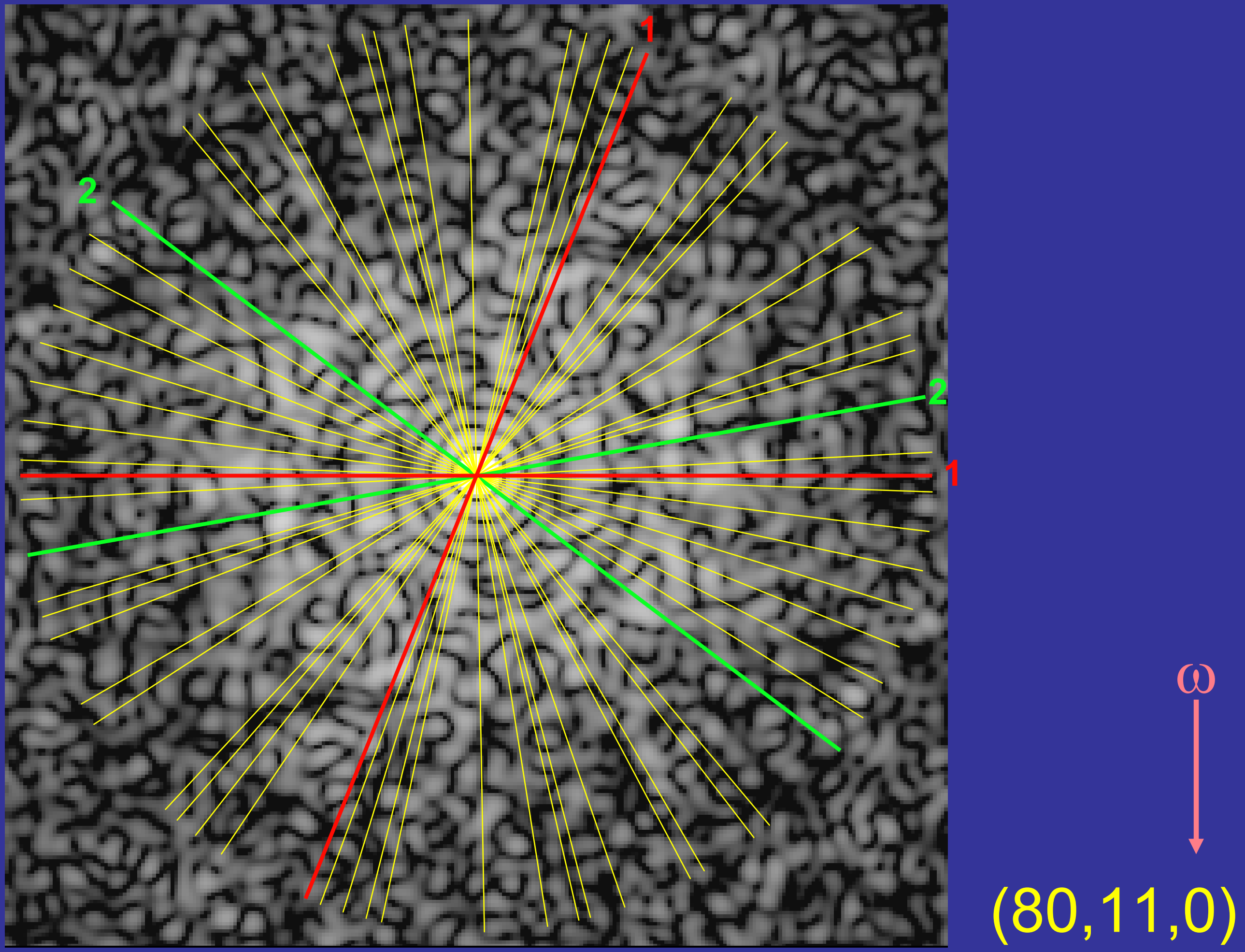
(80,11)

What is (θ,ϕ,ω) for this particle?

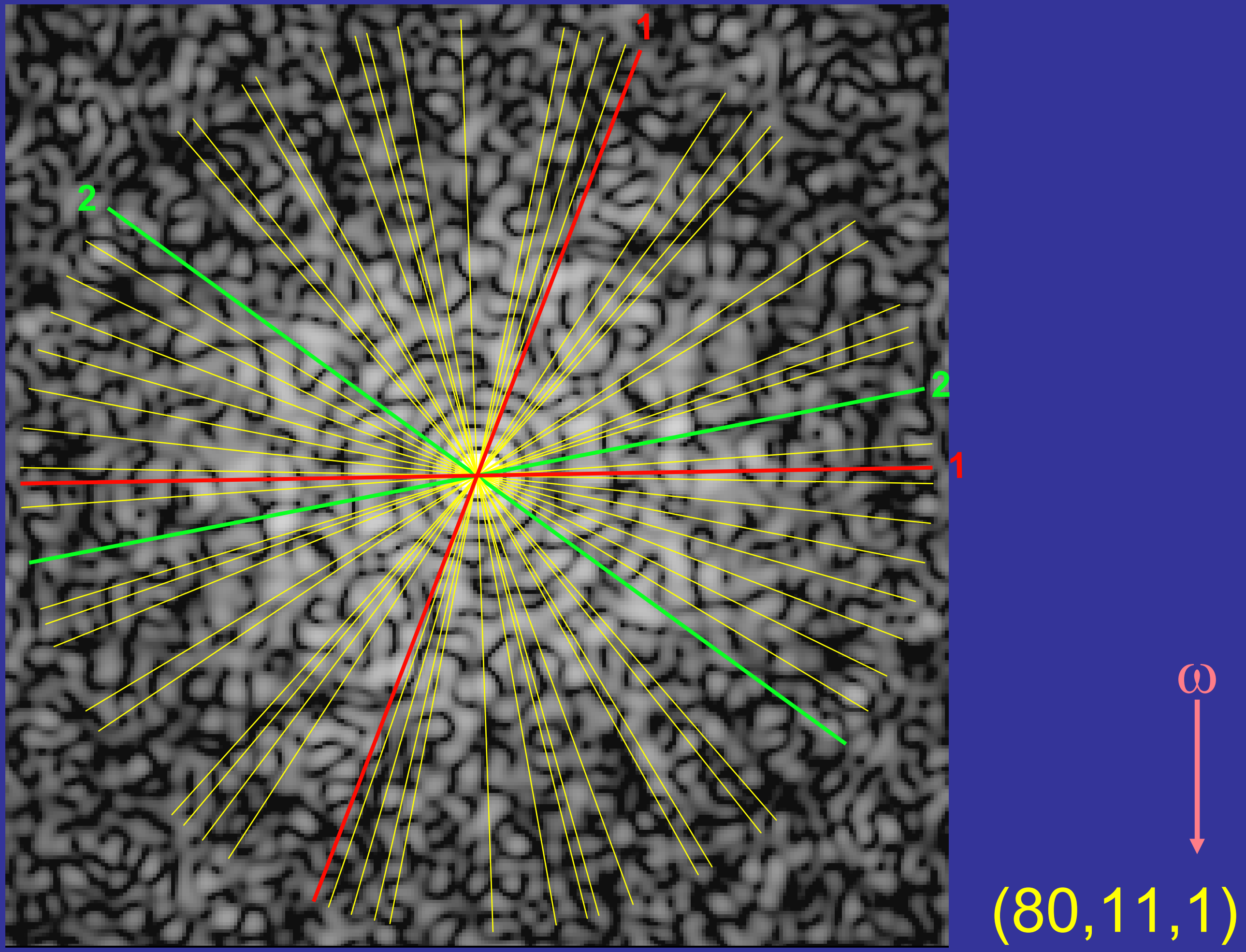
Orientation Determination by Common Lines



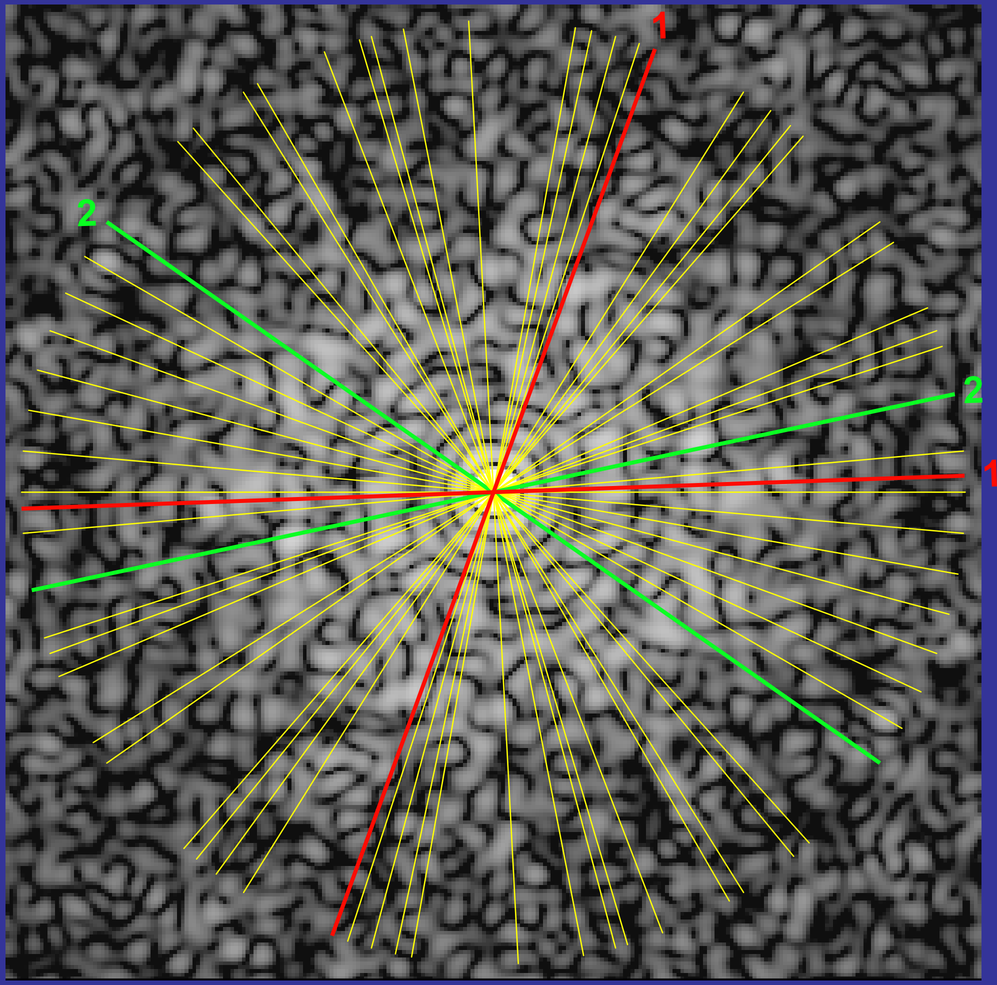
Orientation Determination by Common Lines



Orientation Determination by Common Lines



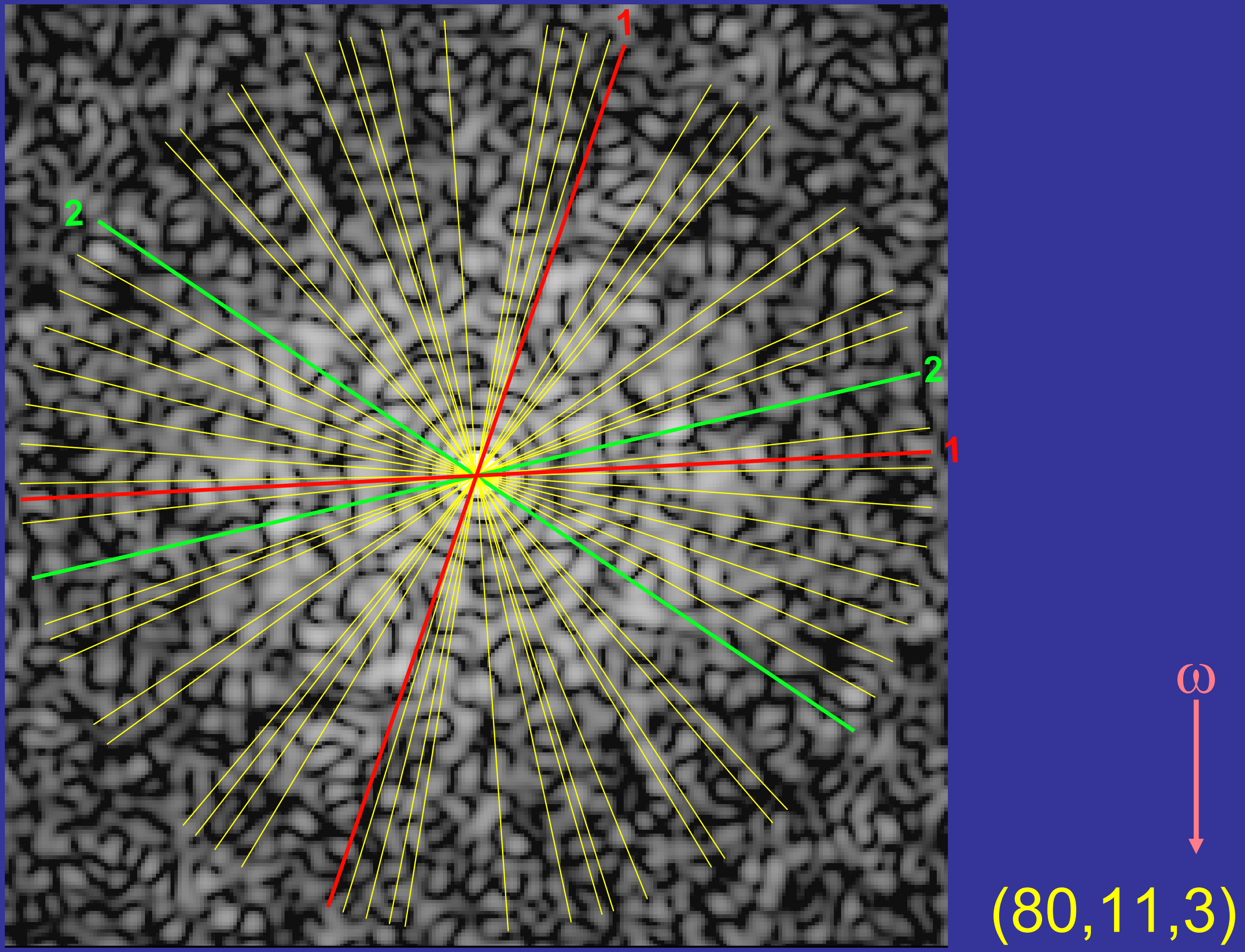
Orientation Determination by Common Lines



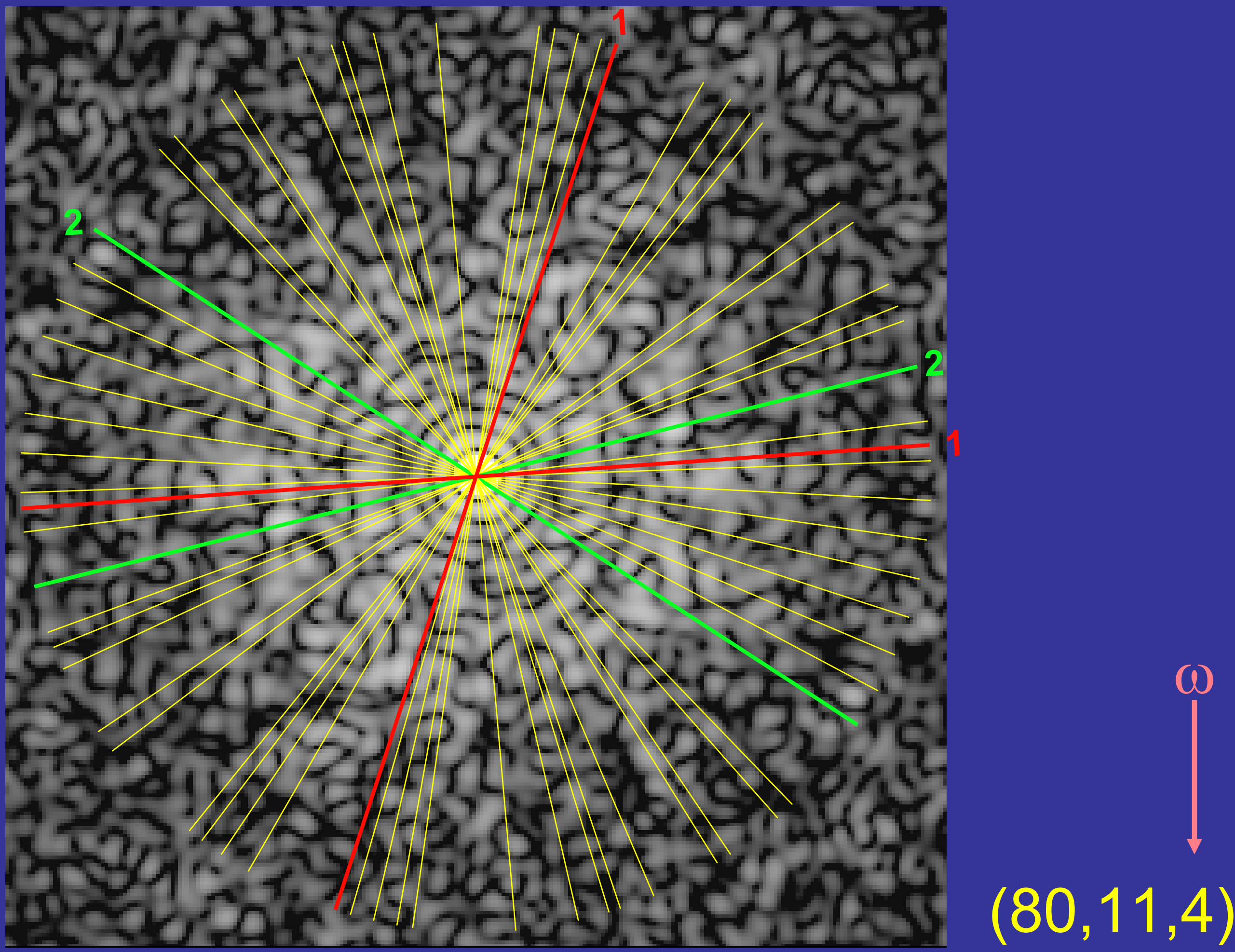
ω

(80,11,2)

Orientation Determination by Common Lines



Orientation Determination by Common Lines



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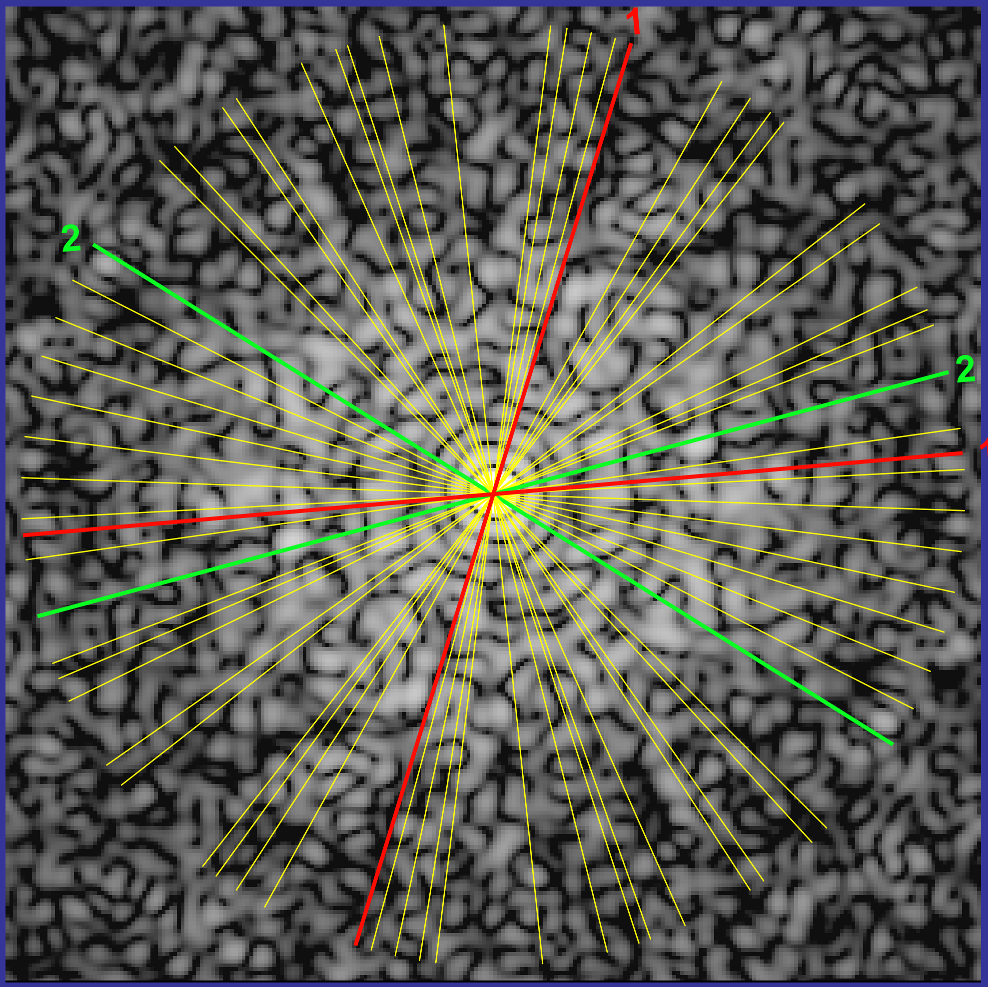
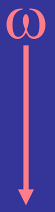
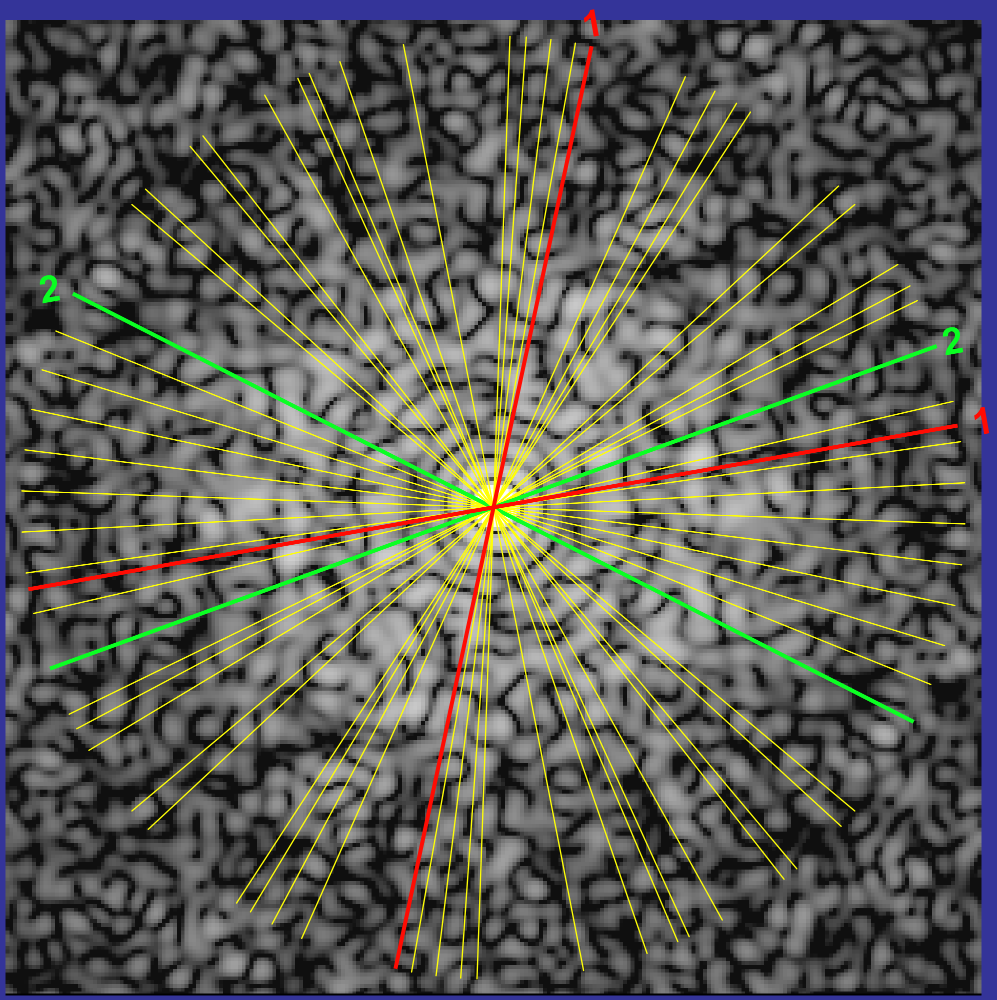




(80,11,5)

Orientation Determination by Common Lines



(80,11,10)

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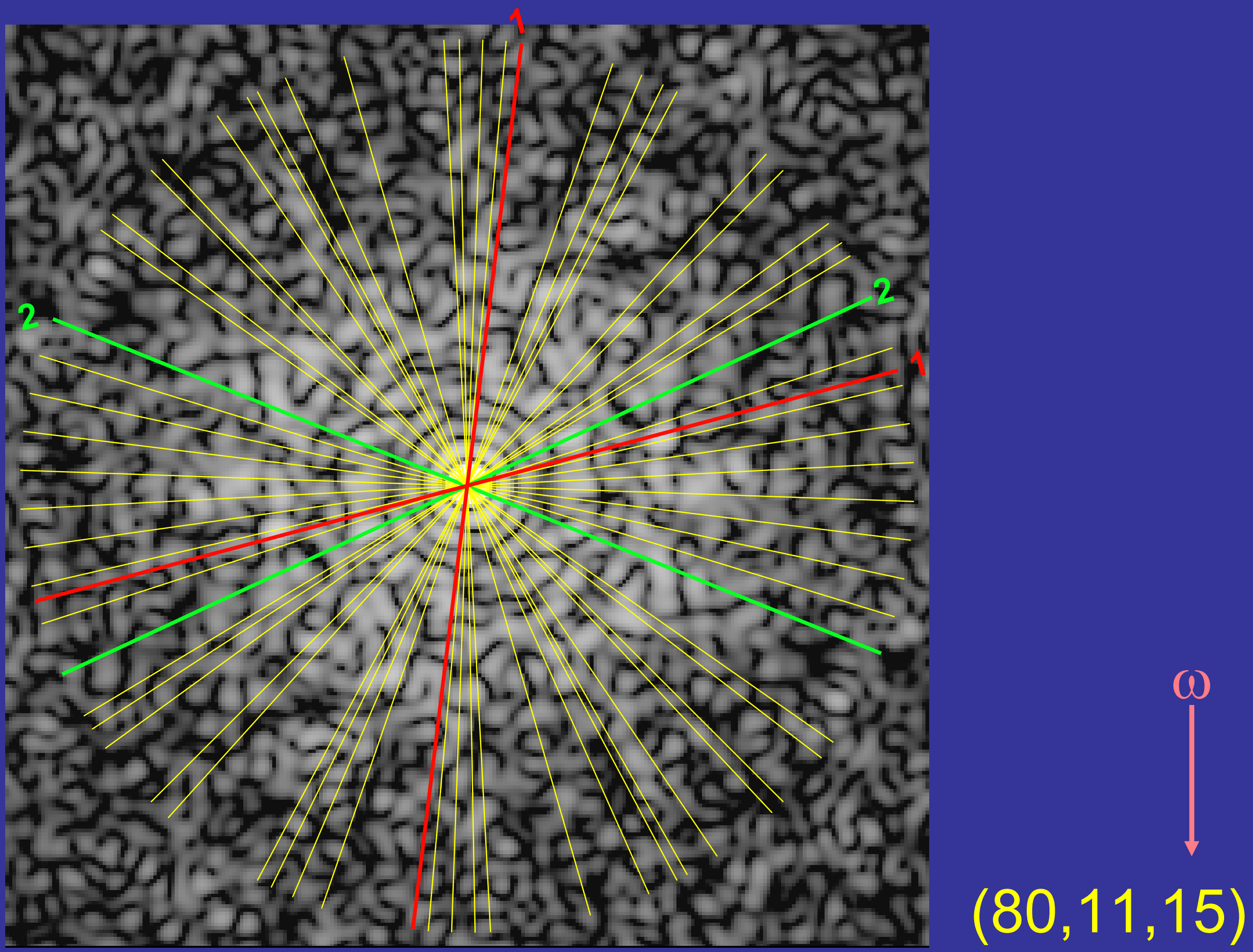

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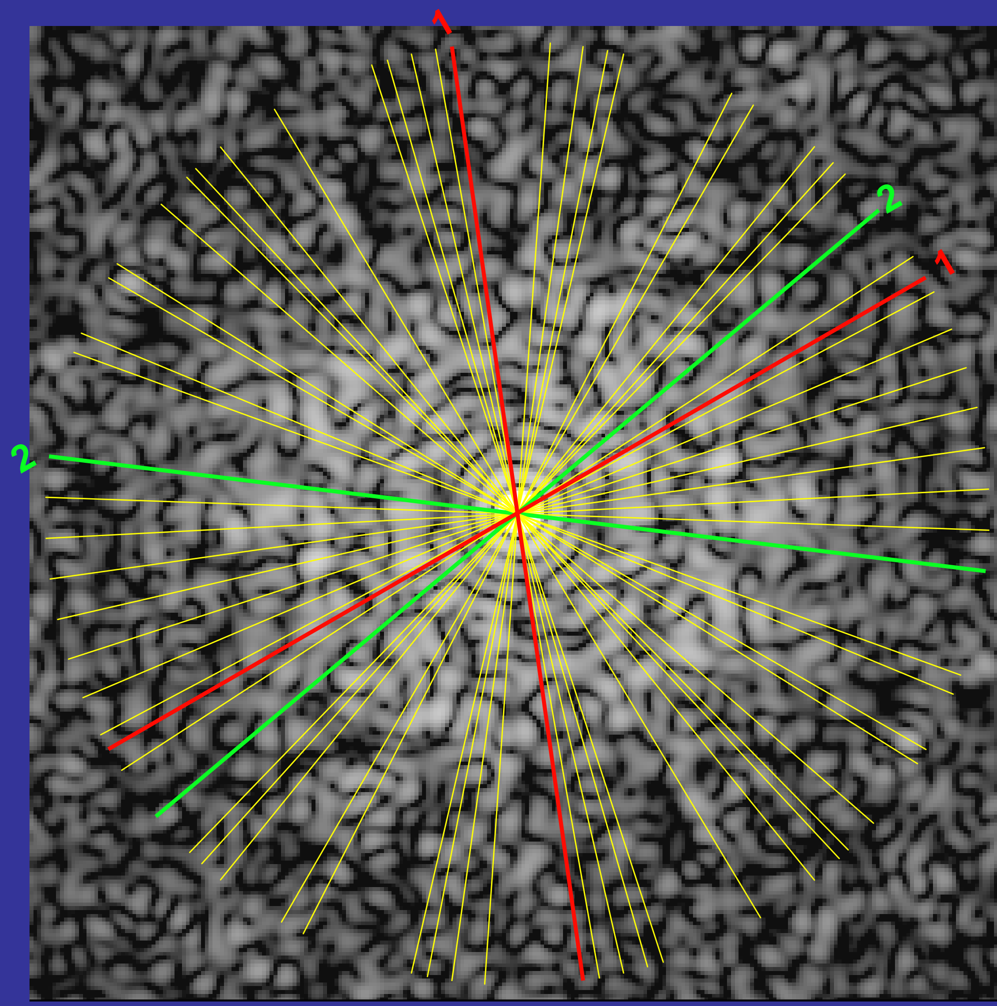
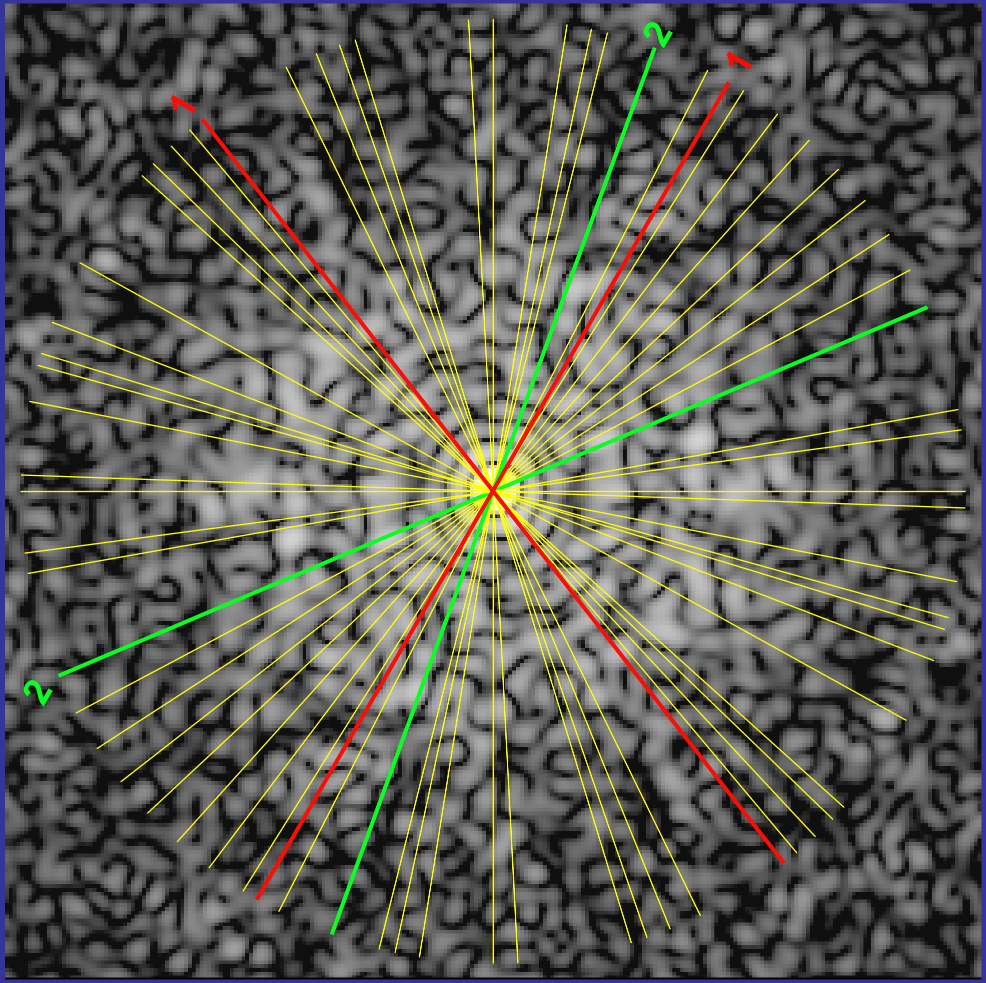


ω

(80,11,30)

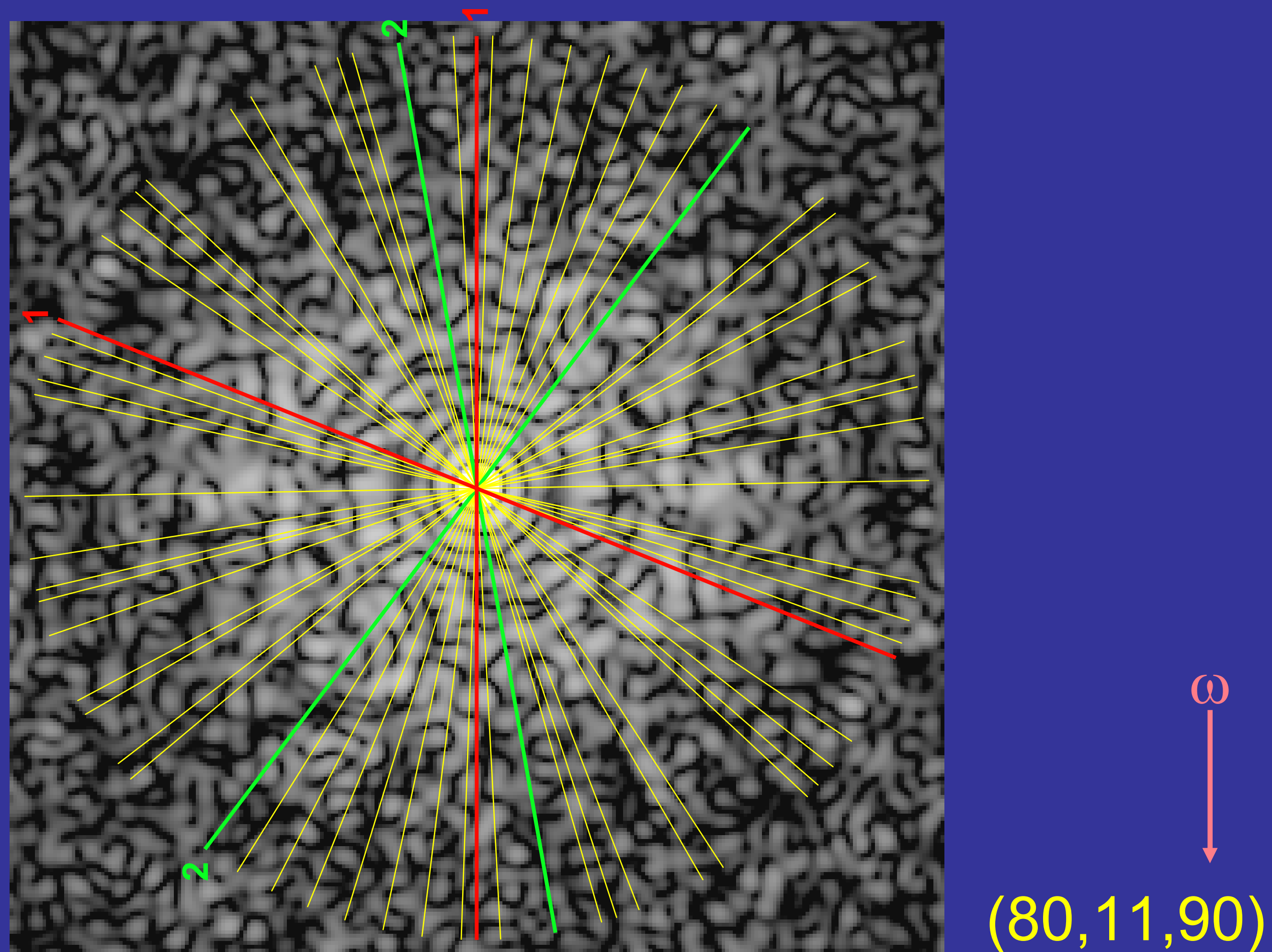
Orientation Determination by Common Lines



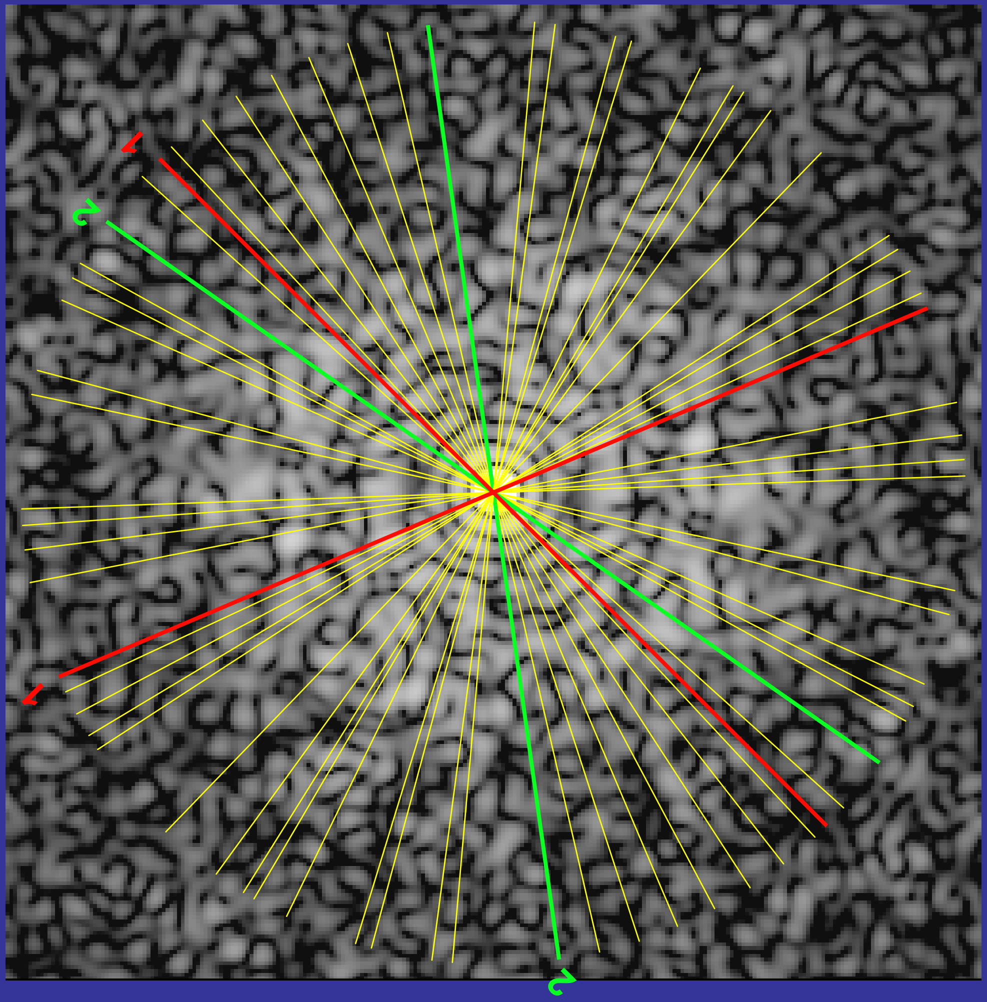
ω

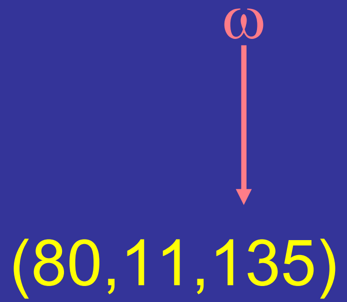
(80,11,60)

Orientation Determination by Common Lines

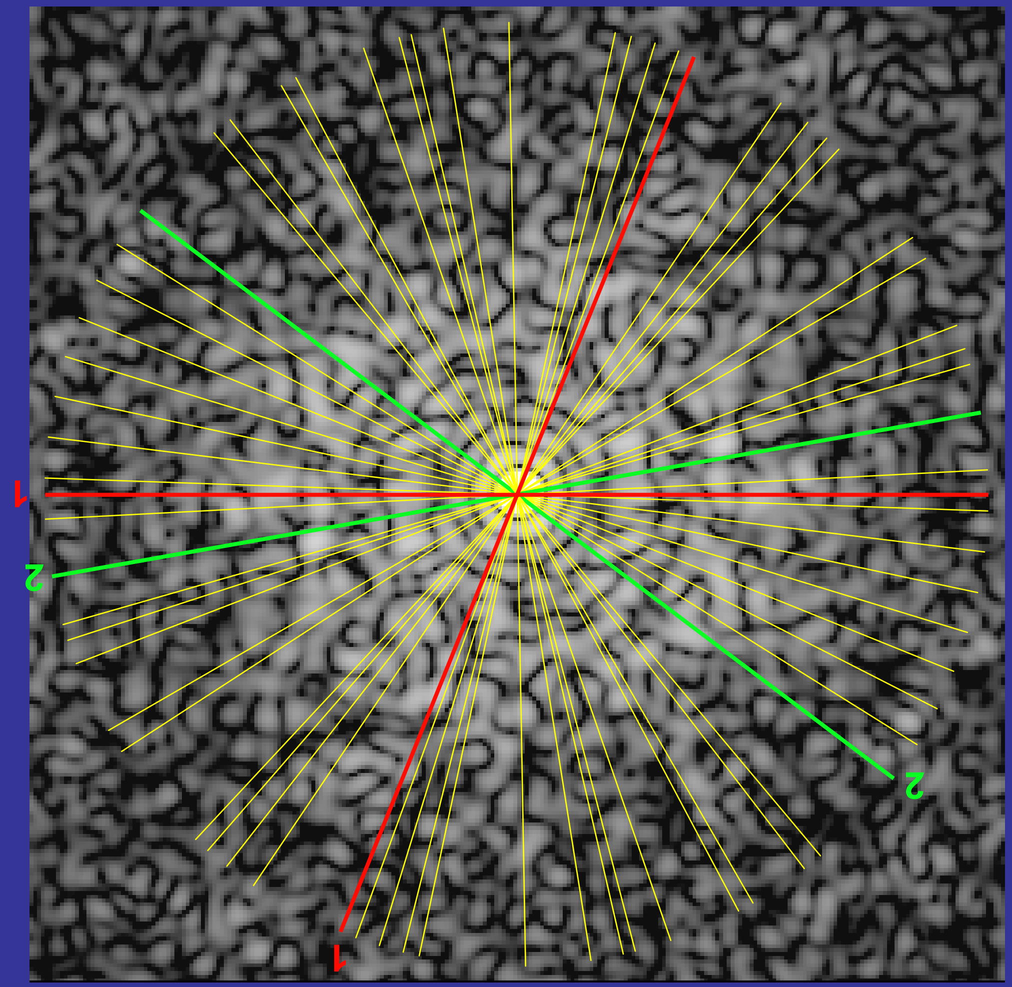


Orientation Determination by Common Lines





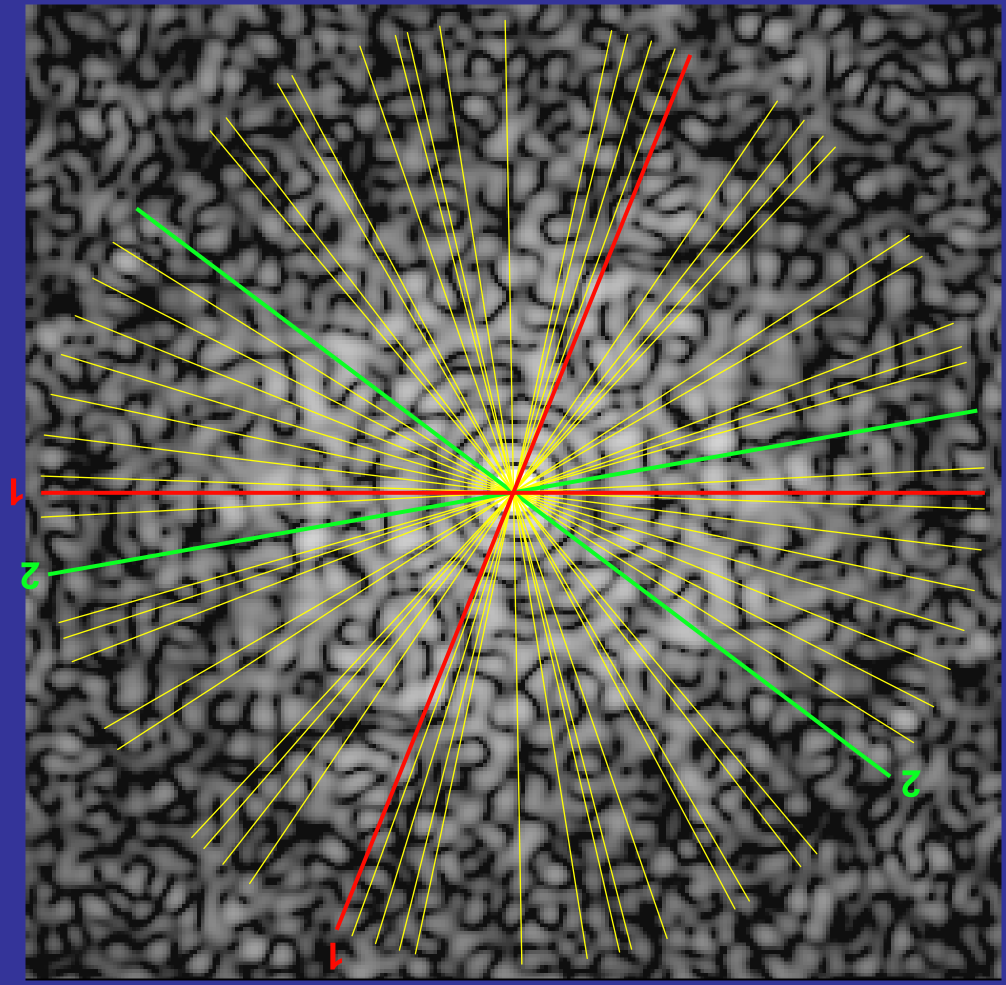
Orientation Determination by Common Lines



ω

(80,11,180)

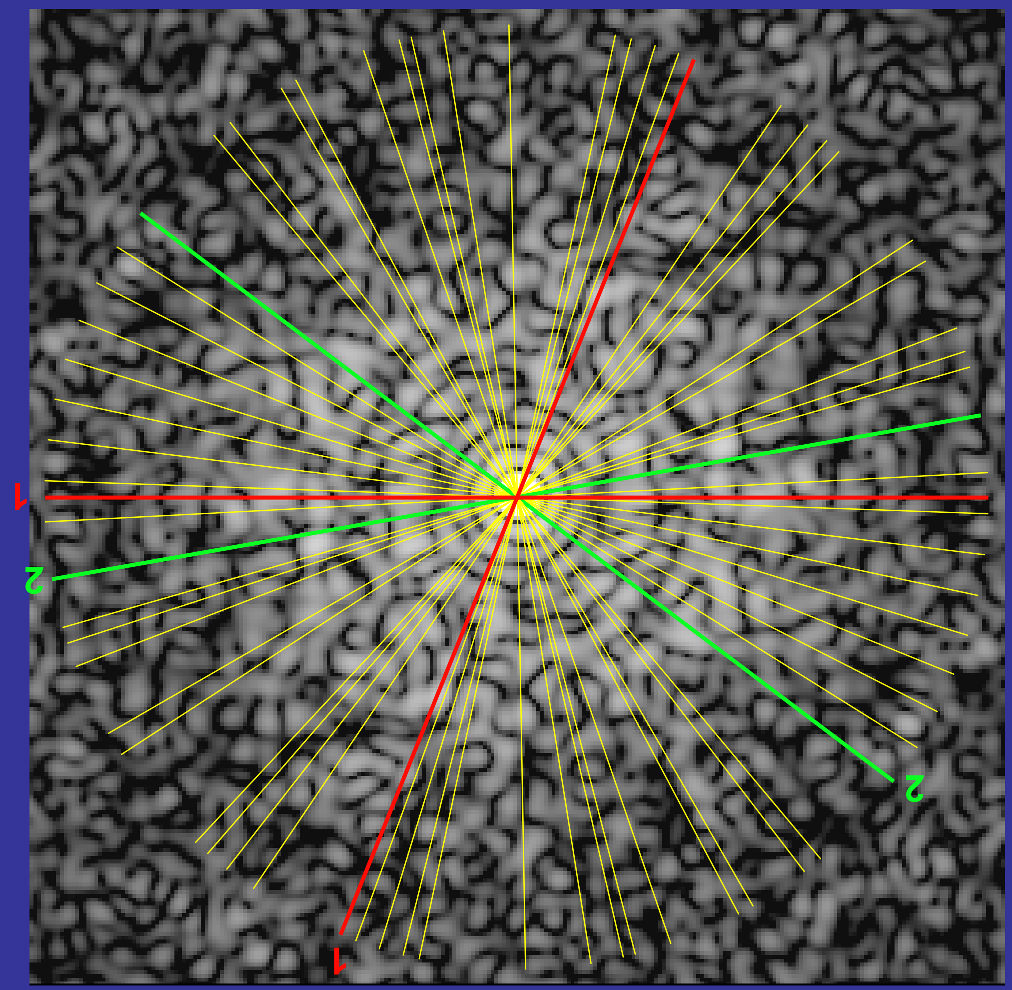
Orientation Determination by Common Lines



(80,11,ω)

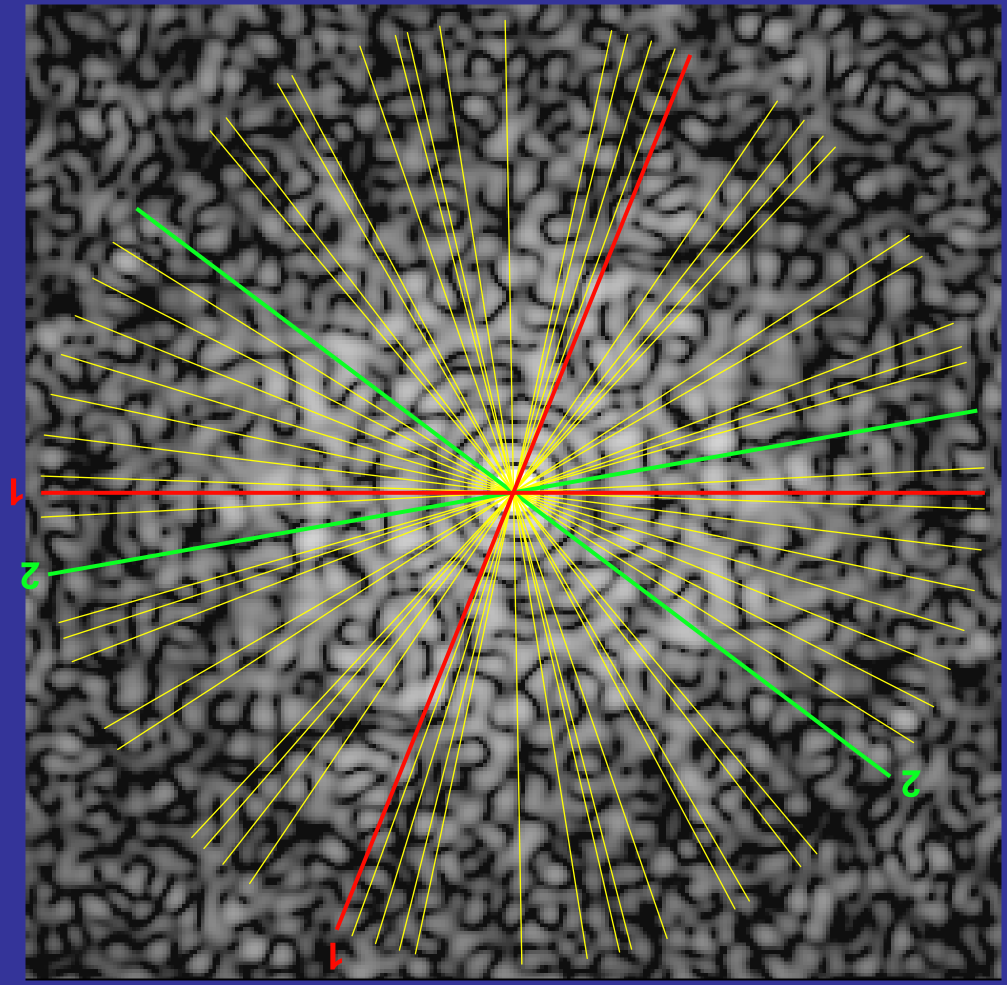
Metric: Identify ω that gives lowest phase residual

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

Determine Origin and Orientation (θ,ϕ,ω,x,y)

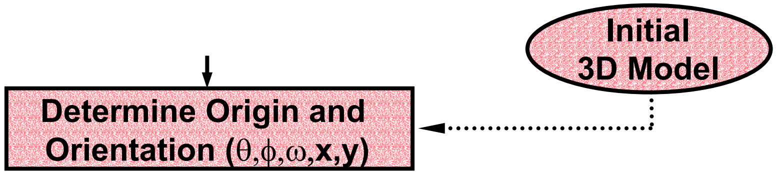
Common Lines

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 (θ, ϕ, ω, x, y):

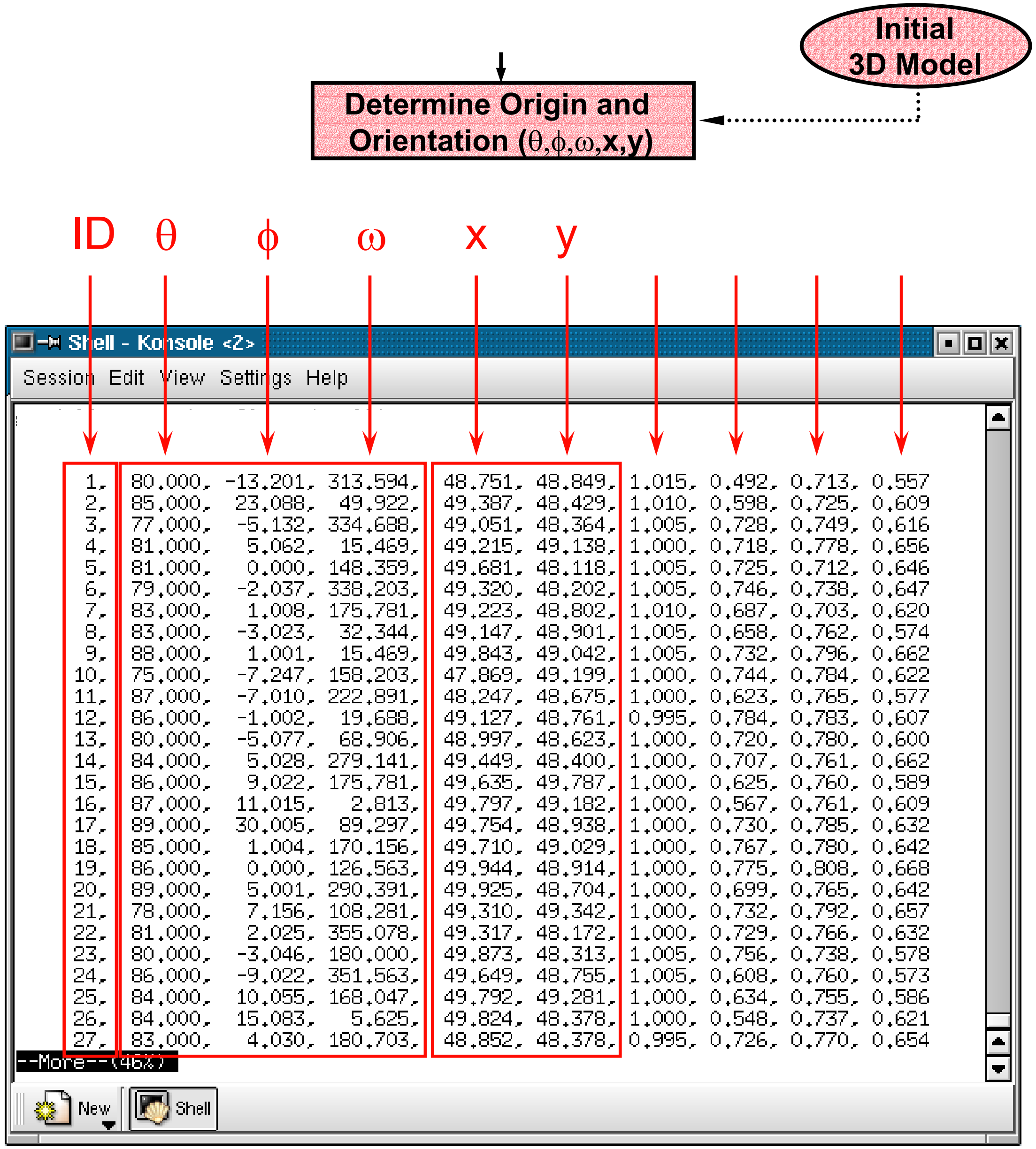
1. Common lines

2. Model-based (template) matching

Bulk of structures now solved this way

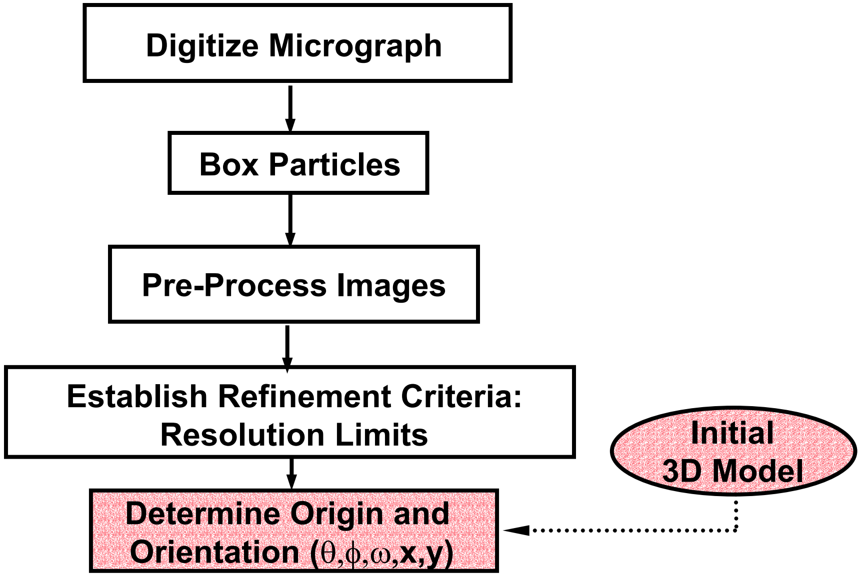
Details discussed in practical session

Icosahedral Virus 3D Reconstruction Scheme

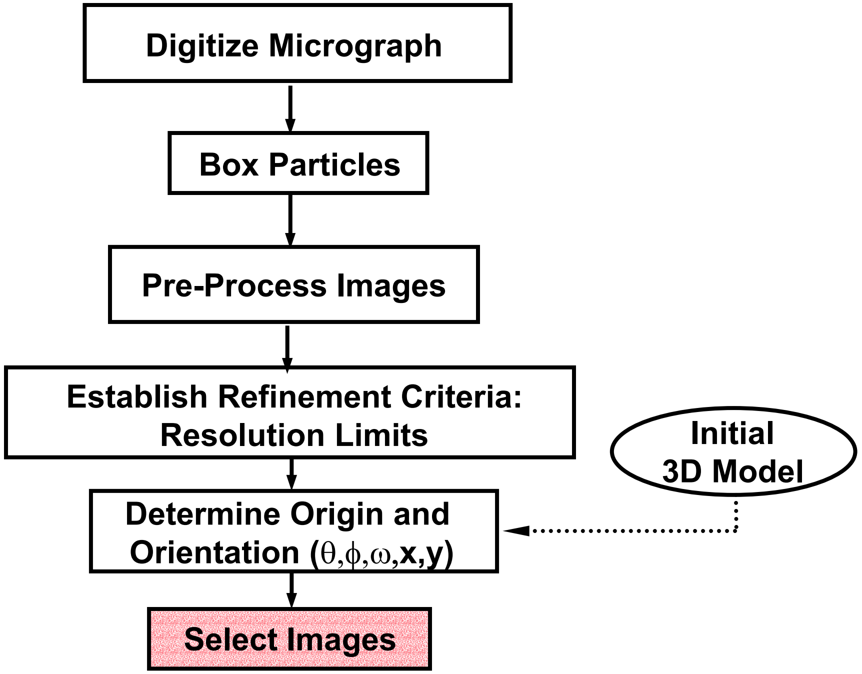


1,	80.000,	-13.201,	313.594,	48.751,	48.849,	1.015,	0.492,	0.713,	0.557
2,	85.000,	23.088,	49.922,	49.387,	48.429,	1.010,	0.598,	0.725,	0.609
3,	77.000,	-5.132,	334.688,	49.051,	48.364,	1.005,	0.728,	0.749,	0.616
4,	81.000,	5.062,	15.469,	49.215,	49.138,	1.000,	0.718,	0.778,	0.656
5,	81.000,	0.000,	148.359,	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



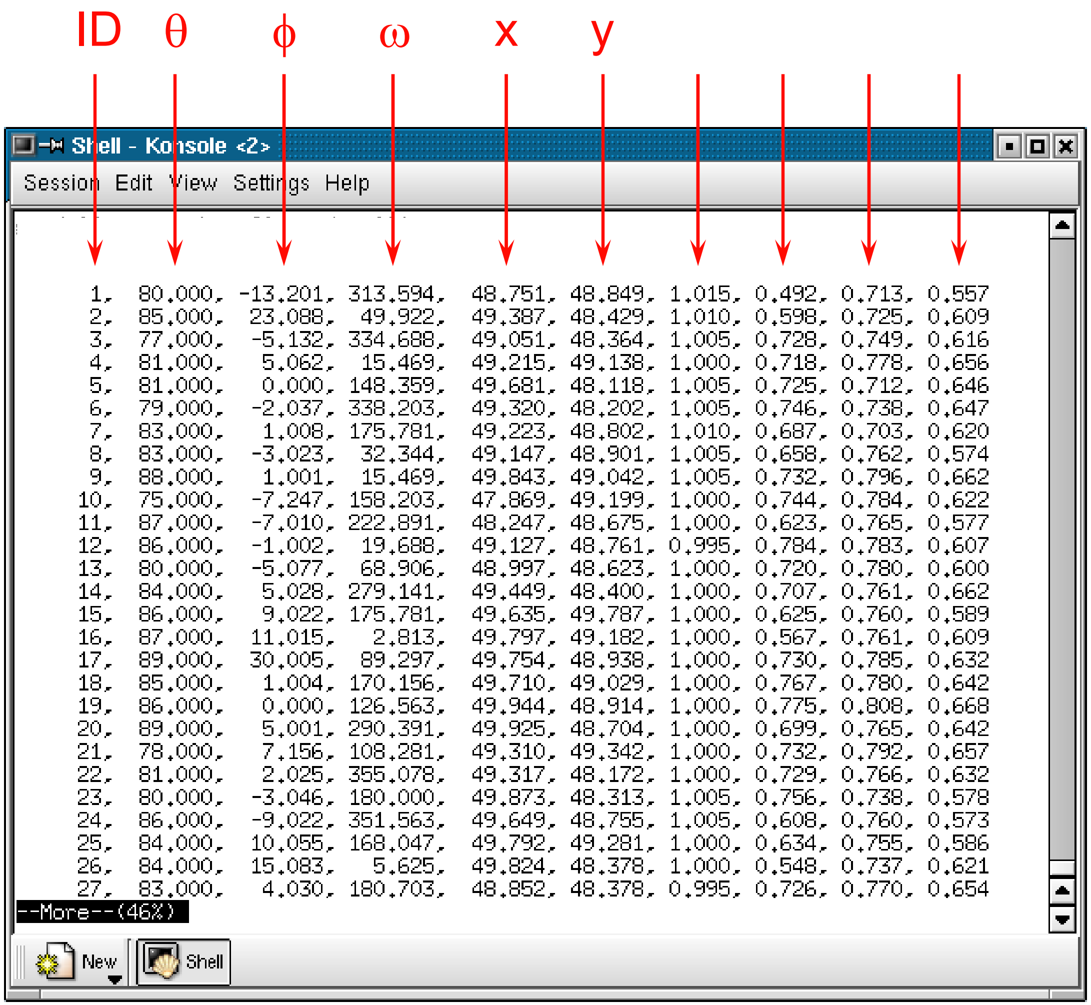
Icosahedral Virus 3D Reconstruction Scheme

Select Images

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
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
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20,	89.000,	5.001,	290.391,	49.925,	48.704,	1.000,	0.699,	0.765,	0.642
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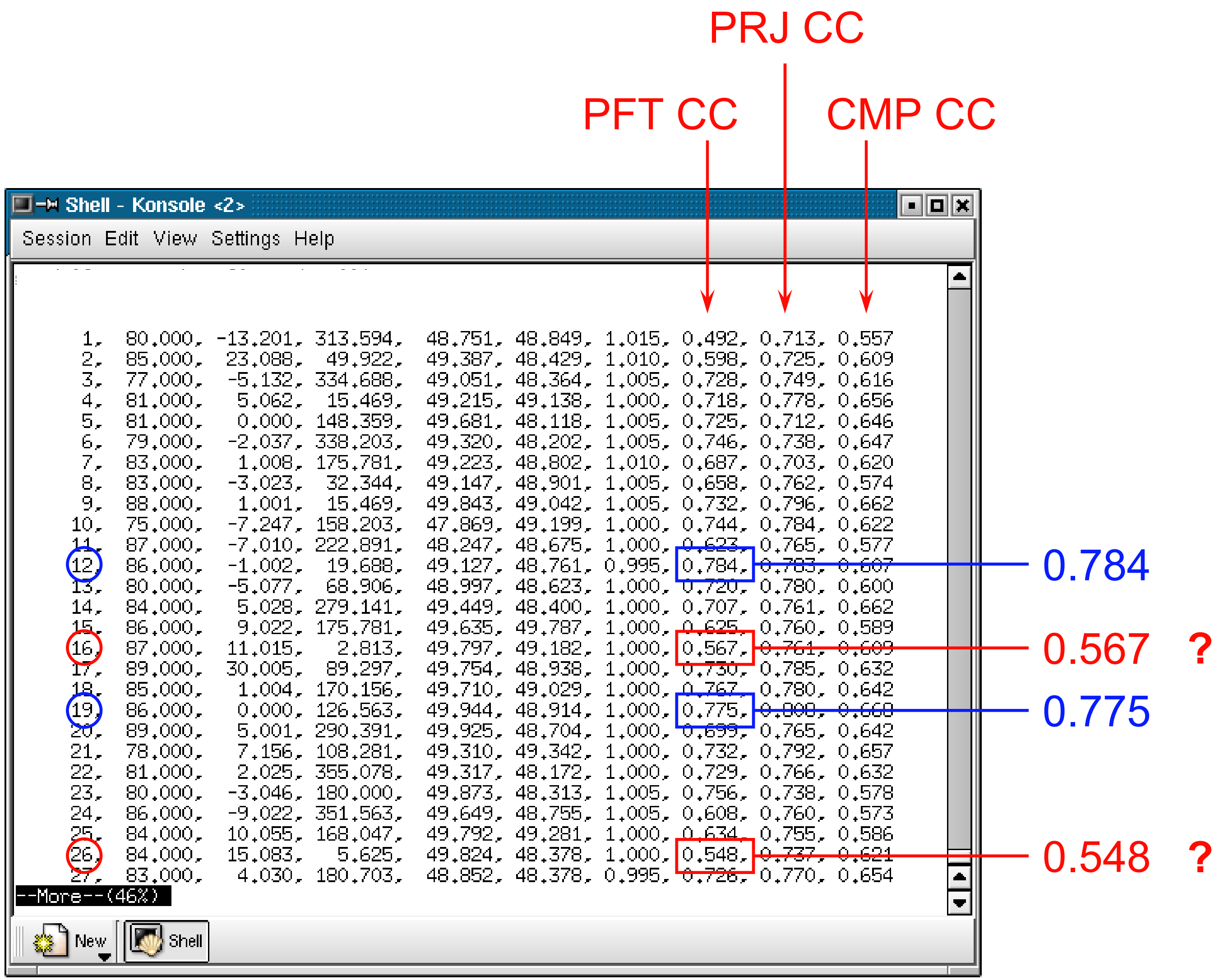
27,	83.000,	4.030,	180.703,	48.852,	48.378,	0.995,	0.726,	0.770,	0.654

--More--(46%)

Icosahedral Virus 3D Reconstruction Scheme

Select Images



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3,	77,000,	-5,132,	334,688,	49,051,	48,364,	1,005,	0,728,	0,749,	0,616
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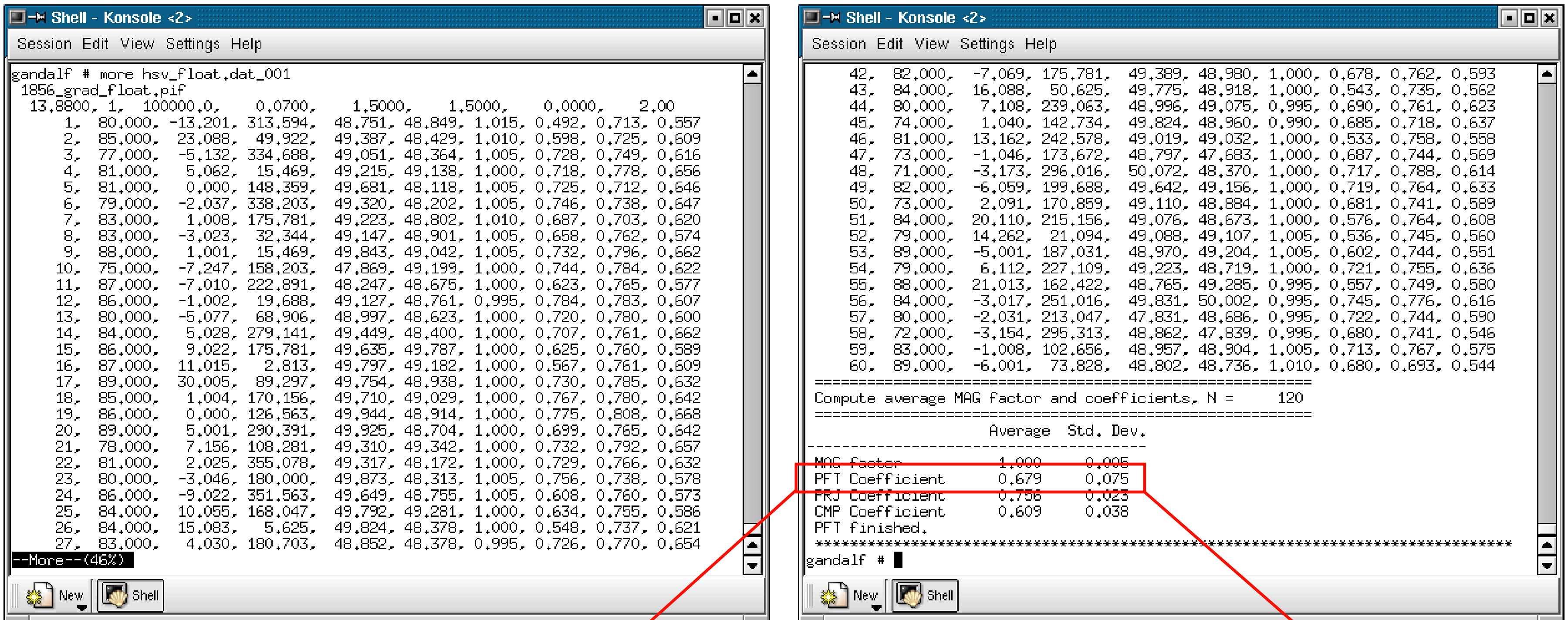
26,	84,000,	15,083,	5,625,	49,824,	48,378,	1,000,	0,548,	0,737,	0,621
27,	83,000,	4,030,	180,703,	48,852,	48,378,	0,995,	0,726,	0,770,	0,654

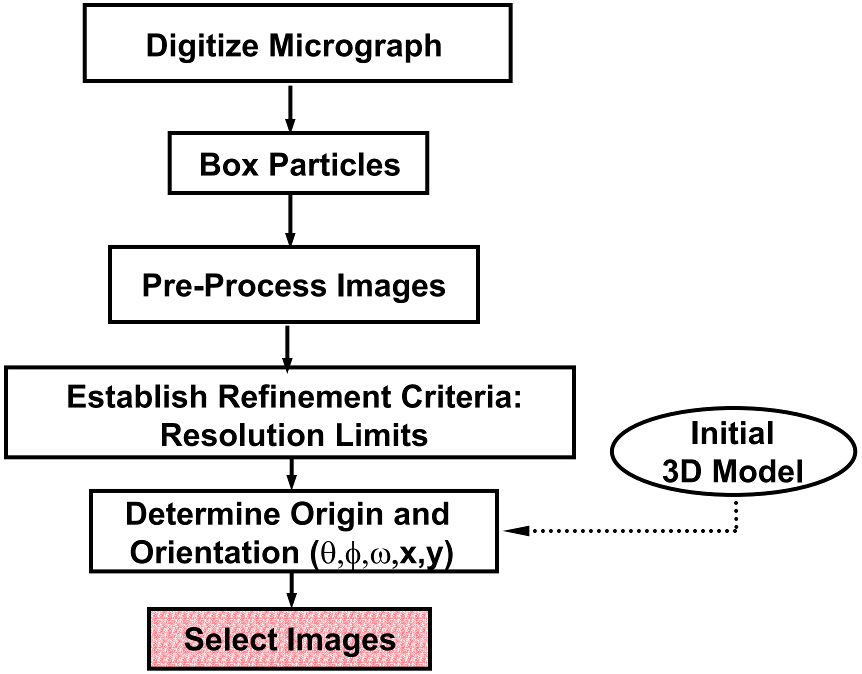
Icosahedral Virus 3D Reconstruction Scheme

Select Images

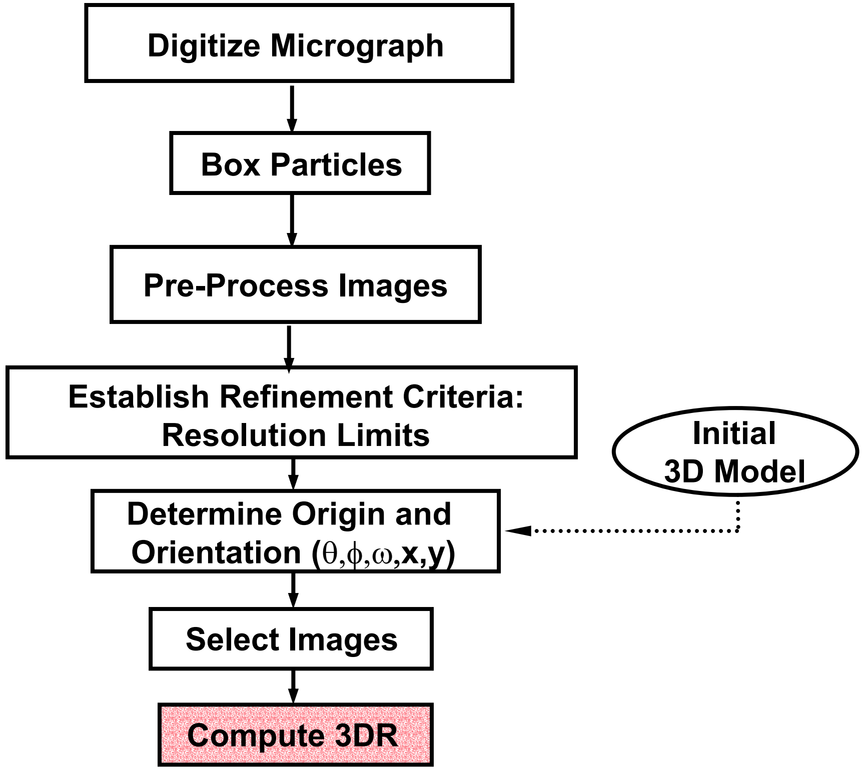


PFT Coefficient 0.679 ± 0.075

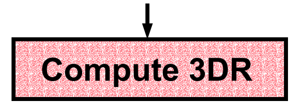
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



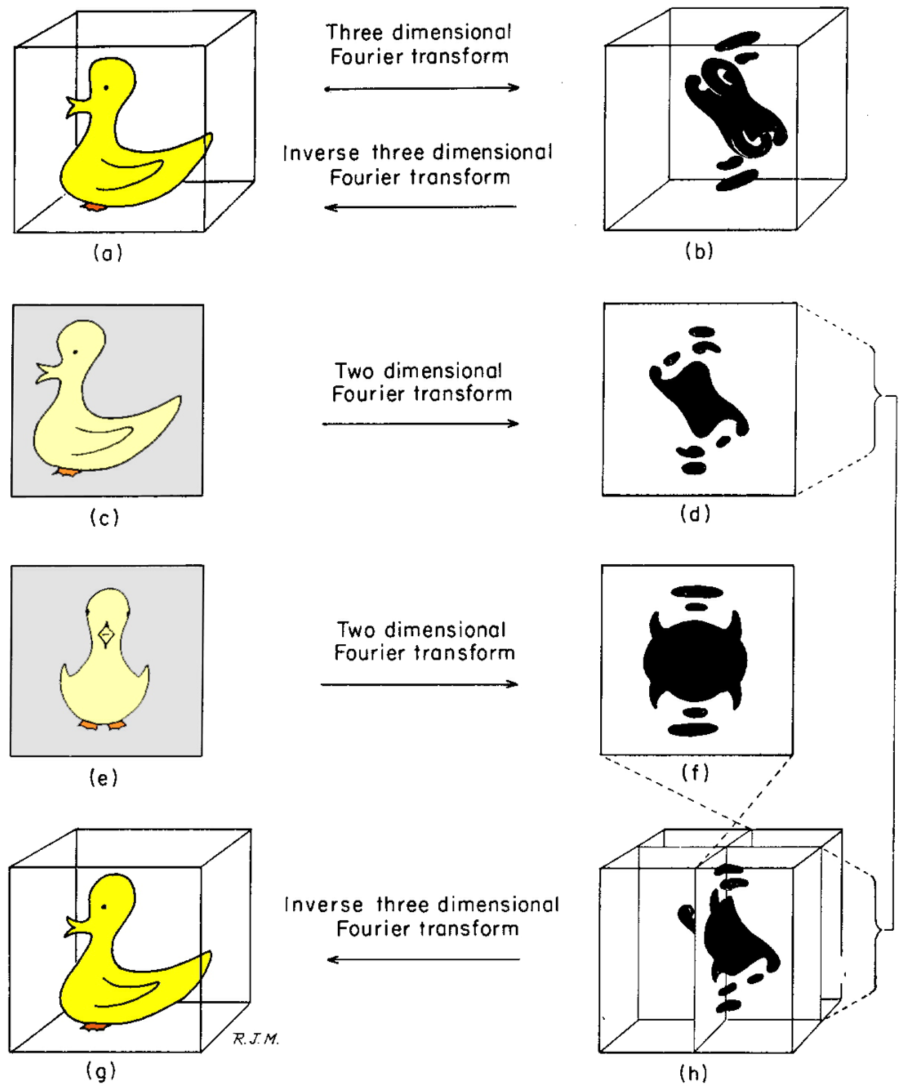
Goal: combine “good” particle images to compute a 3D density map

Icosahedral Virus 3D Reconstruction Scheme

Compute 3DR

In theory

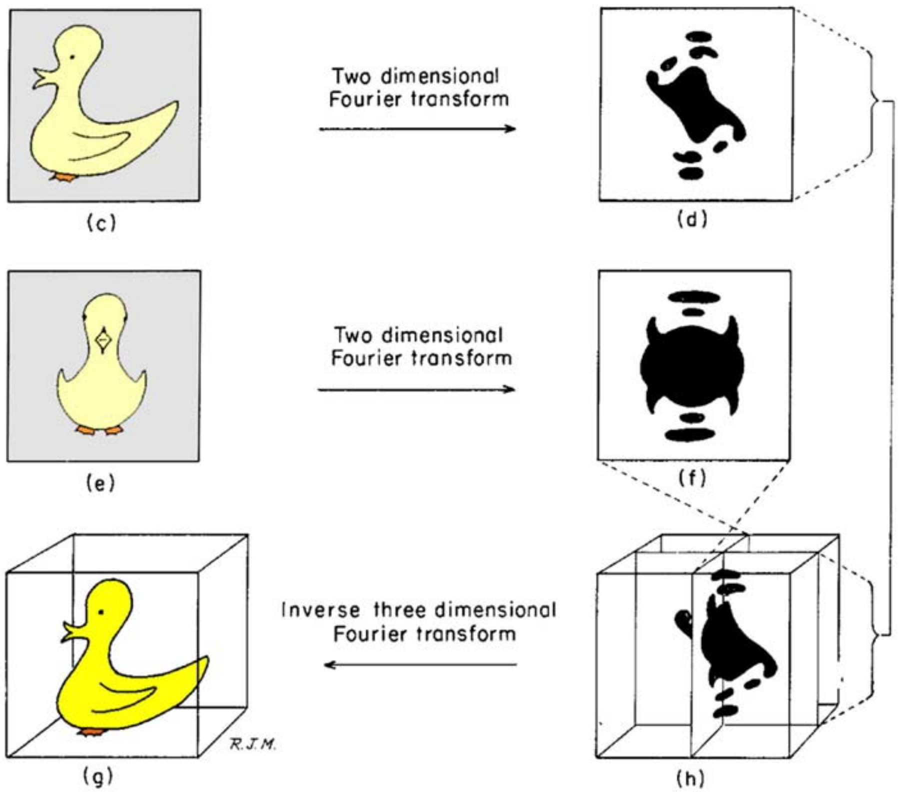
In practice



From Lake (1972), p.174

Icosahedral Virus 3D Reconstruction Scheme

Compute 3DR



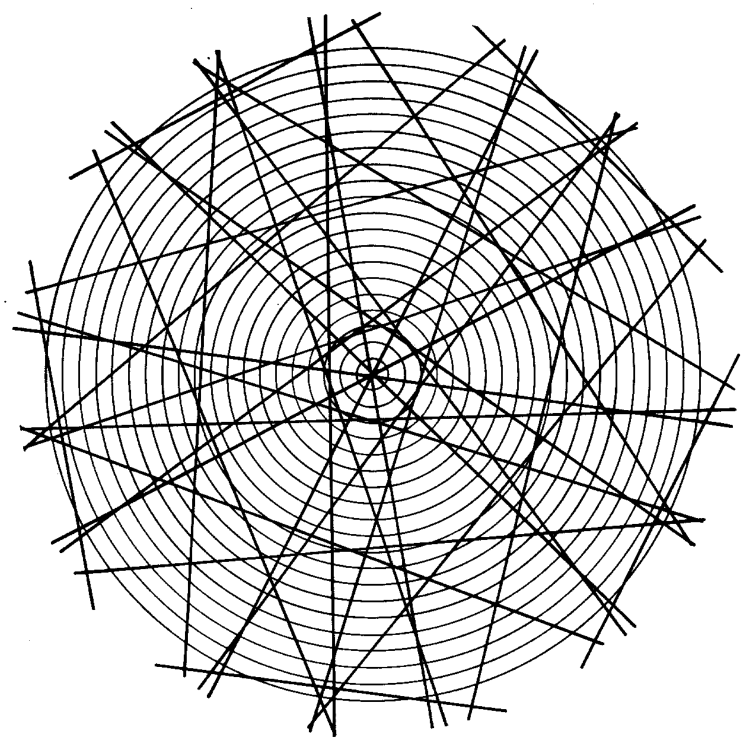
Overall scheme: $\rho \leftarrow \mathbf{g} \leftarrow \mathbf{G} \leftarrow \mathbf{F}$

Icosahedral Virus 3D Reconstruction Scheme

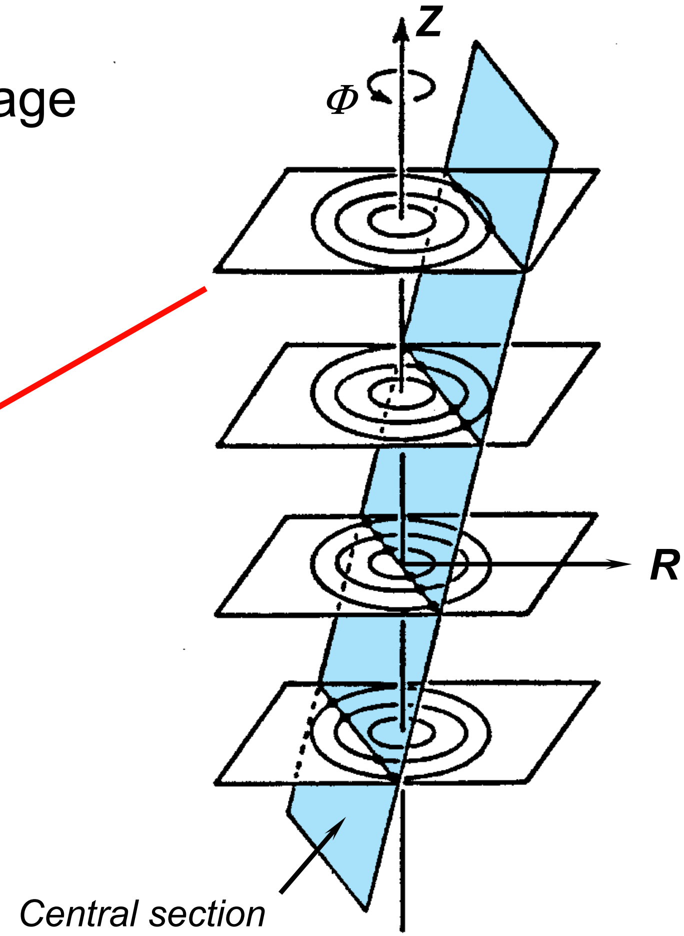
Compute 3DR

Steps:

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



Crowther, DeRosier and Klug, 1970, p.329



$F(R,\Phi,Z)$

Adapted from Crowther (1971) Fig. 4, p.223

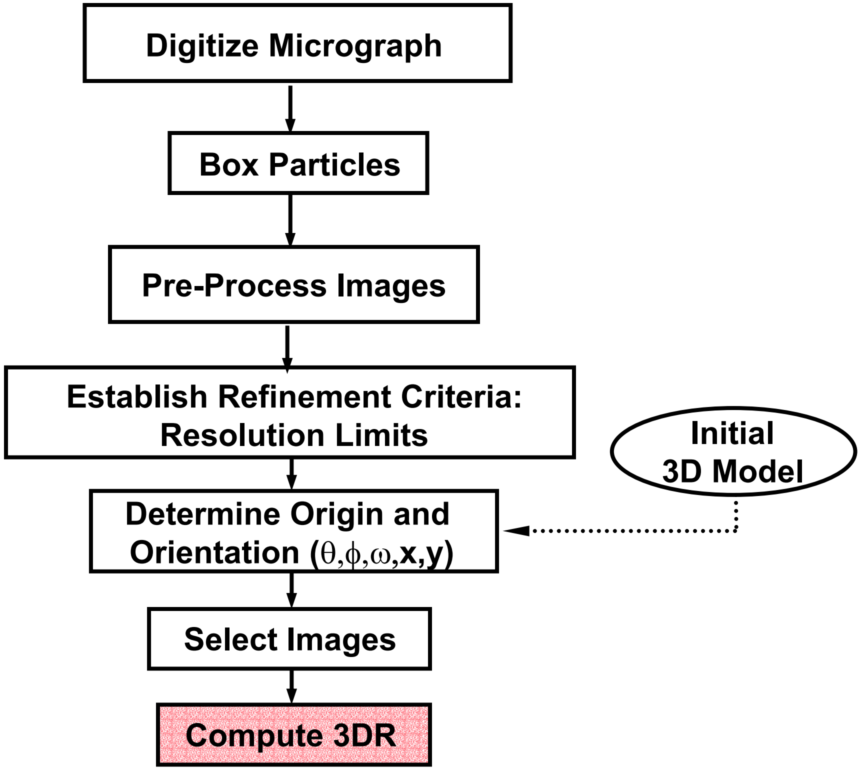
Icosahedral Virus 3D Reconstruction Scheme

$\rho \leftarrow \mathbf{g} \leftarrow \mathbf{G} \leftarrow \mathbf{F}$

Steps:

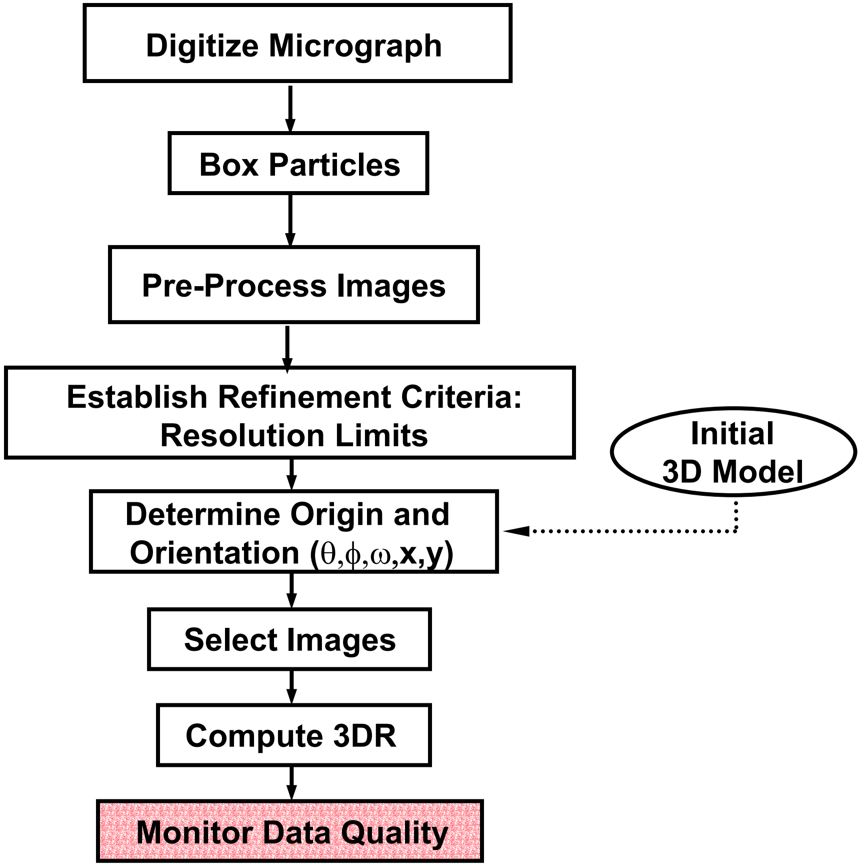
1. Compute 2D FFT of each particle image
2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform
3. Compute G_n's on each annulus $G = \left(B^{\dagger}B\right)^{-1} B^{\dagger}F$
4. Compute g_n's from G_n's (Fourier-Bessel transform)
5. Compute polar density map ($\rho(r,\phi,z)$) from g_n's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

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

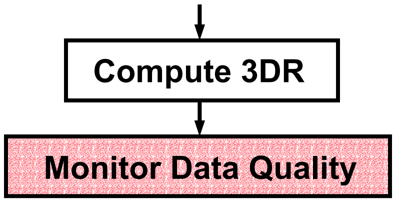


Icosahedral Virus 3D Reconstruction Scheme

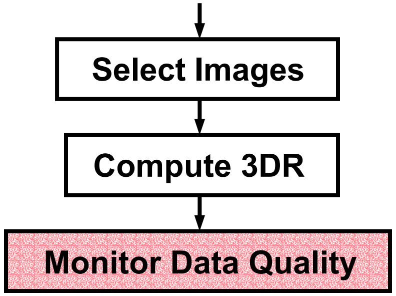
Monitor Data Quality

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

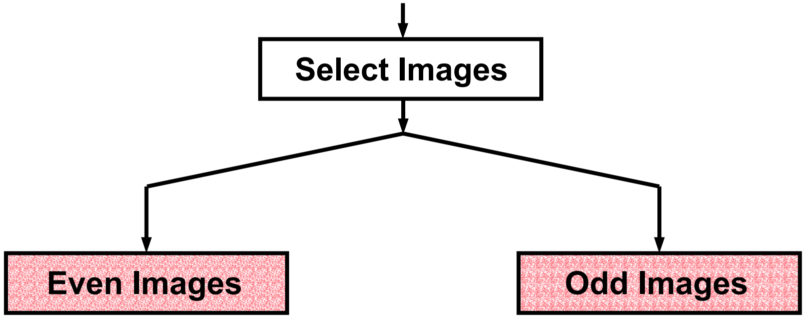
Icosahedral Virus 3D Reconstruction Scheme



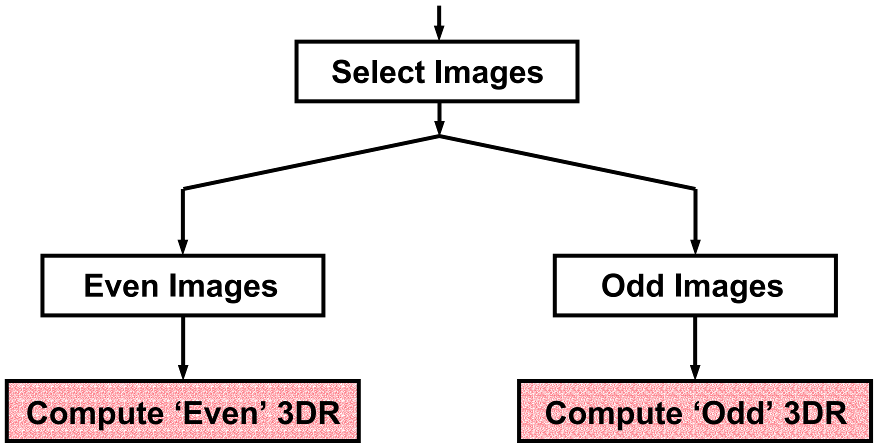
Icosahedral Virus 3D Reconstruction Scheme



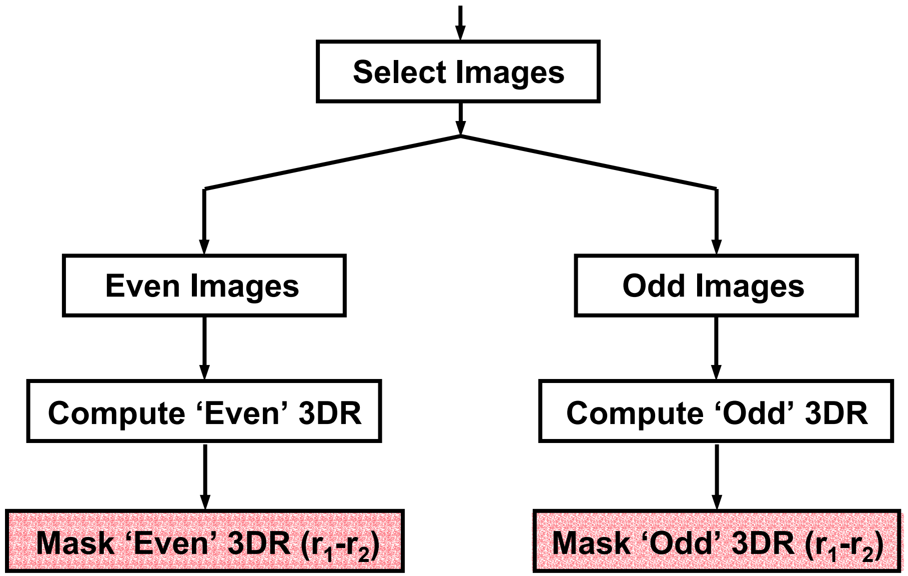
Icosahedral Virus 3D Reconstruction Scheme

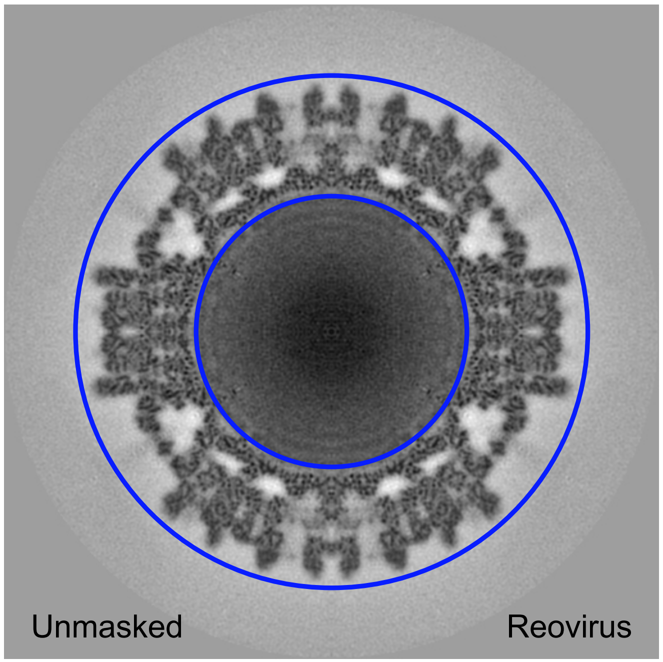


Icosahedral Virus 3D Reconstruction Scheme

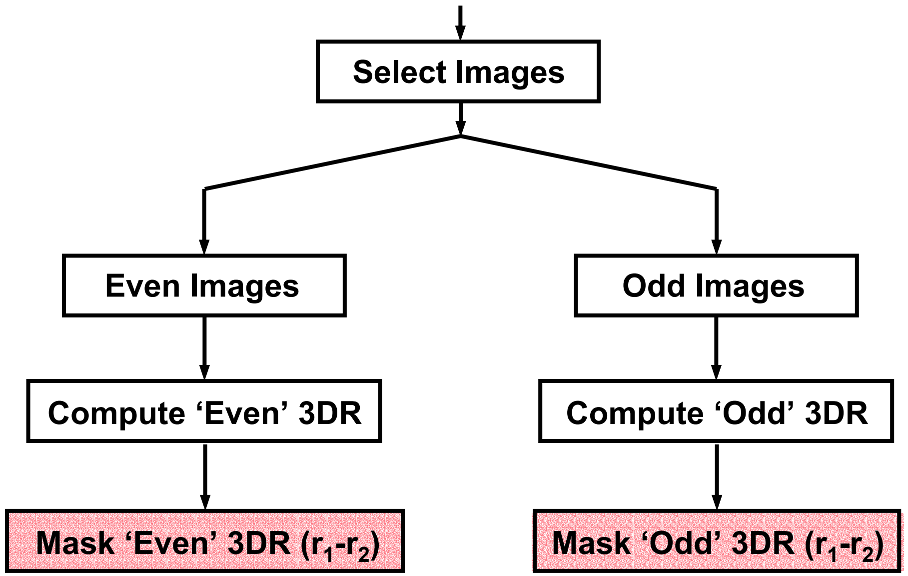


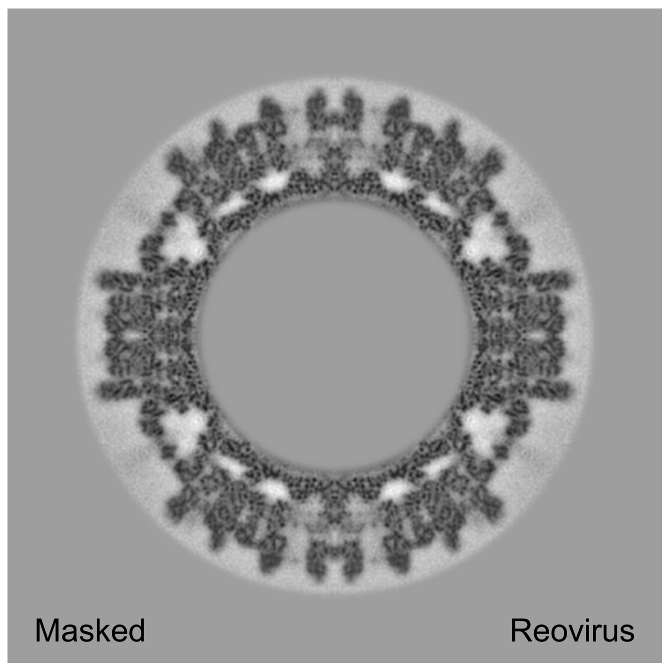
Icosahedral Virus 3D Reconstruction Scheme



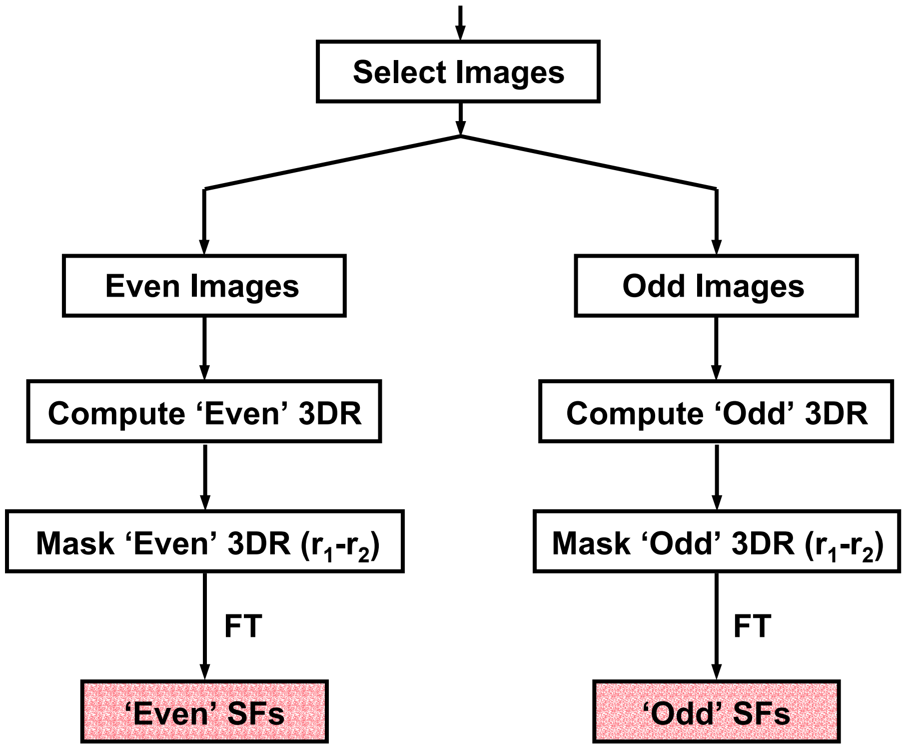


Icosahedral Virus 3D Reconstruction Scheme

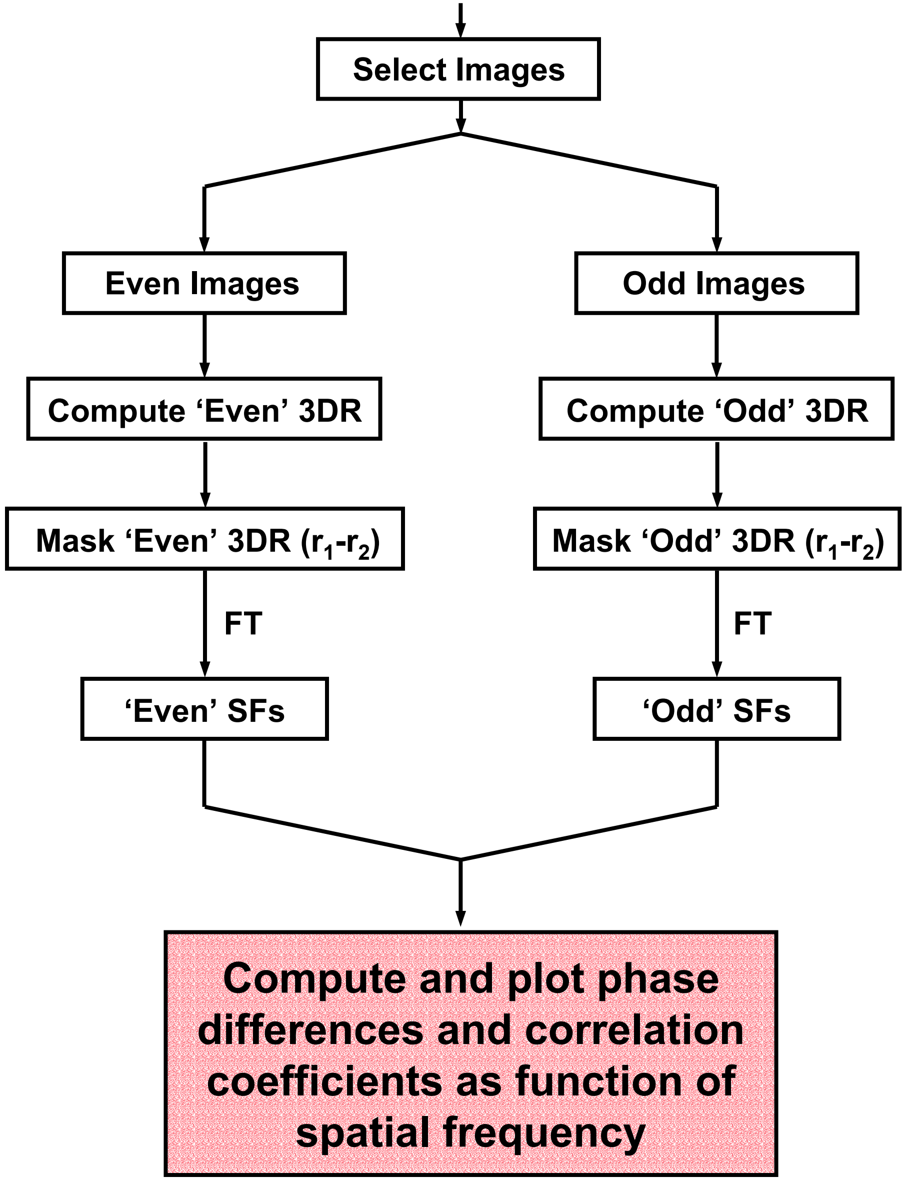




Icosahedral Virus 3D Reconstruction Scheme

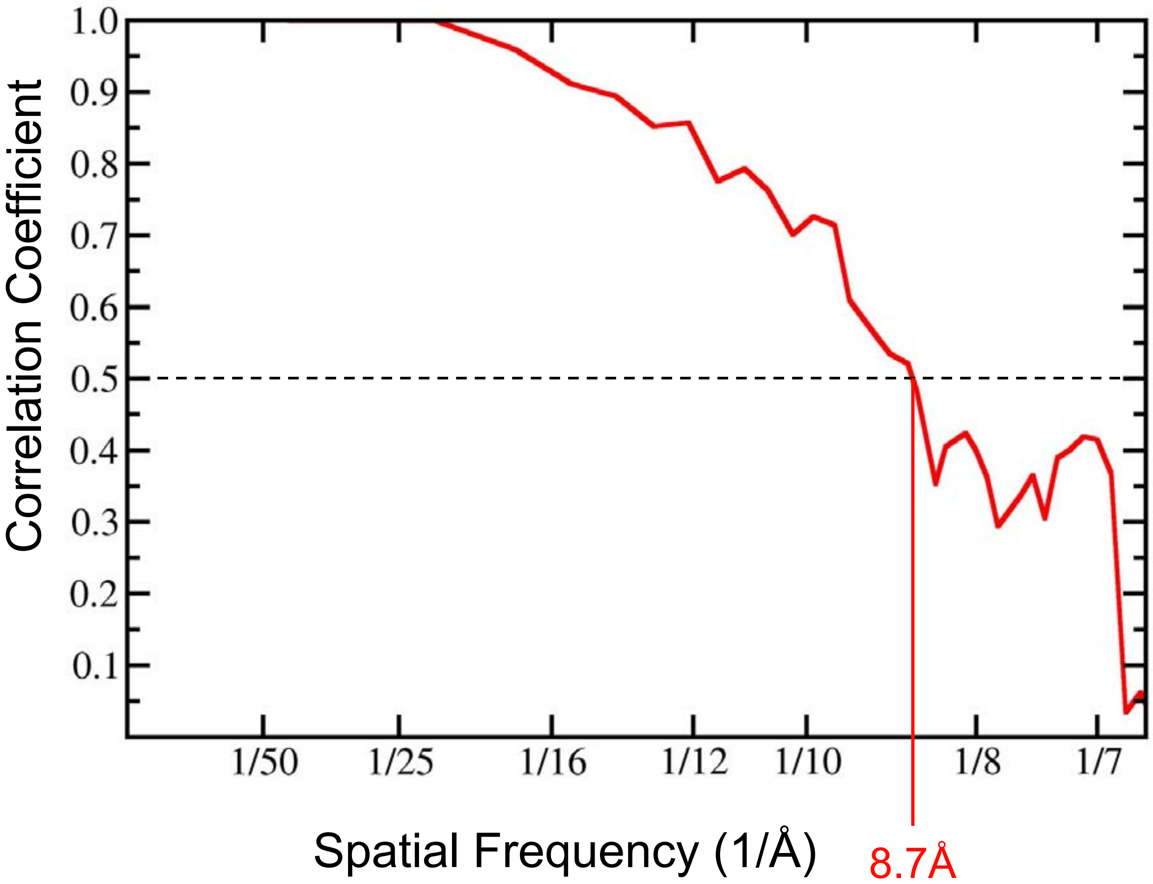


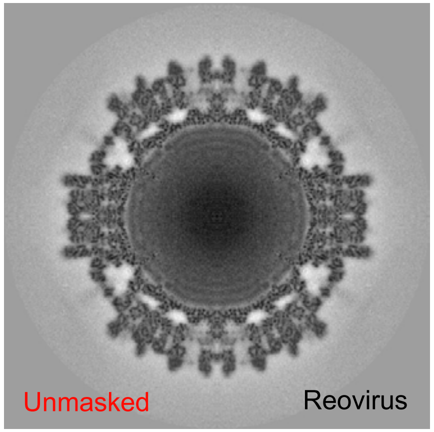
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality





Icosahedral Virus 3D Reconstruction Scheme

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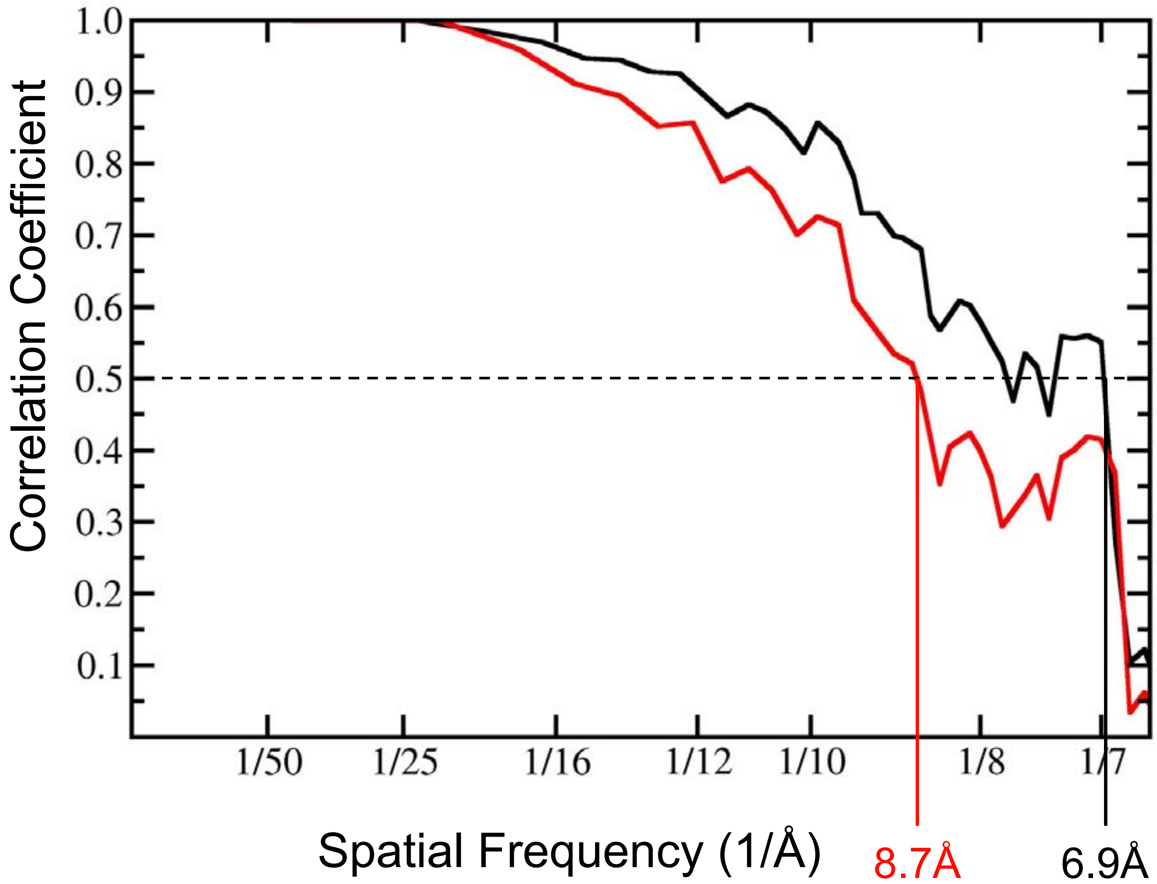
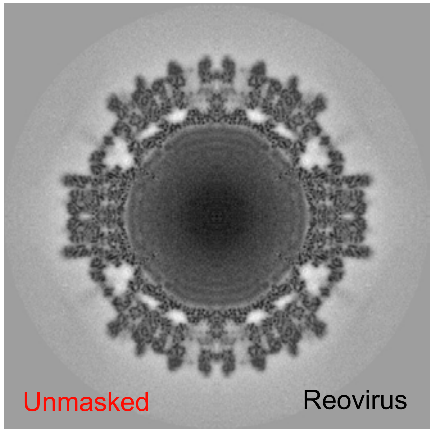
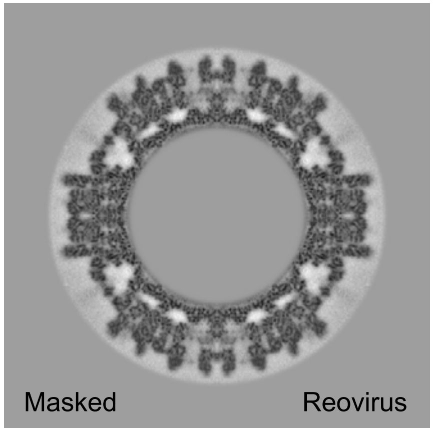






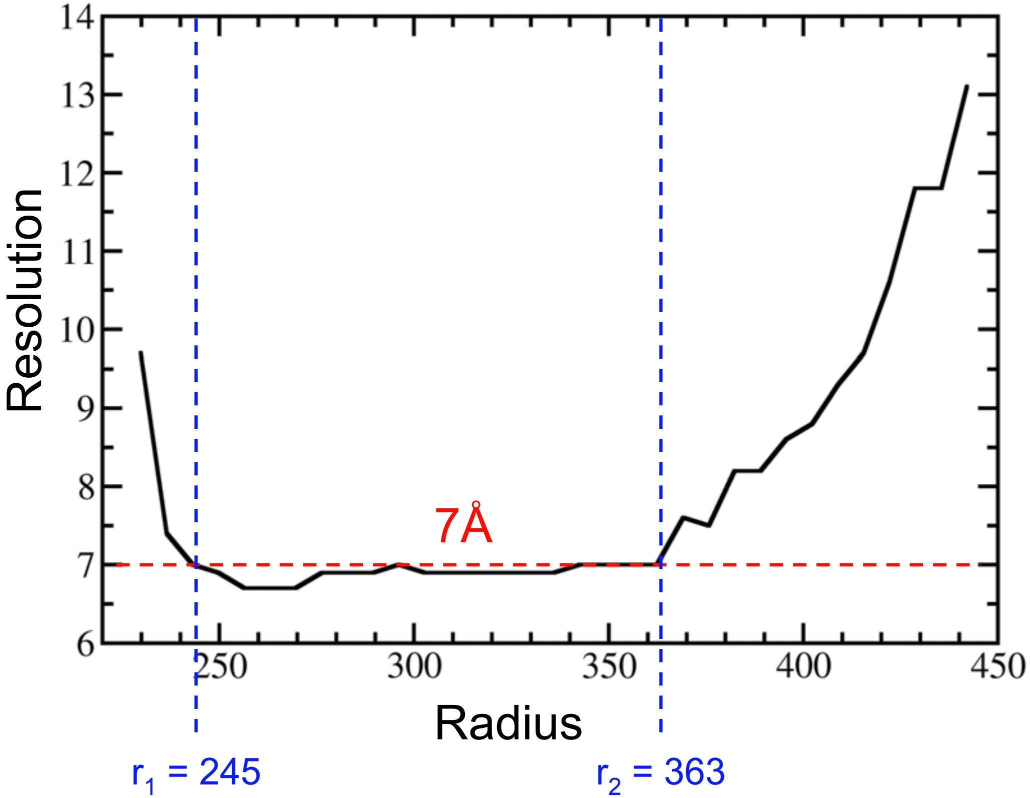
Icosahedral Virus 3D Reconstruction Scheme

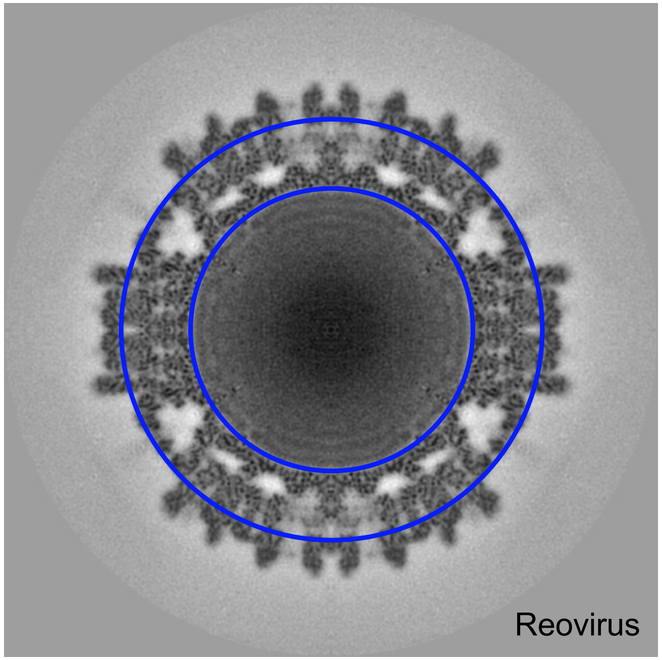
Monitor Data Quality

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

Icosahedral Virus 3D Reconstruction Scheme

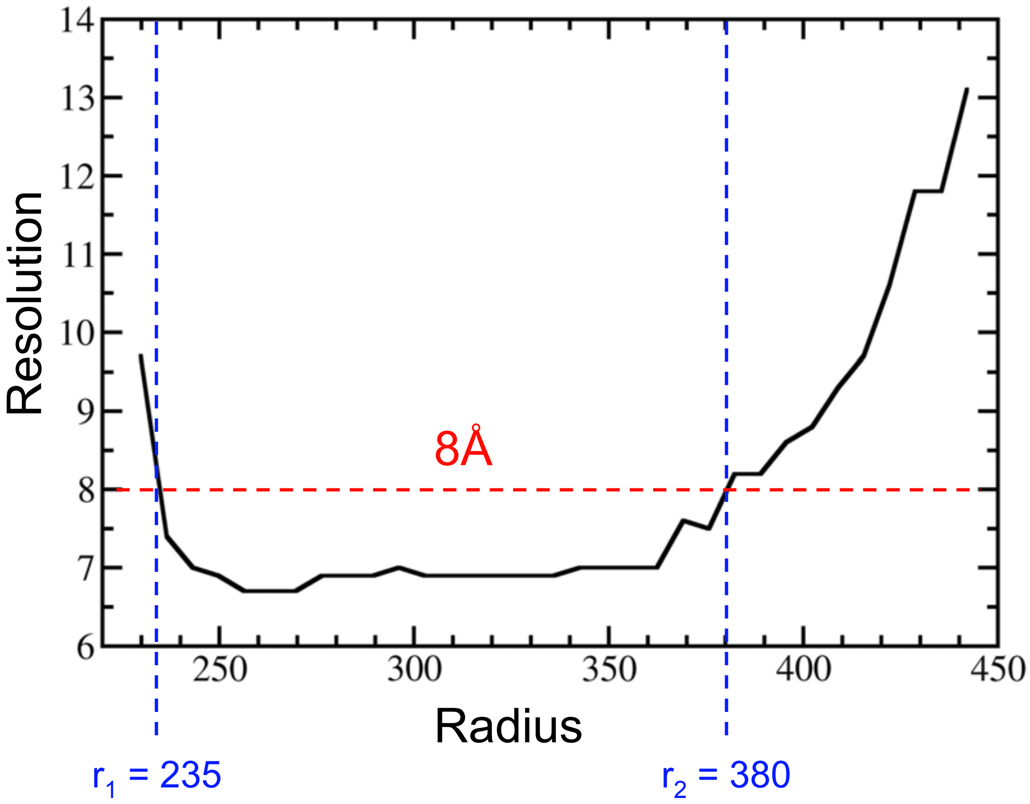
Monitor Data Quality

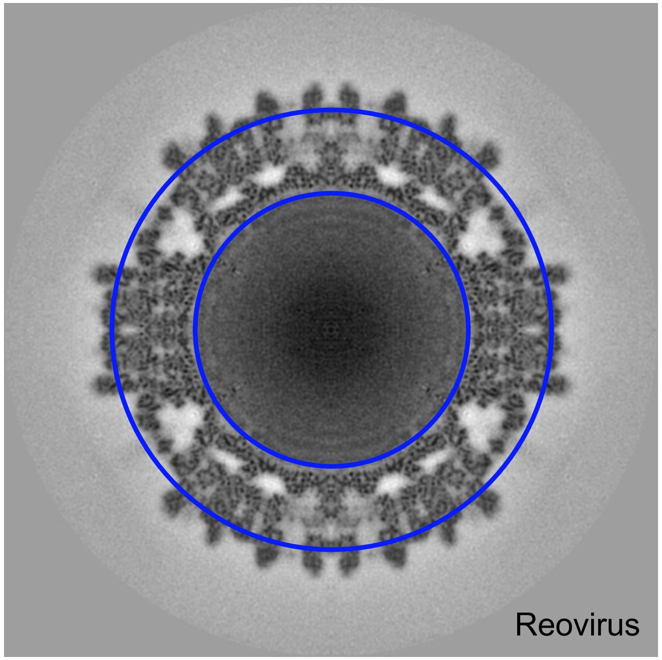




Icosahedral Virus 3D Reconstruction Scheme

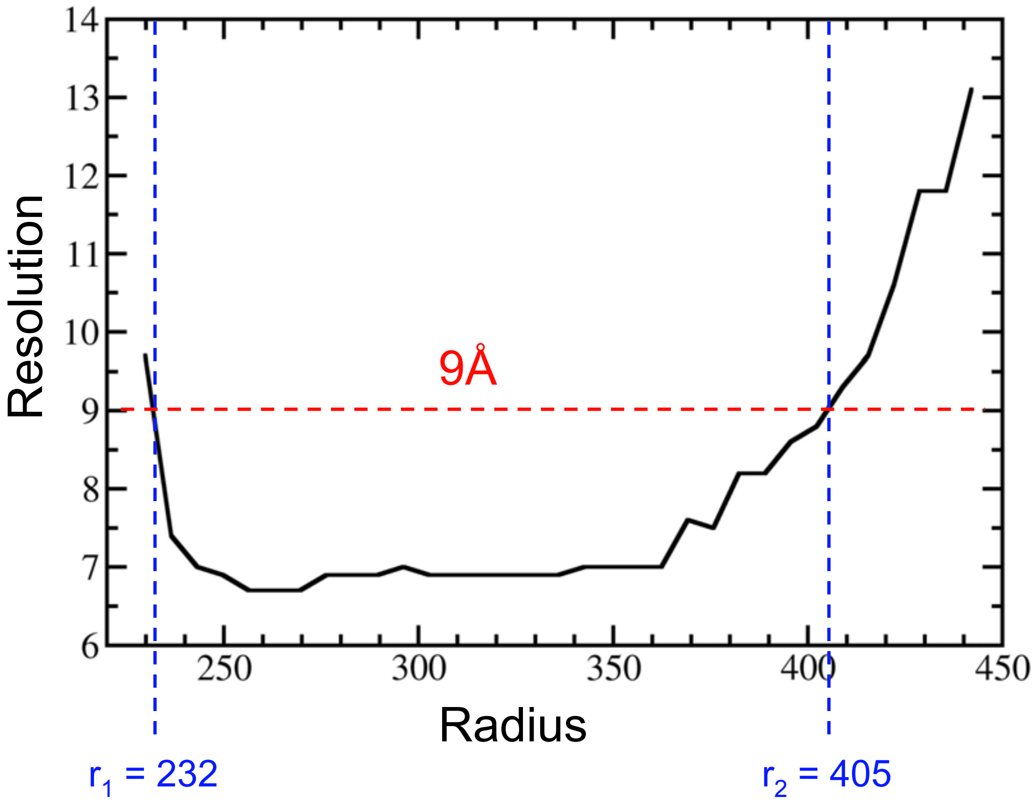
Monitor Data Quality

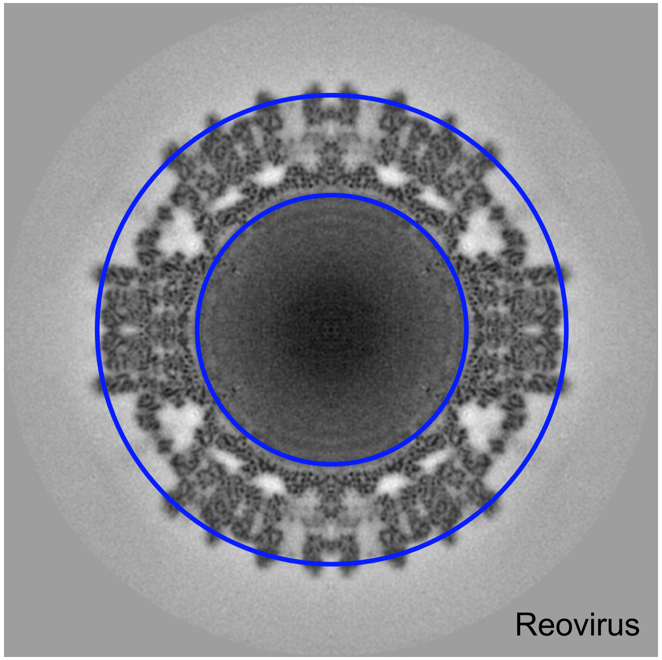




Icosahedral Virus 3D Reconstruction Scheme

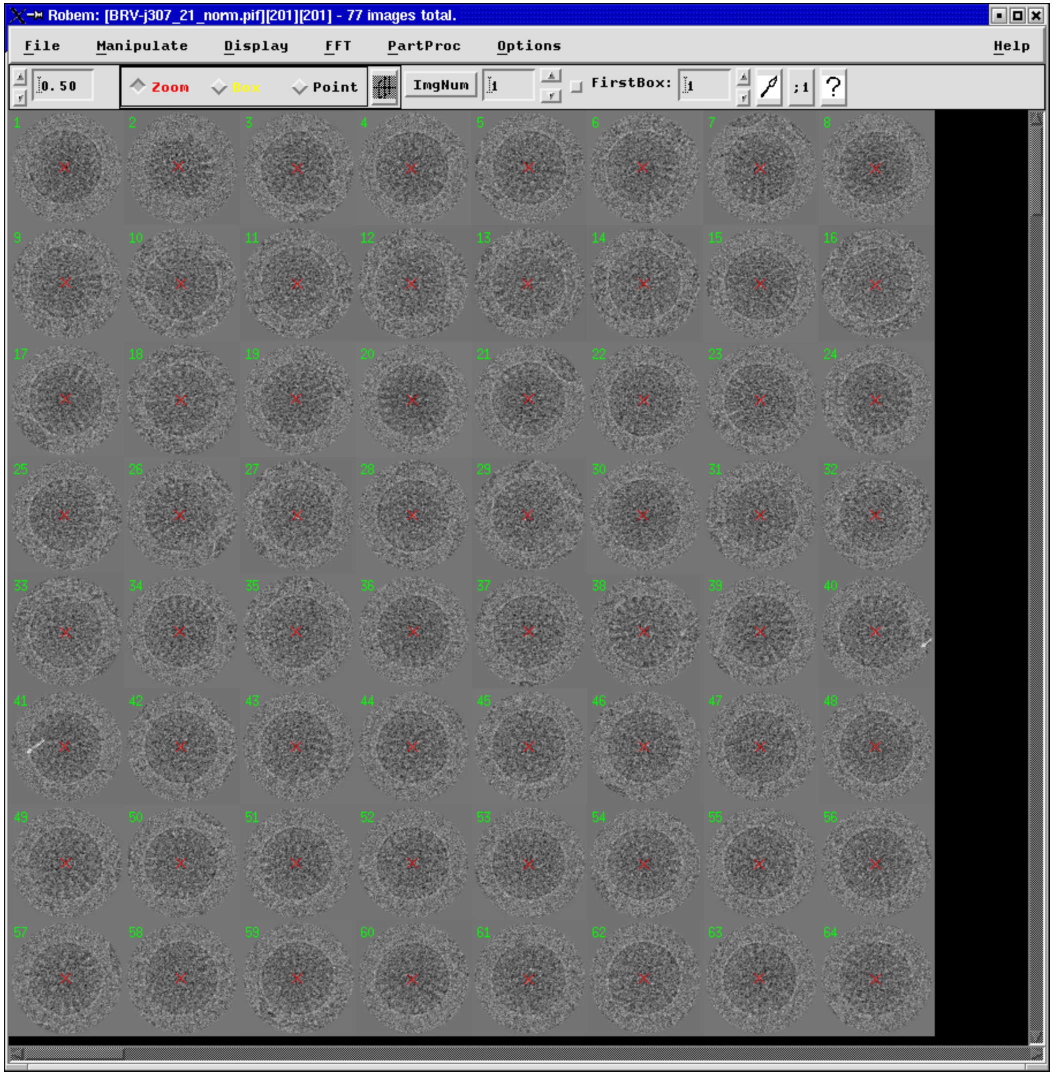
Monitor Data Quality





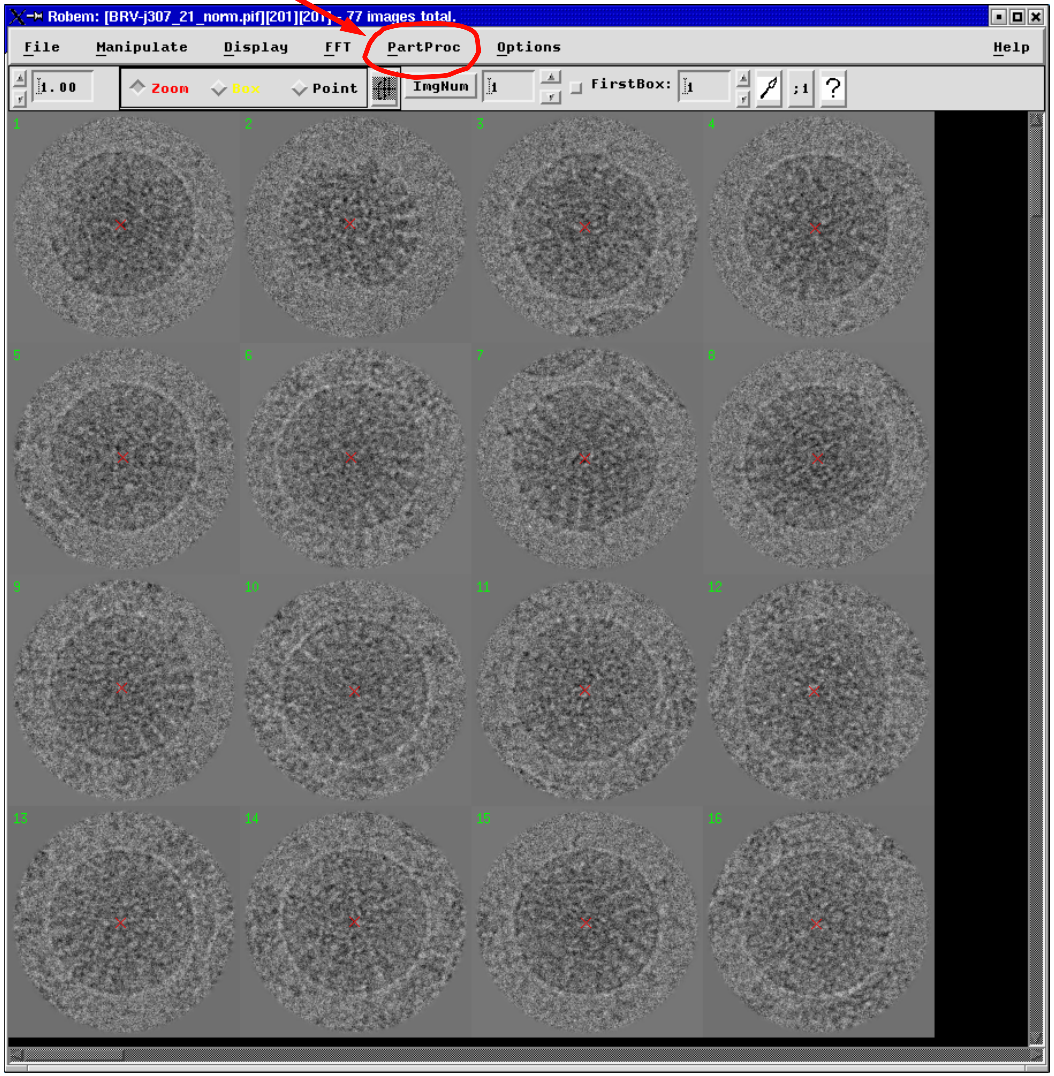
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



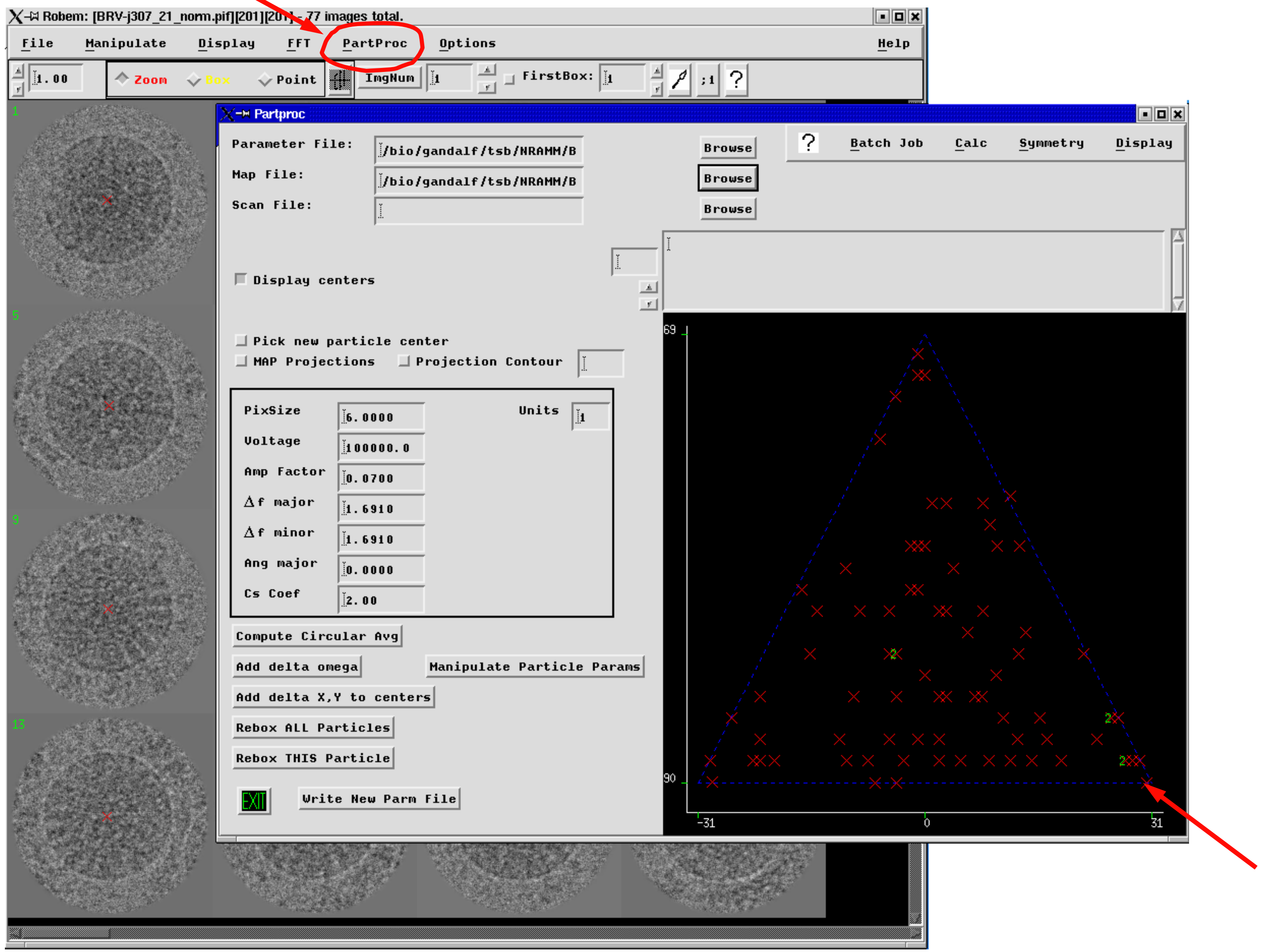
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme

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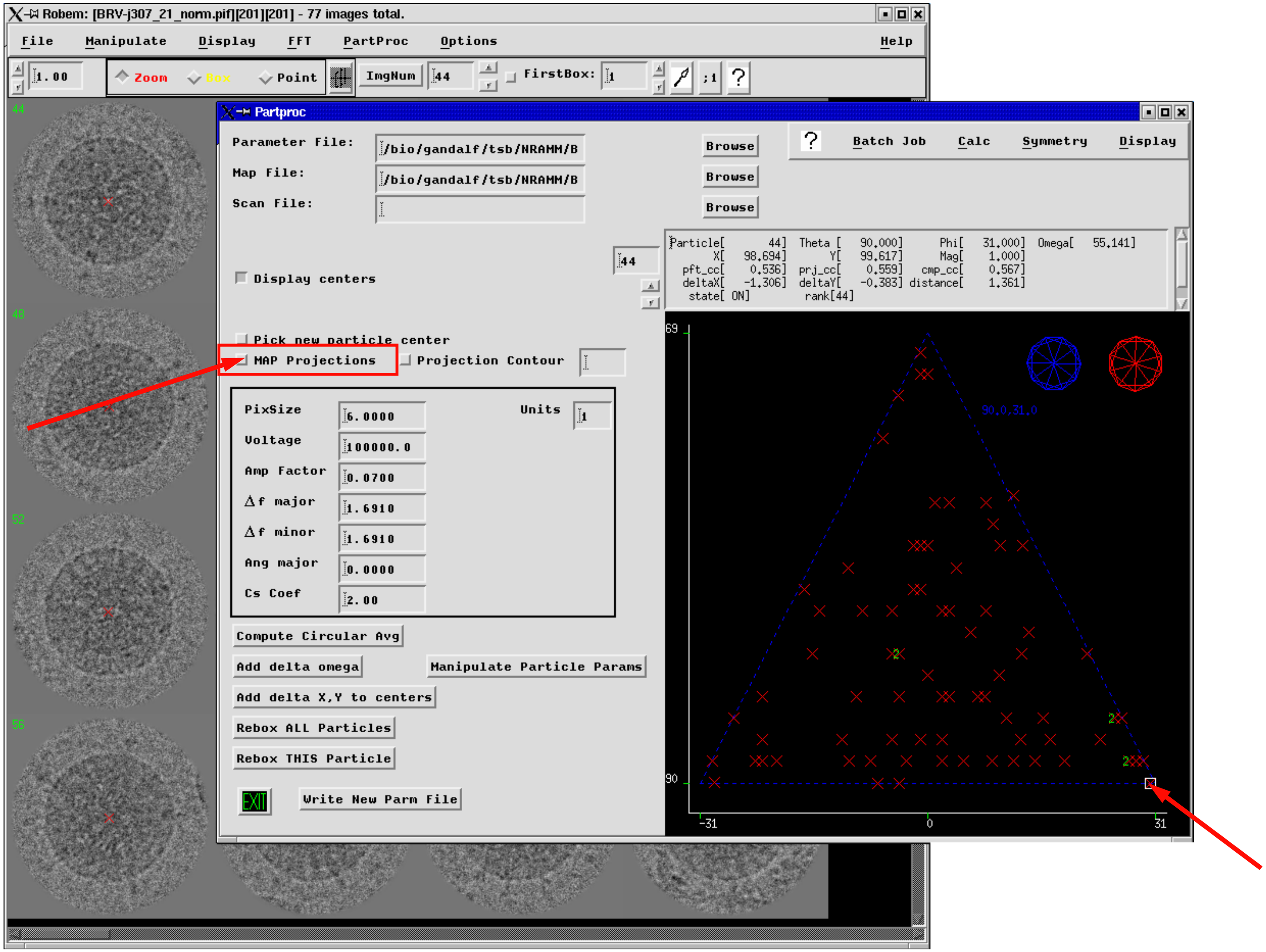

Icosahedral Virus 3D Reconstruction Scheme

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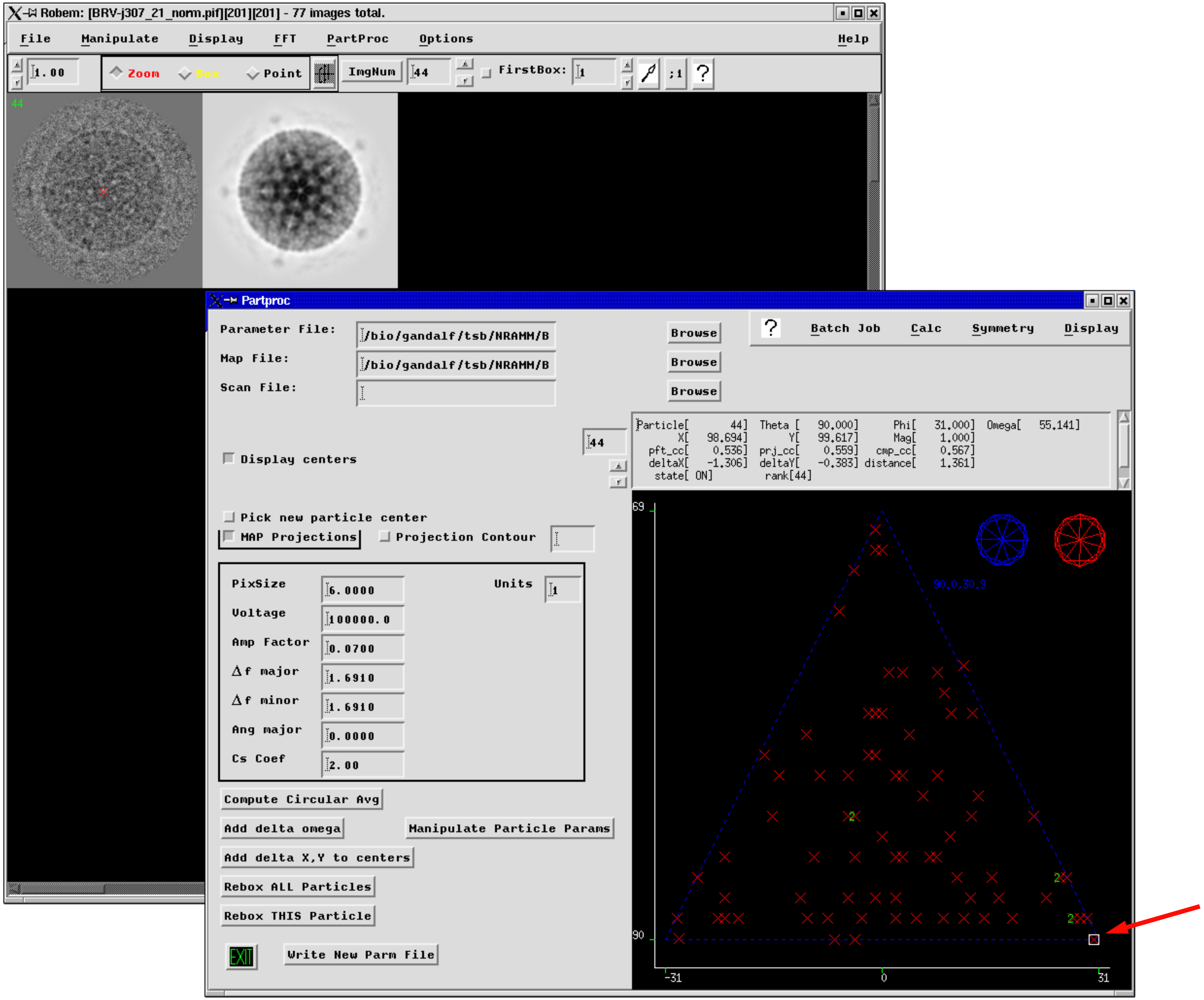

Icosahedral Virus 3D Reconstruction Scheme

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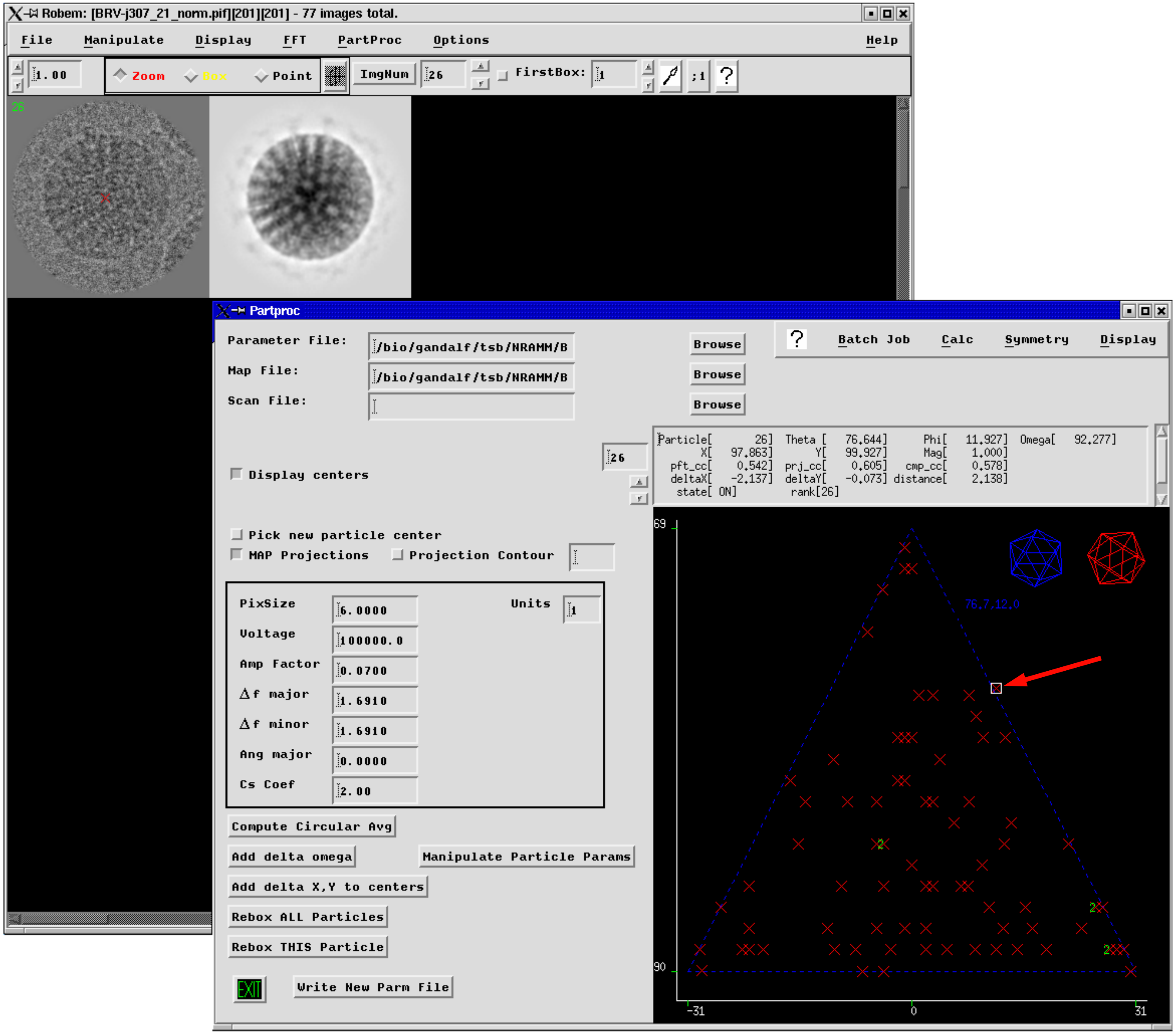

Icosahedral Virus 3D Reconstruction Scheme

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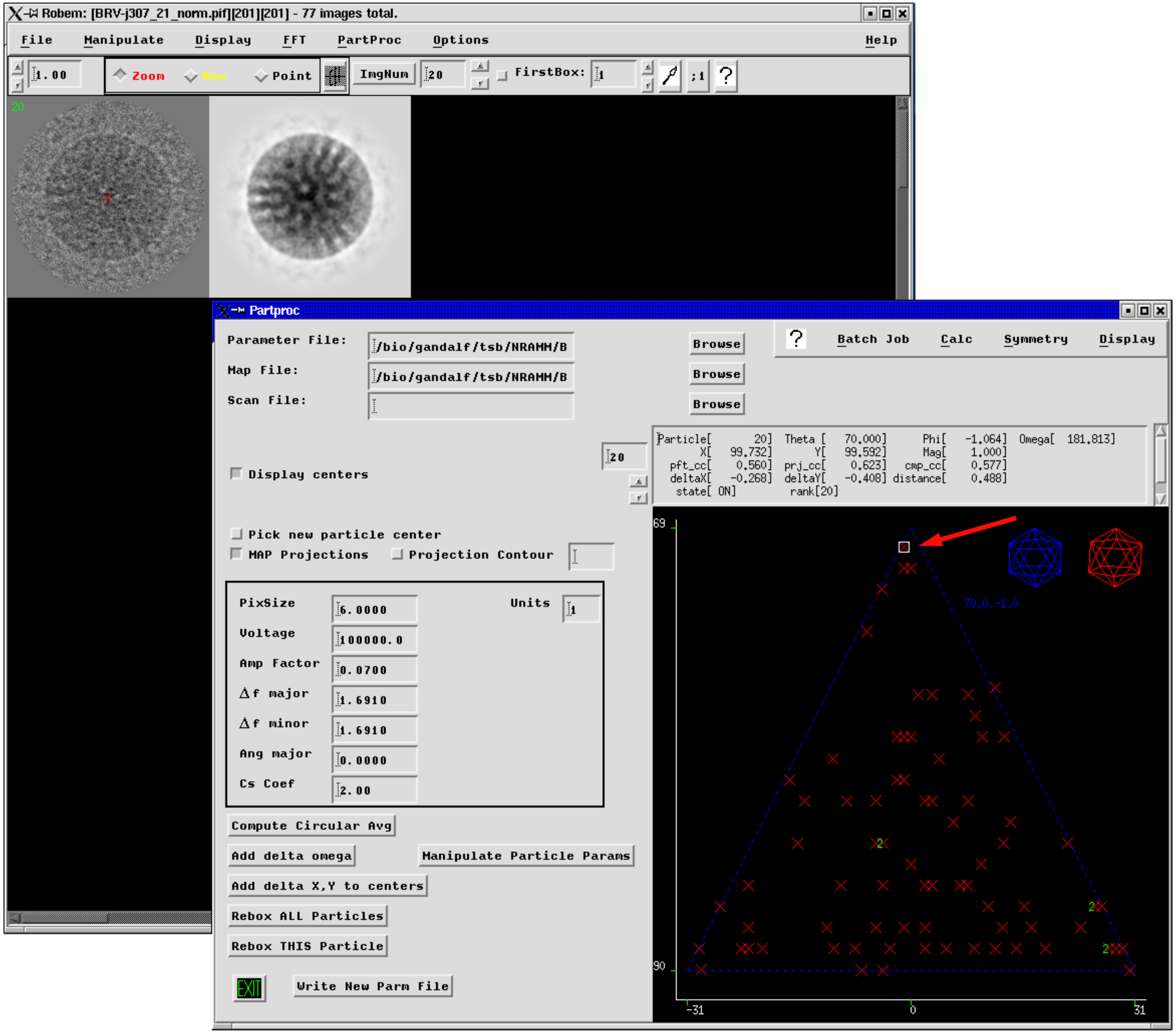
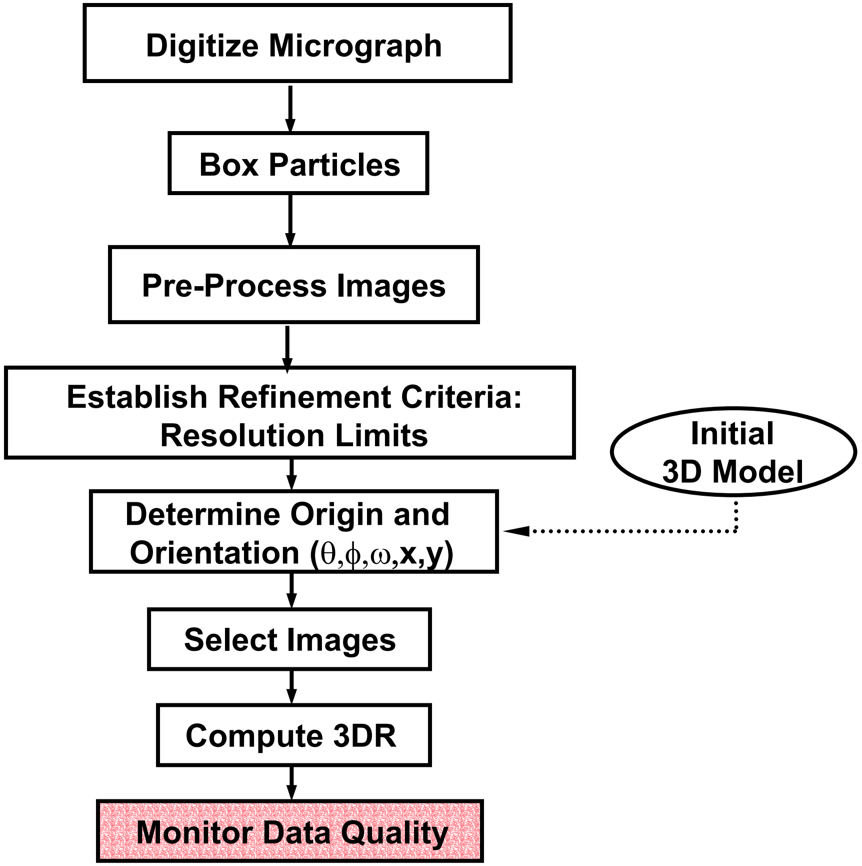
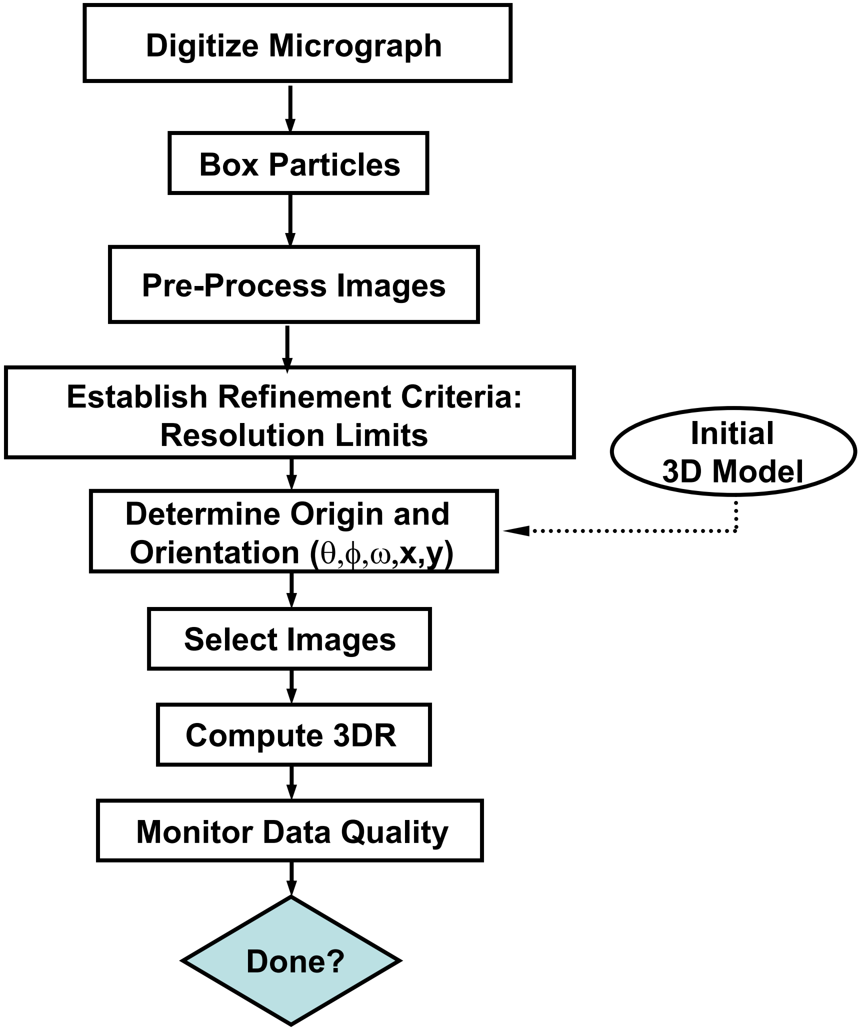


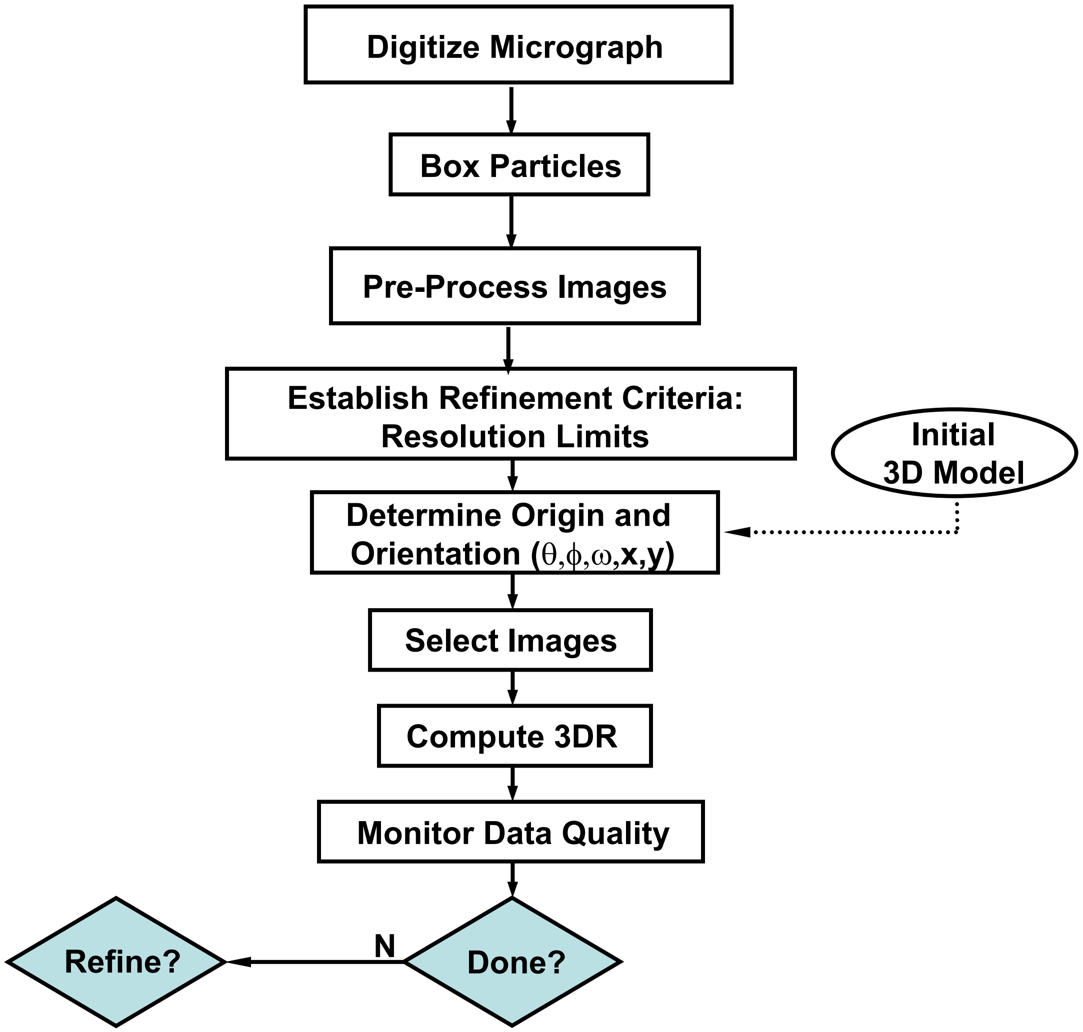
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



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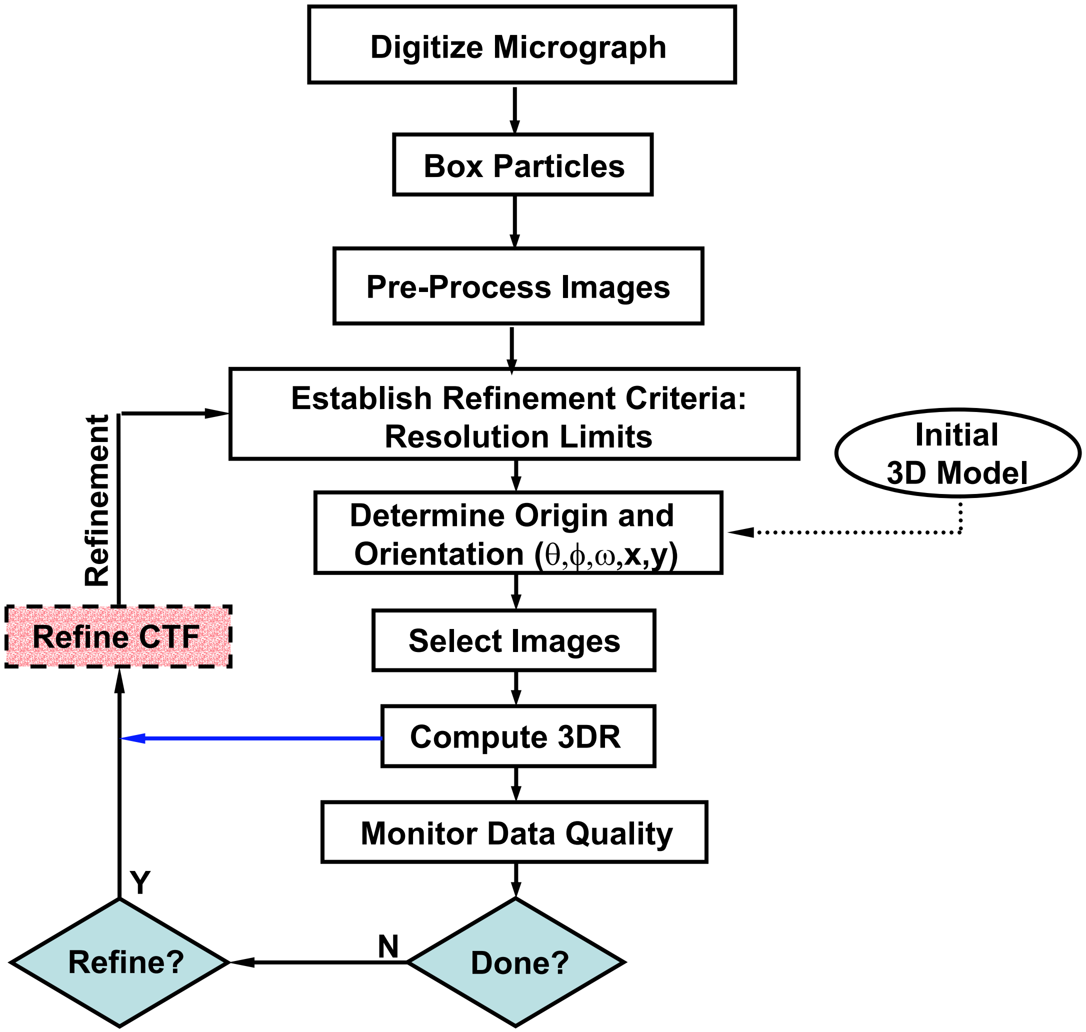
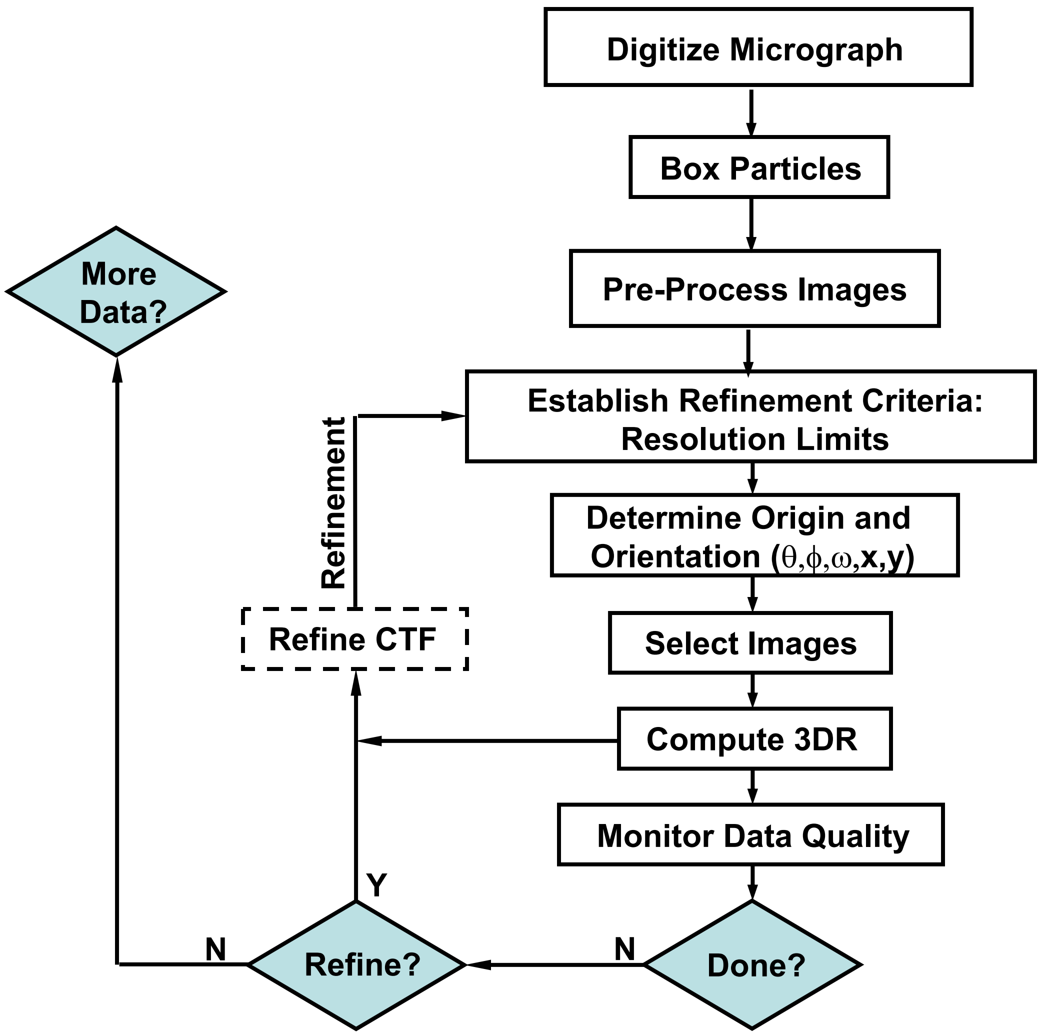


Icosahedral Virus 3D Reconstruction Scheme



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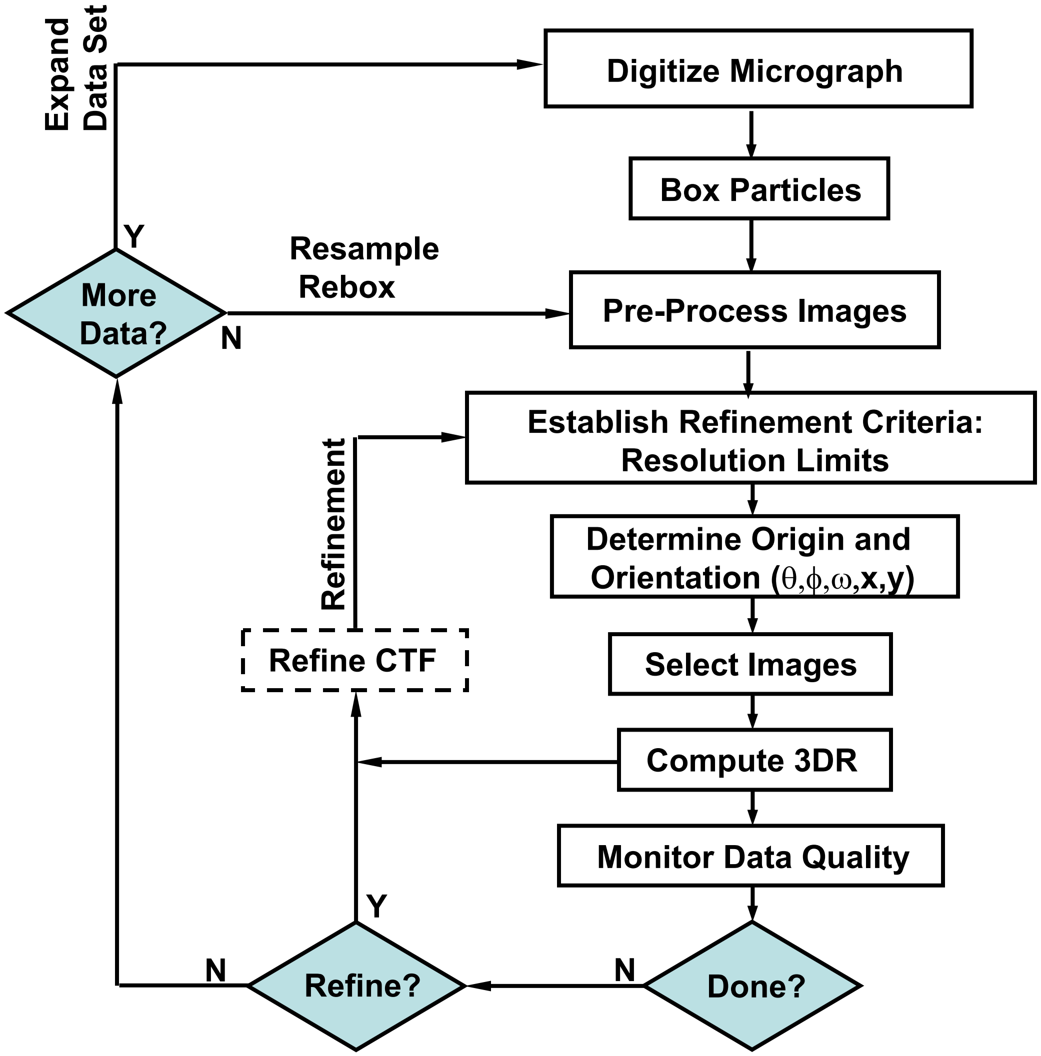

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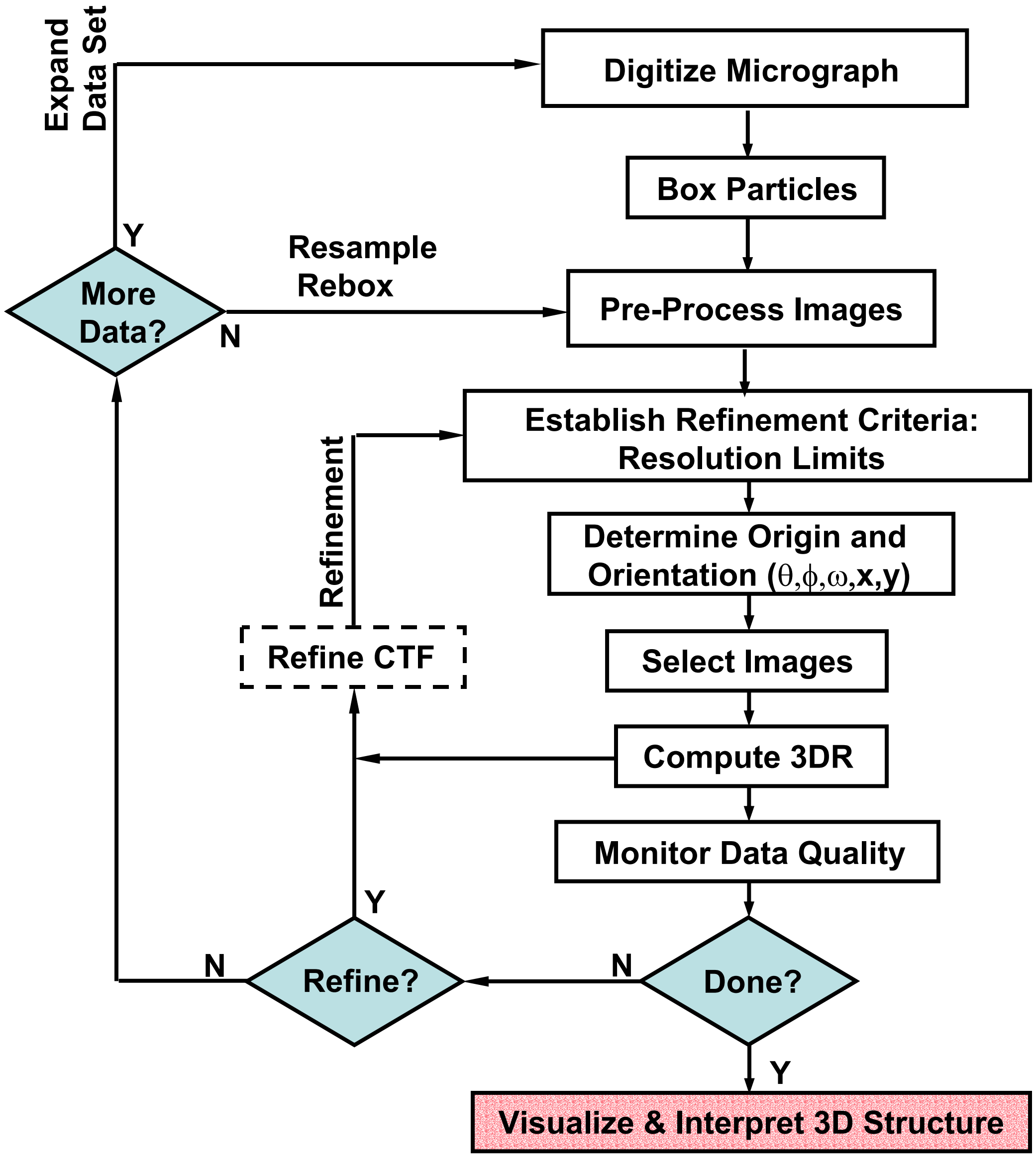

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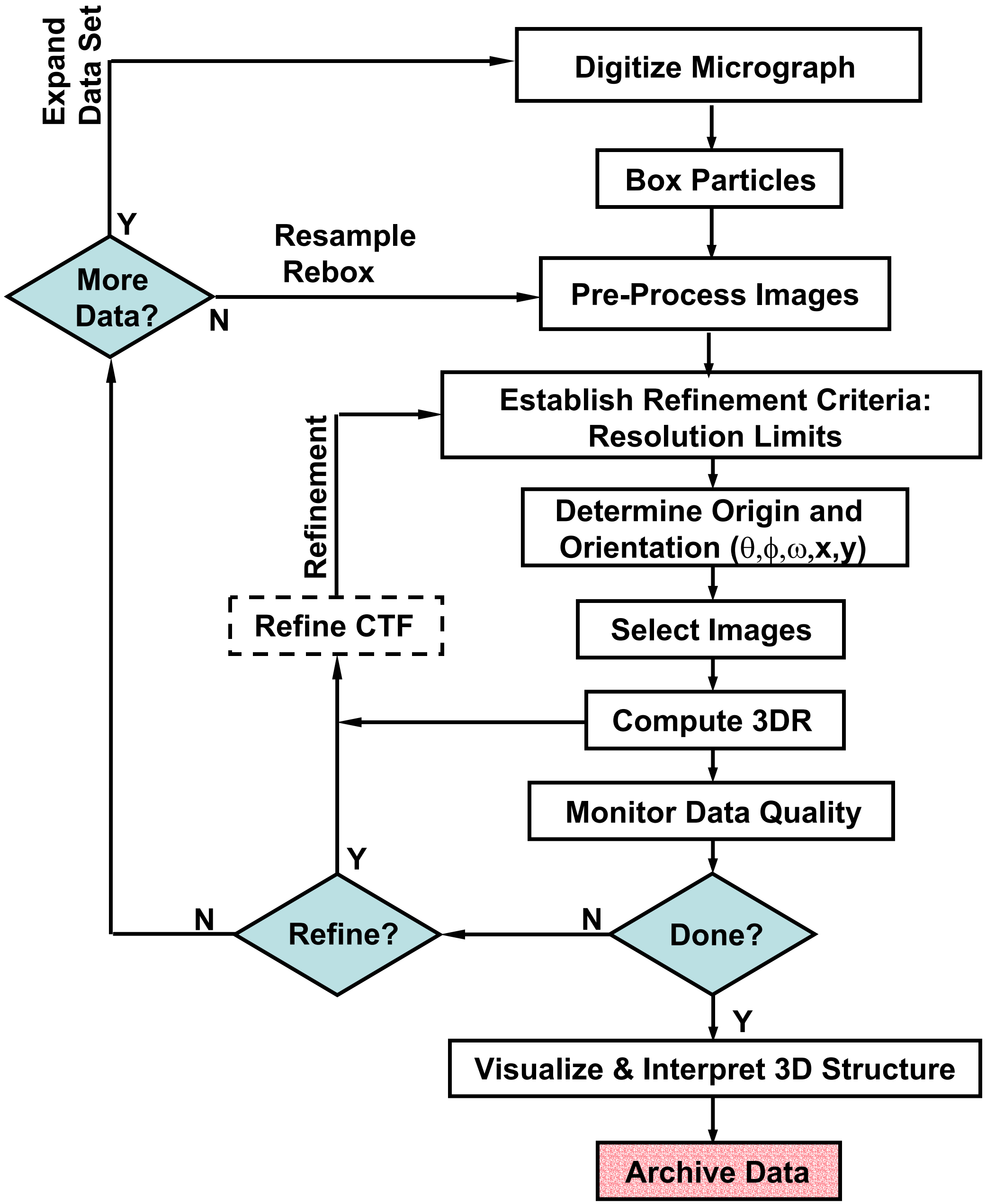

3D Reconstruction of Icosahedral Particles

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

Current and Future Strategies

- Parallelization and new algorithms
    - “Parallel” versions of EM3DR, PFTSEARCH, OOR
    - EM3DR ---> P3DR
    - OOR -------> POOR
- Automation
    - Semi-auto boxing
    - Automated origin/orientation refinement
- Split data set processing
    - Divide image data at very beginning and refine ‘even’ and ‘odd’ data independently.
    - Virtually eliminates any bias in resolution assessment
    - Combine independent reconstructions to obtain ‘final’ 3DR with highest S/N

3D Reconstruction of Icosahedral Particles

Time for lunch???