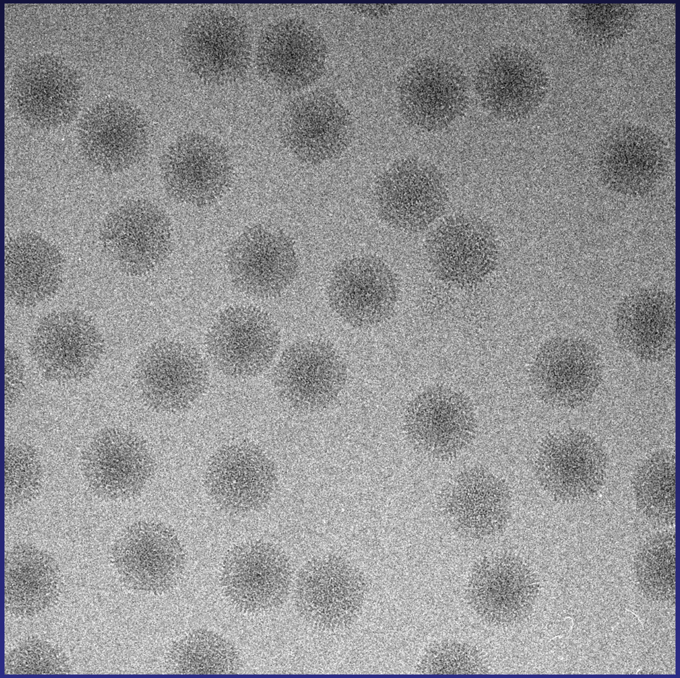
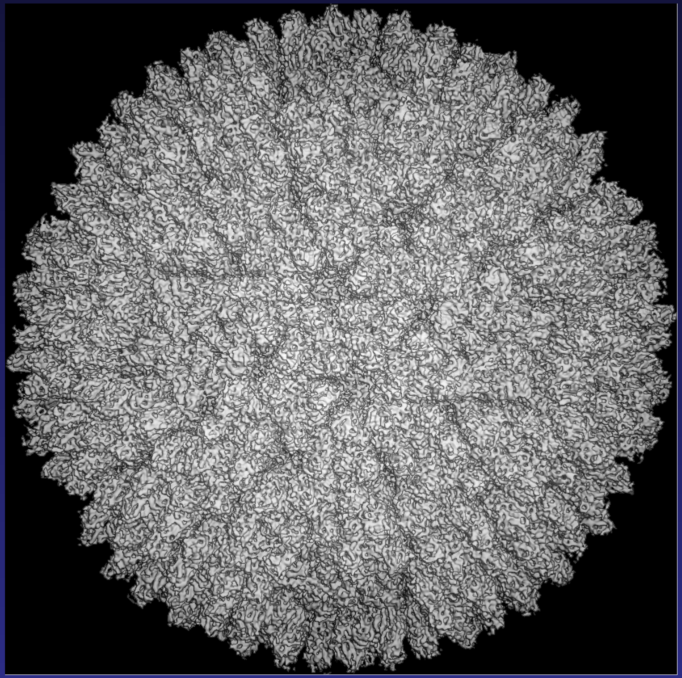
3D Reconstruction of Icosahedral Particles

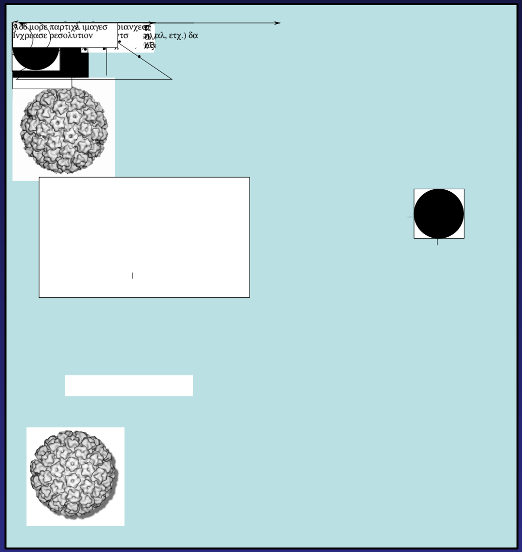




3D Reconstruction of Icosahedral Particles

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

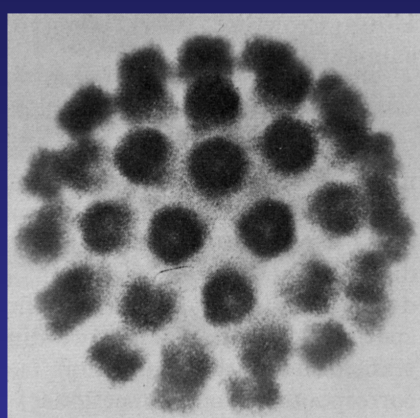
REFERENCES

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**

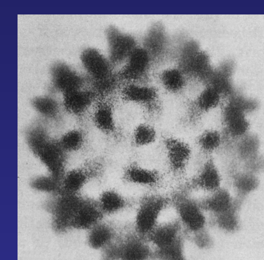
First 3D reconstructions of negatively-stained, spherical viruses:

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

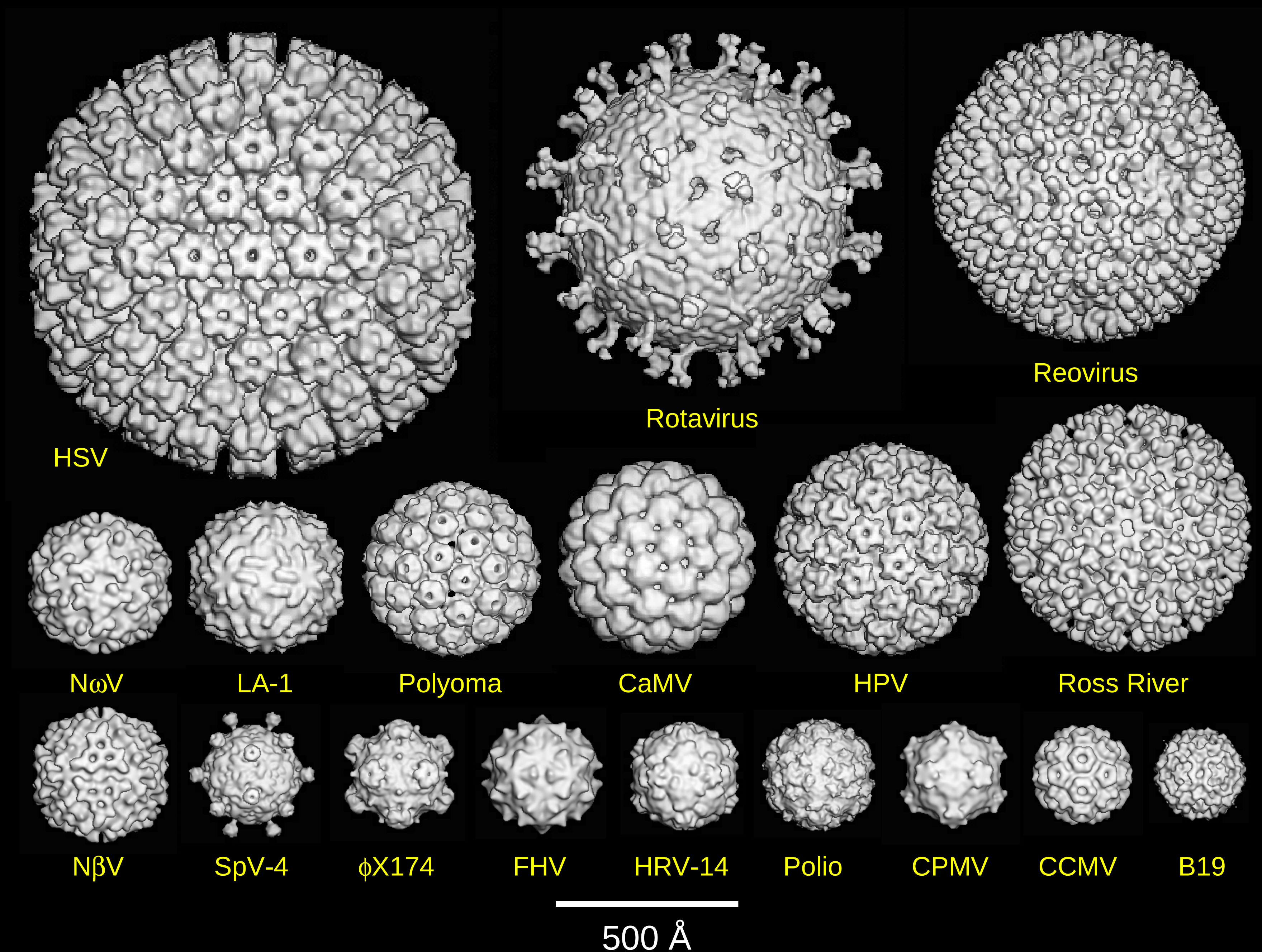
3D Reconstruction of Icosahedral Particles

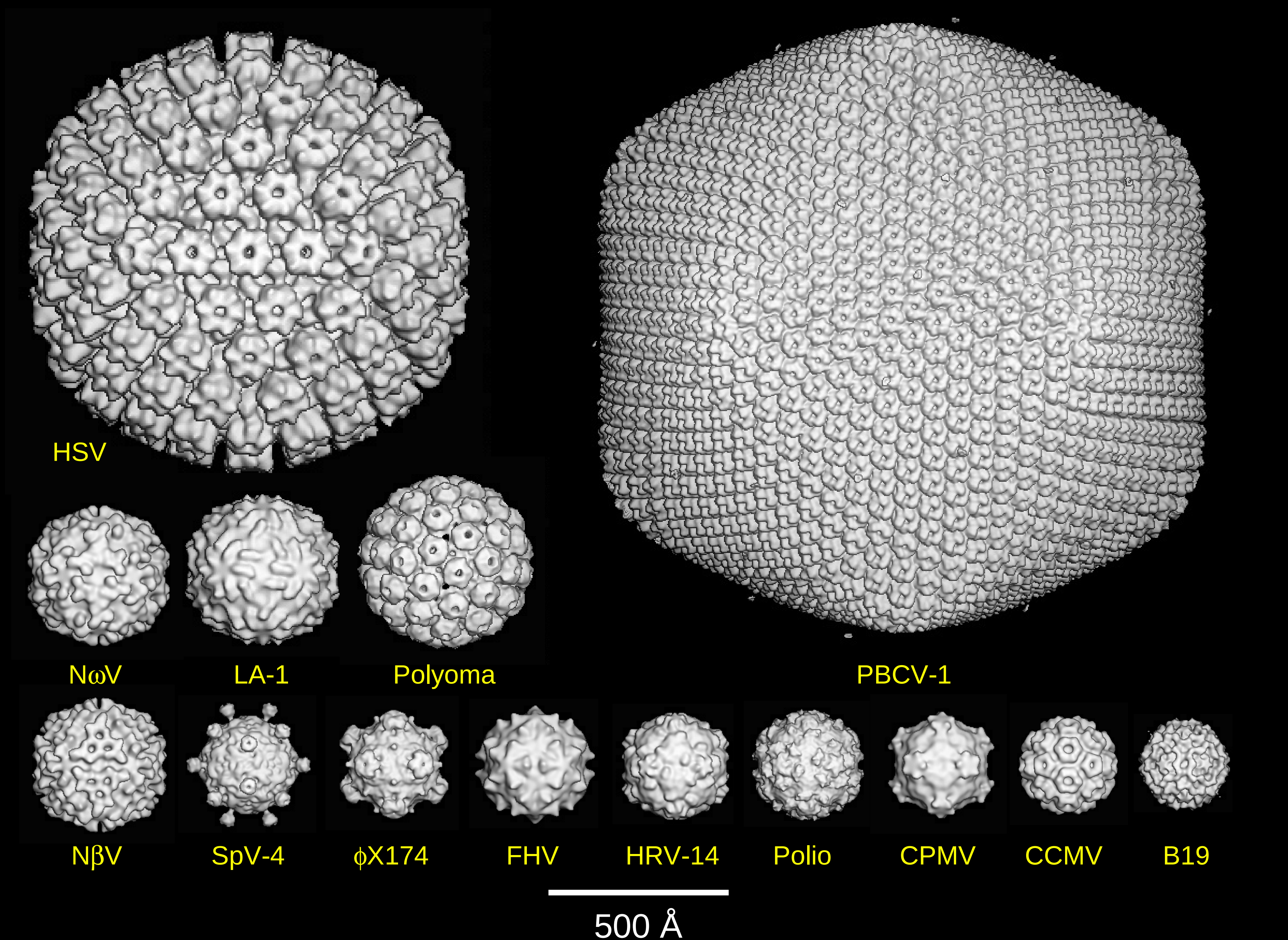
REFERENCES

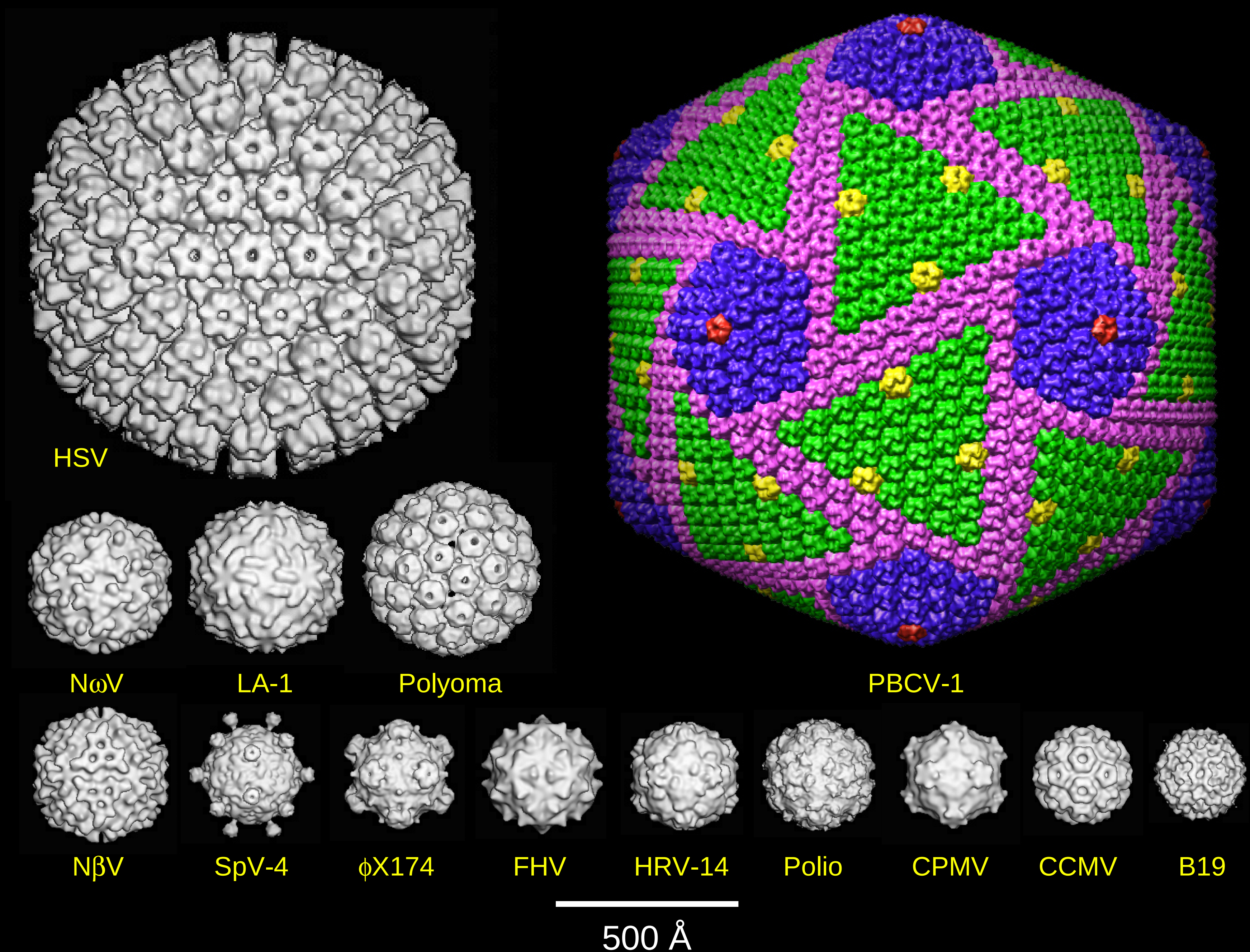
- Reference list available as handout

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


HSV
PBCV-1
NωV
LA-1
Polyoma
NβV
SpV-4
φX174
FHV
HRV-14
Polio
CPMV
CCMV
B19
500 Å


HSV
PBCV-1
NωV
LA-1
Polyoma
NβV
SpV-4
φX174
FHV
HRV-14
Polio
CPMV
CCMV
B19
500 Å

3D Reconstruction of Icosahedral Particles

Outline

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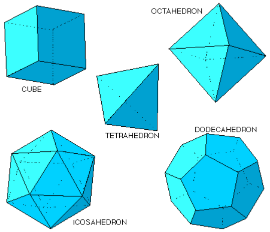
3D Reconstruction of Icosahedral Particles

Symmetry

1. Icosahedral (532) point group symmetry

2. Triangulation symmetry



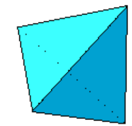
Regular Polyhedra

(Platonic Solids)

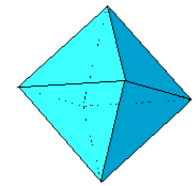
There are just five platonic solids:

From **equilateral triangles** you can make:

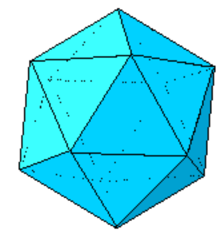
with 3 faces at each vertex, a **tetrahedron**



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

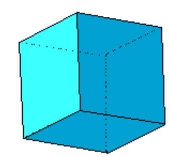


with 5 faces at each vertex, an **icosahedron**



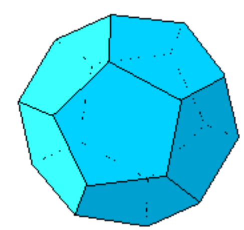
From **squares** you can make:

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

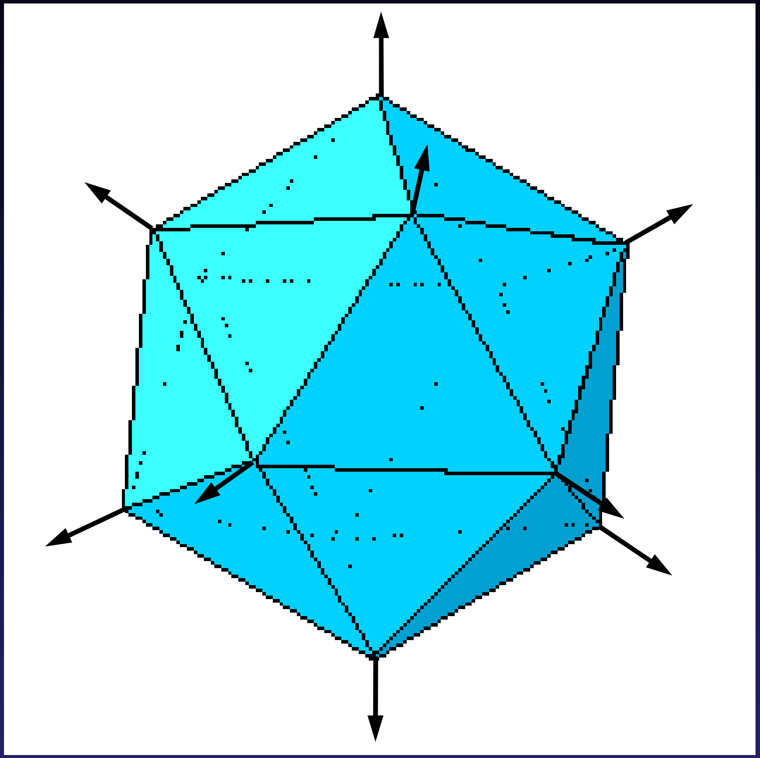


From **pentagons** you can make:

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

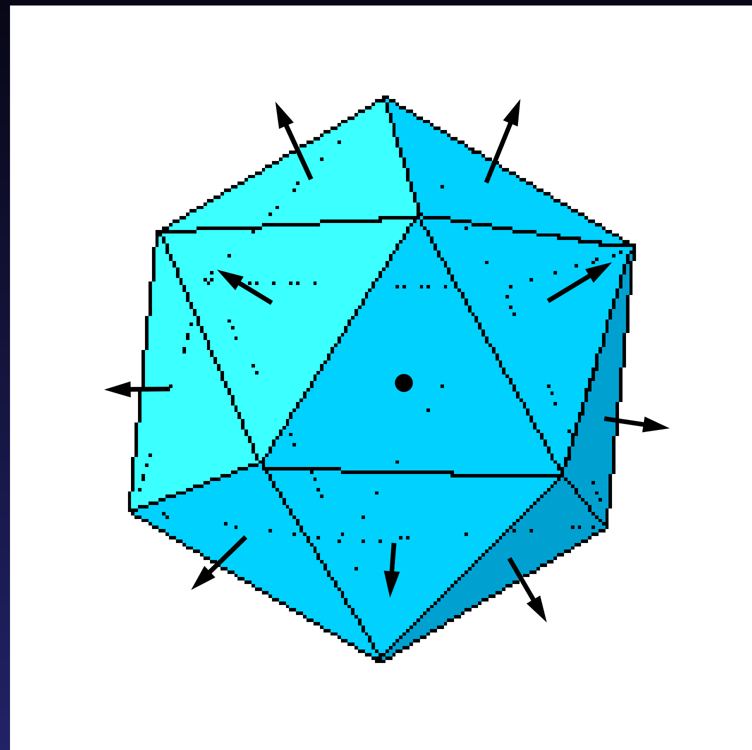


Icosahedral (532) Point Group Symmetry



12 vertices (5-fold)

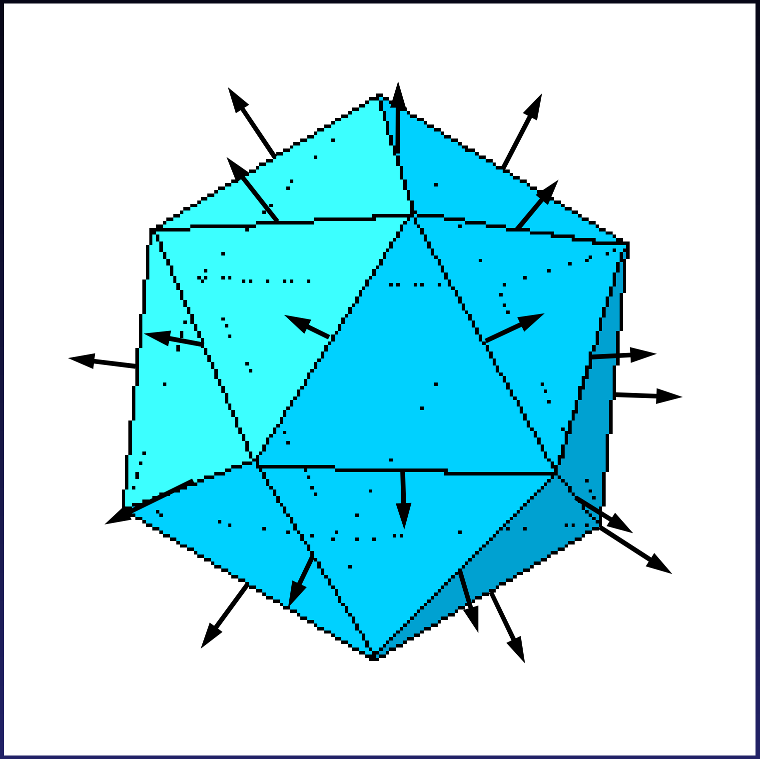
Icosahedral (532) Point Group Symmetry



12 vertices (5-fold)

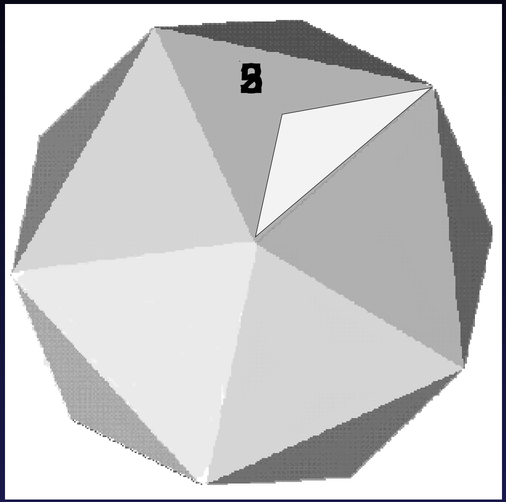
20 faces (3-fold)

Icosahedral (532) Point Group Symmetry

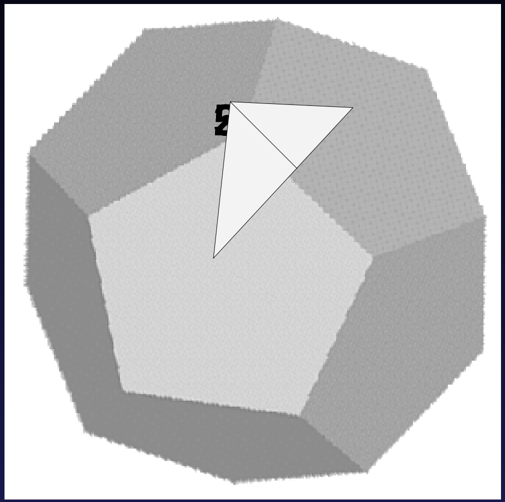


- 12 vertices (5-fold)
- 20 faces (3-fold)
- 30 edges (2-fold)

Icosahedron



Dodecahedron



Different shapes, but both have 532 symmetry

12 vertices, 20 faces, 30 edges

(6 5-folds, 10 3-folds, 15 2-folds)

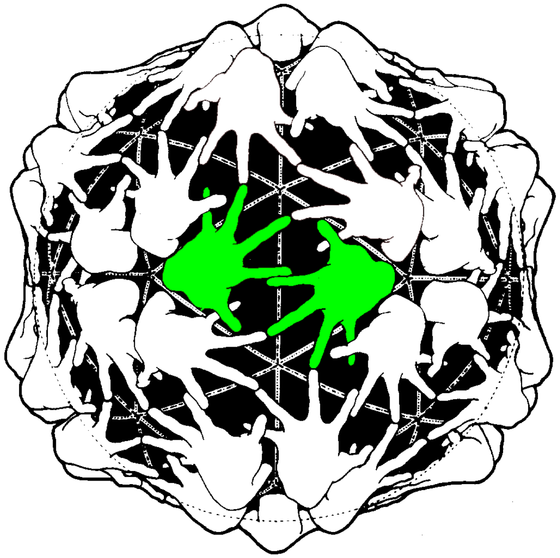
20 vertices, 12 faces, 30 edges

(10 3-folds, 6 5-folds, 15 2-folds)

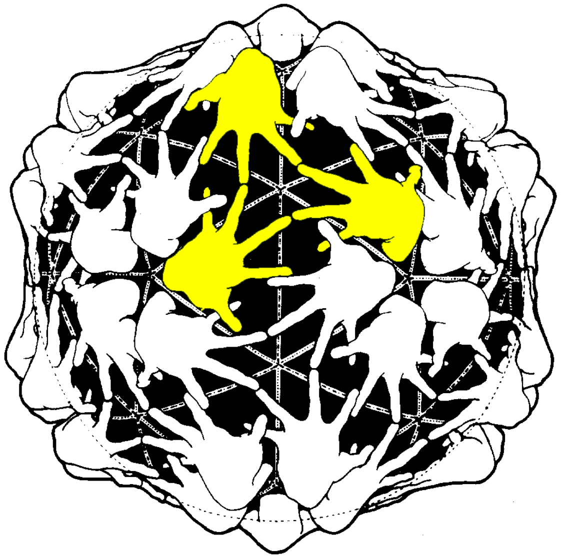
Asymmetric unit is $1/60^{th}$ of whole object

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

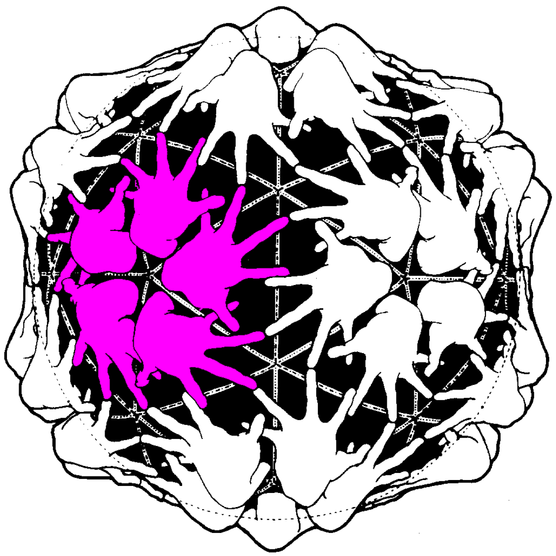
Icosahedral (532) Point Group Symmetry



30 dimers



20 trimers



12 pentamers

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

3D Reconstruction of Icosahedral Particles

Symmetry

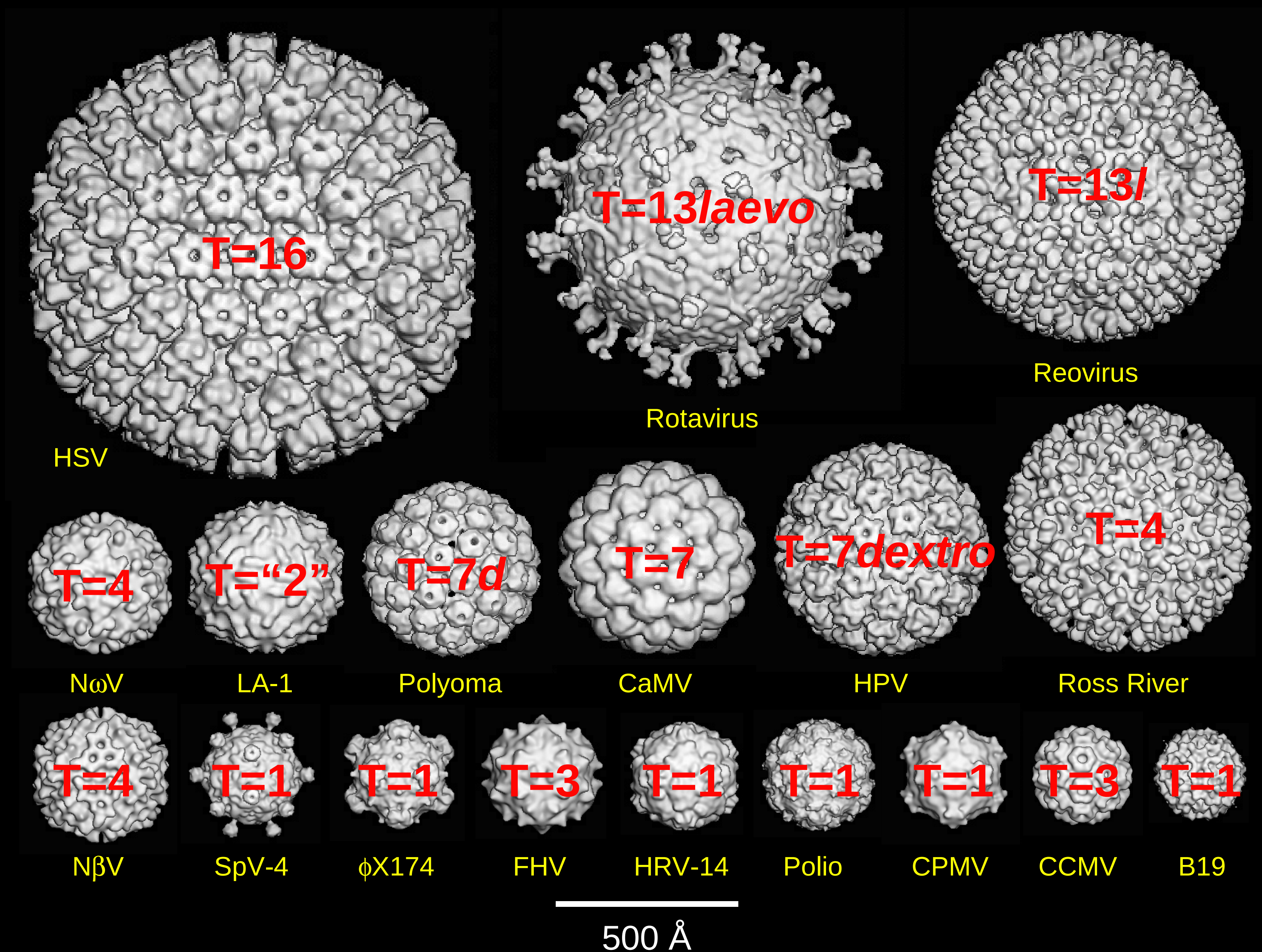
1. Icosahedral (532) point group symmetry

2. Triangulation symmetry

Purely mathematical concept (concerns lattices)

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

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


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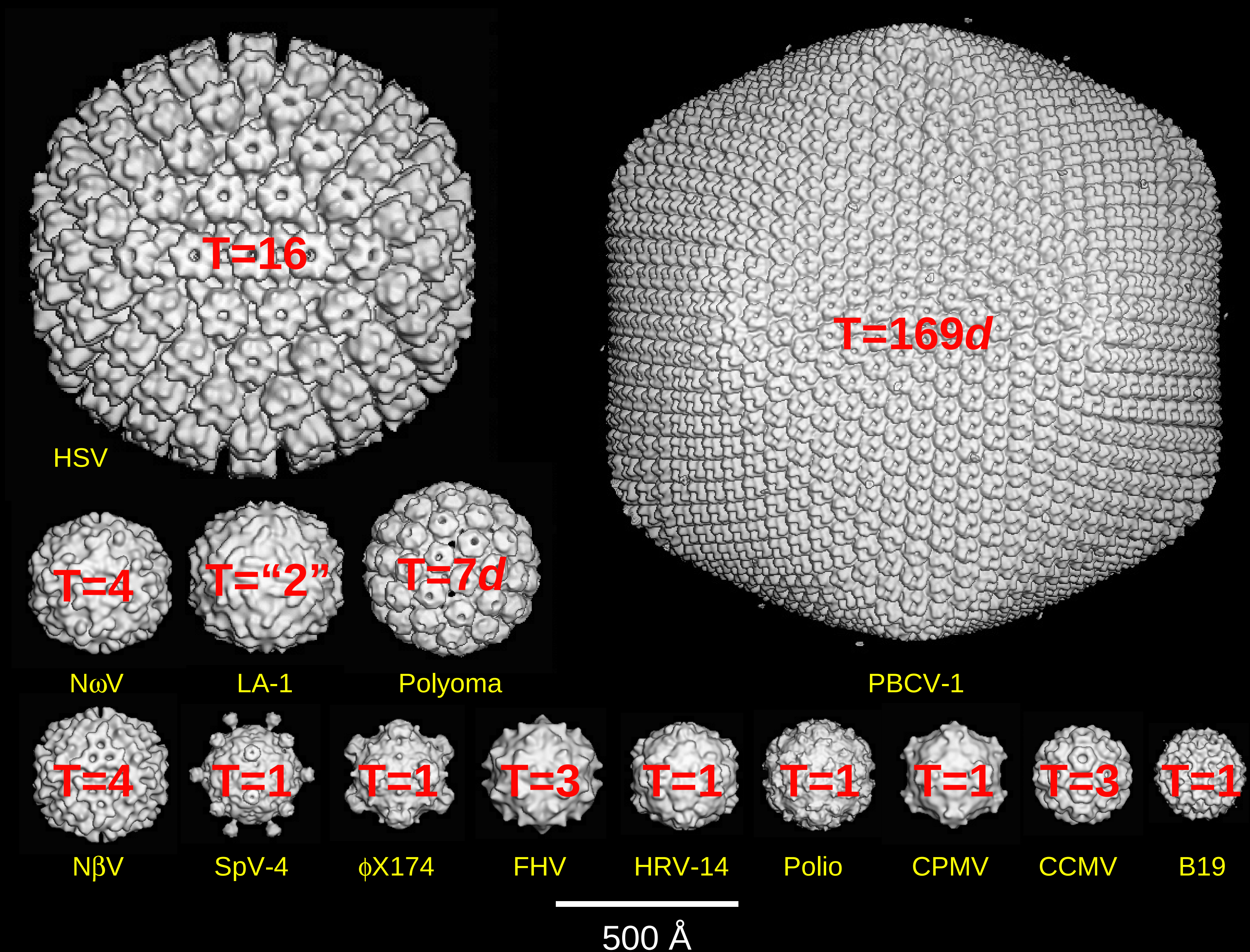
HRV-14
T=1
Polio
T=1
CPMV
T=3
CCMV
T=1
B19
500 Å


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

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

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

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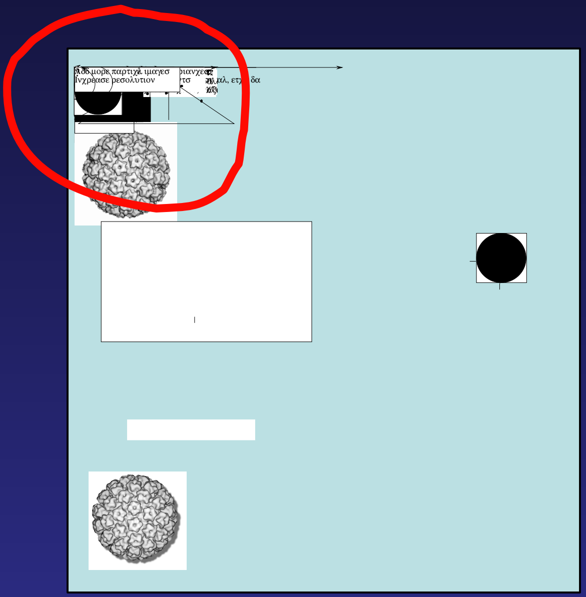


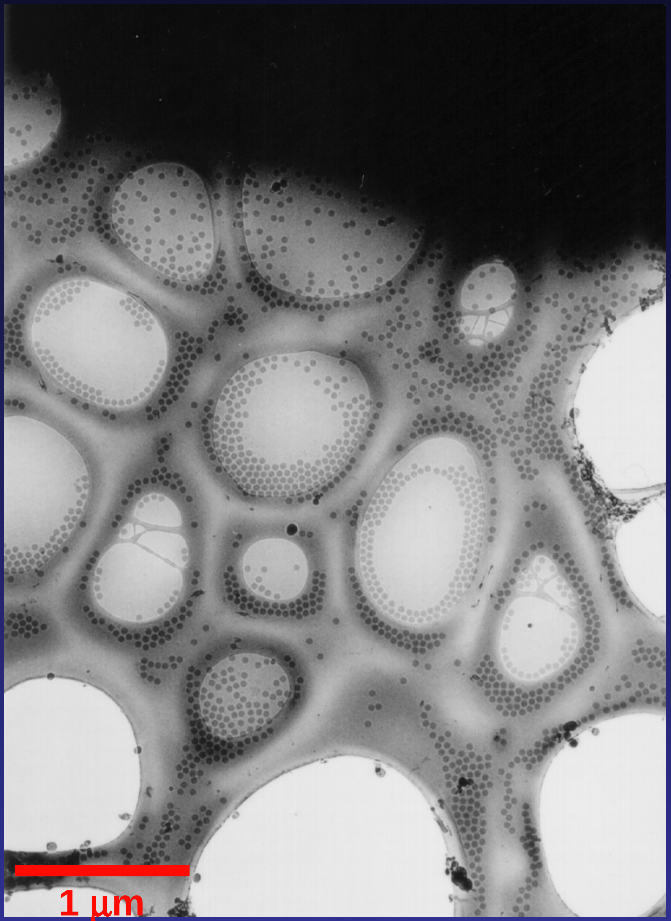
3D Reconstruction of Icosahedral Particles

Protocol

Electron Cryo-Microscopy

Sample : ~2-3 µl at 1-5 mg/ml

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



3D Reconstruction of Icosahedral Particles

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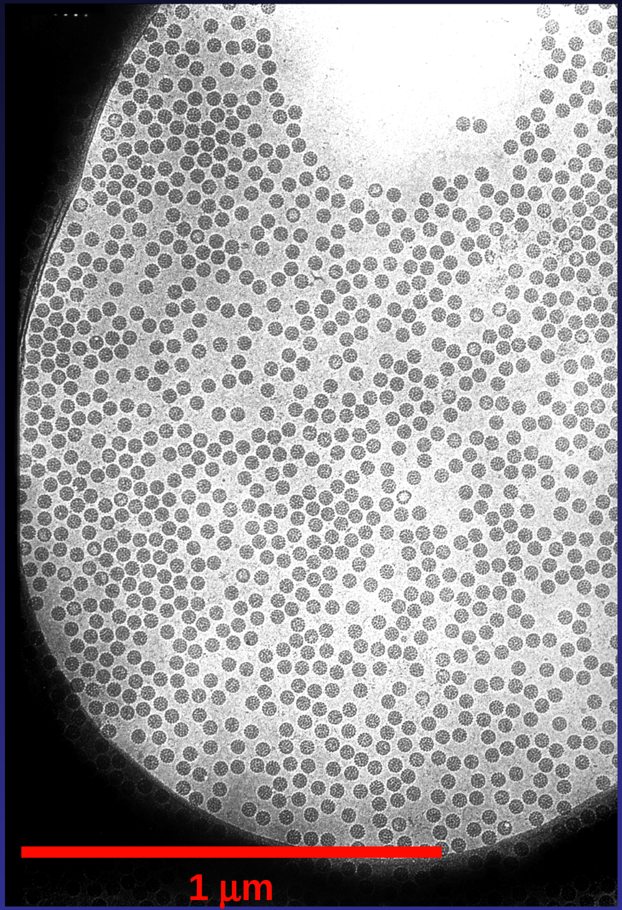

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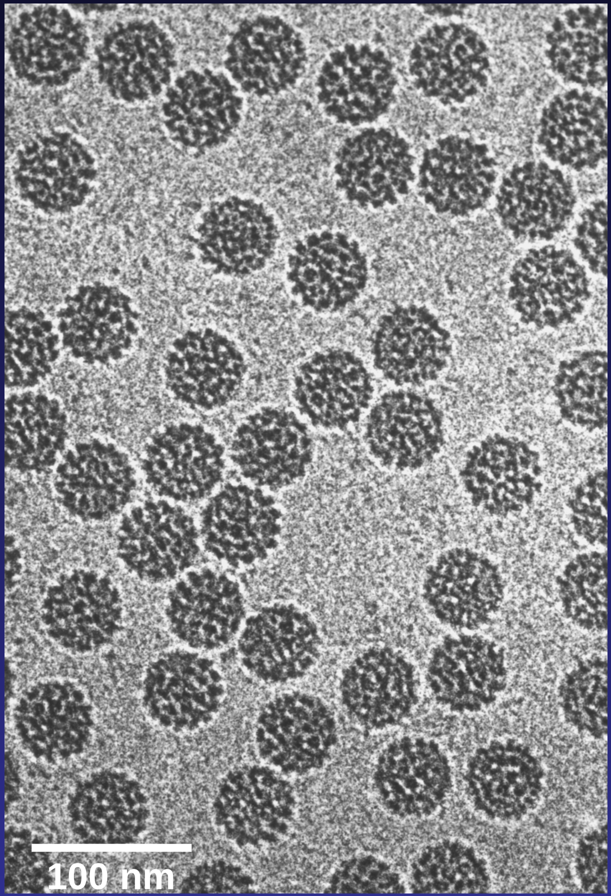

3D Reconstruction of Icosahedral Particles

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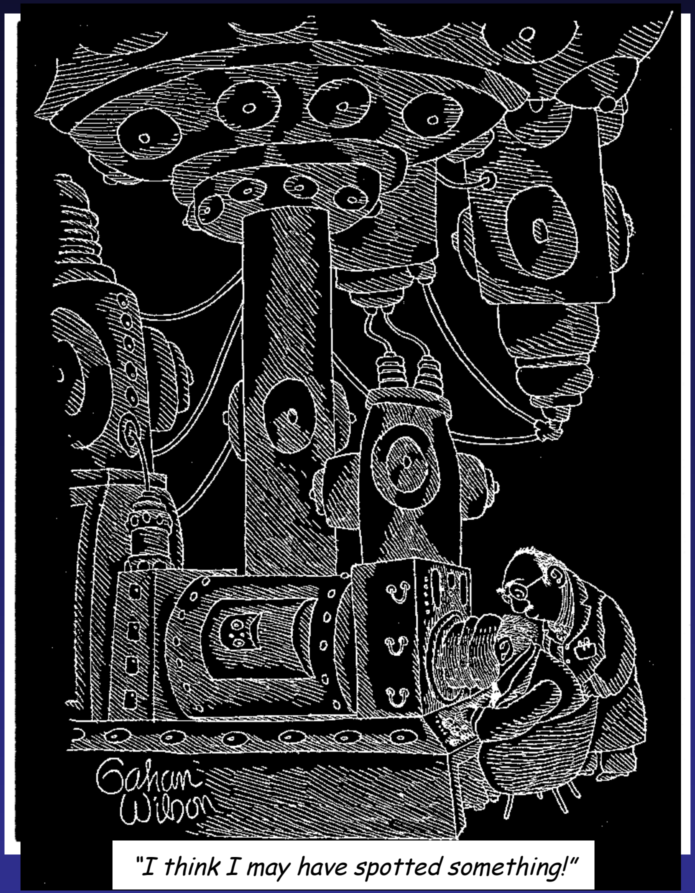
Electron Cryo-Microscopy

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 $e^-/Å^2$

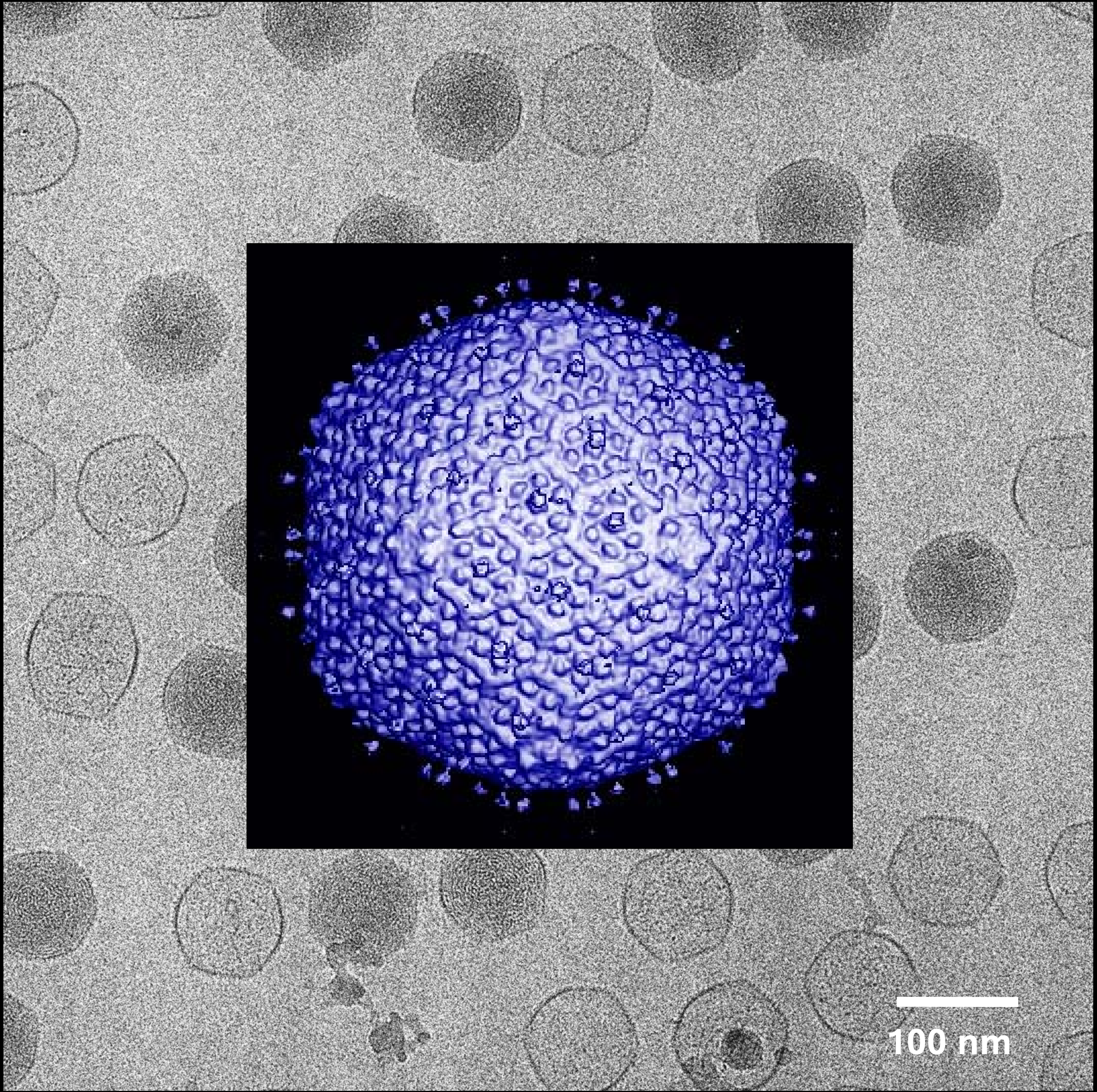
Film: SO-163 (12 min, full strength)

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

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

Target resolution: 10 - 6 Å --> 4Å (?)

FEI Tecnai F30 Polara


100 nm

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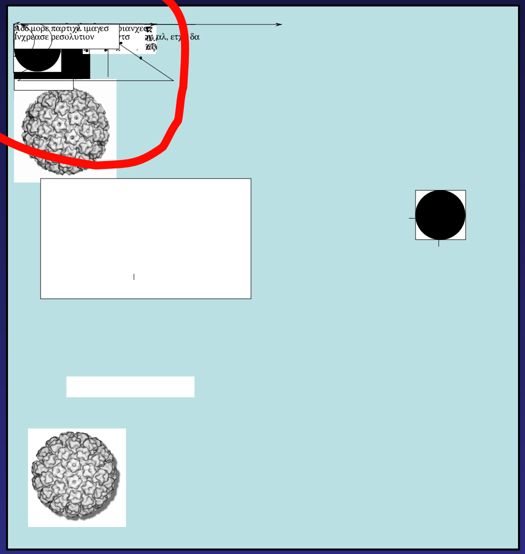
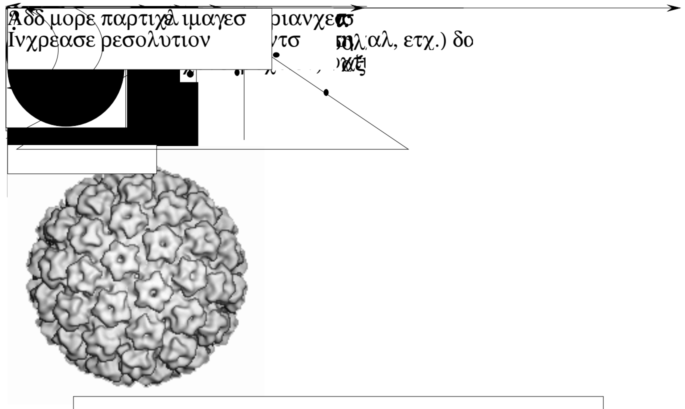
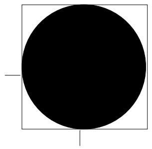
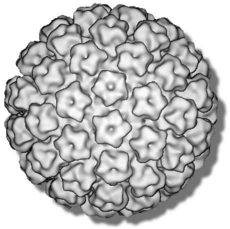


Icosahedral Particle Image Reconstruction Scheme

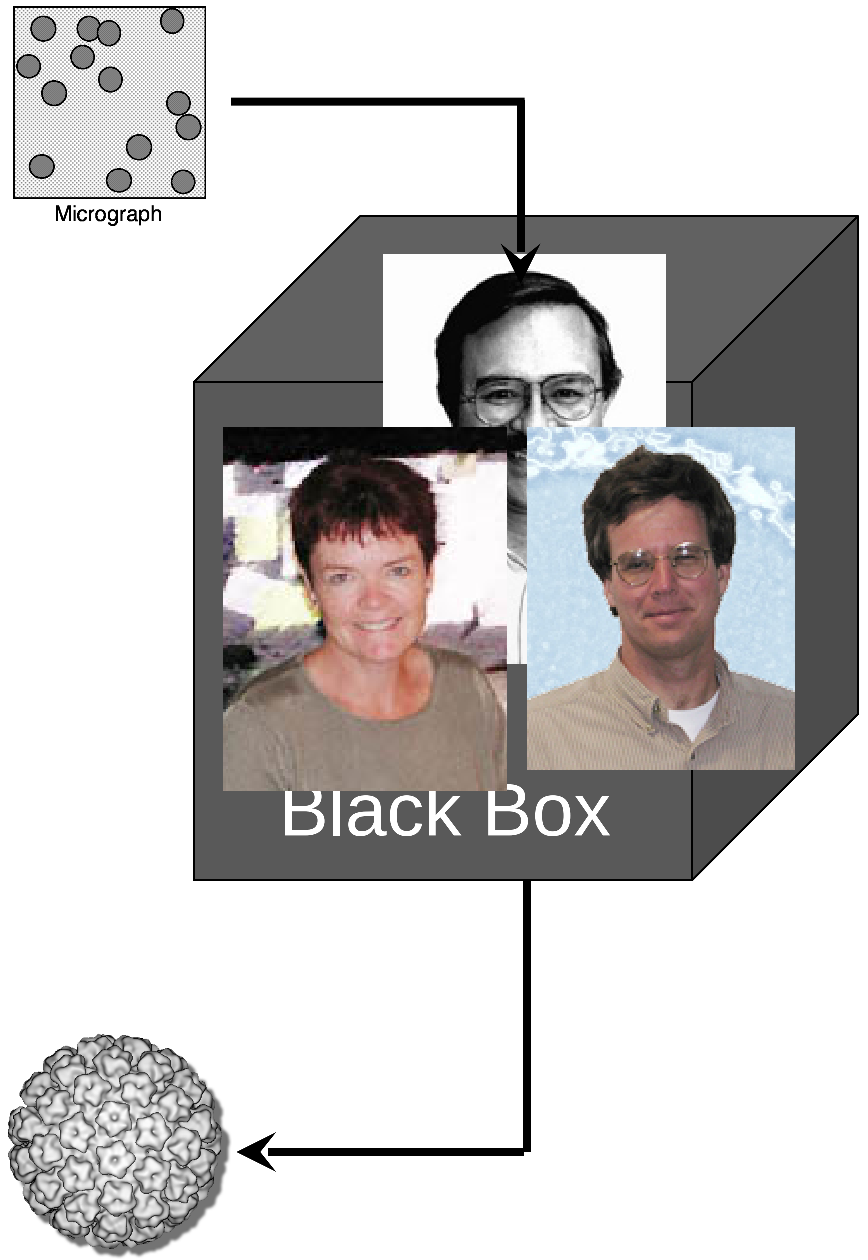
Icosahedral Particle Image Reconstruction Scheme



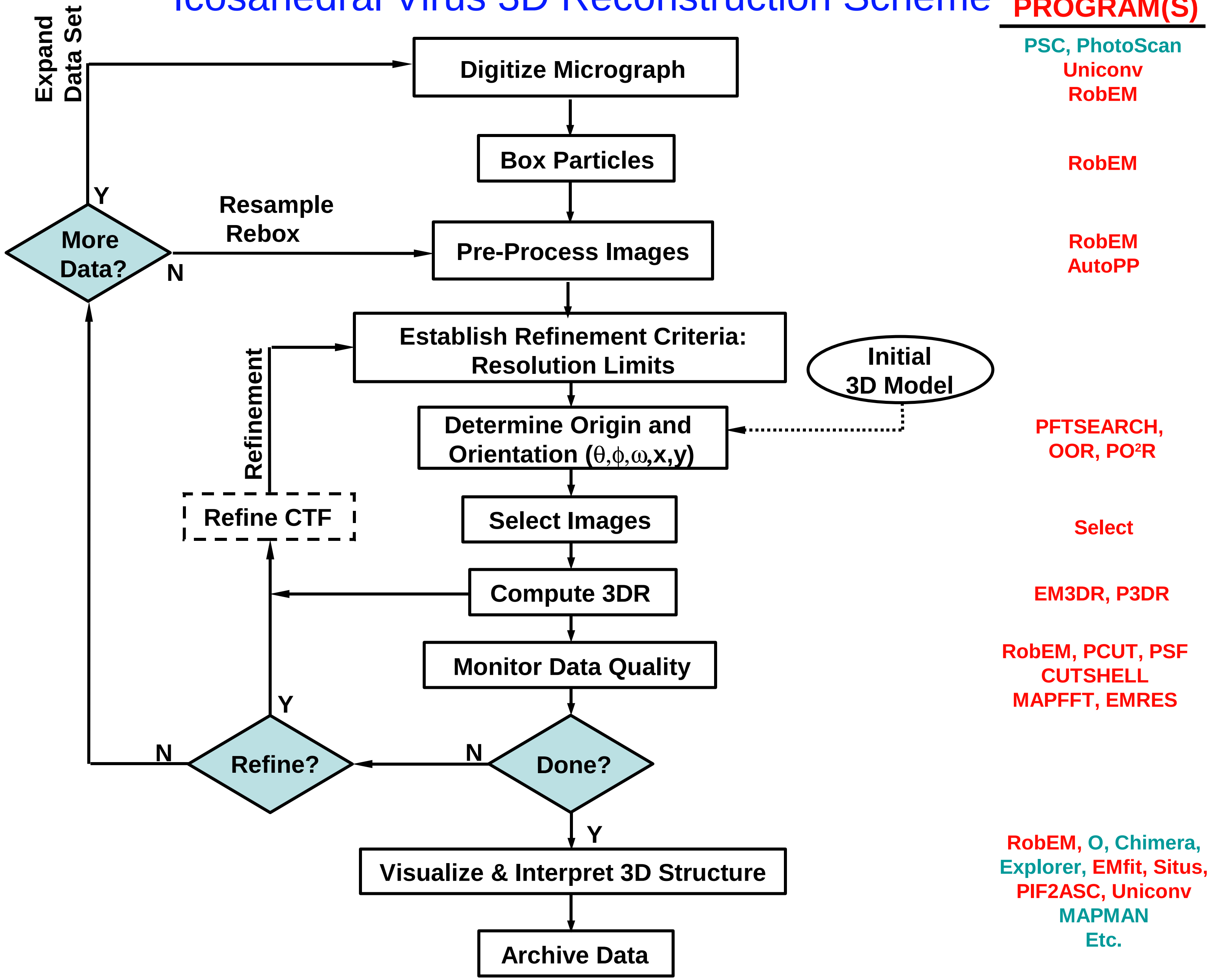




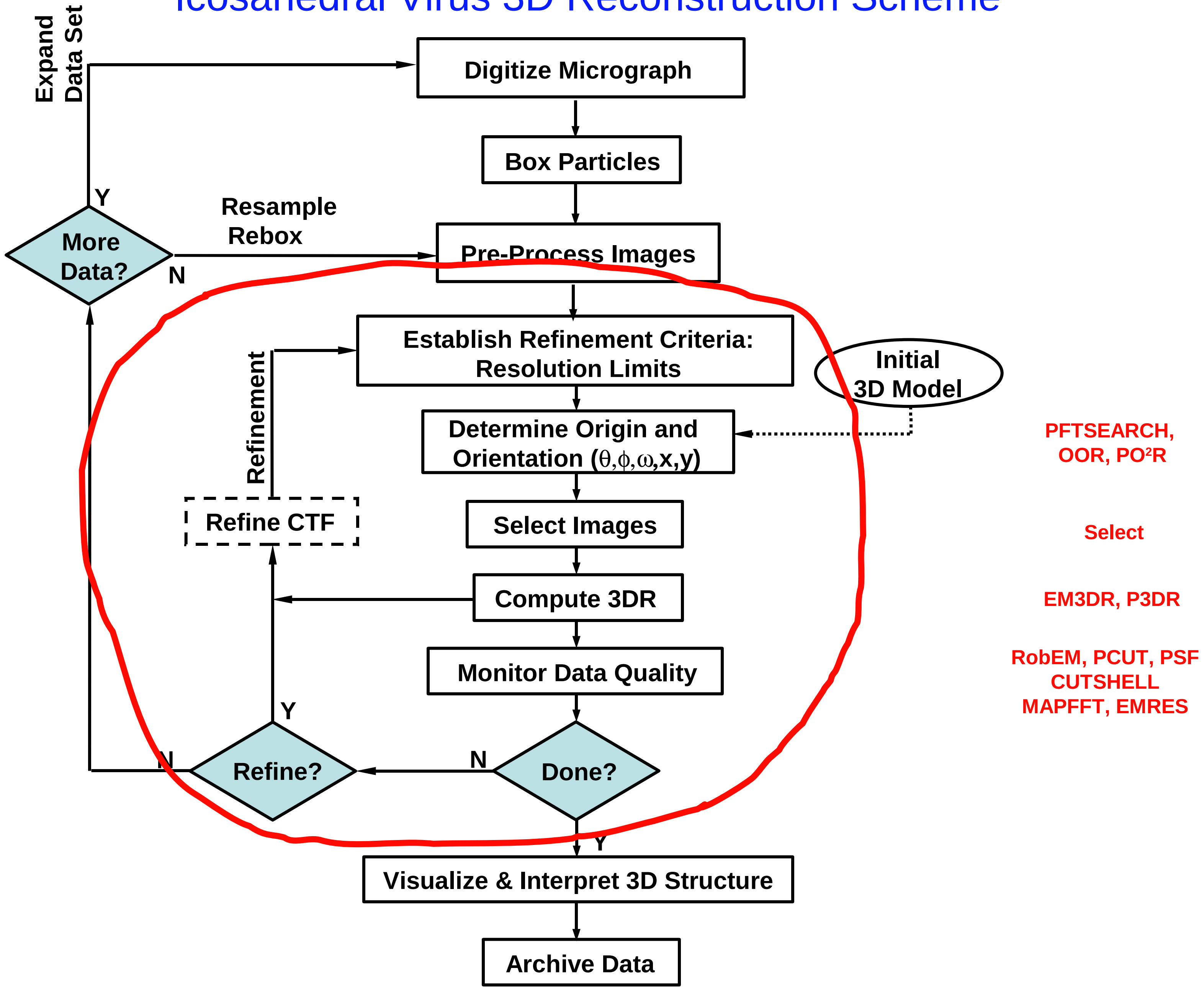
Icosahedral Particle Image Reconstruction Scheme



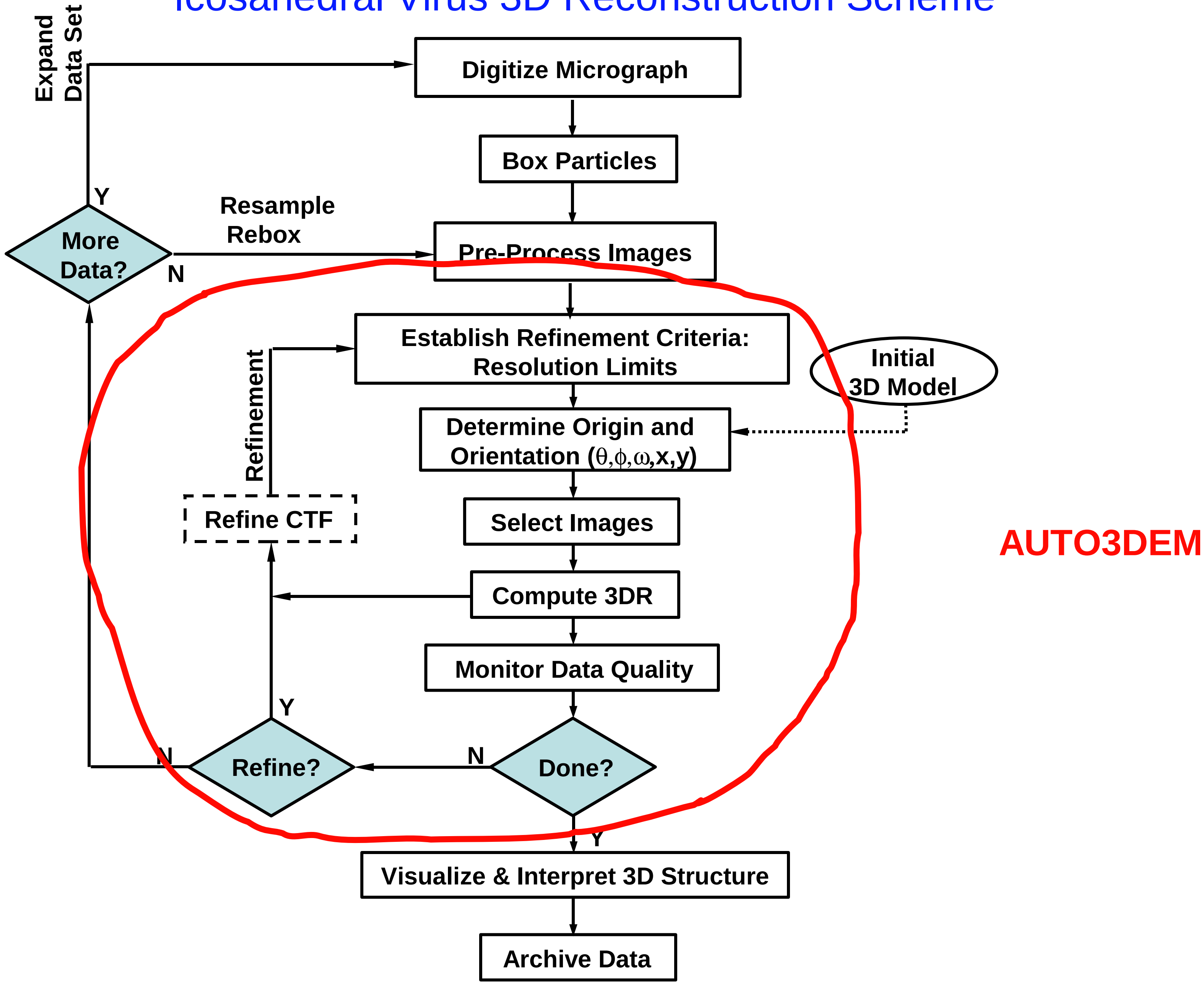
Icosahedral Virus 3D Reconstruction Scheme



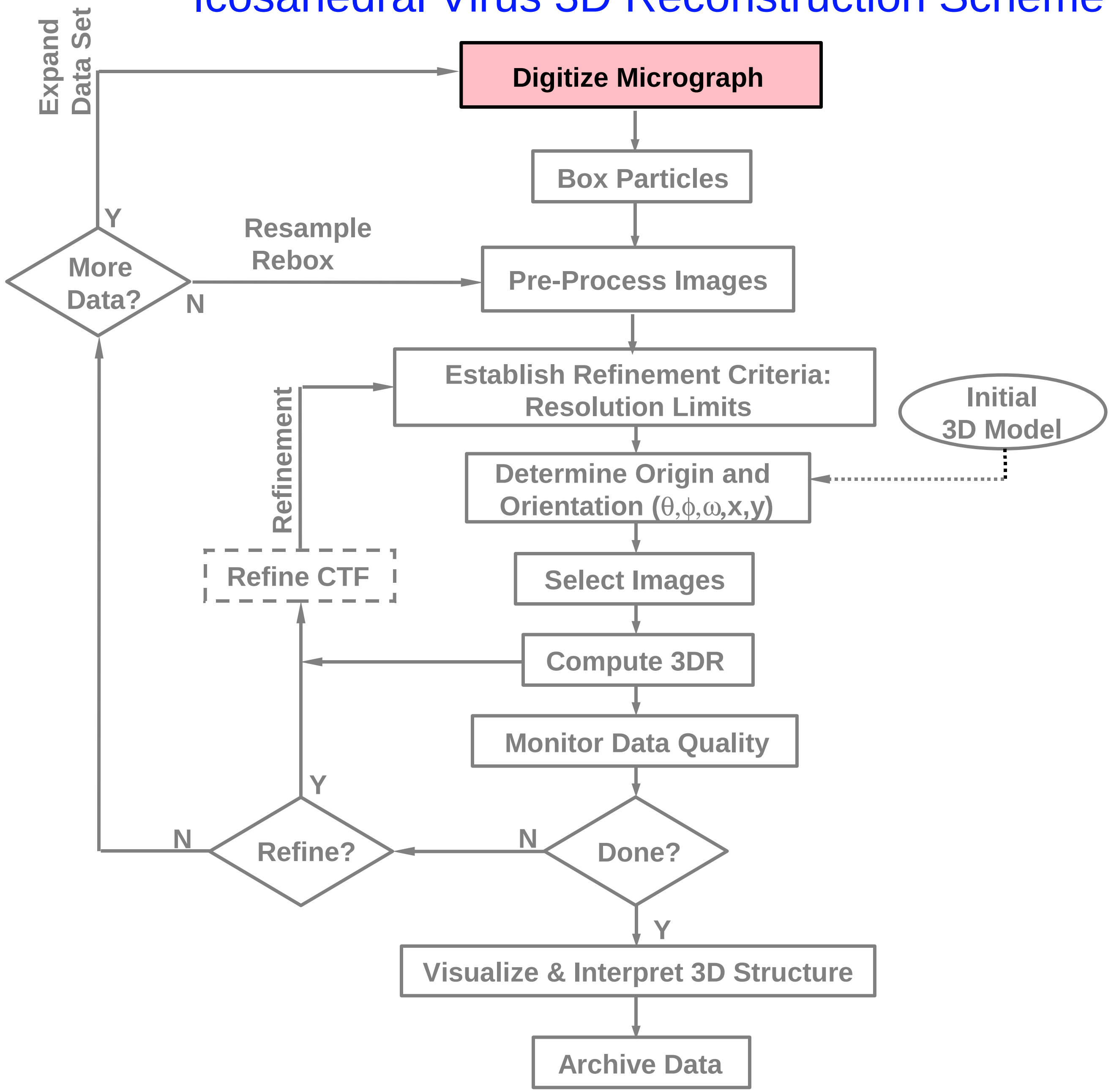
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



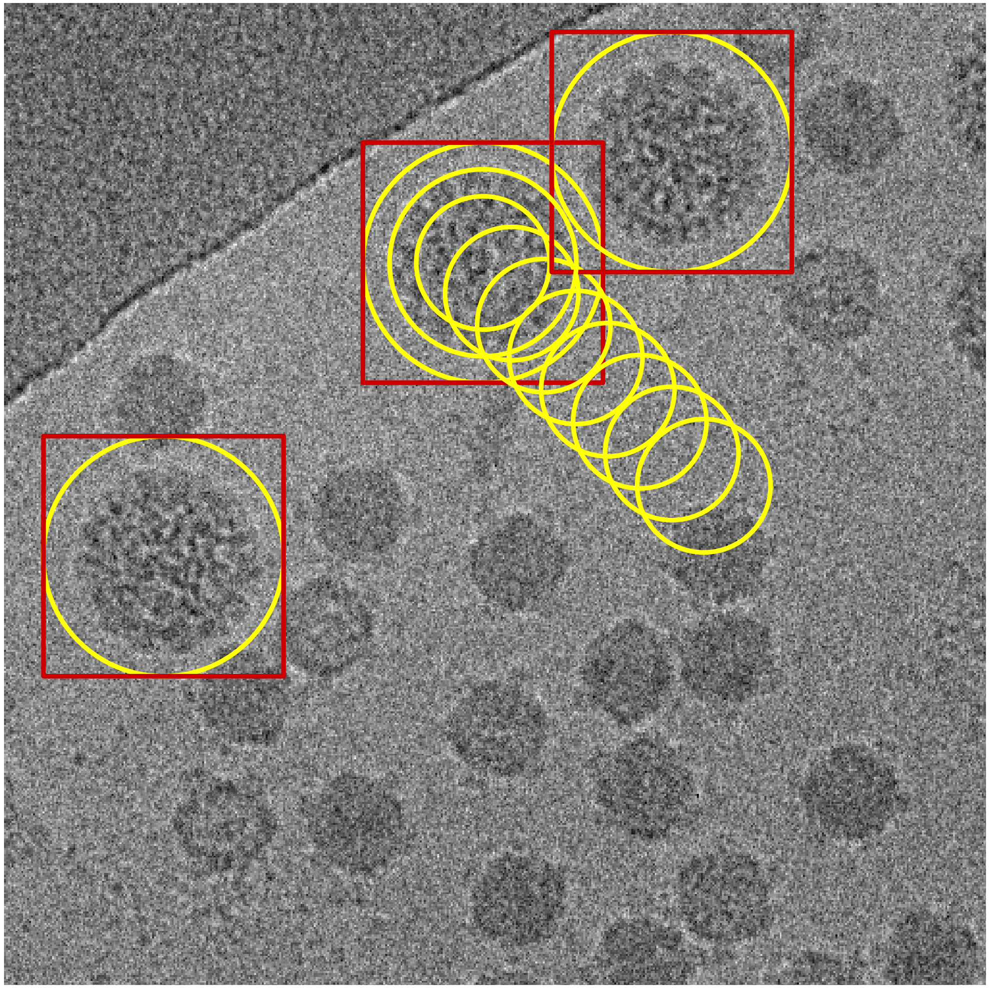
Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

Box Particles

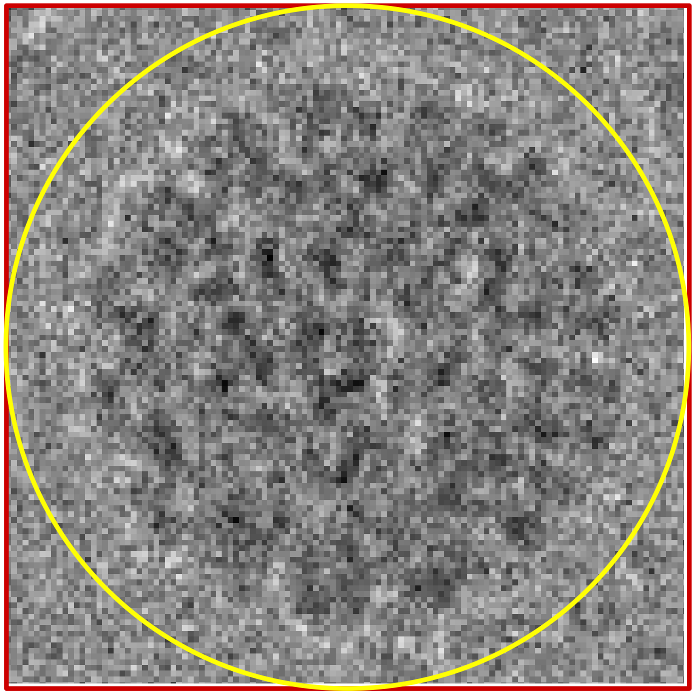


Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles



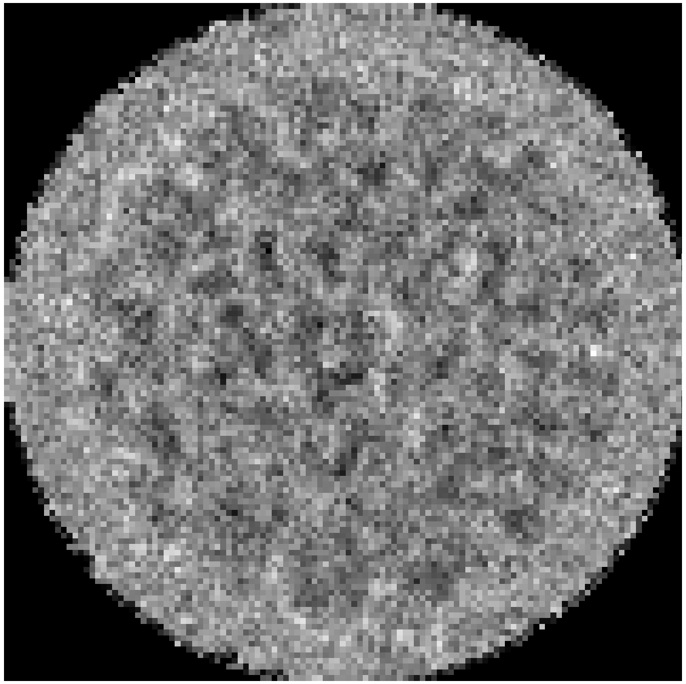
Extracted

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles



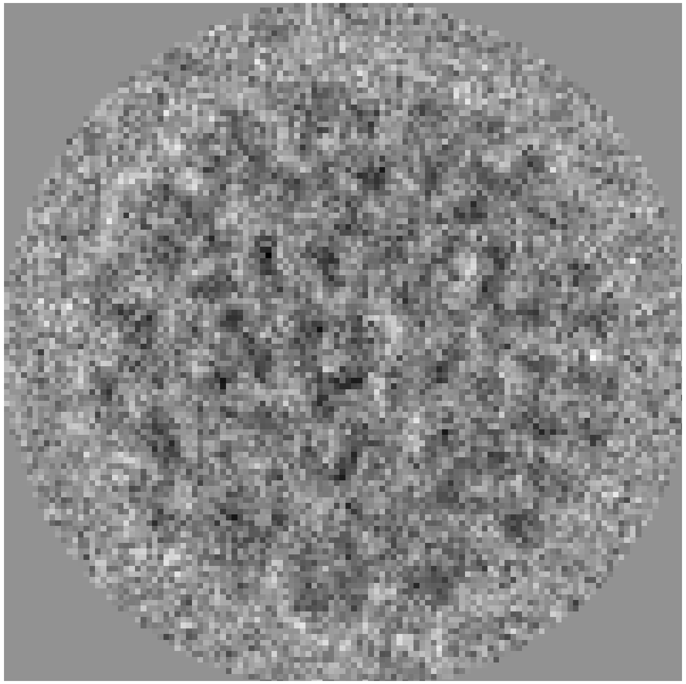
Masked

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles



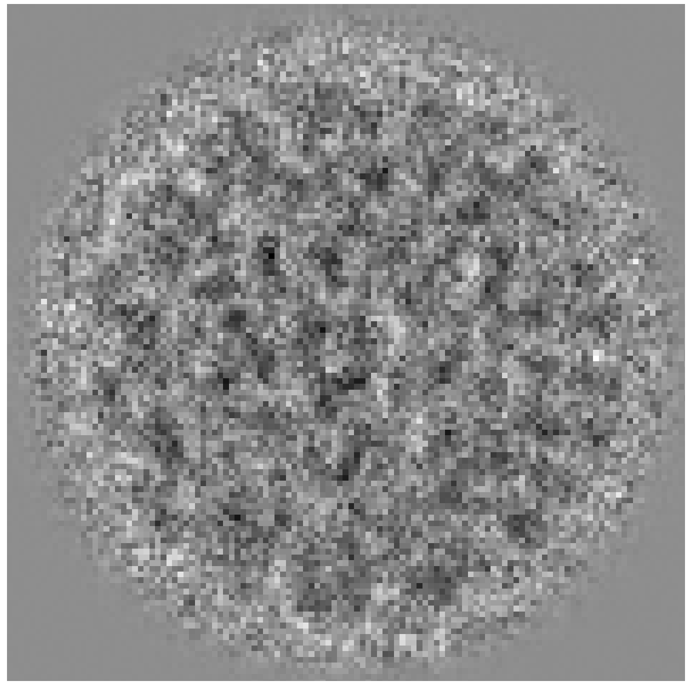
Floated

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles



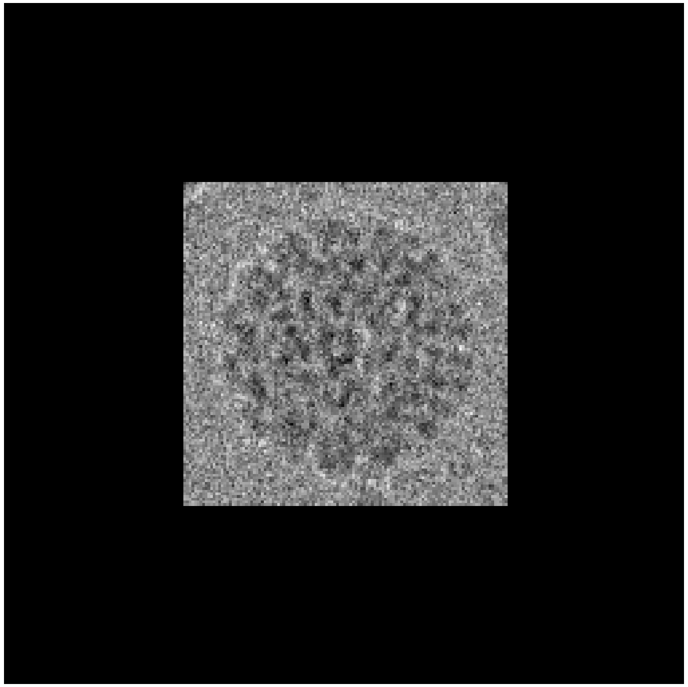
Apodized

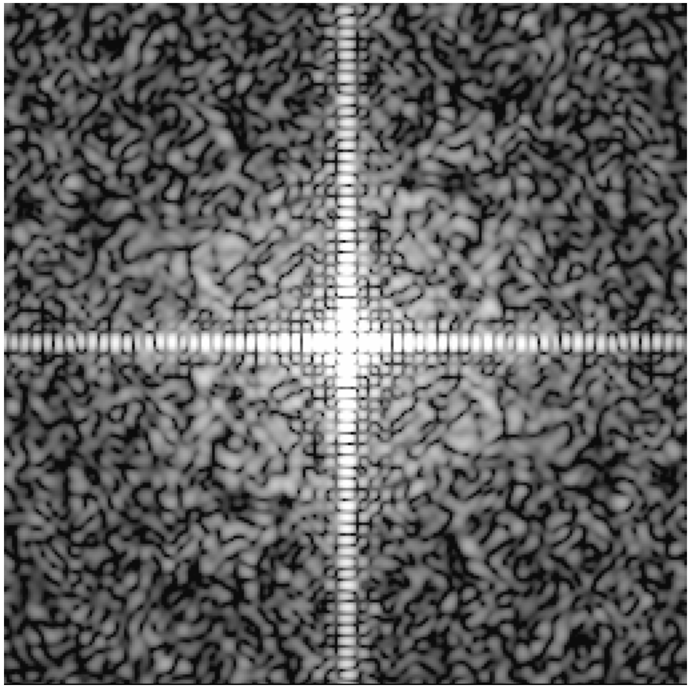
Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles





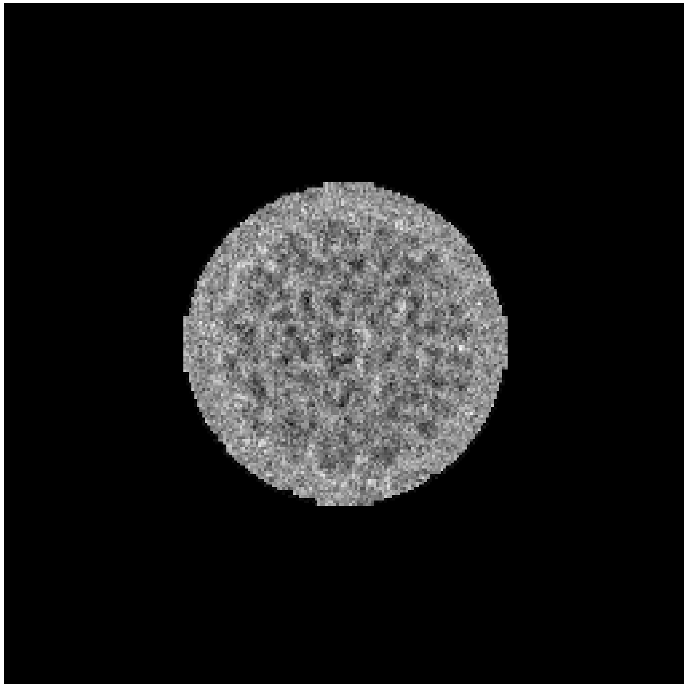
Square mask; **<u>un</u>**floated

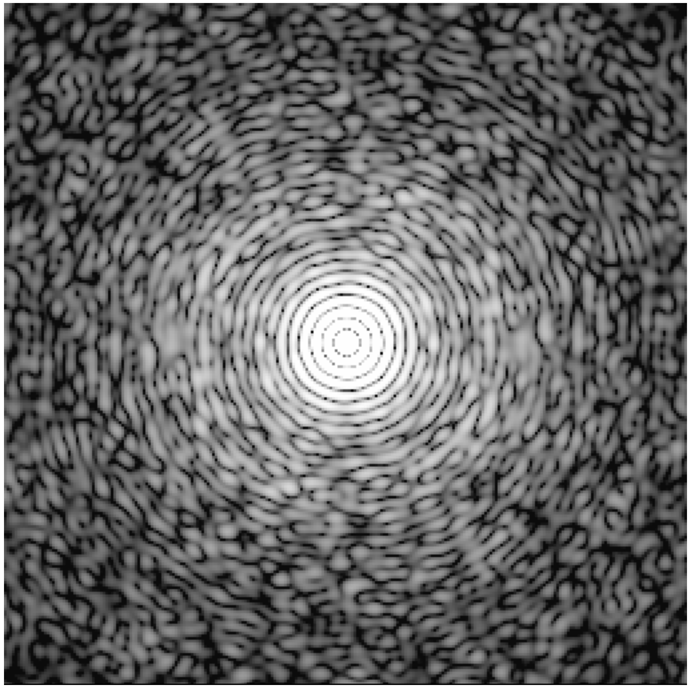
Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles





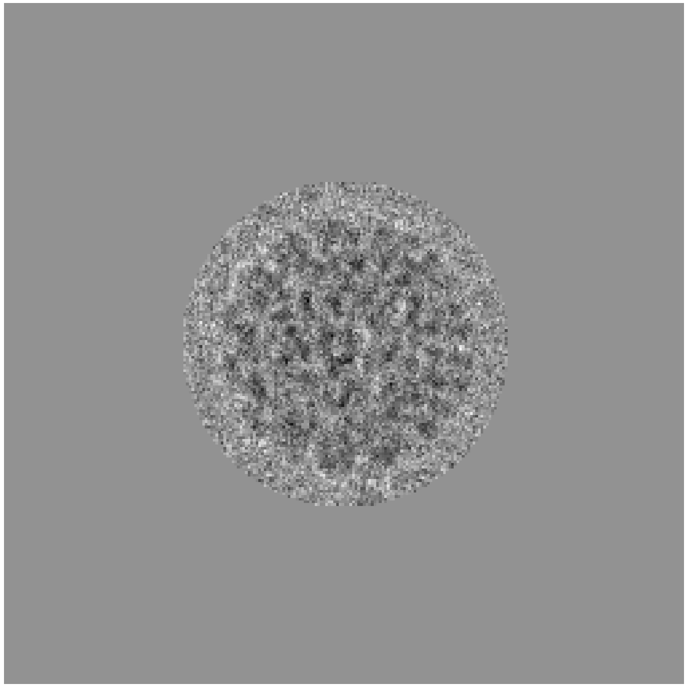
Circular mask; **<u>un</u>**floated

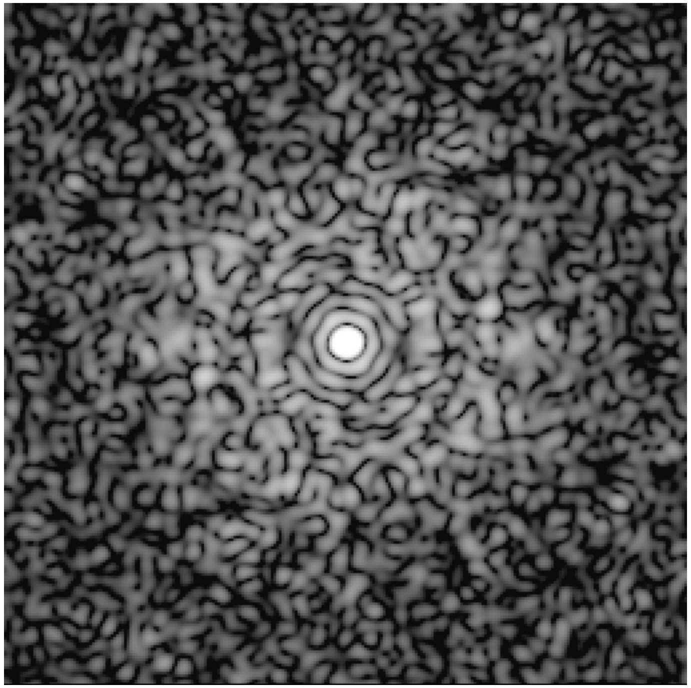
Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles





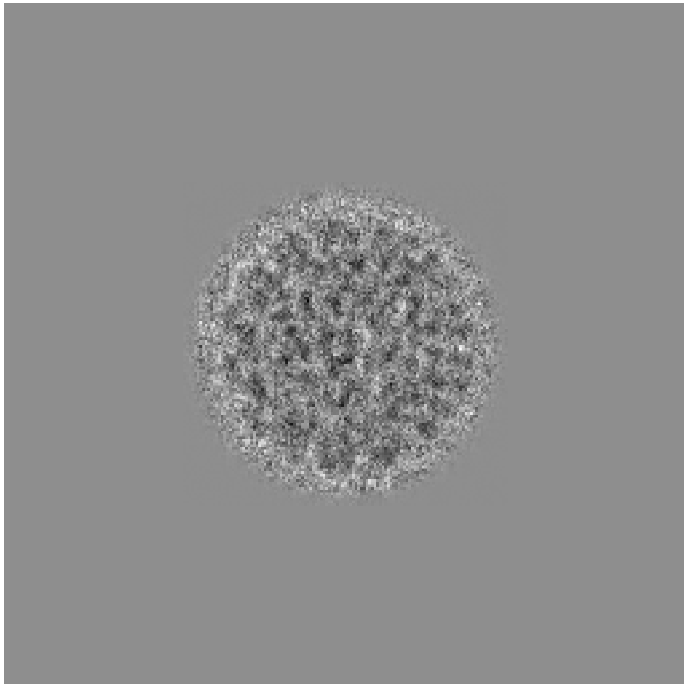
Circular mask; floated

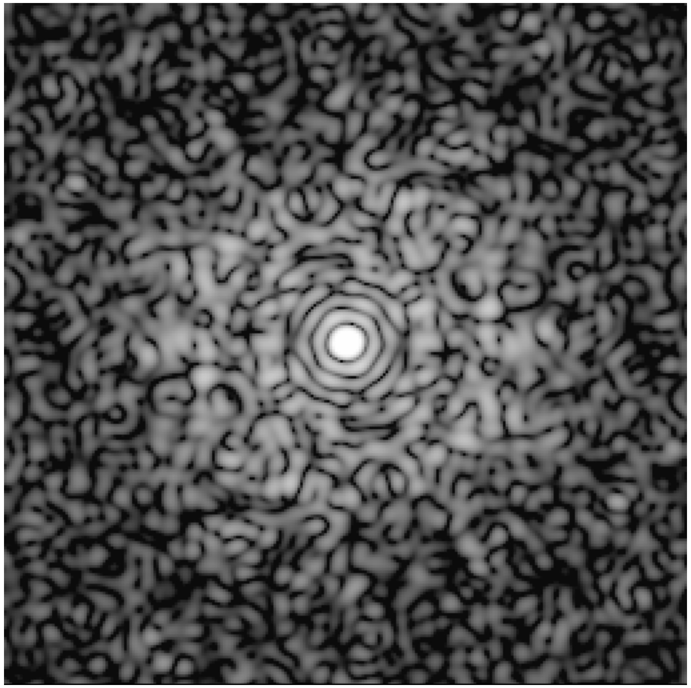
Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles





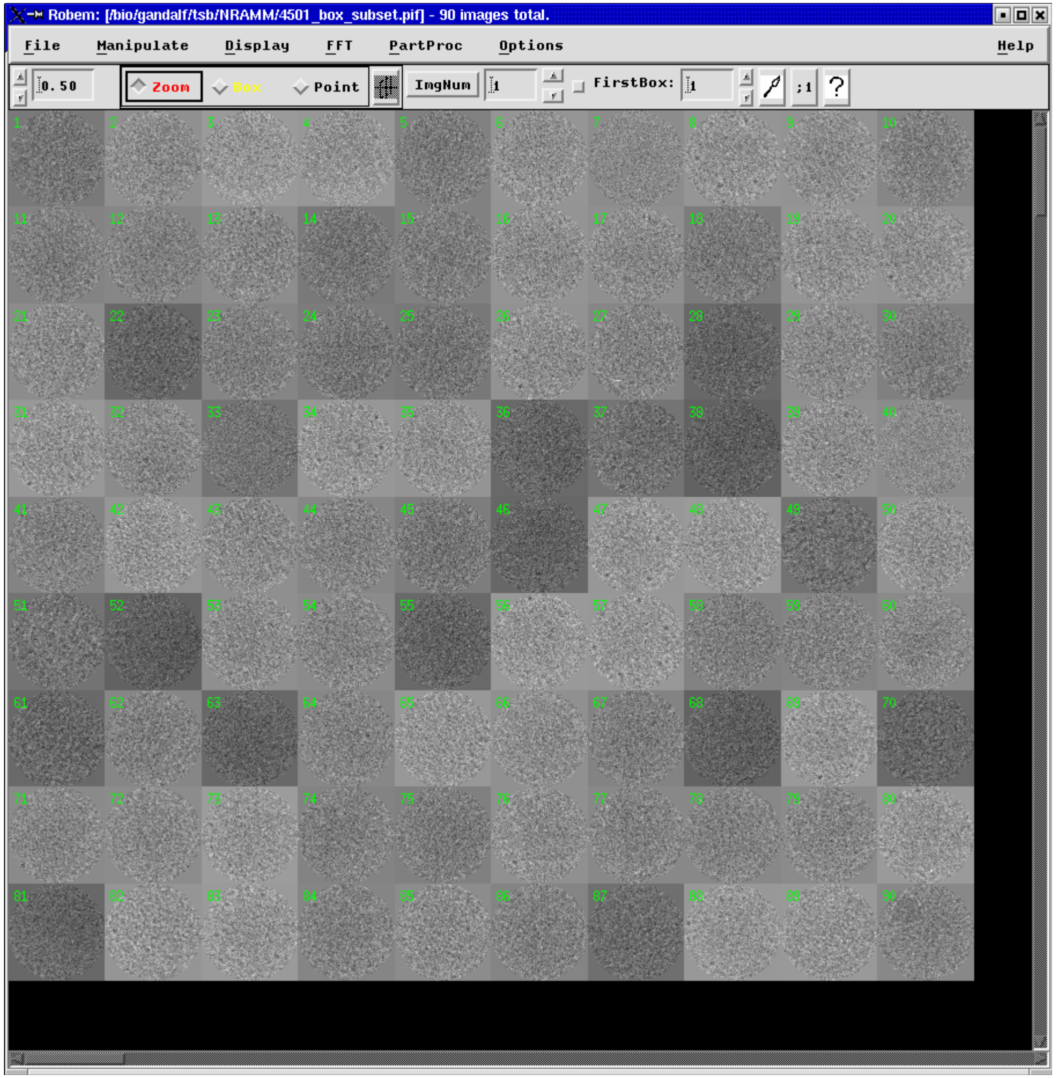
Circular mask; floated & apodized

Icosahedral Virus 3D Reconstruction Scheme

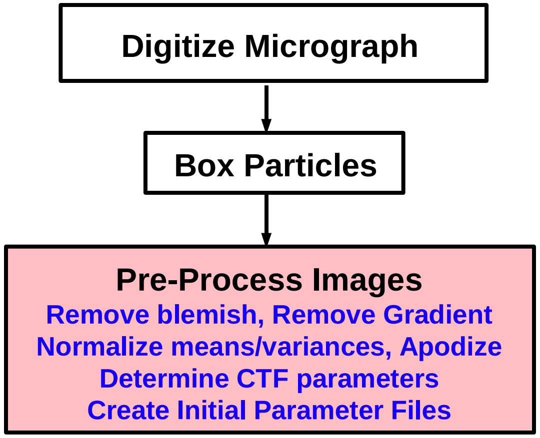
Digitize Micrograph

↓

Box Particles



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

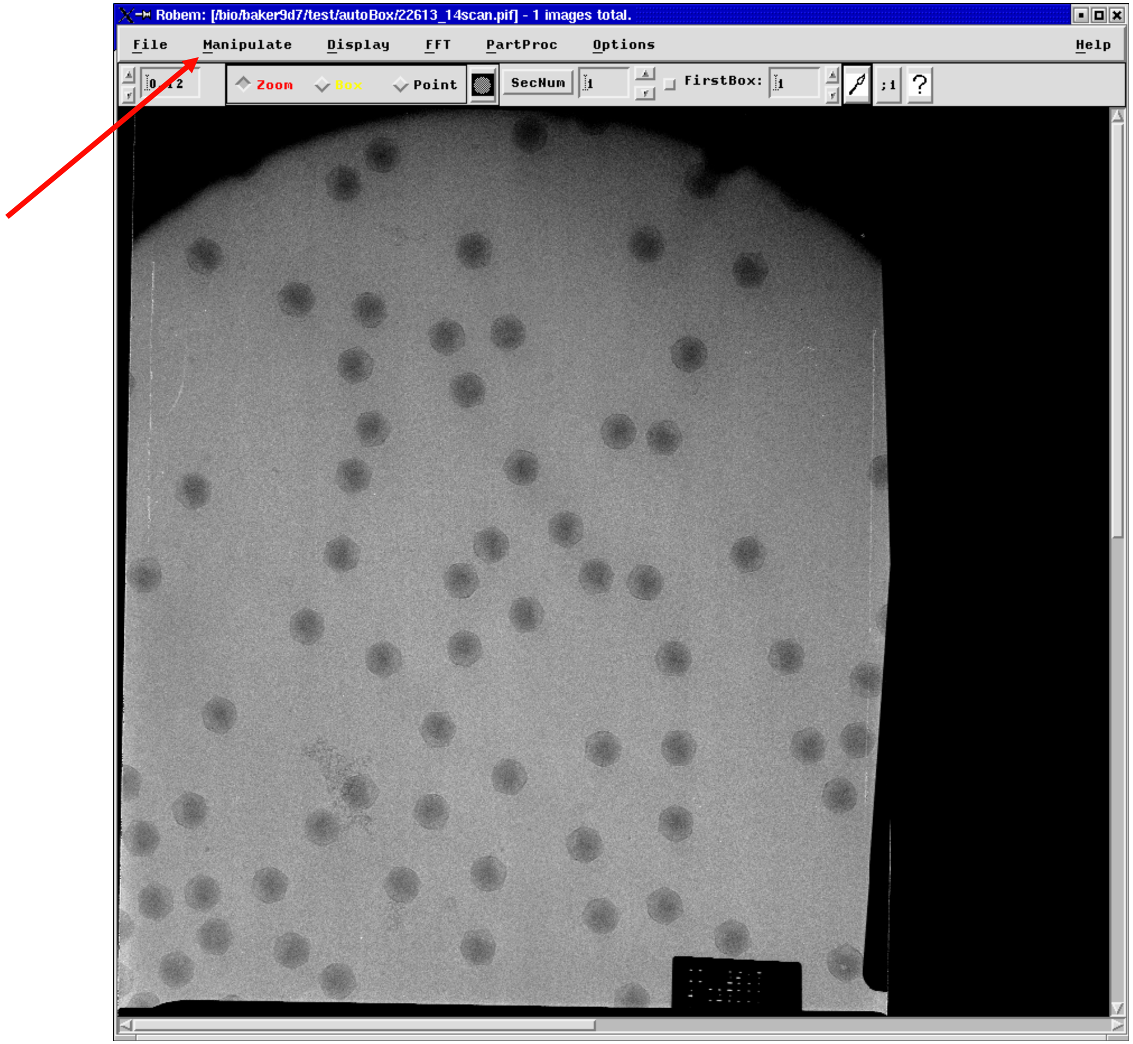
Pre-Process Images

Remove blemish, Remove Gradient

Normalize means/variances, Apodize

Determine CTF parameters

Create Initial Parameter Files



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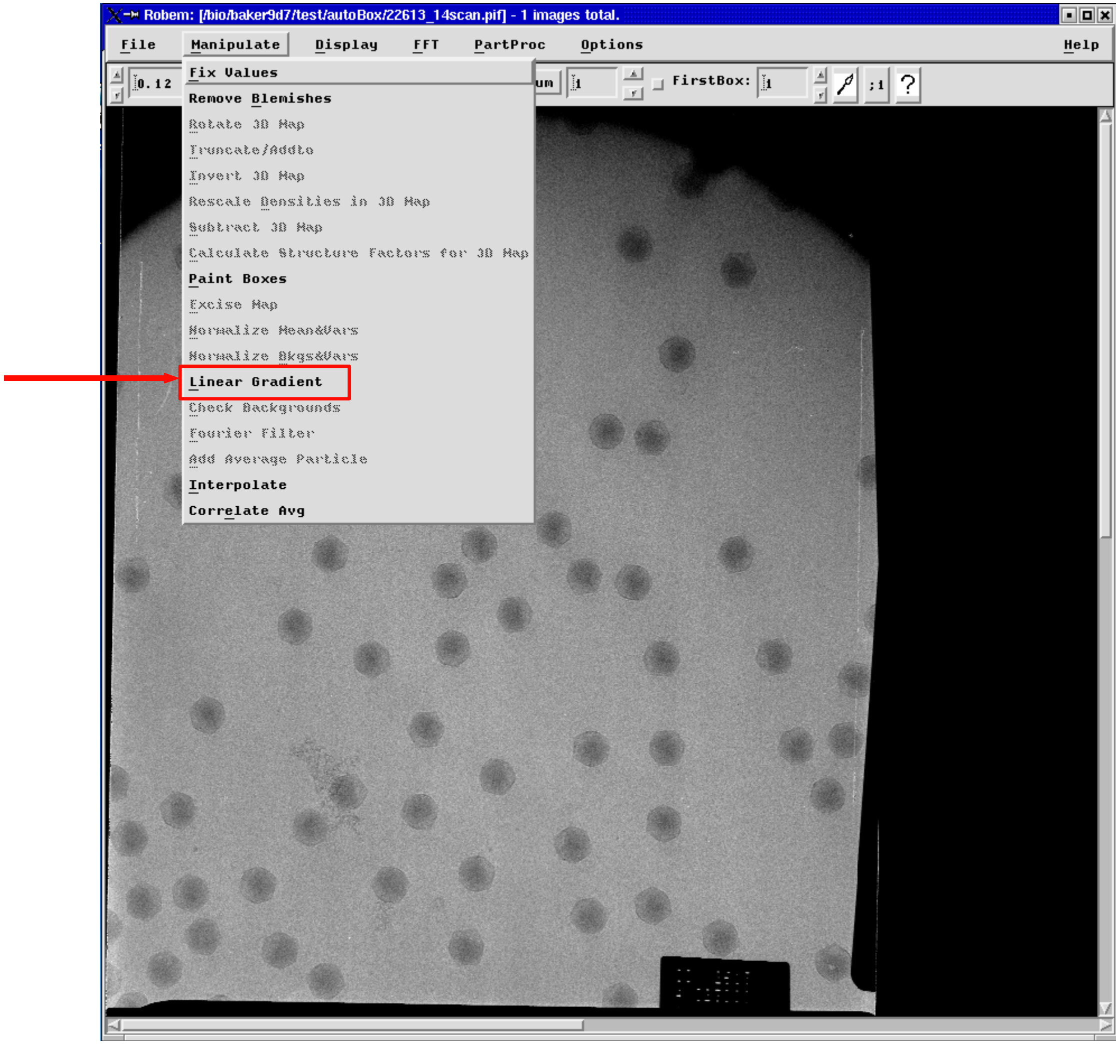

Icosahedral Virus 3D Reconstruction Scheme

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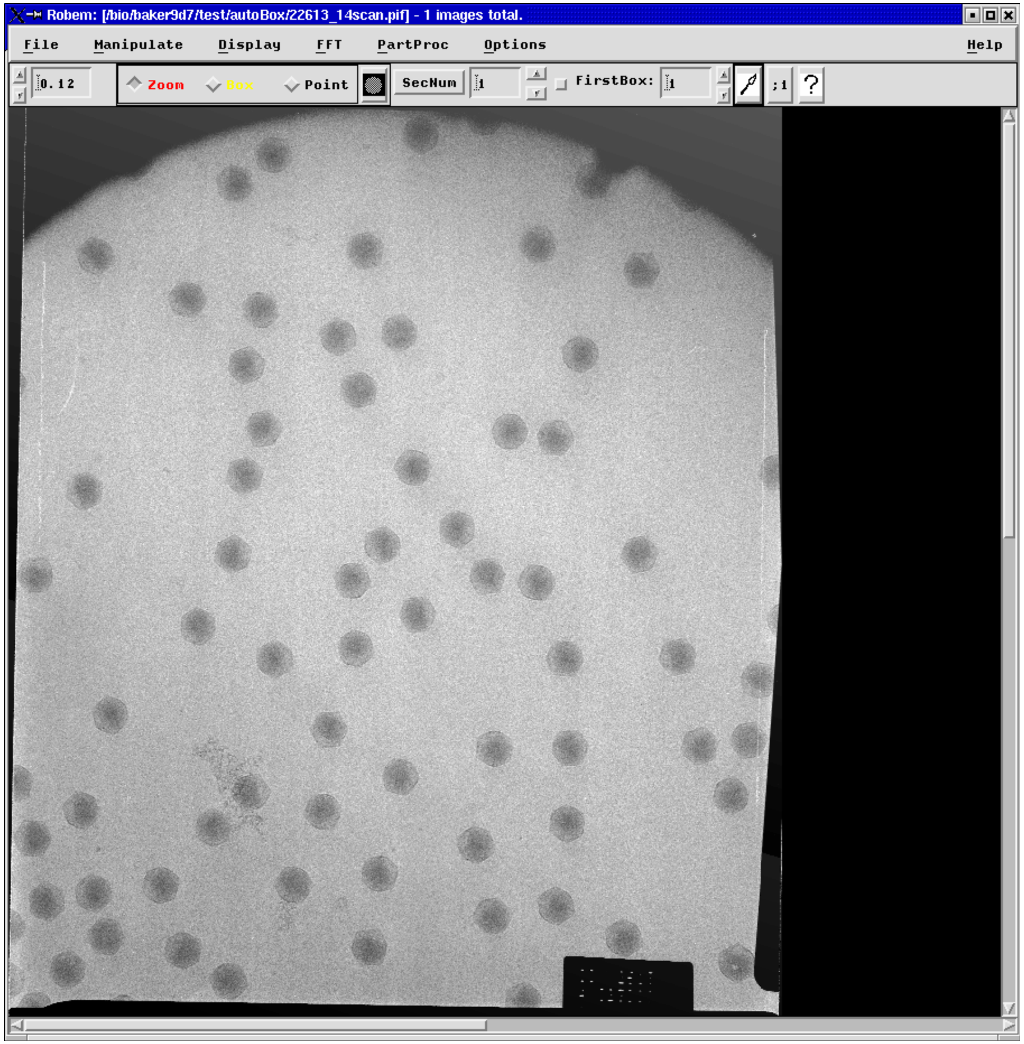

Gradient removed

Icosahedral Virus 3D Reconstruction Scheme

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Remove blemish, Remove Gradient
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Determine CTF parameters
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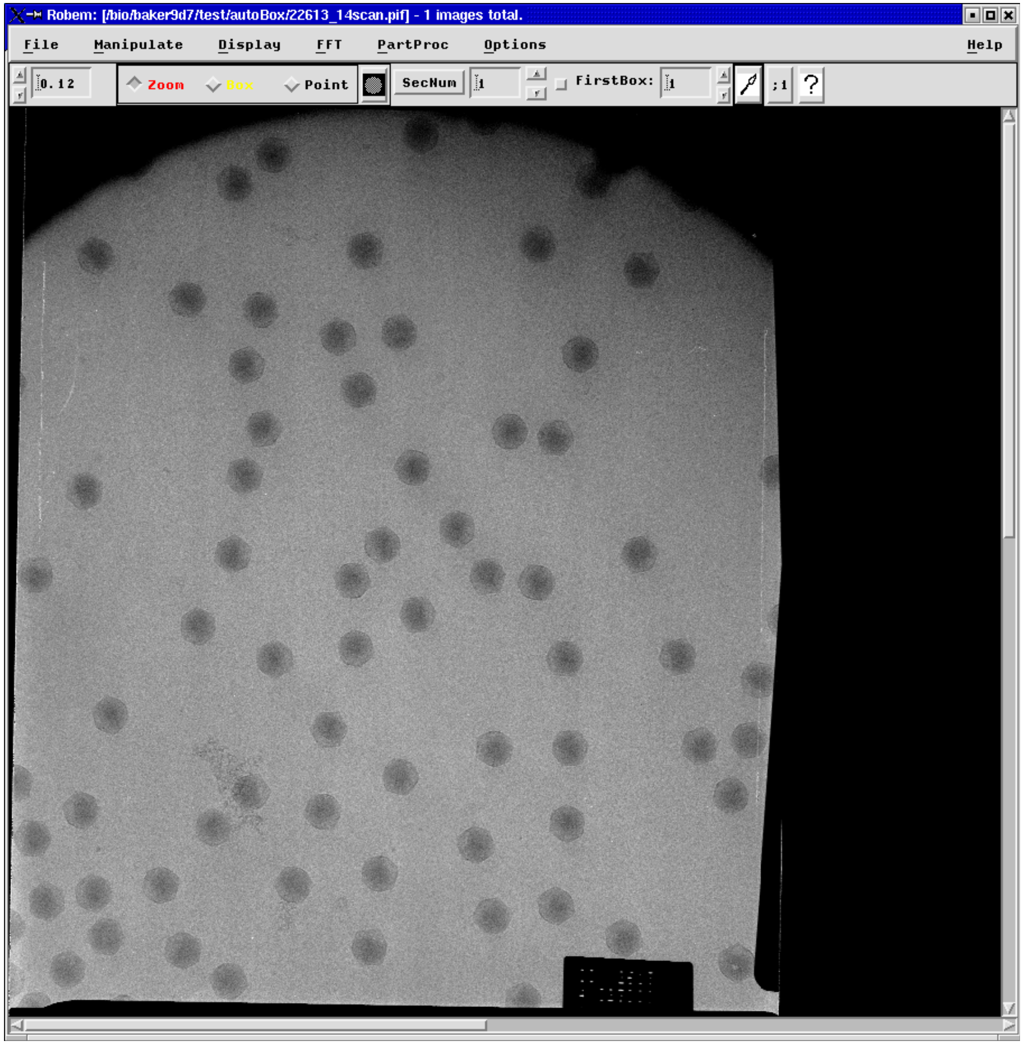

Gradient
not removed

Icosahedral Virus 3D Reconstruction Scheme

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Remove blemish, Remove Gradient
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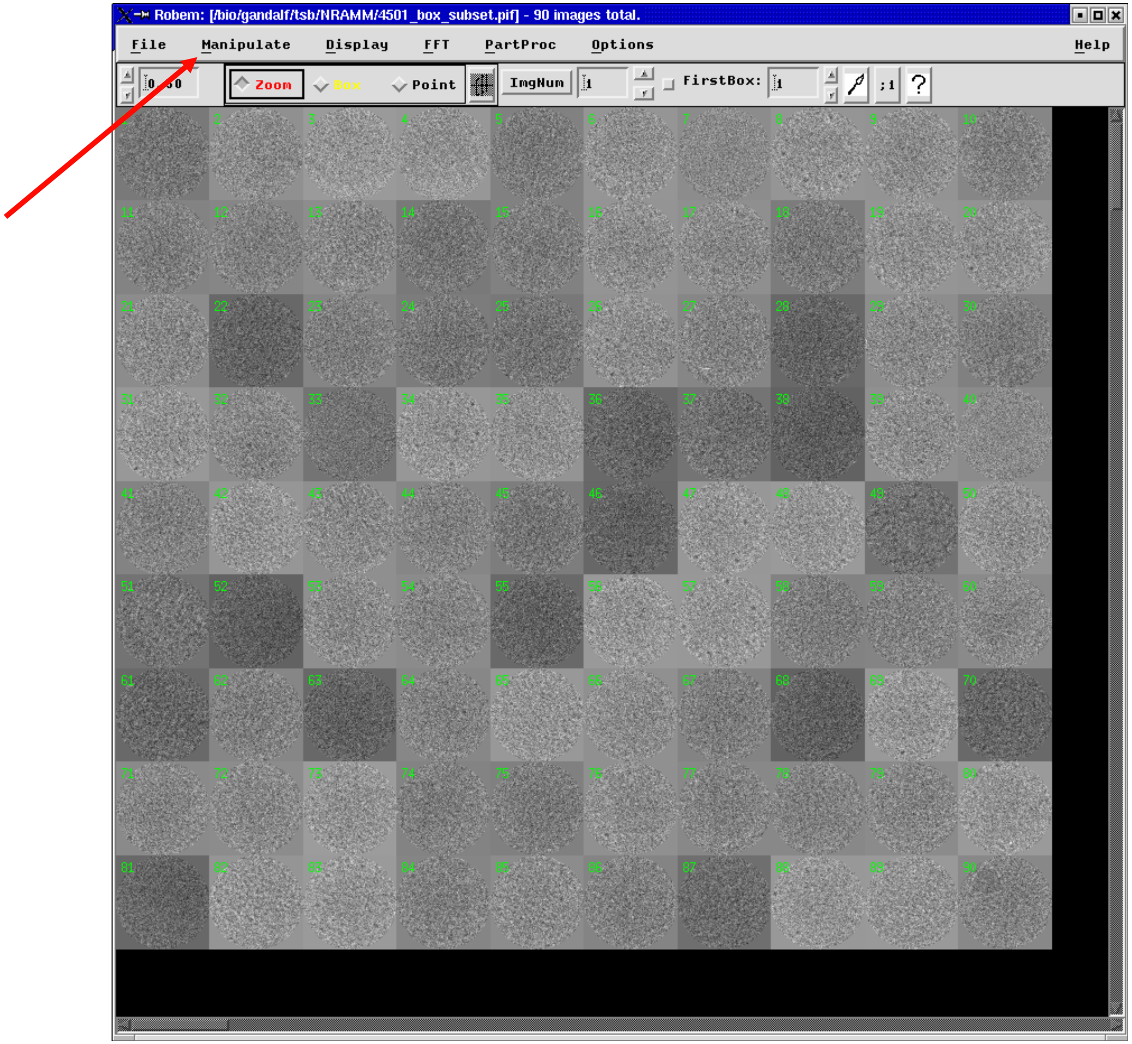

Icosahedral Virus 3D Reconstruction Scheme

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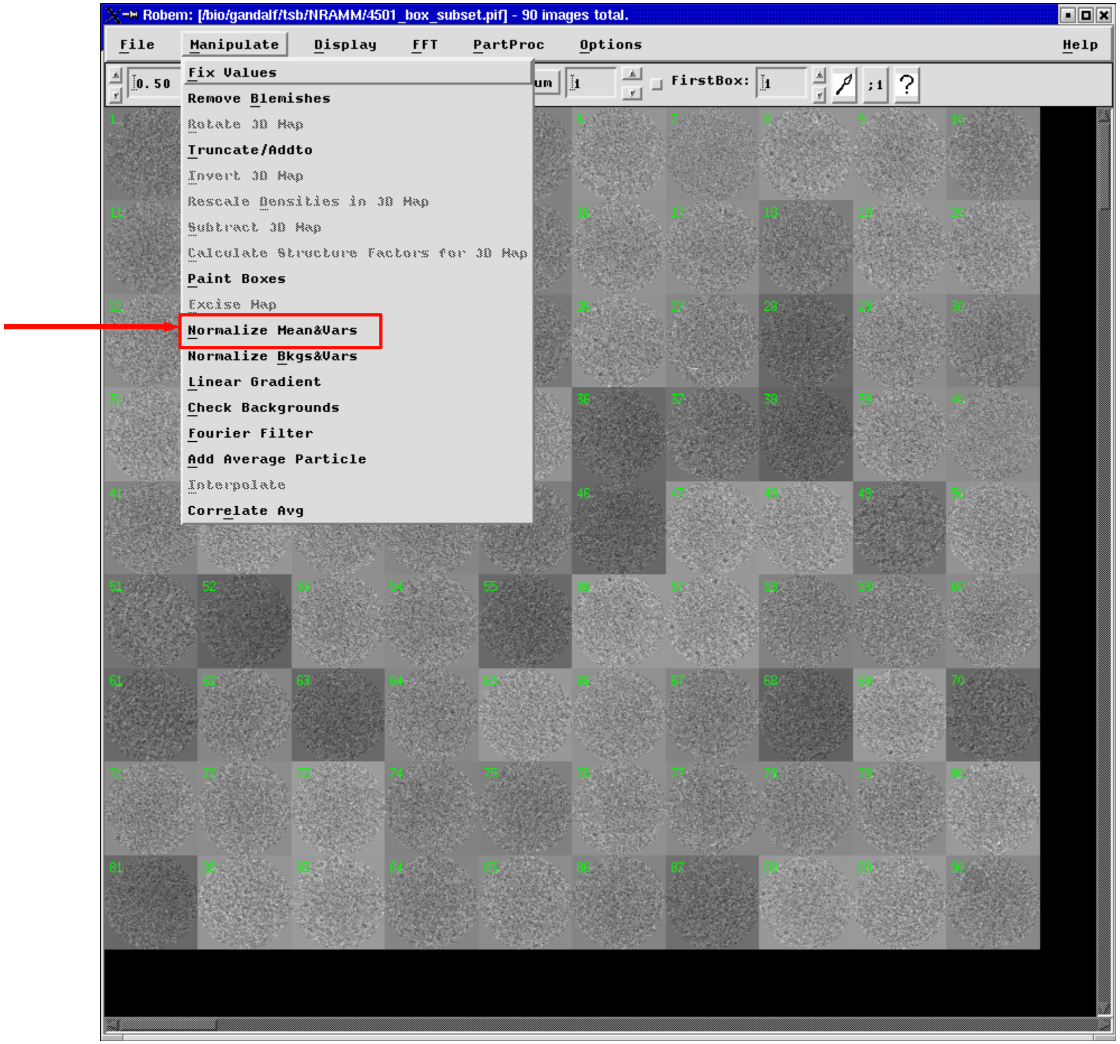

Icosahedral Virus 3D Reconstruction Scheme

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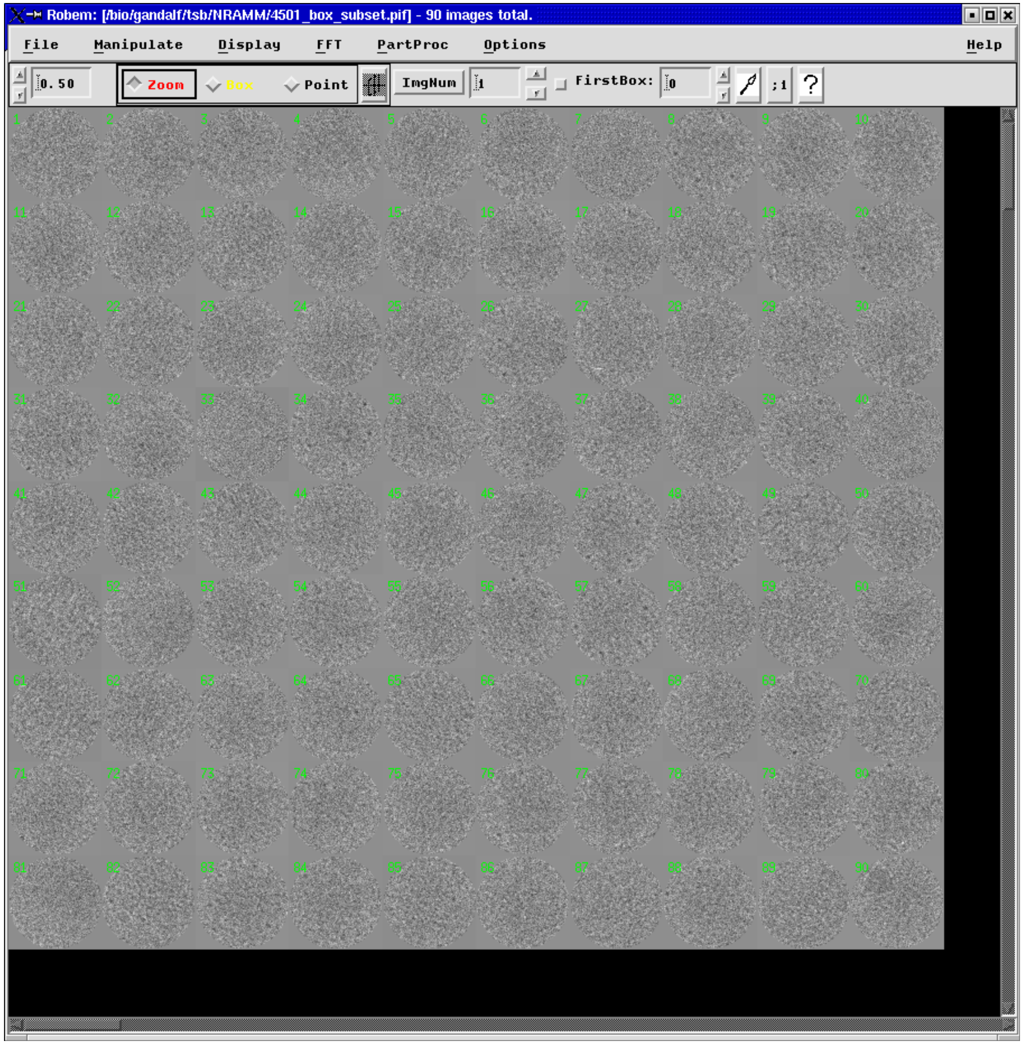

Icosahedral Virus 3D Reconstruction Scheme

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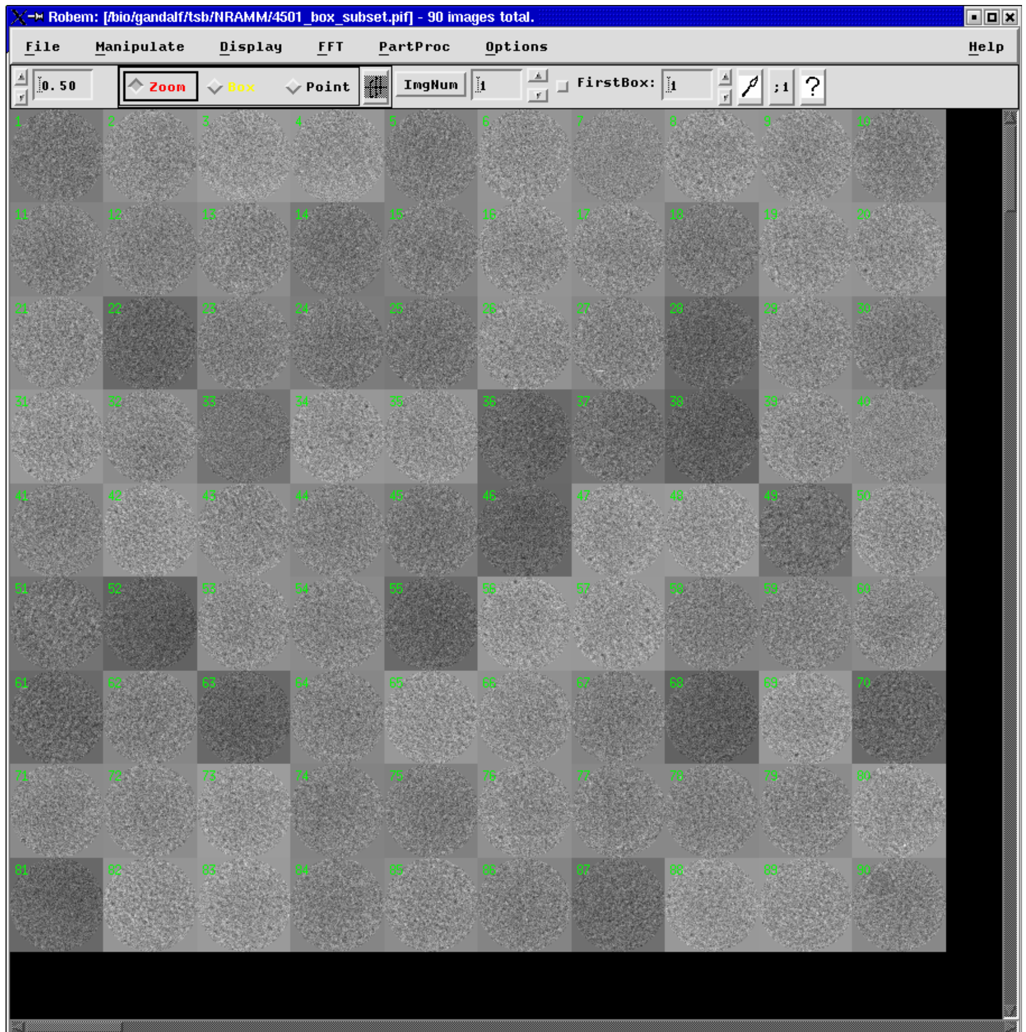

Icosahedral Virus 3D Reconstruction Scheme

Pre-Process Images

Remove blemish, Remove Gradient
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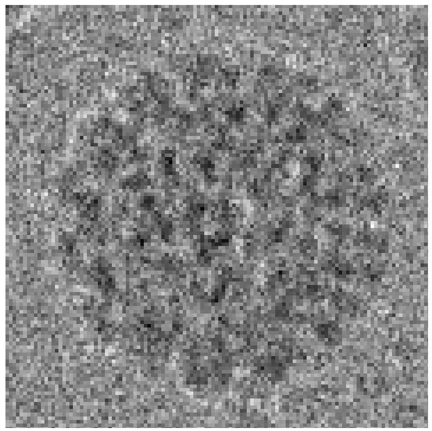
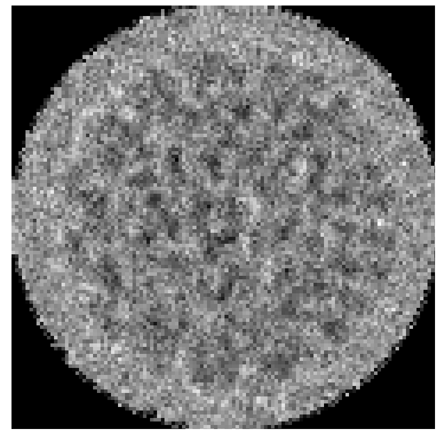
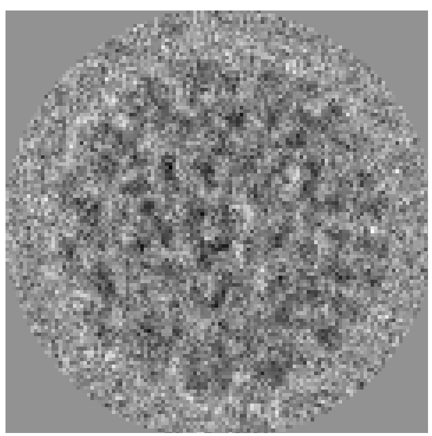


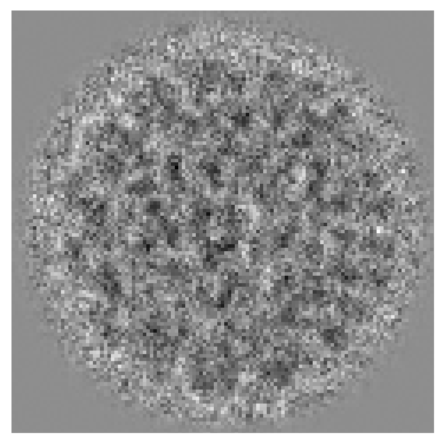
Extracted



Masked



Floated



Apodized

Icosahedral Virus 3D Reconstruction Scheme

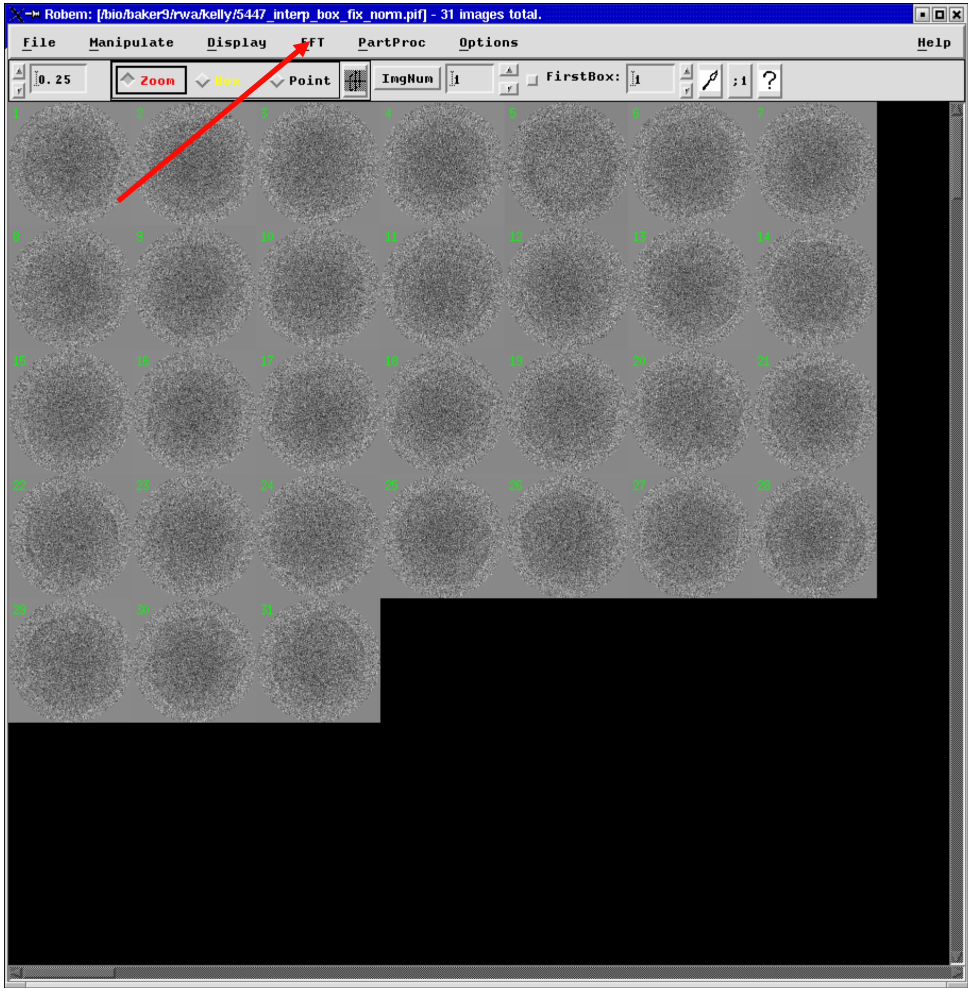
Pre-Process Images

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
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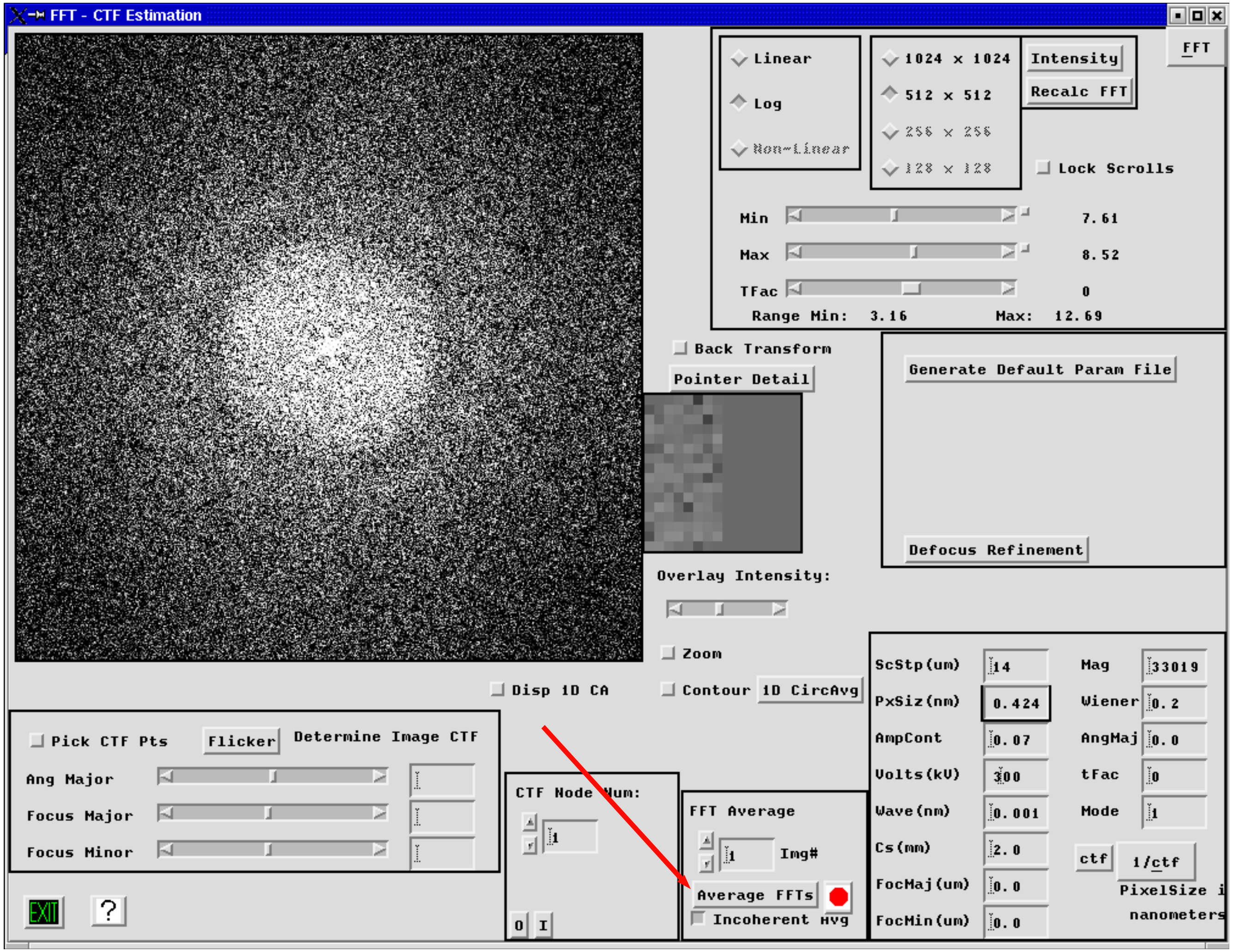

Icosahedral Virus 3D Reconstruction Scheme

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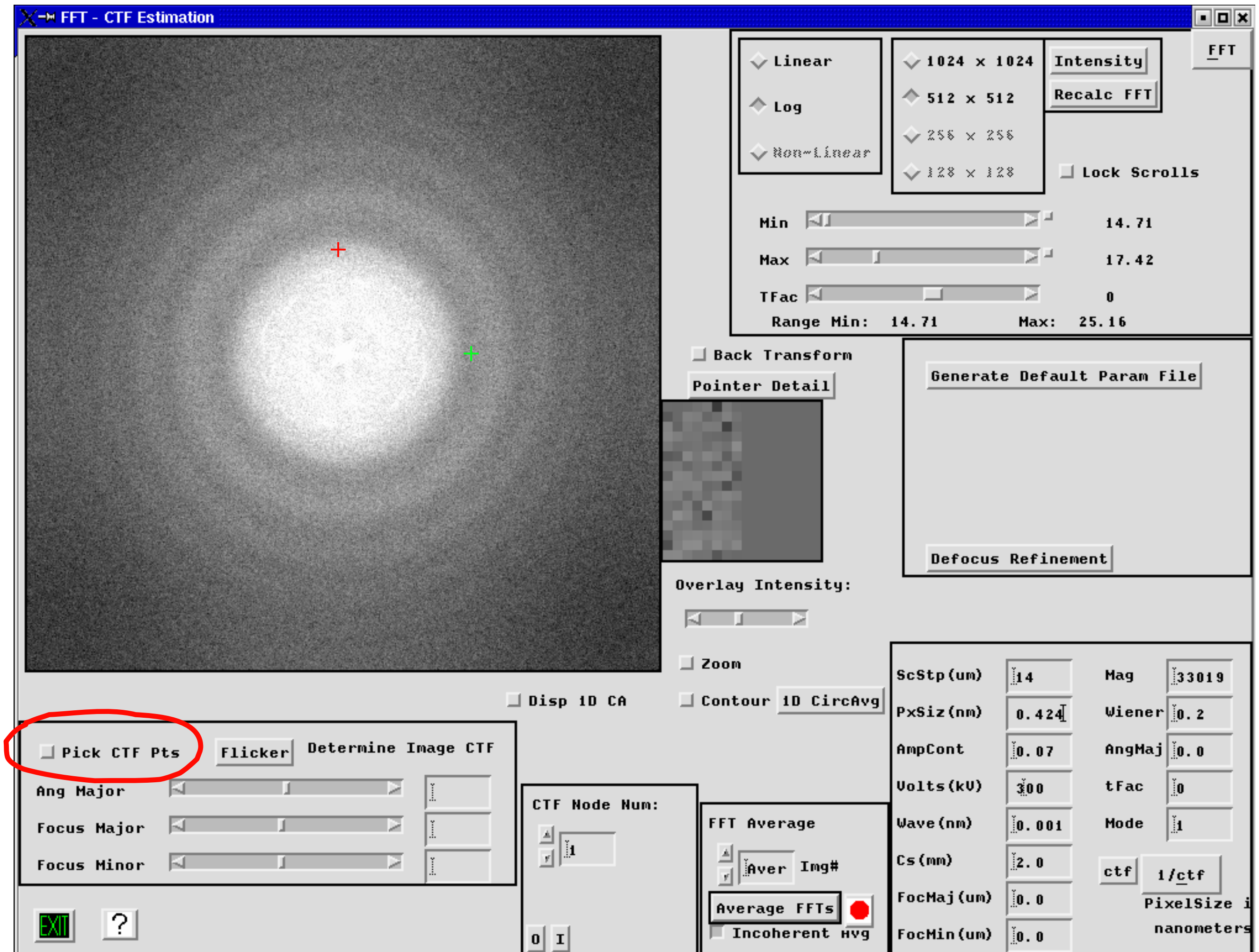

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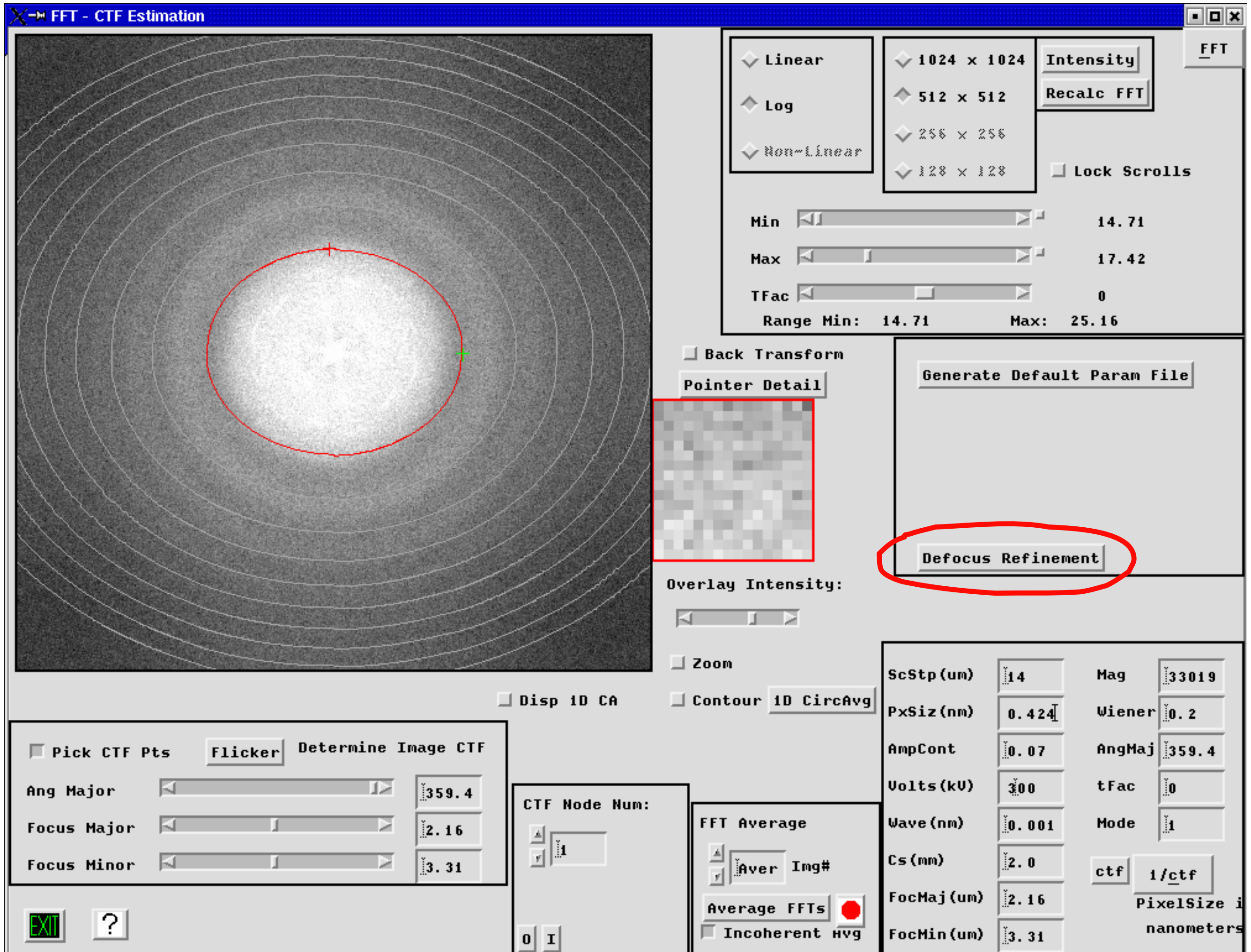

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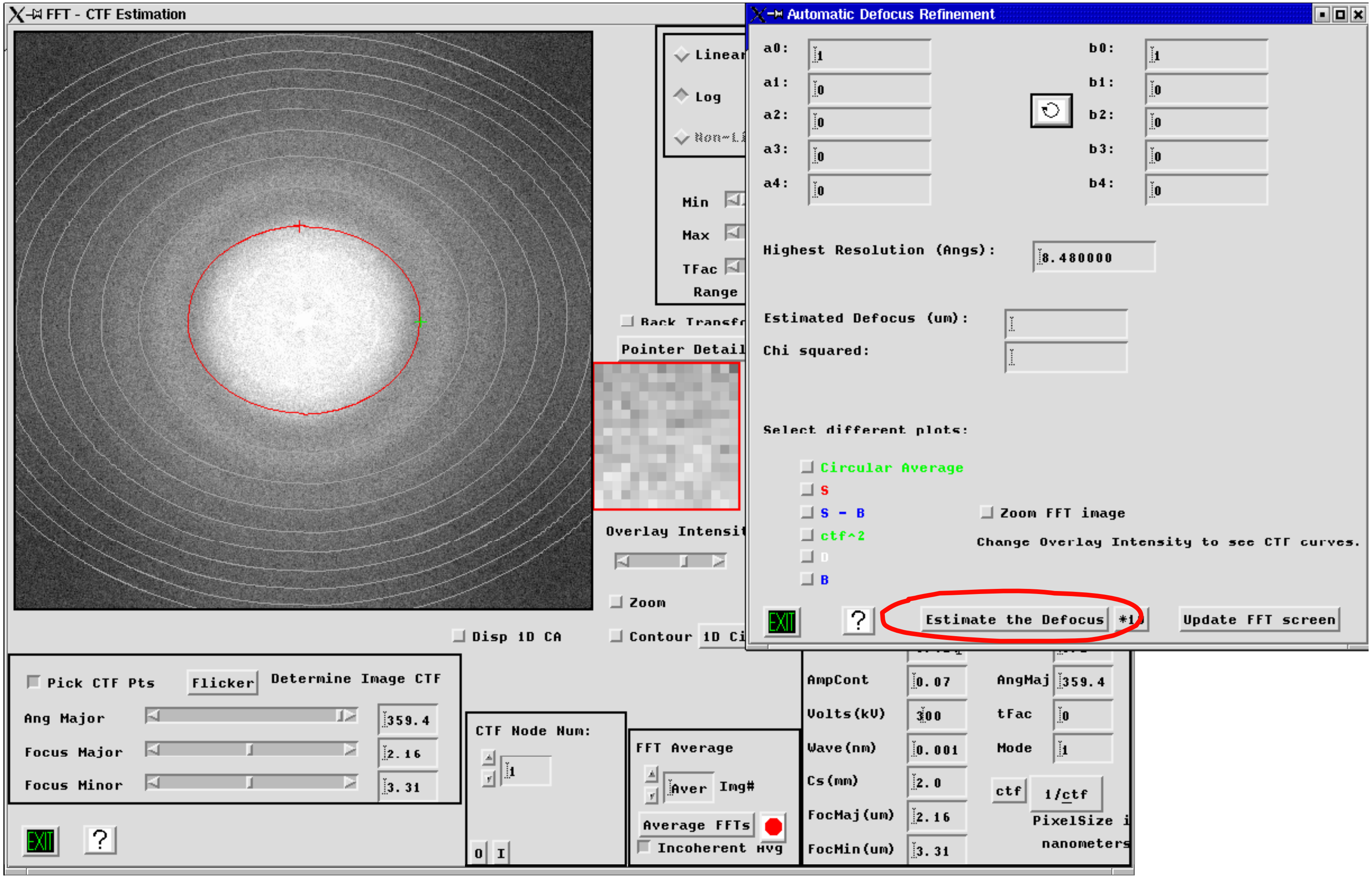

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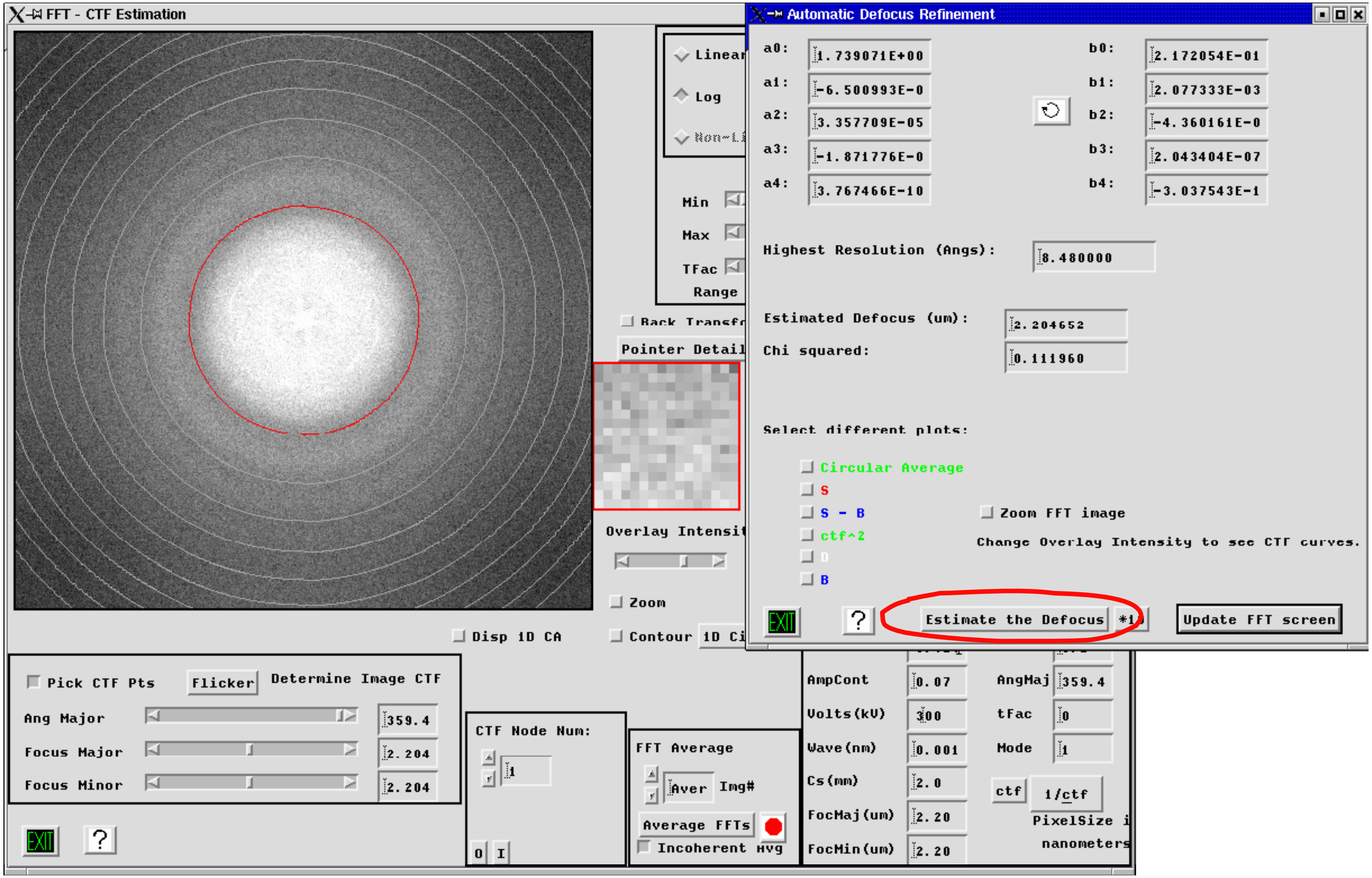

Icosahedral Virus 3D Reconstruction Scheme

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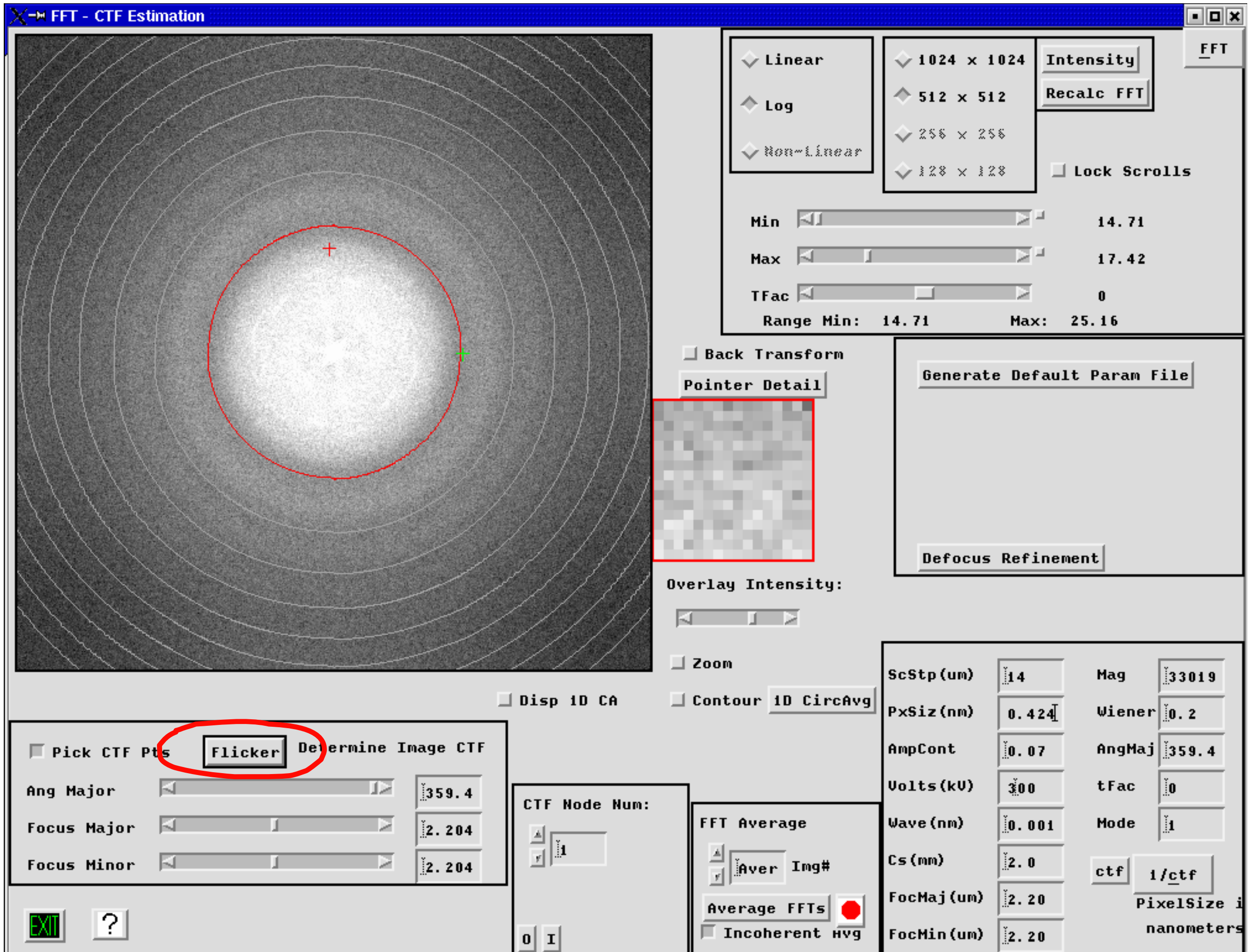

Icosahedral Virus 3D Reconstruction Scheme

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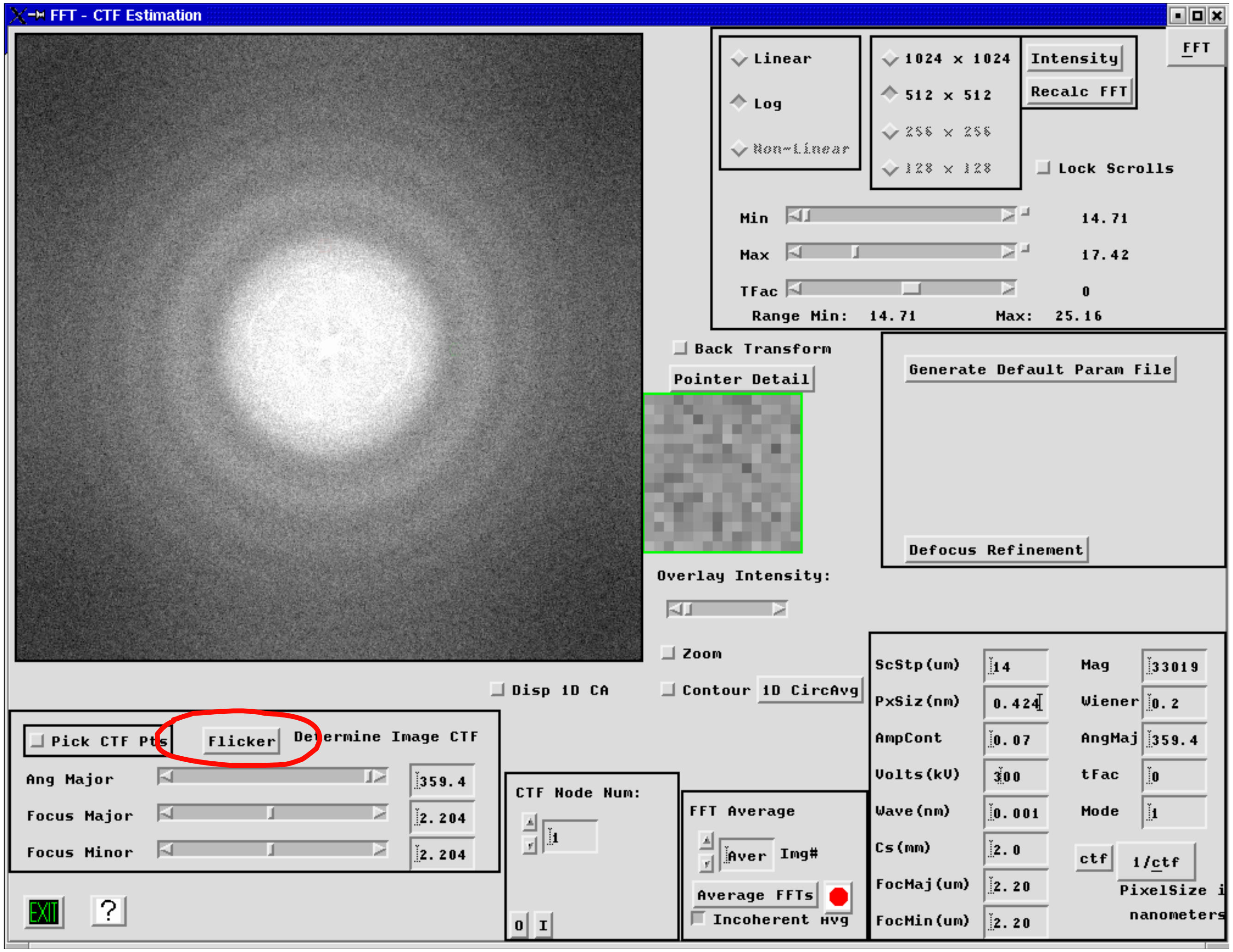
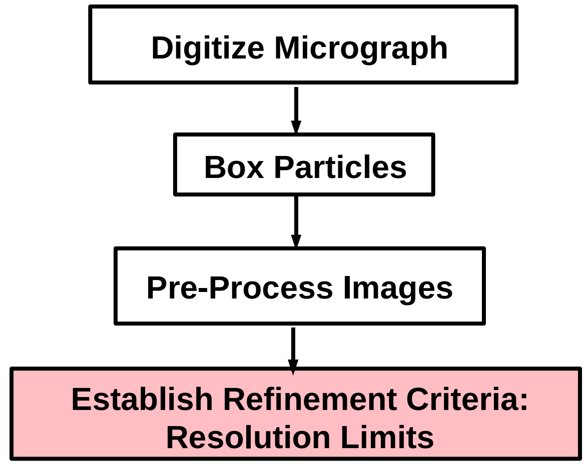
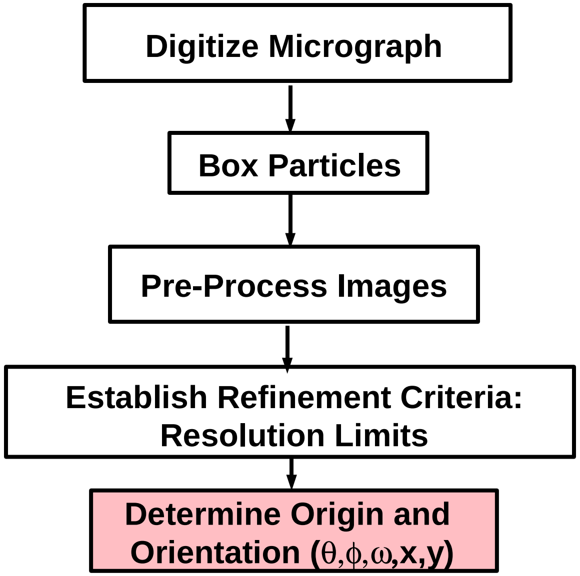


Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

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

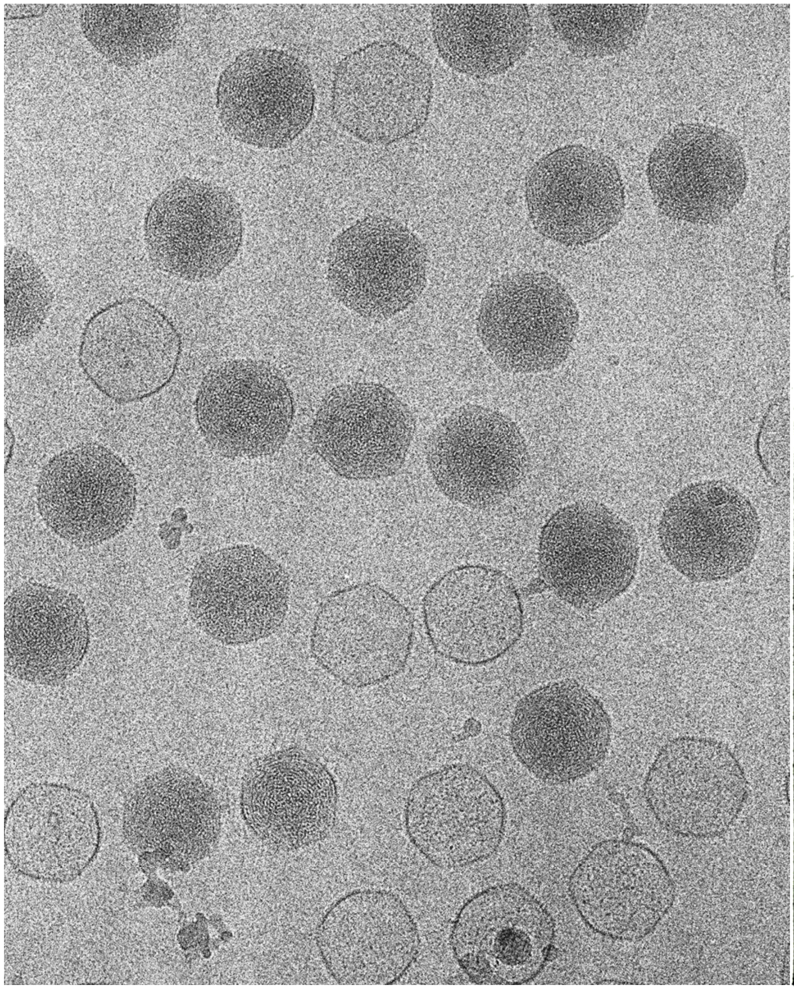
Goal: determine phase origin and view orientation for each boxed particle

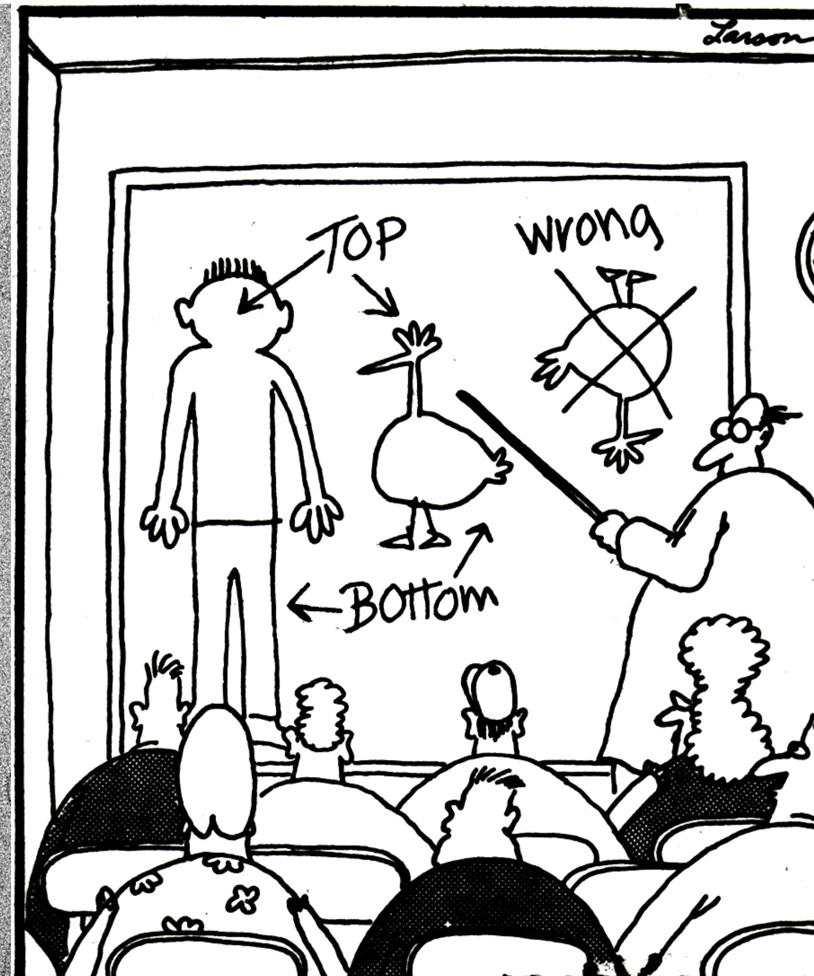
MOST IMPORTANT STEP?

Garbage in ------> garbage out

Icosahedral Virus 3D Reconstruction Scheme

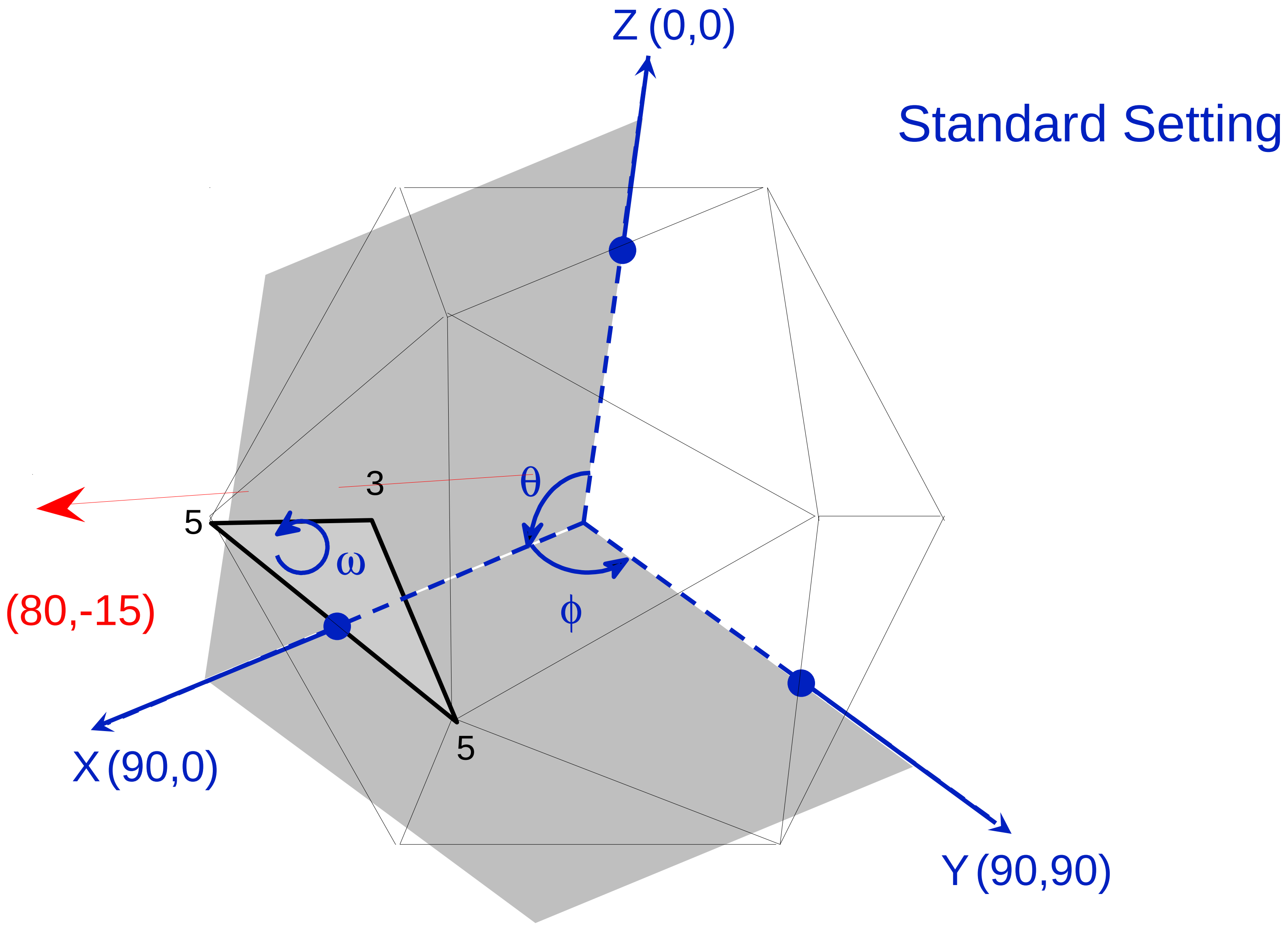
Determine Origin and Orientation (θ,ϕ,ω,x,y)



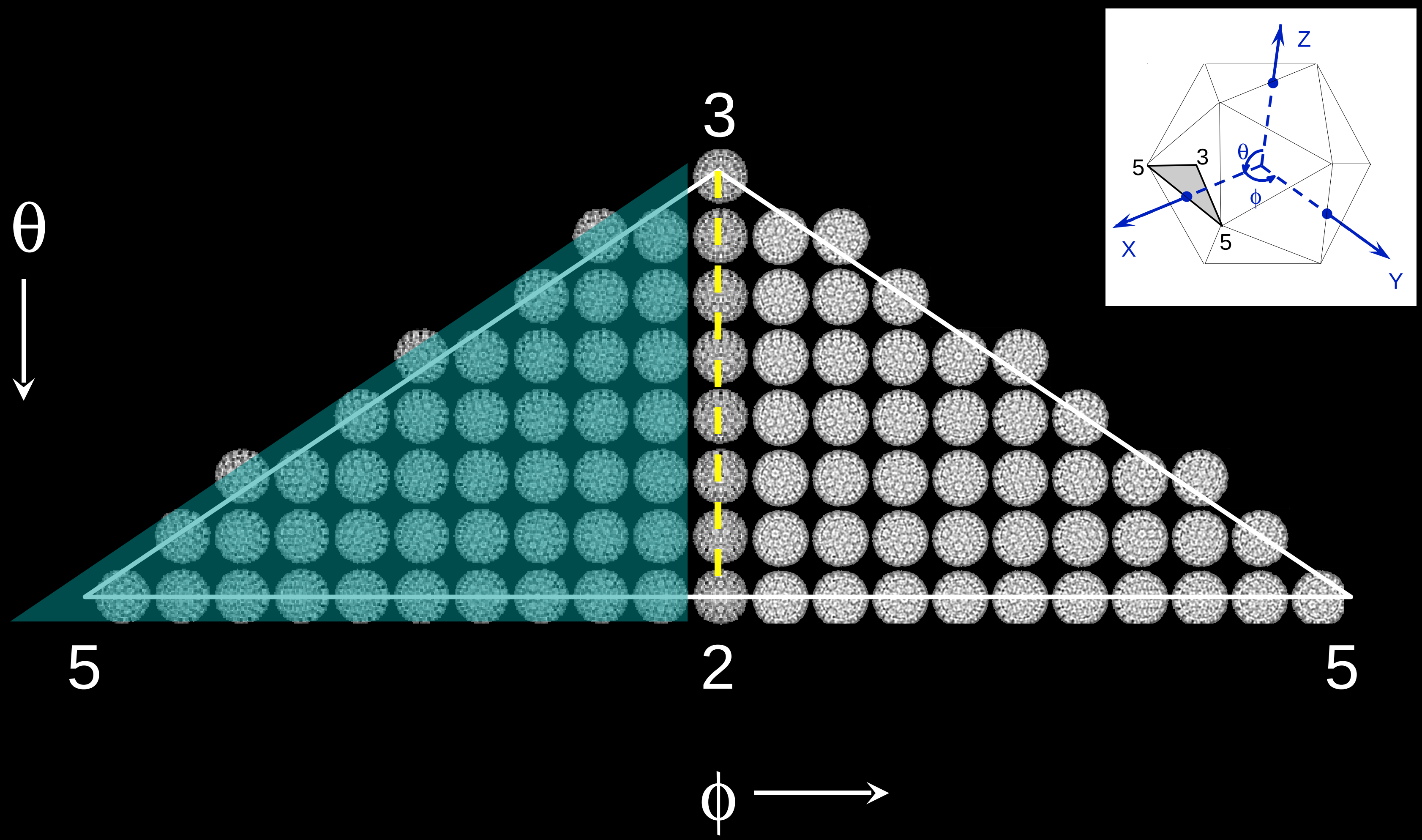


People who don't know which end is up

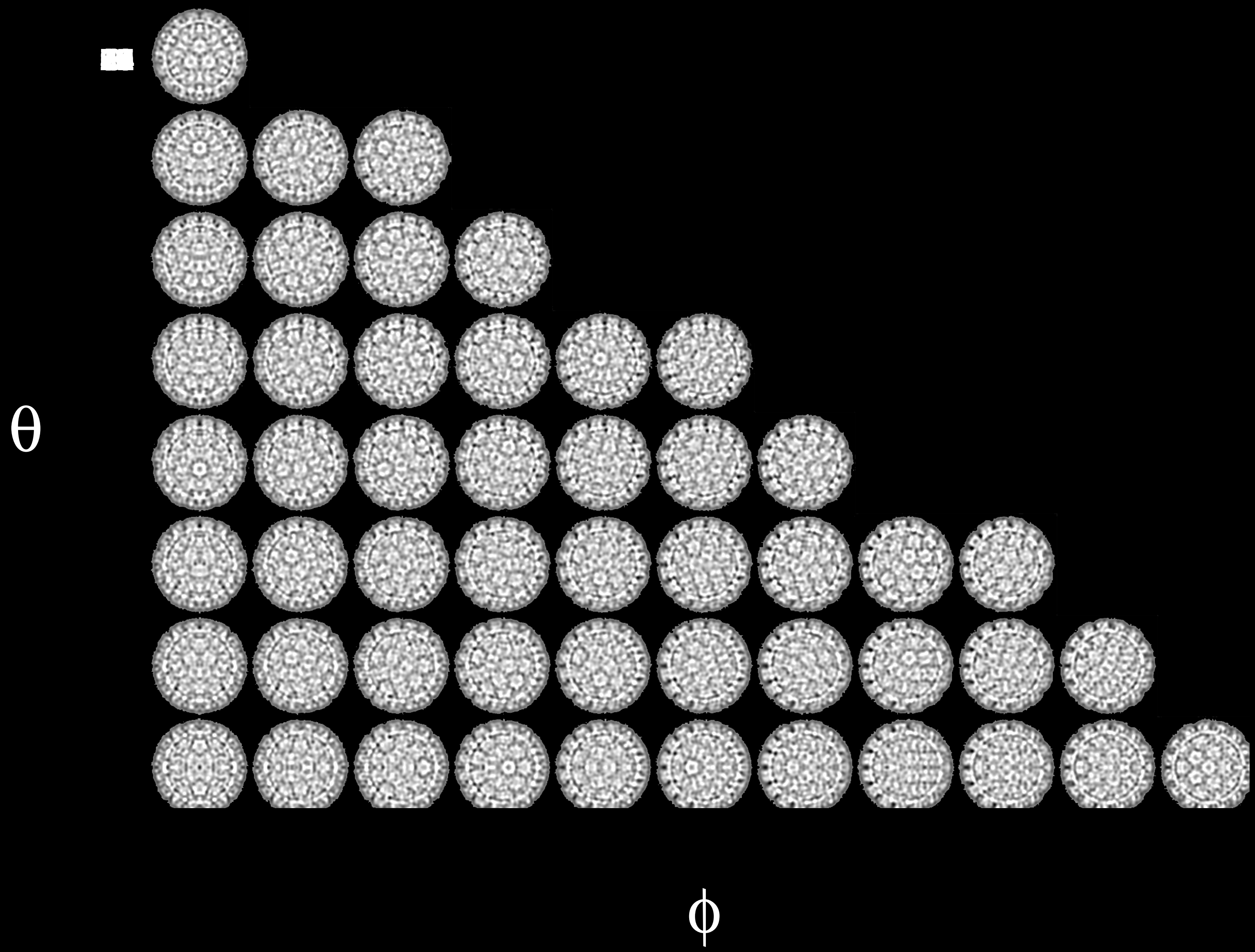
Specifying Direction of View: (θ,ϕ,ω) Orientation



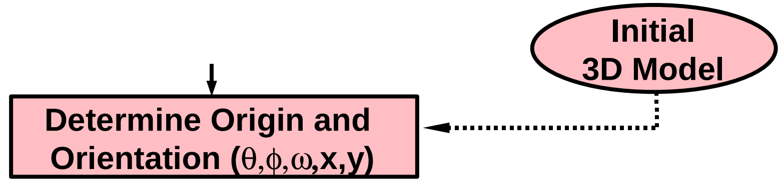
BPV Projections: Icosahedral ASU



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)

How do we determine the (θ, ϕ, ω, x, y) parameters?

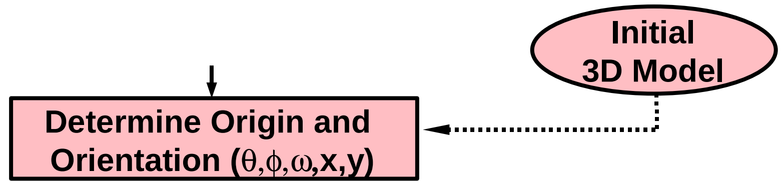
Two methods:

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 (θ, ϕ, ω, 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)

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."

Icosahedral Virus 3D Reconstruction Scheme

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

Common Lines

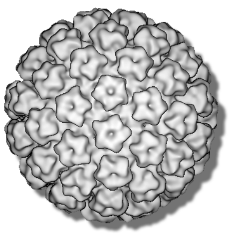
The ‘gospel’ continued:

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

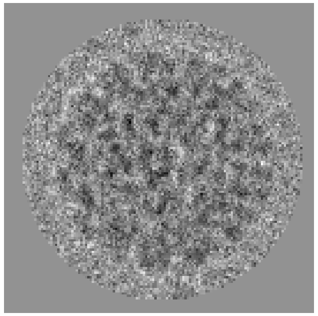
“A similar pair of lines will be generated by each possible choice of pairs of symmetry operations”

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

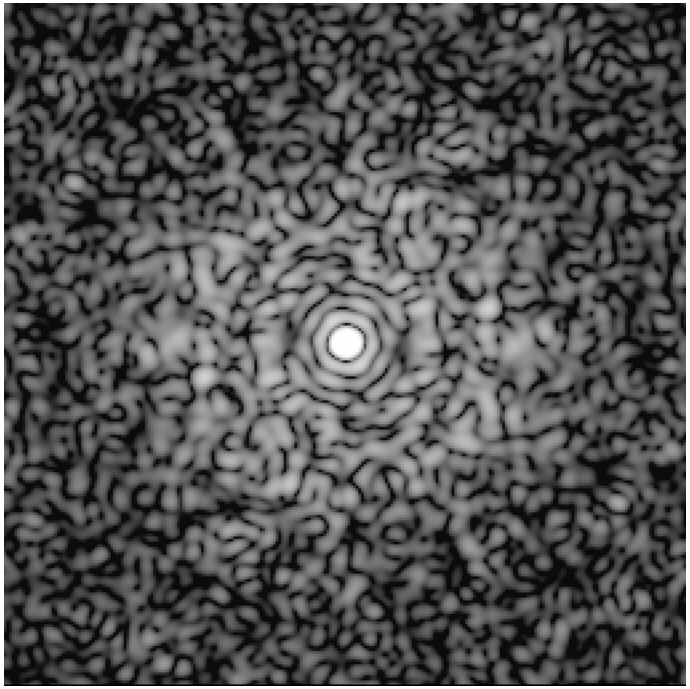
Orientation Determination by Common Lines



3D Object



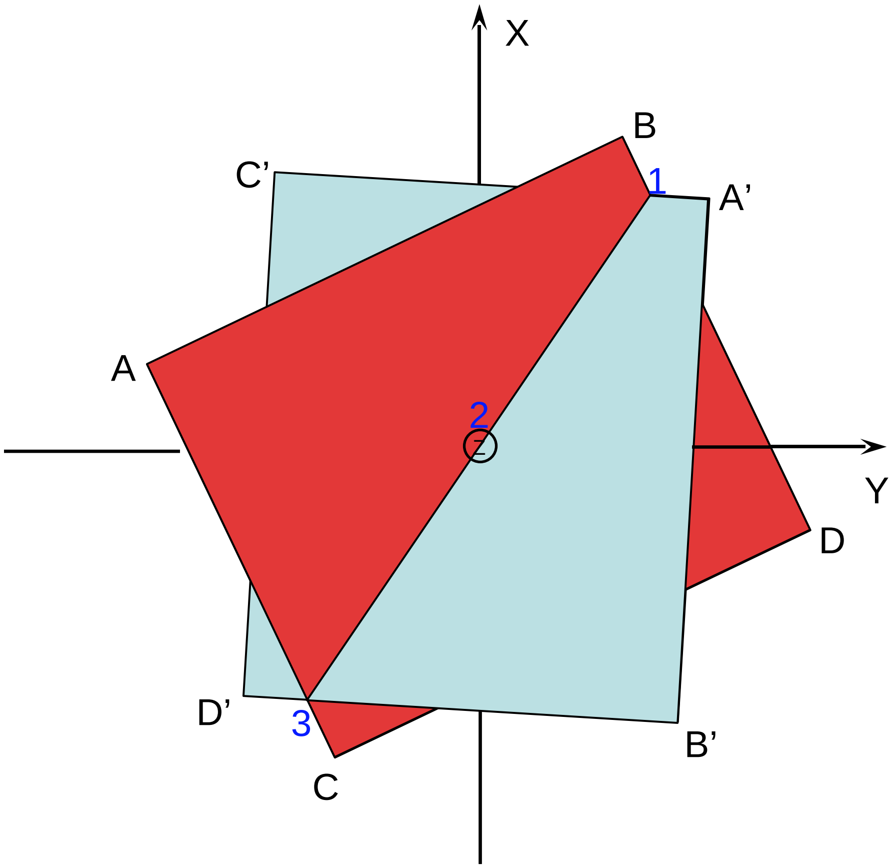
2D Projection
(θ,ϕ,ω)



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

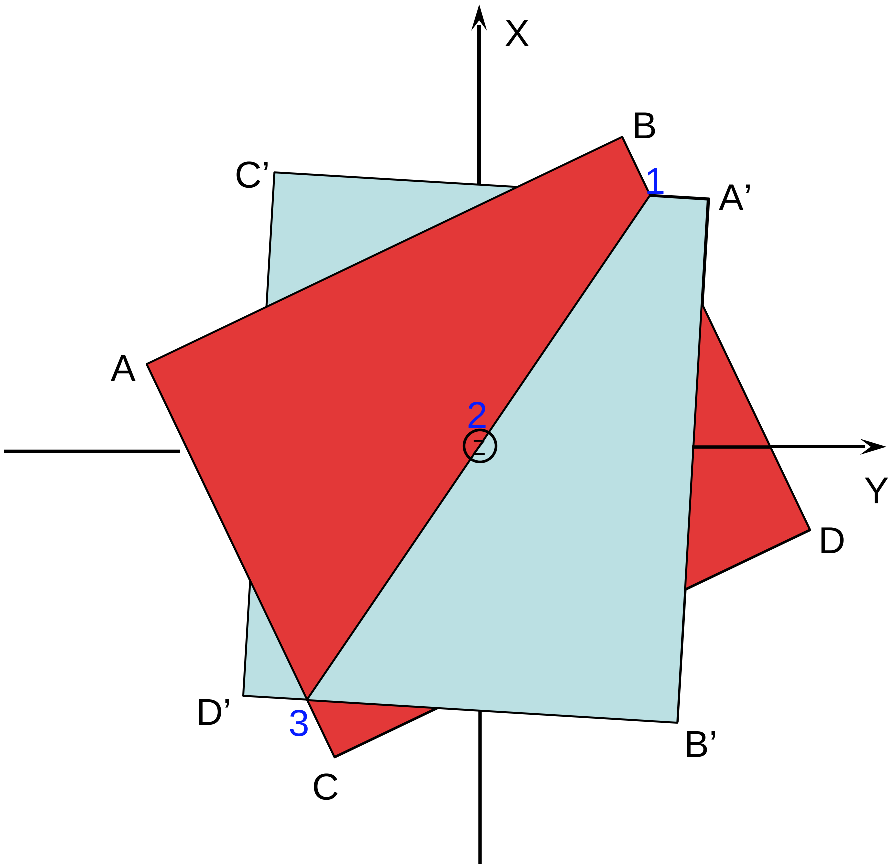
Let Z-direction coincide with **3-fold** axis of symmetry

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

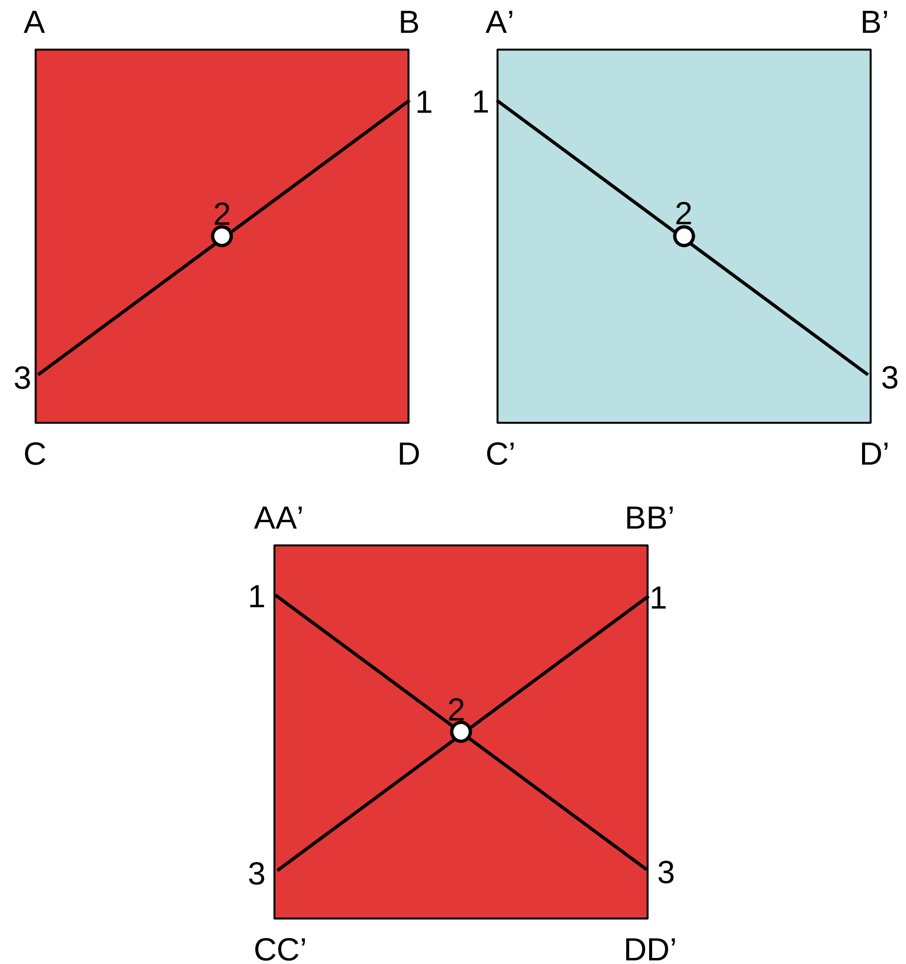
Both planes have **common values** along the **line** (1,2,3) of their intersection

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

Orientation Determination by Common Lines

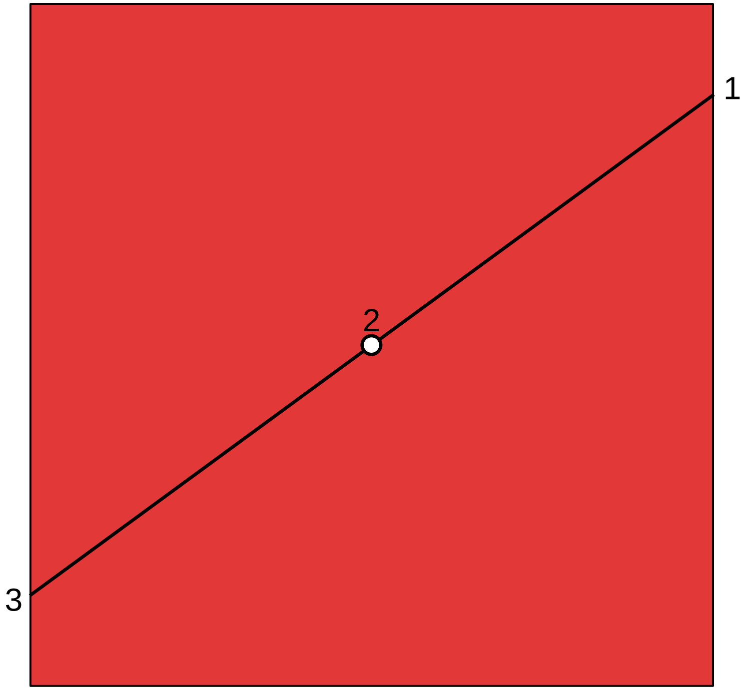


Adapted from Moody (1990) Fig. 7.68, p.245

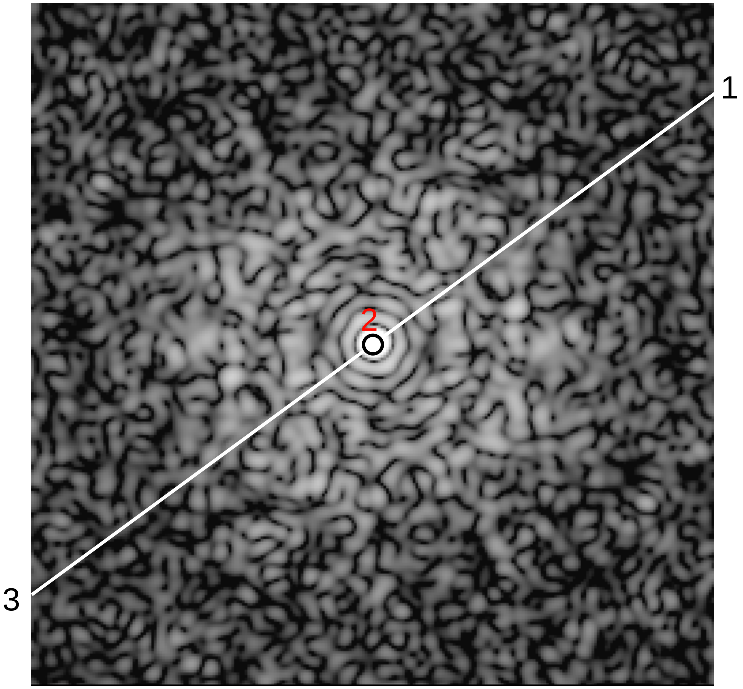


Adapted from Moody (1990) Fig. 7.69, p.246

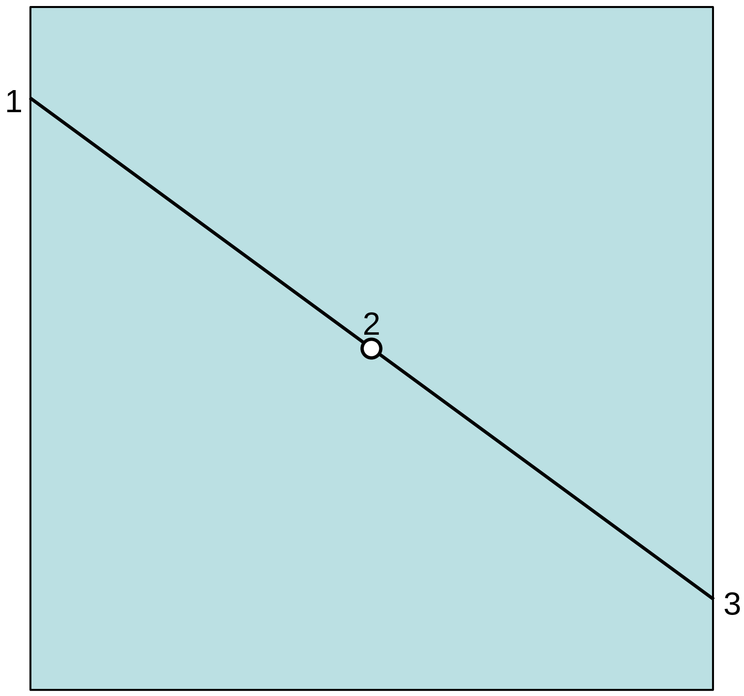
Orientation Determination by Common Lines



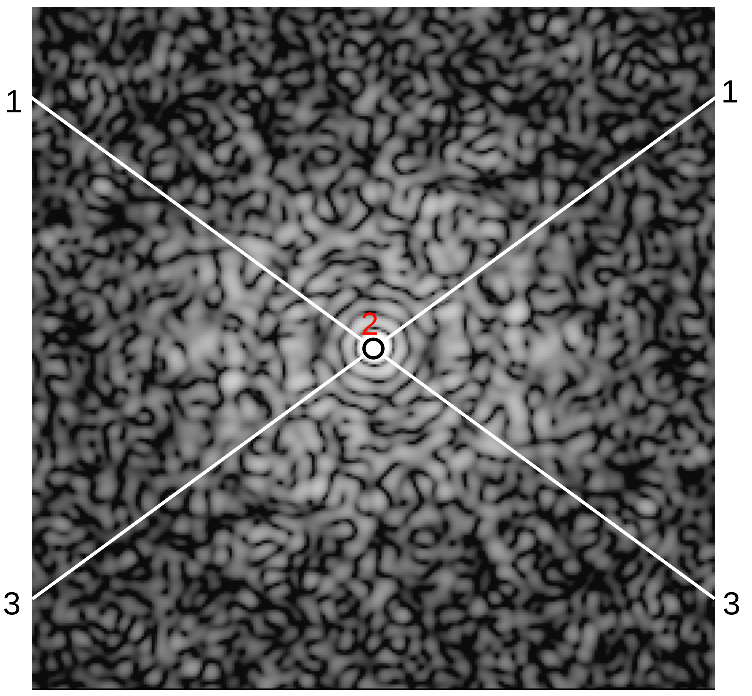
Original Transform Plane



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:

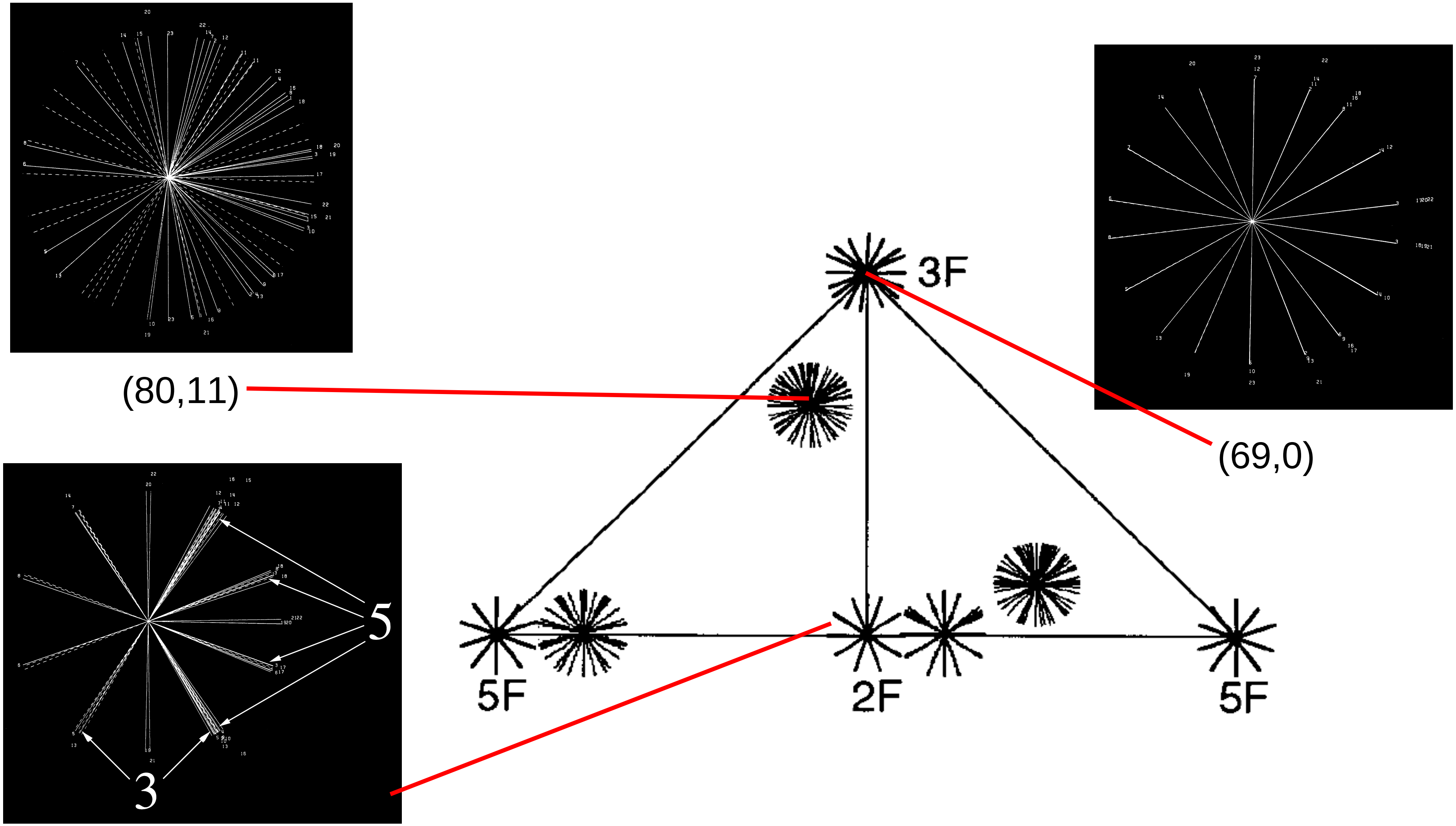
5-folds: $\frac{12}{2}$ x 2 = 12 pairs

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

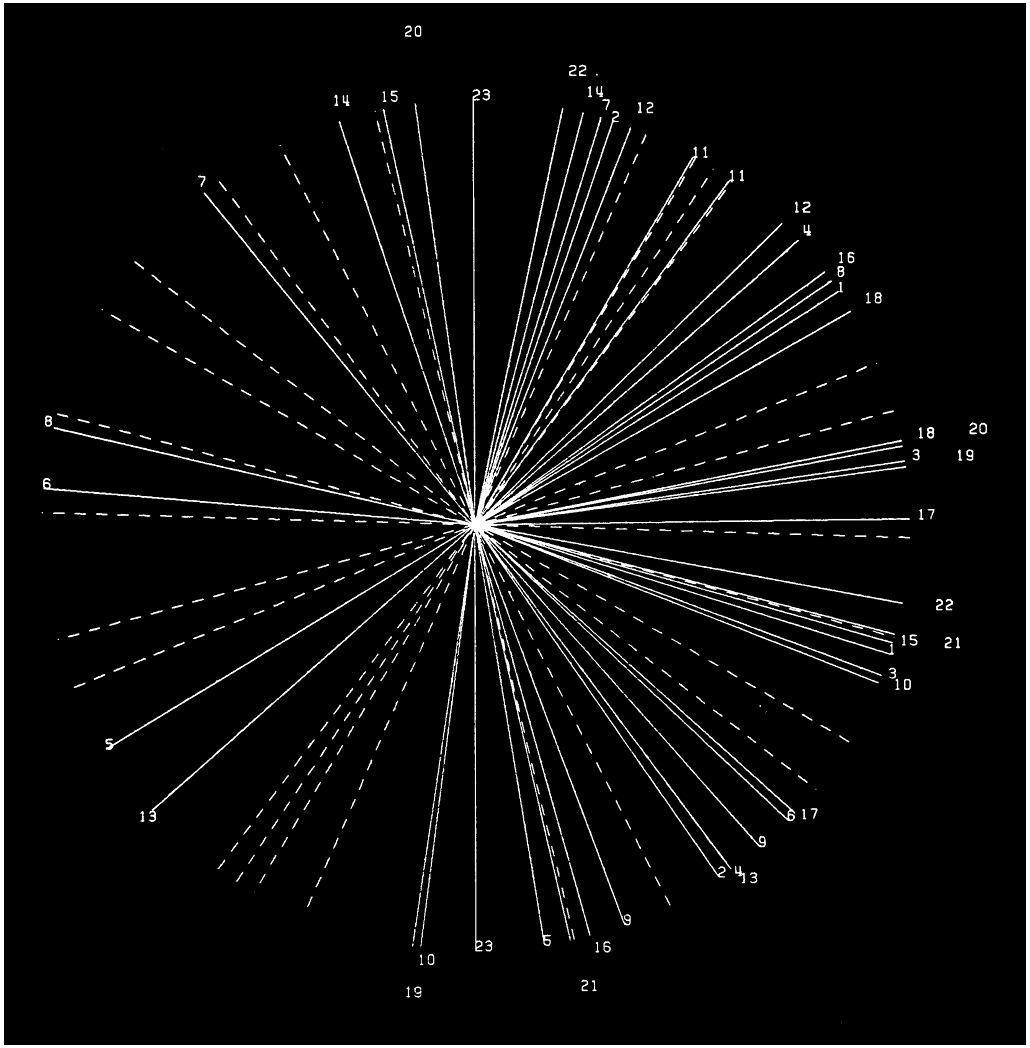
2-folds: $\frac{30}{2}$ x 1 = 15 real lines

37 common lines

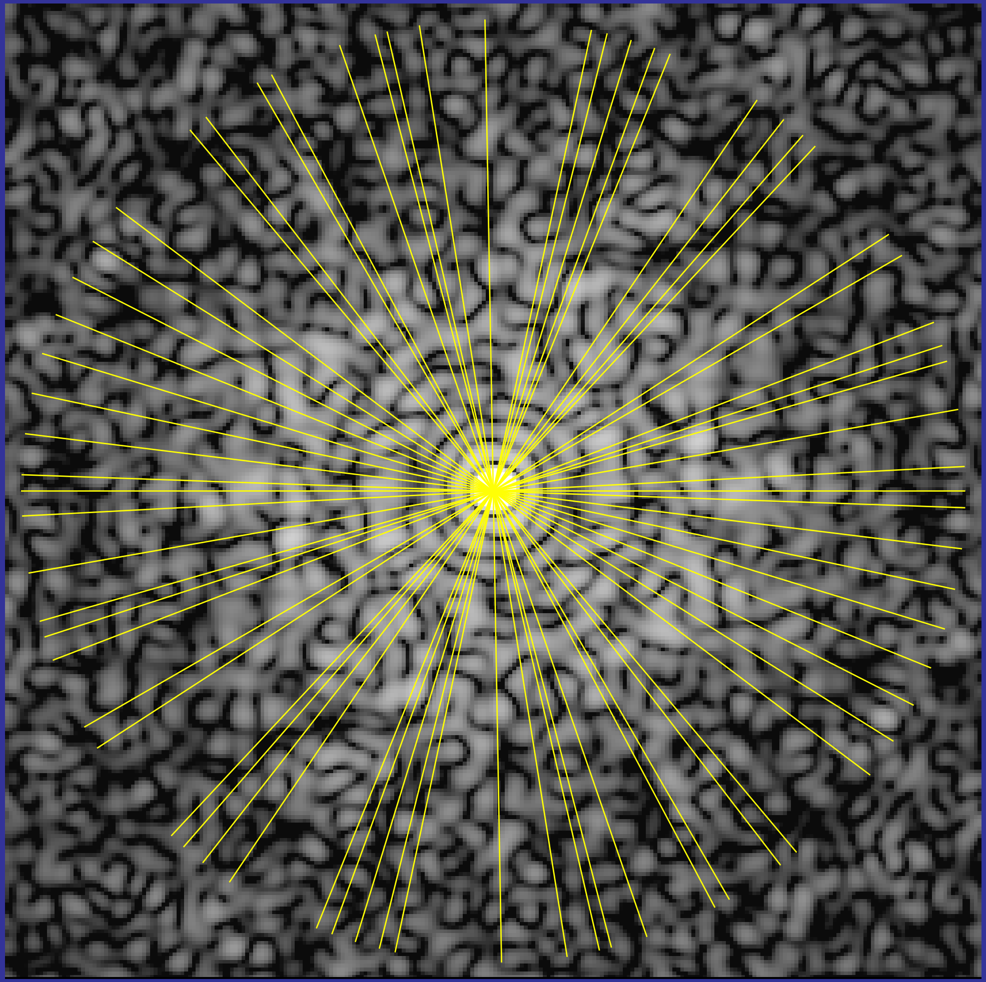
Orientation Determination by Common Lines



Orientation Determination by Common Lines



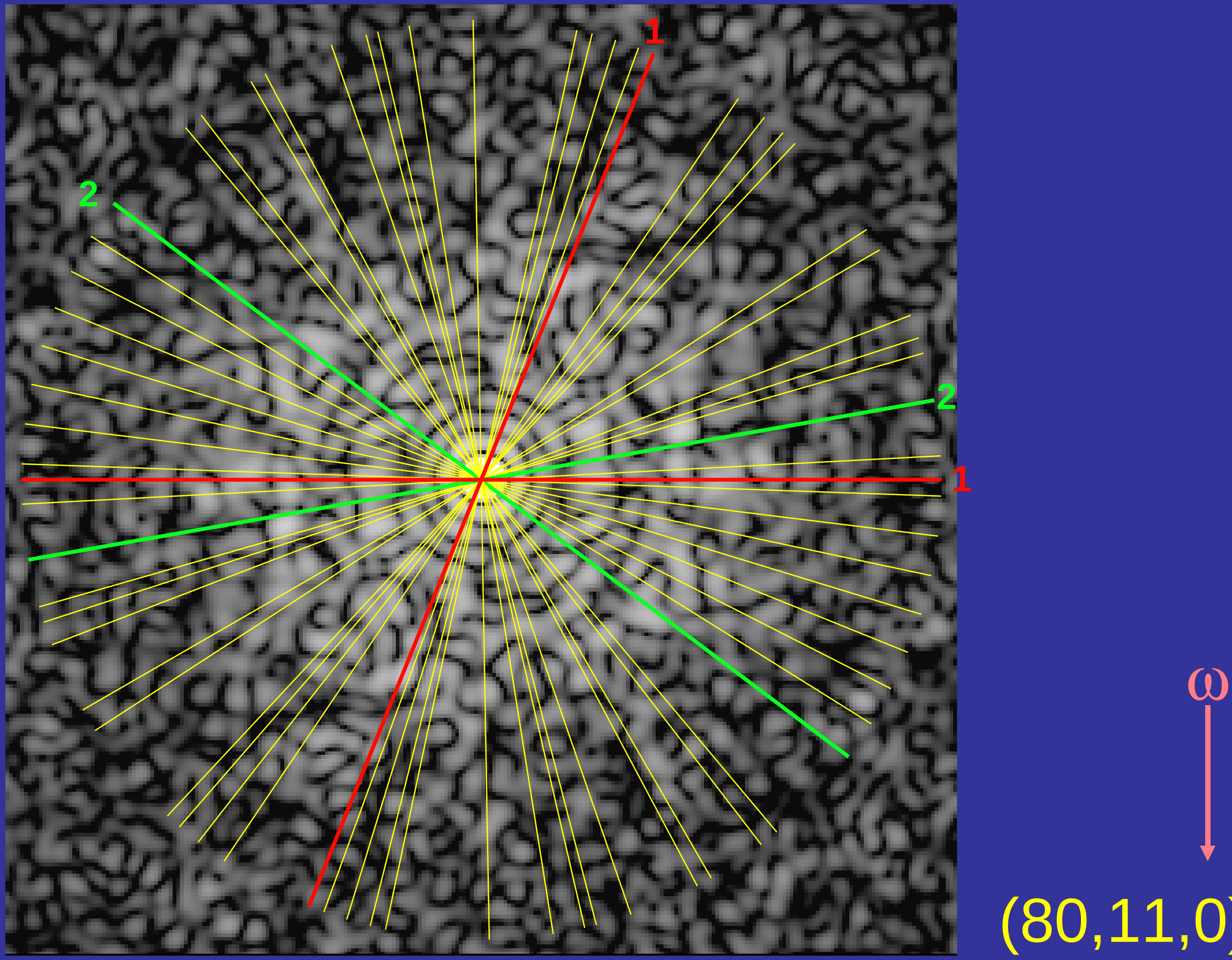
Orientation Determination by Common Lines



(80,11)

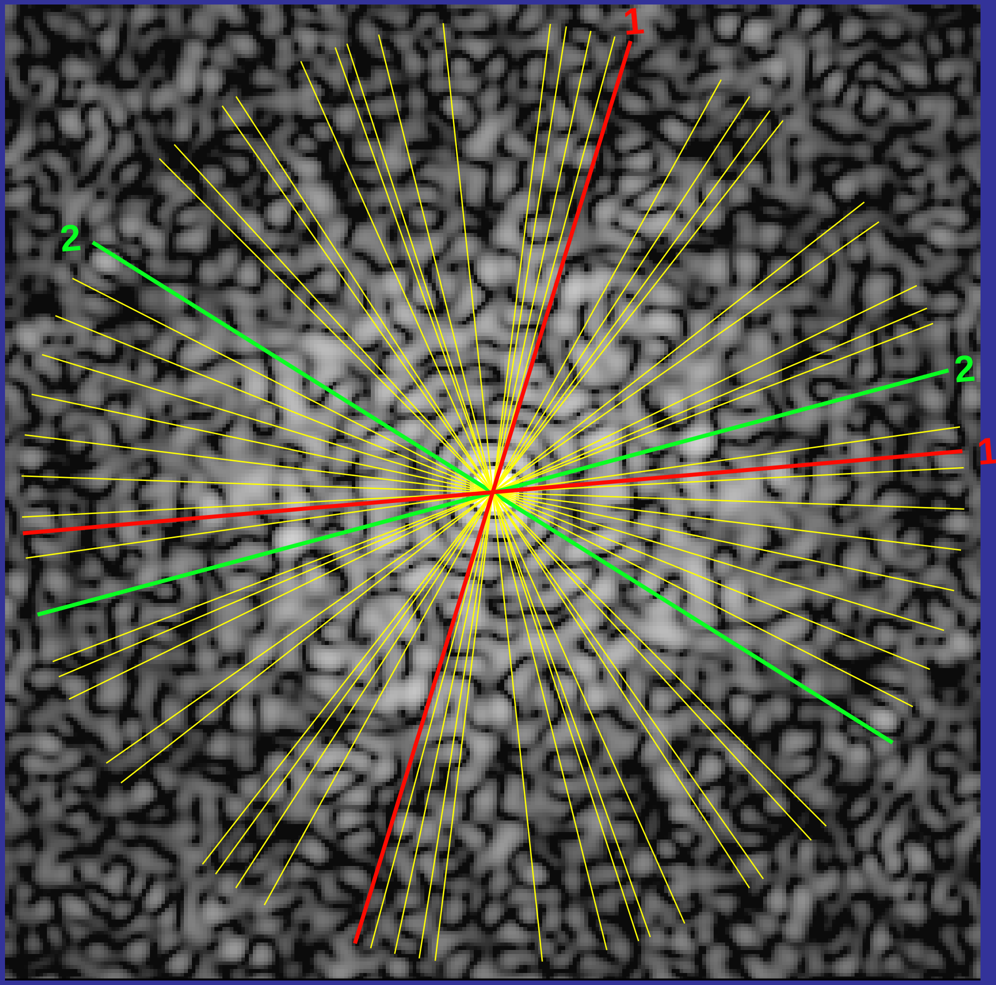
What is (θ,ϕ,ω) for this particle?

Orientation Determination by Common Lines



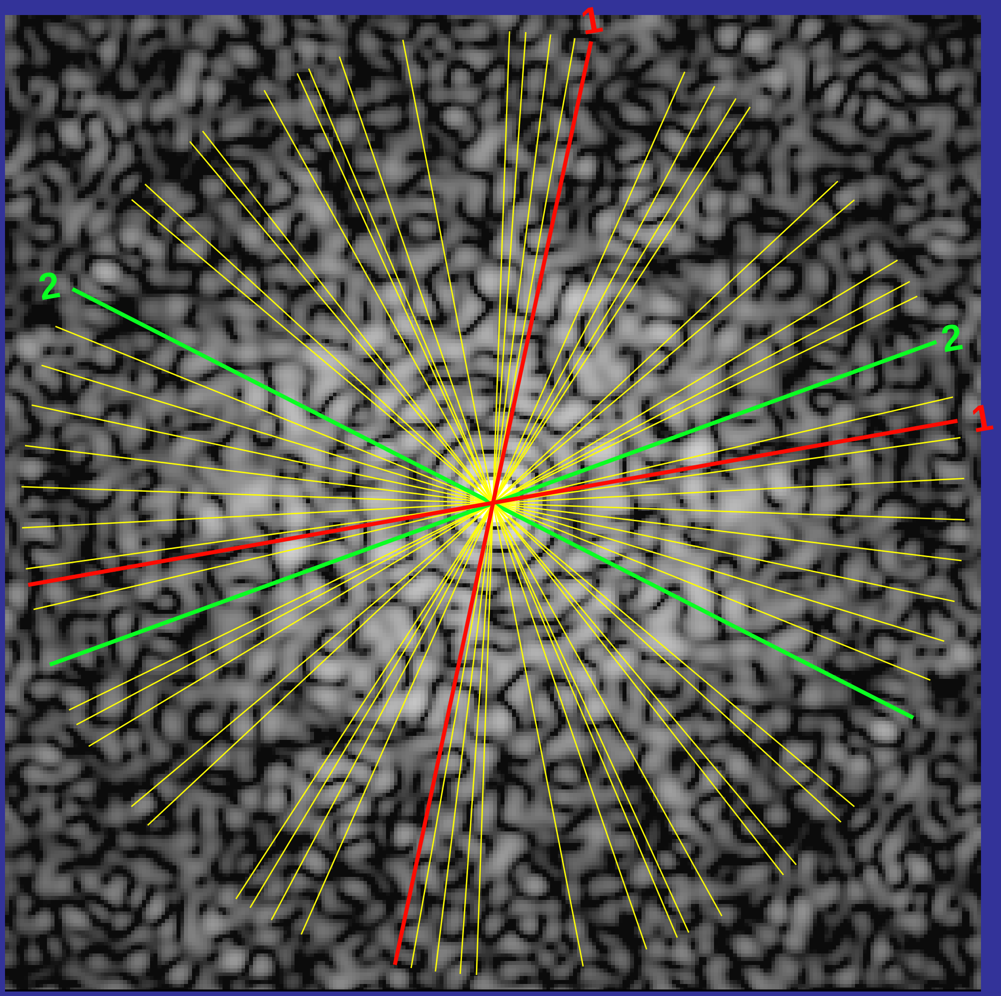
(80,11,0)

Orientation Determination by Common Lines



(80,11,5)

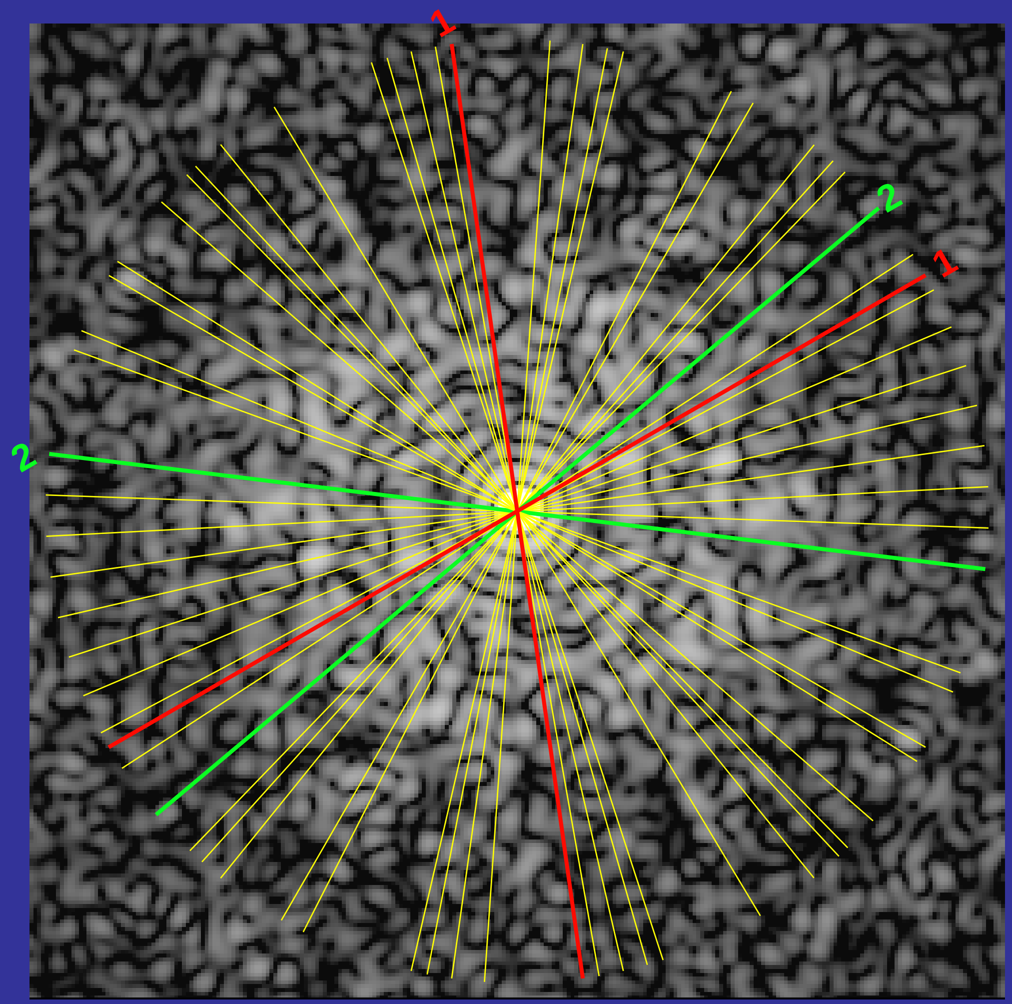
Orientation Determination by Common Lines

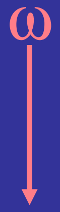


ω

(80,11,10)

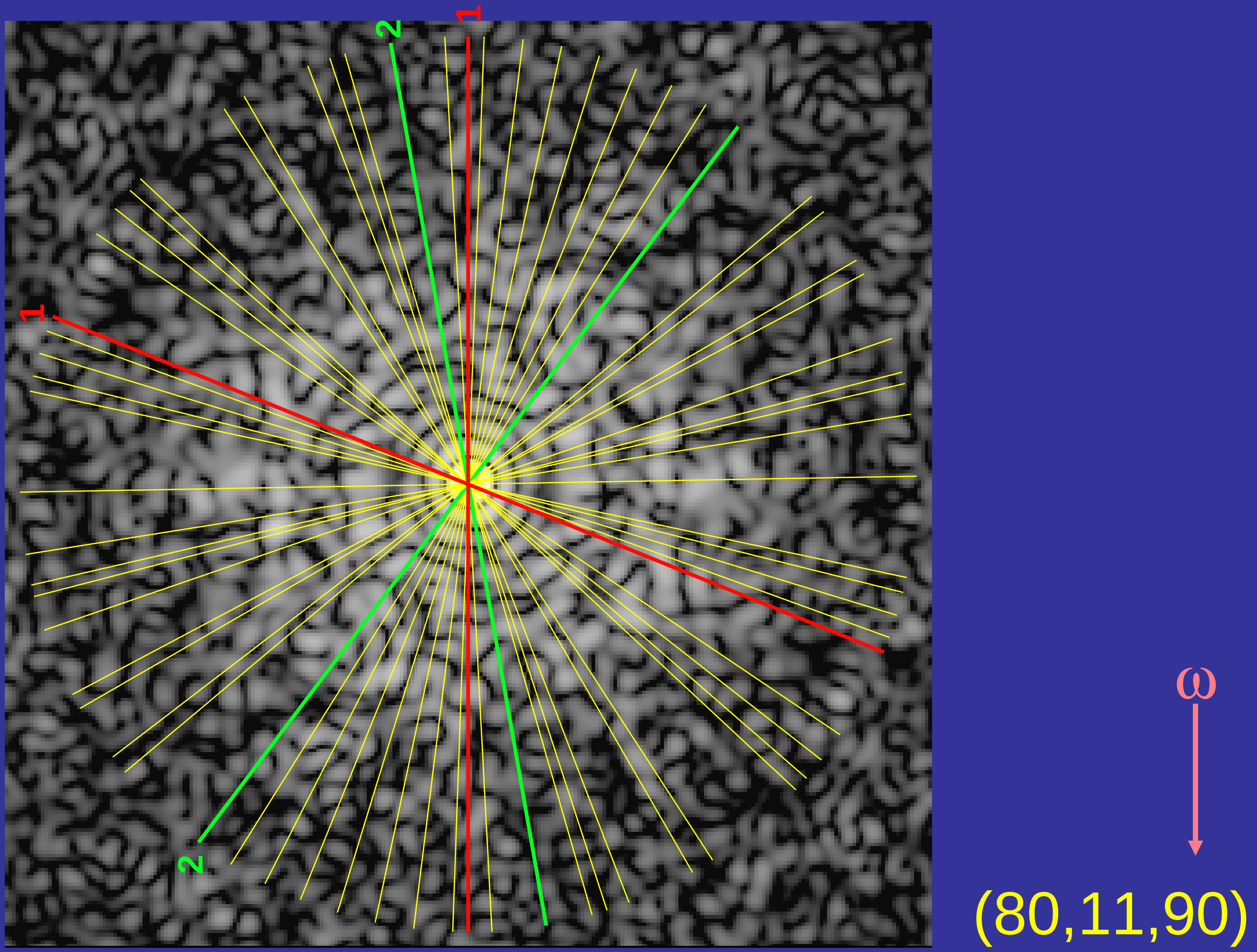
Orientation Determination by Common Lines



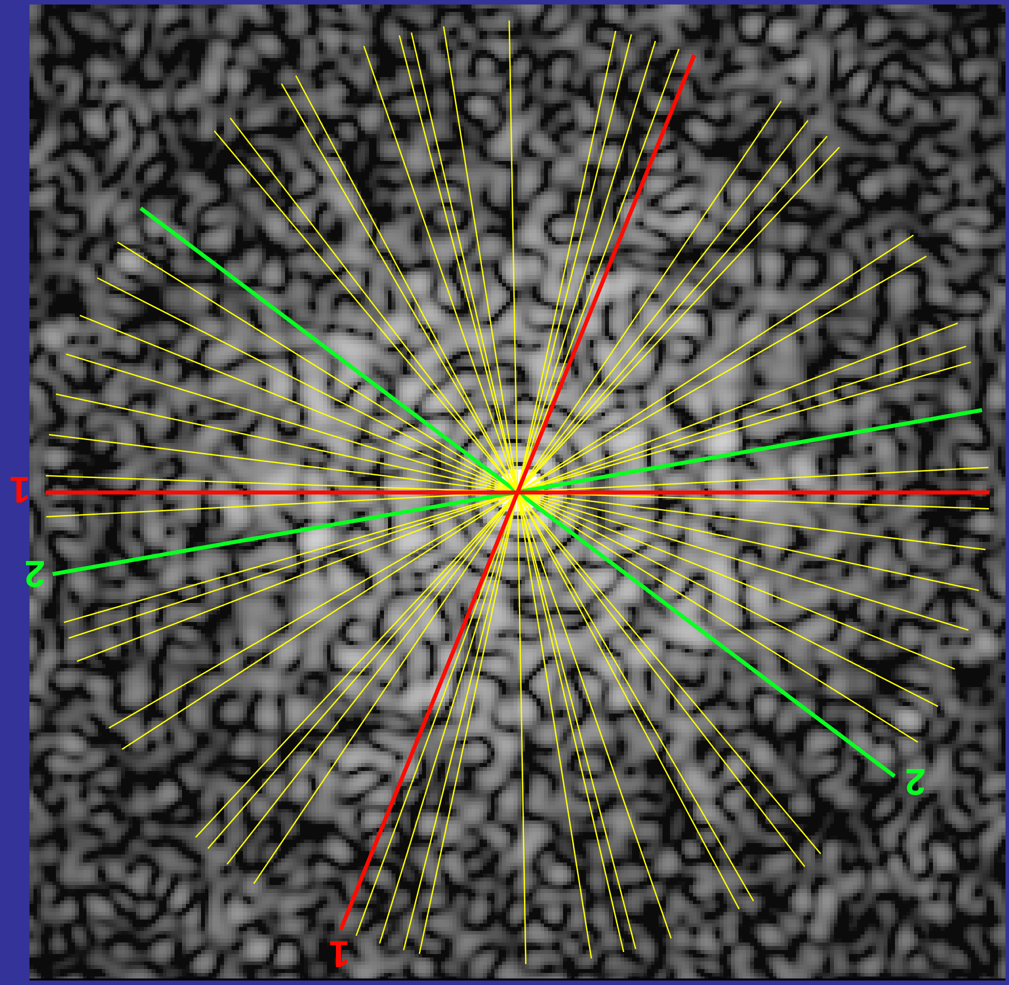


(80,11,30)

Orientation Determination by Common Lines



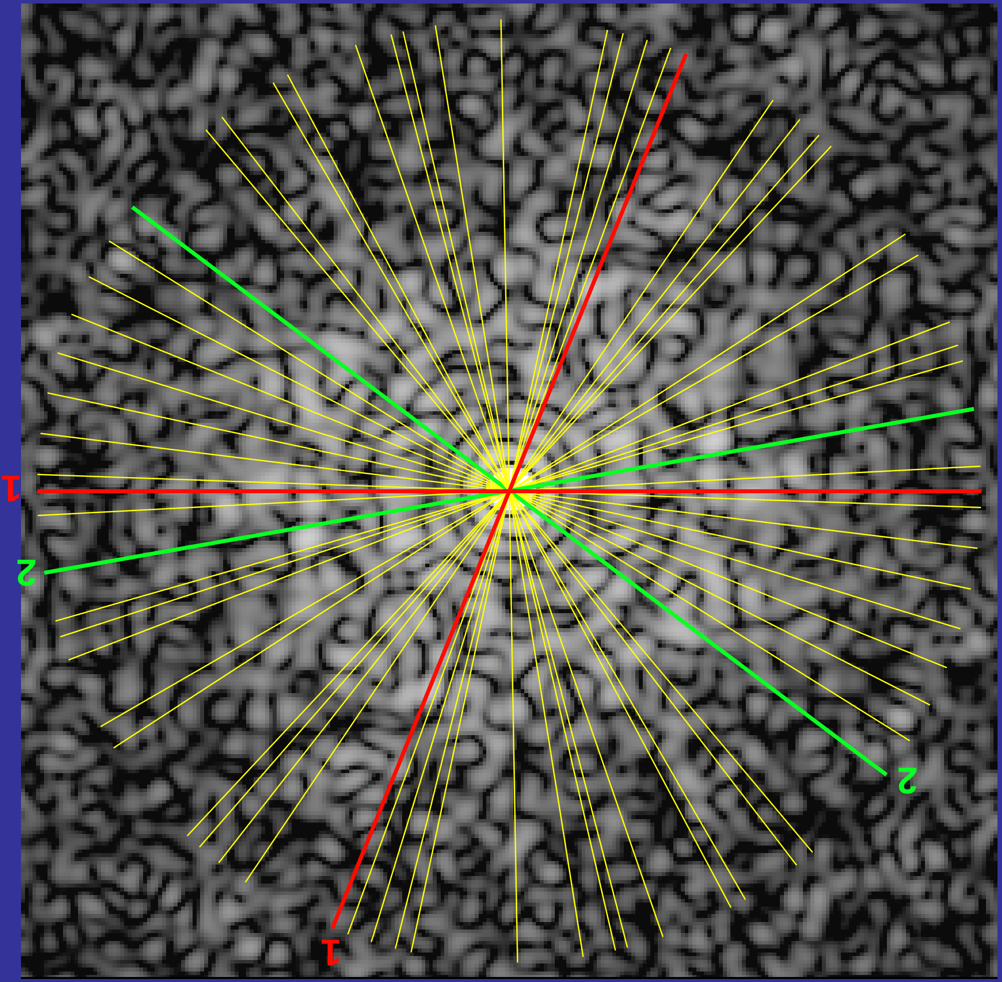
Orientation Determination by Common Lines



ω

(80,11,180)

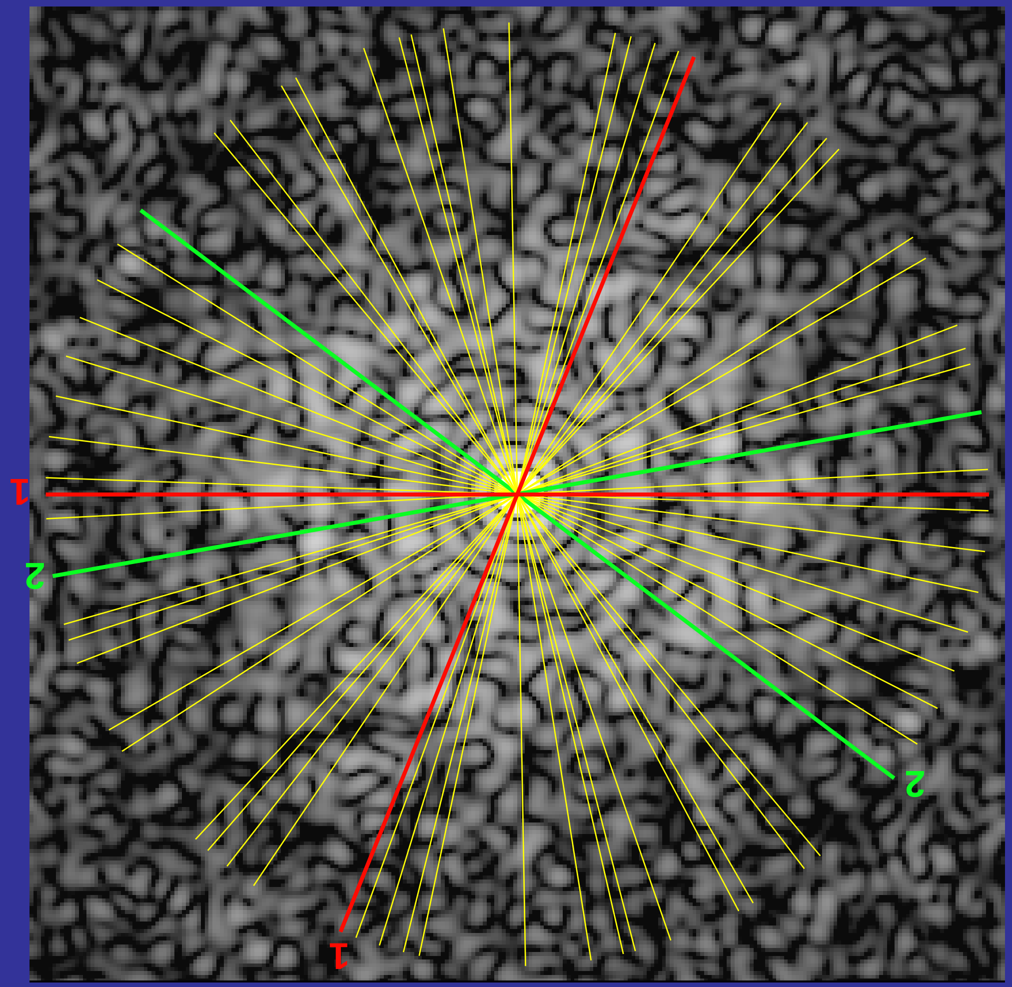
Orientation Determination by Common Lines



(80,11,ω)

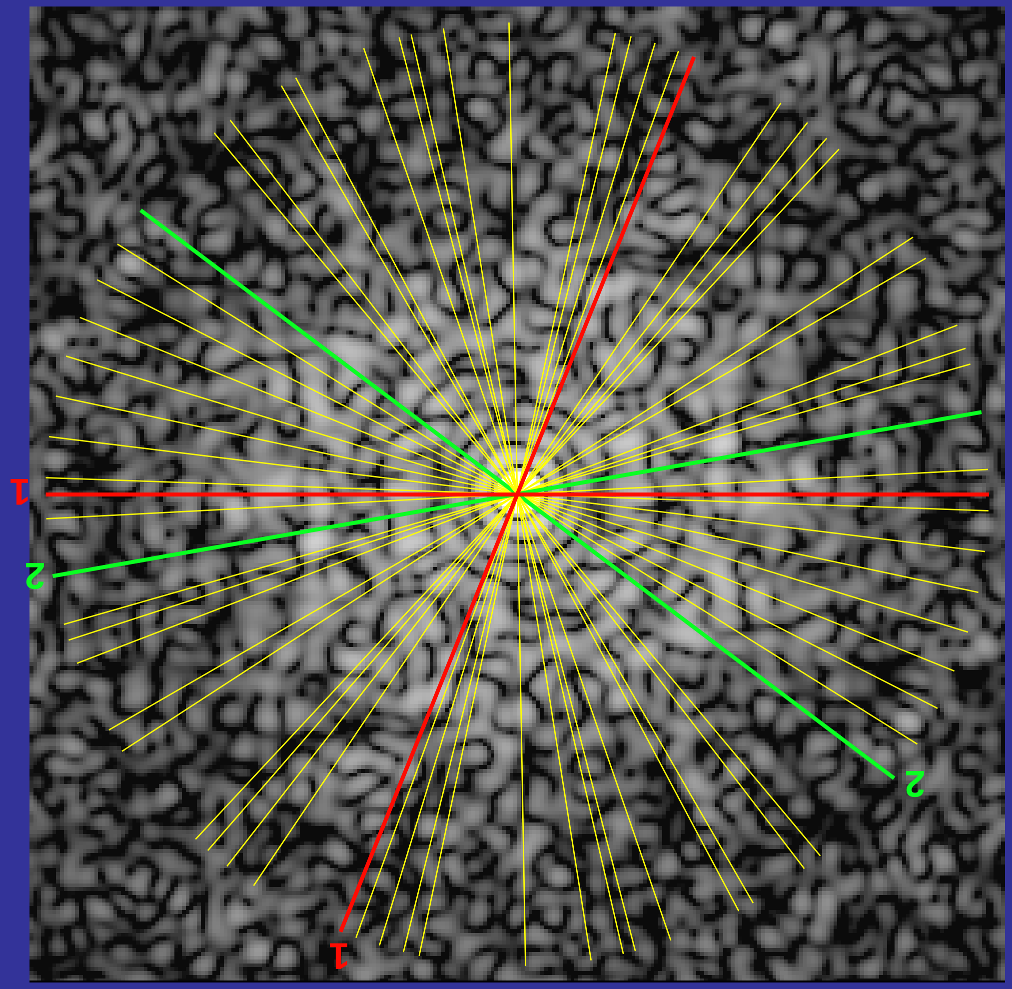
Metric: Identify ω that gives lowest phase residual

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)

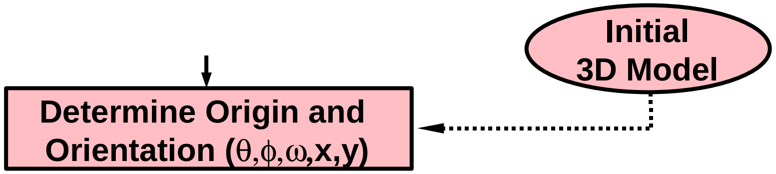
Common Lines

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 ($\theta, \phi, \omega, x, y$):

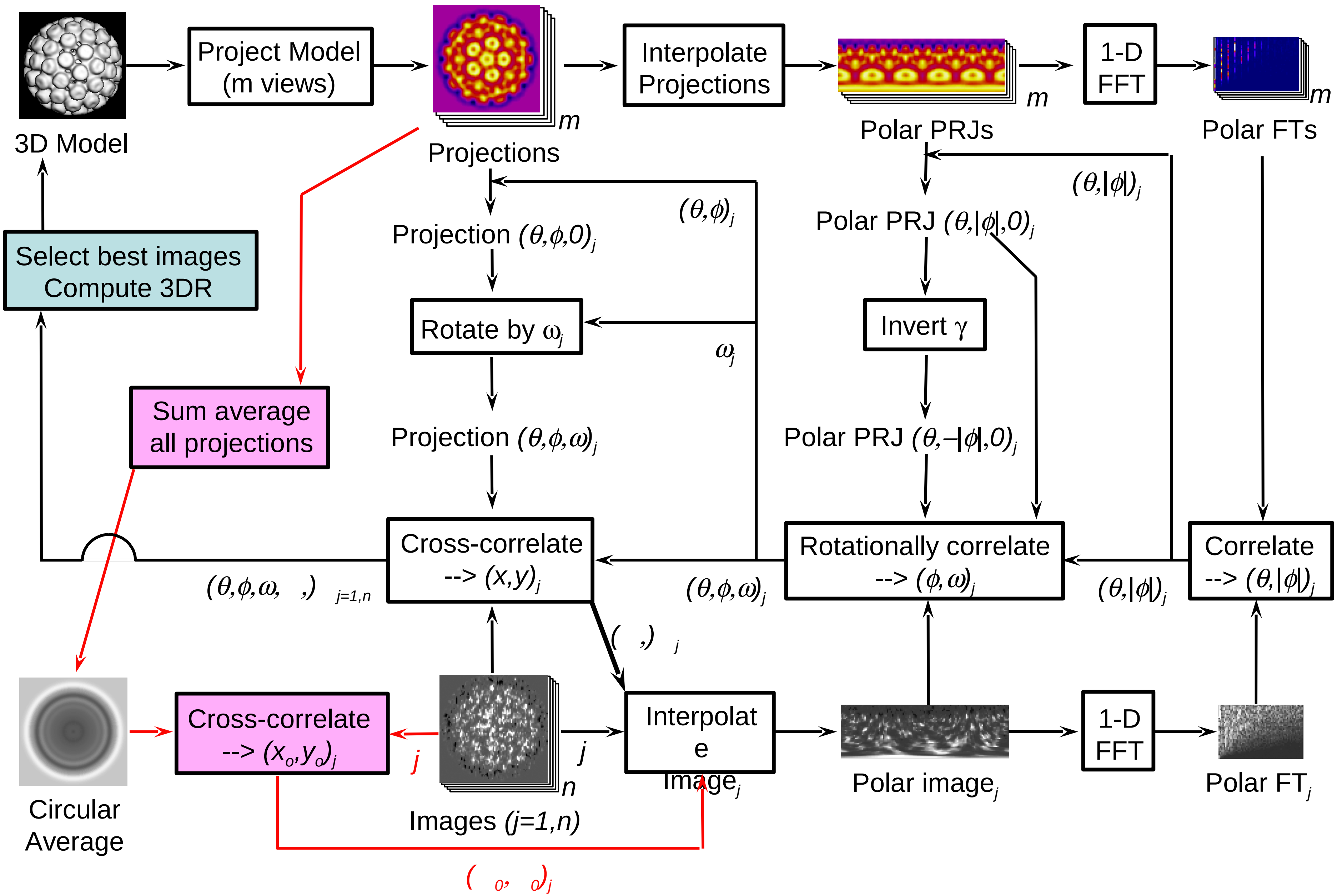
1. Common lines

2. Model-based (template) matching

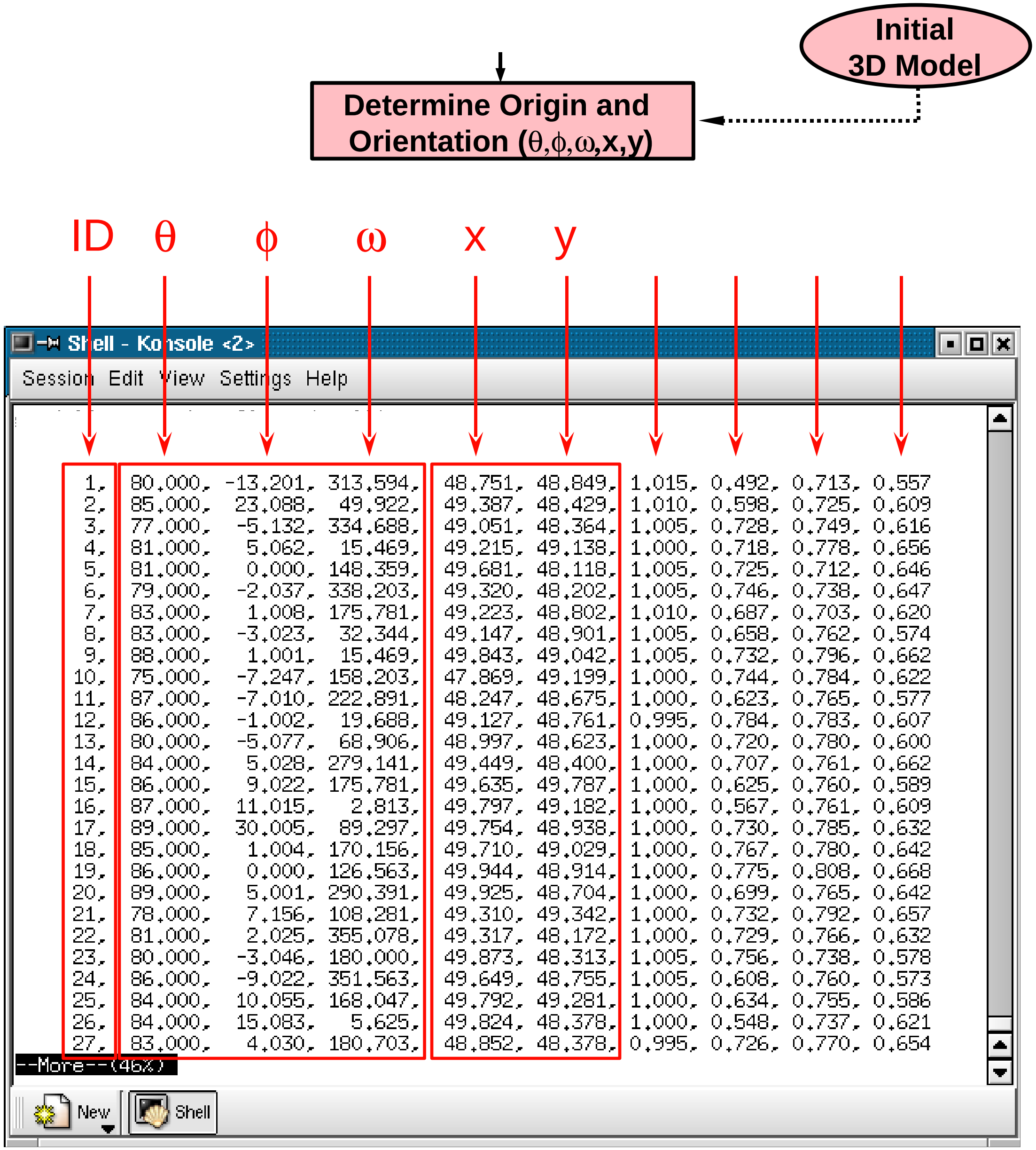
   Bulk of structures now solved this way

   Details discussed in practical session

PFTSEARCH Program Flowchart

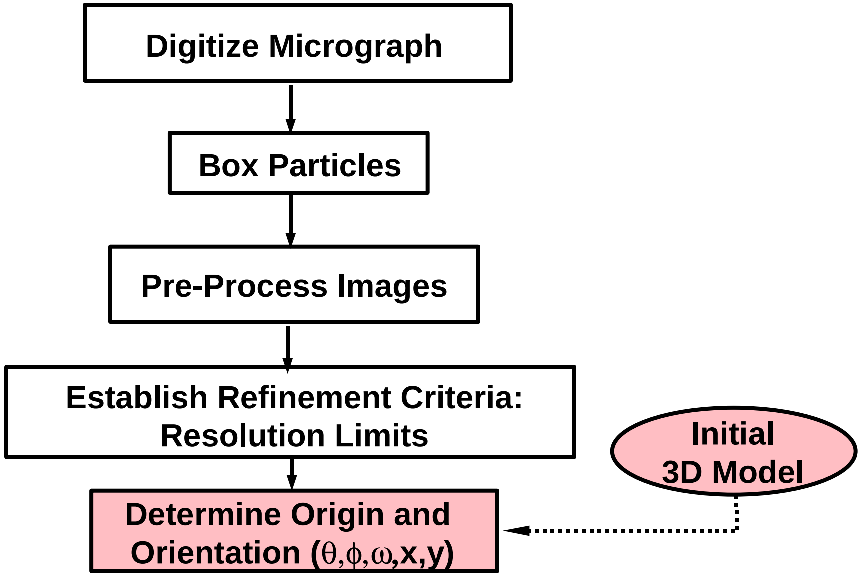


Icosahedral Virus 3D Reconstruction Scheme

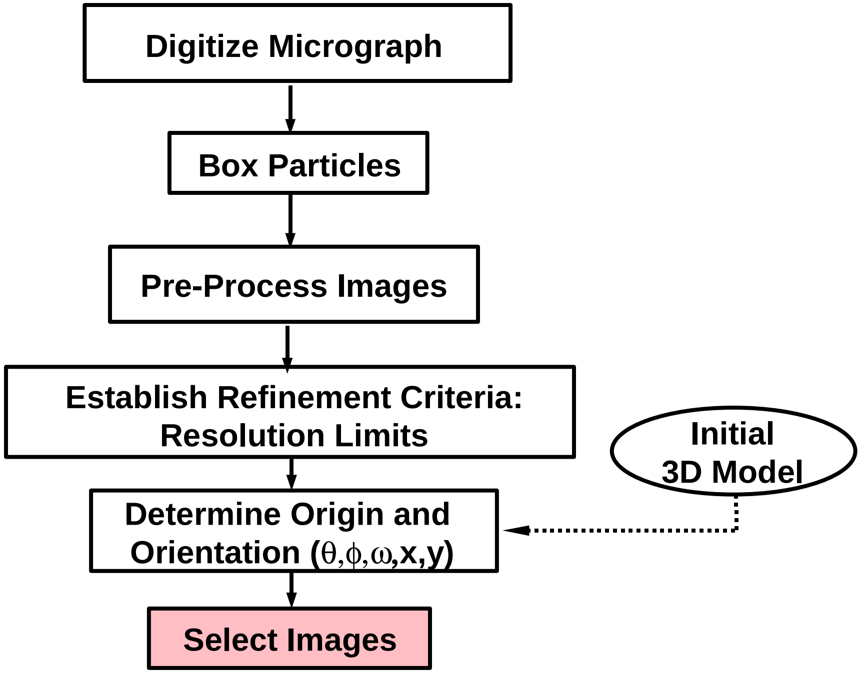


ID	θ	ϕ	ω	x	y				
1,	80.000,	-13.201,	313.594,	48.751,	48.849,	1.015,	0.492,	0.713,	0.557
2,	85.000,	23.088,	49.922,	49.387,	48.429,	1.010,	0.598,	0.725,	0.609
3,	77.000,	-5.132,	334.688,	49.051,	48.364,	1.005,	0.728,	0.749,	0.616
4,	81.000,	5.062,	15.469,	49.215,	49.138,	1.000,	0.718,	0.778,	0.656
5,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

Select Images

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

Icosahedral Virus 3D Reconstruction Scheme

Select Images

Shell - Konsole <2>

Session Edit View Settings Help

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
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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
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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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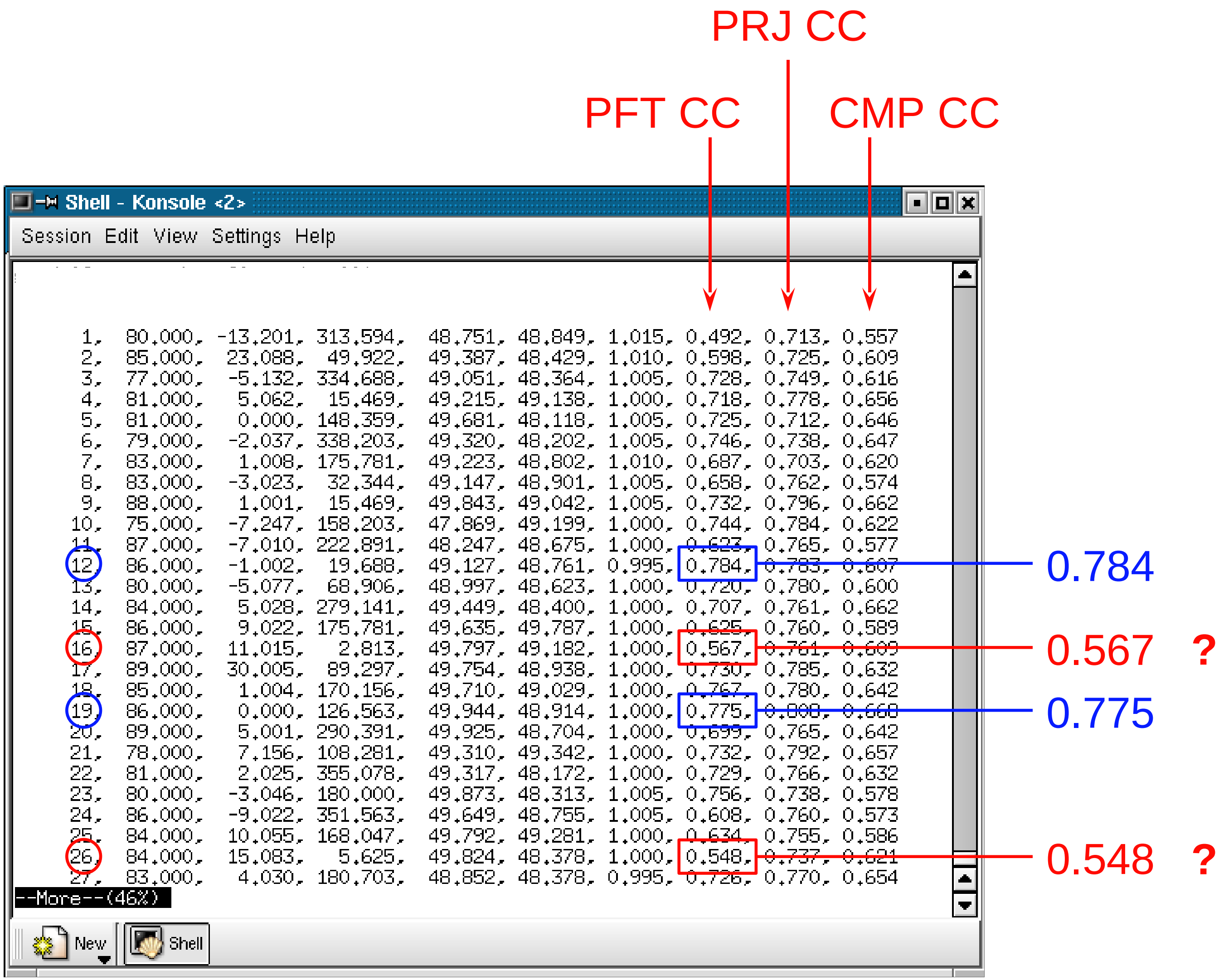
27,	83.000,	4.030,	180.703,	48.852,	48.378,	0.995,	0.726,	0.770,	0.654

--More--(46%)

New Shell

Icosahedral Virus 3D Reconstruction Scheme

Select Images



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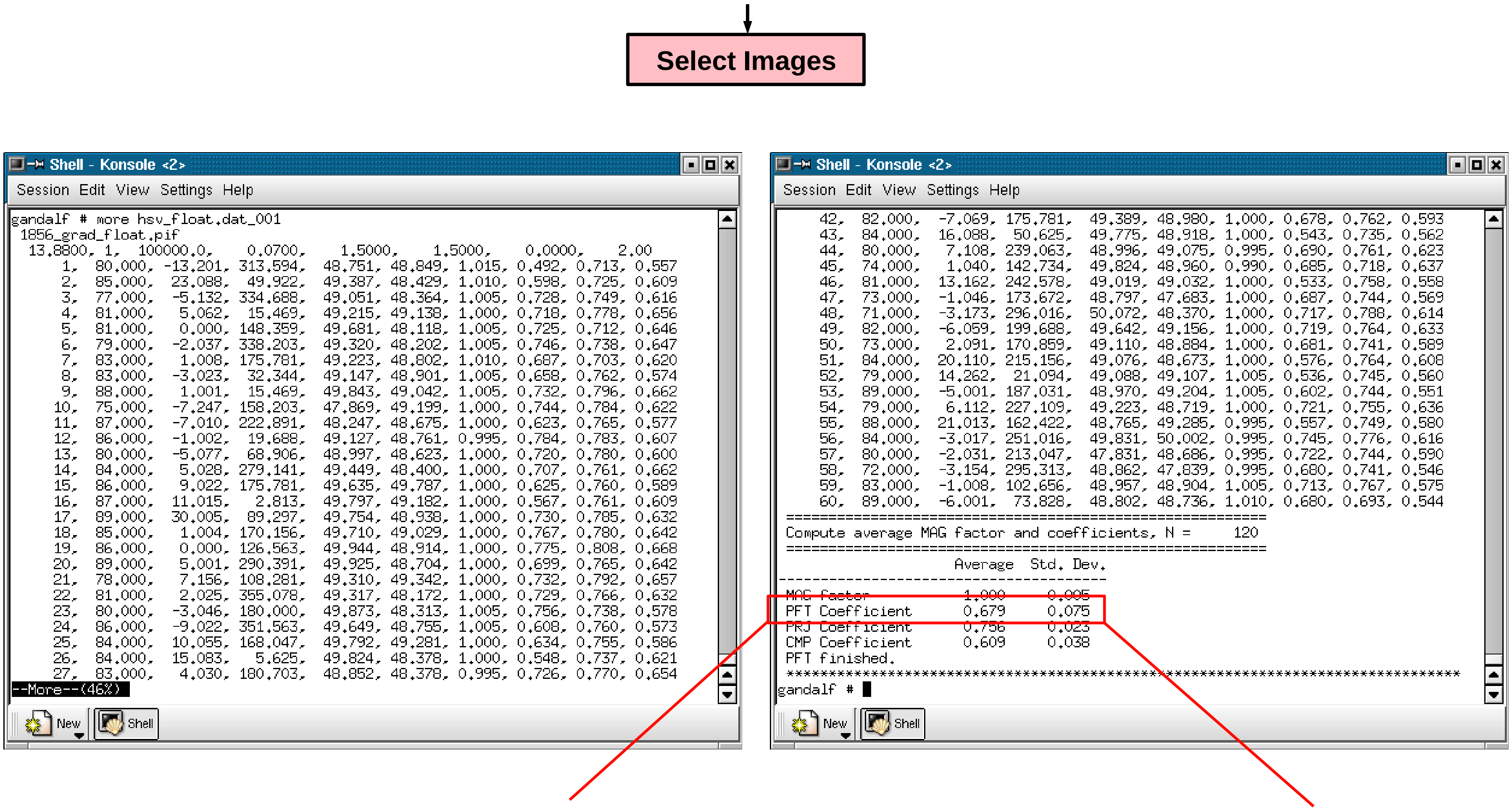
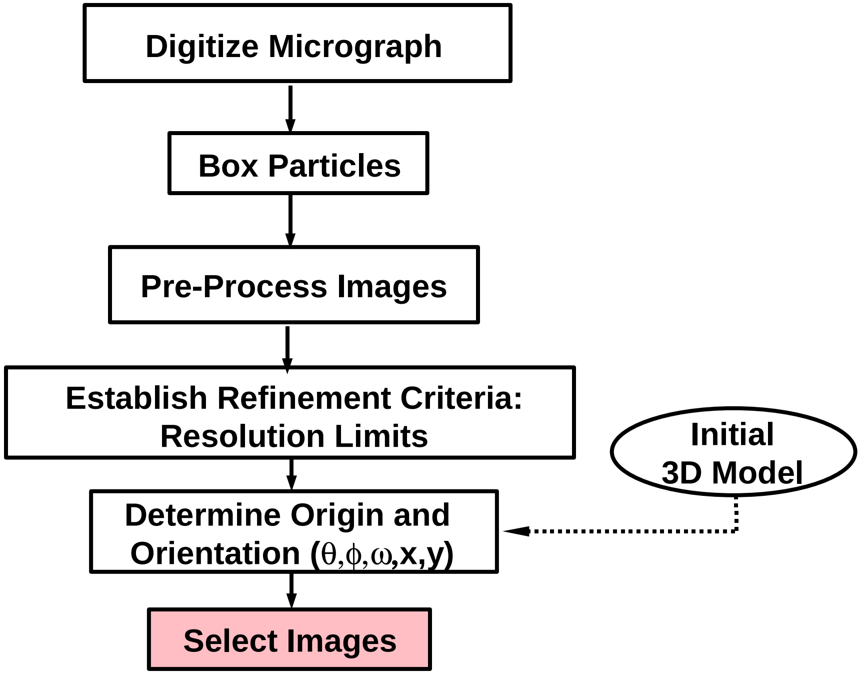
27,	83.000,	4.030,	180.703,	48.852,	48.378,	0.995,	0.726,	0.770,	0.654

Icosahedral Virus 3D Reconstruction Scheme

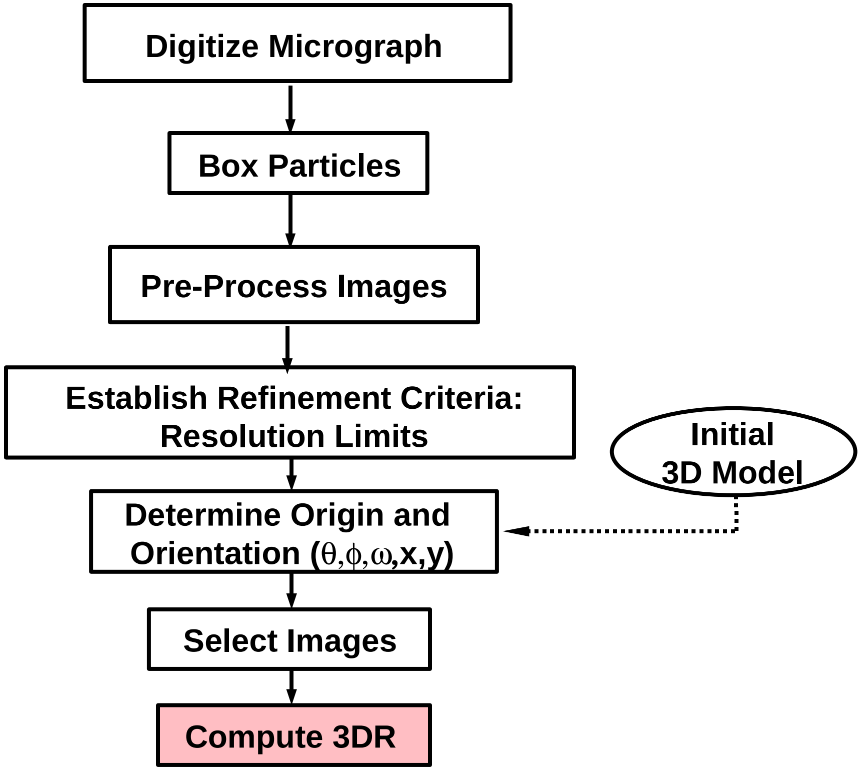


PFT Coefficient 0.679 ± 0.075

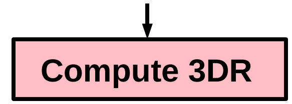
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



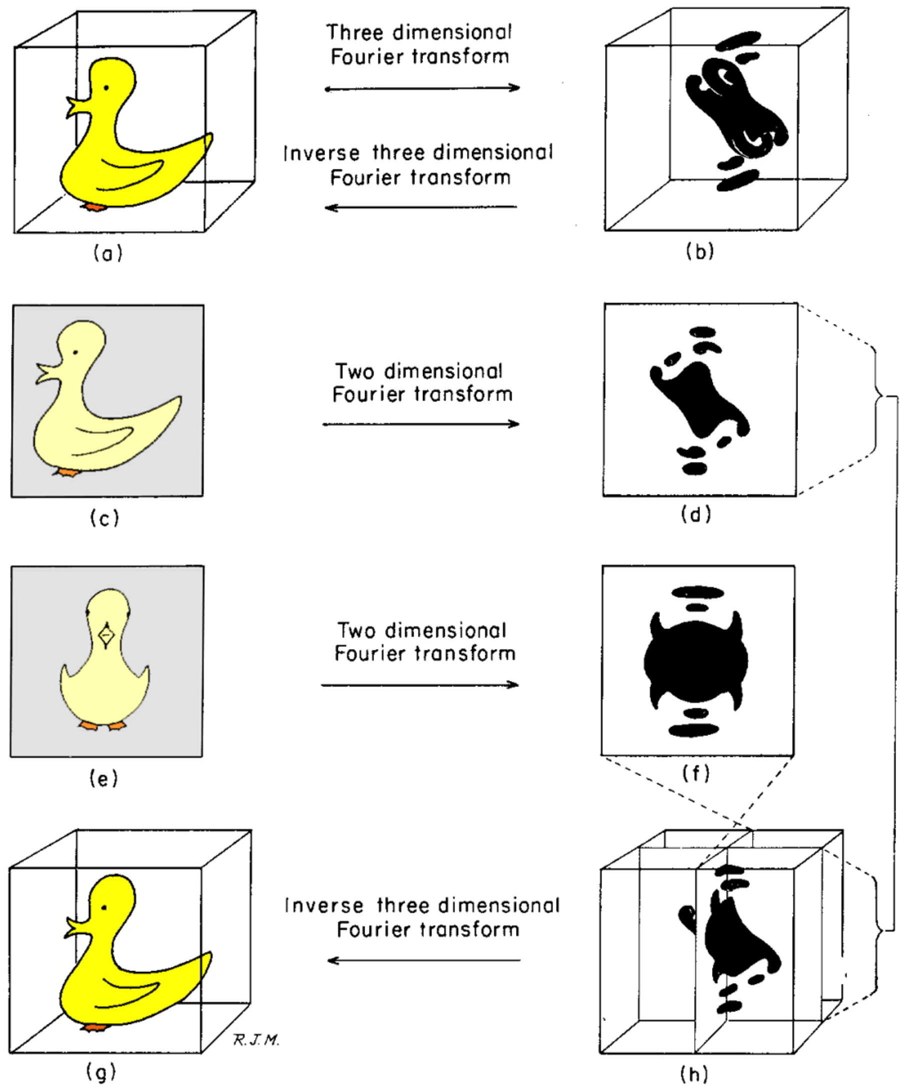
Goal: combine only “good” particle images
to compute a 3D density map

Icosahedral Virus 3D Reconstruction Scheme

Compute 3DR

In theory

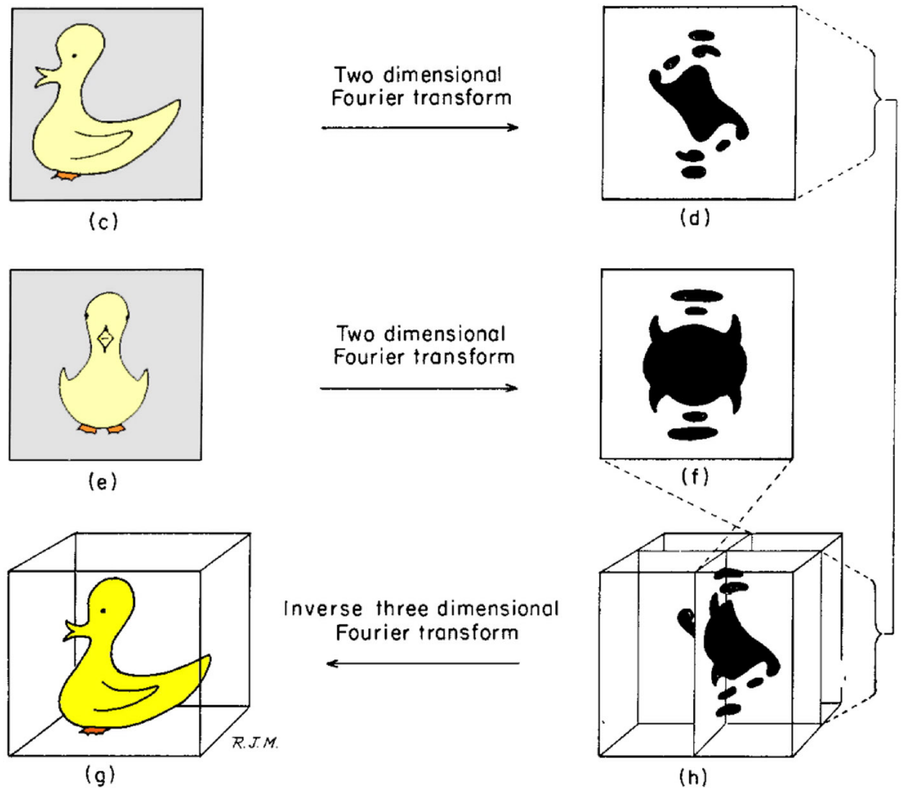
In practice



From Lake (1972), p.174

Icosahedral Virus 3D Reconstruction Scheme

Compute 3DR



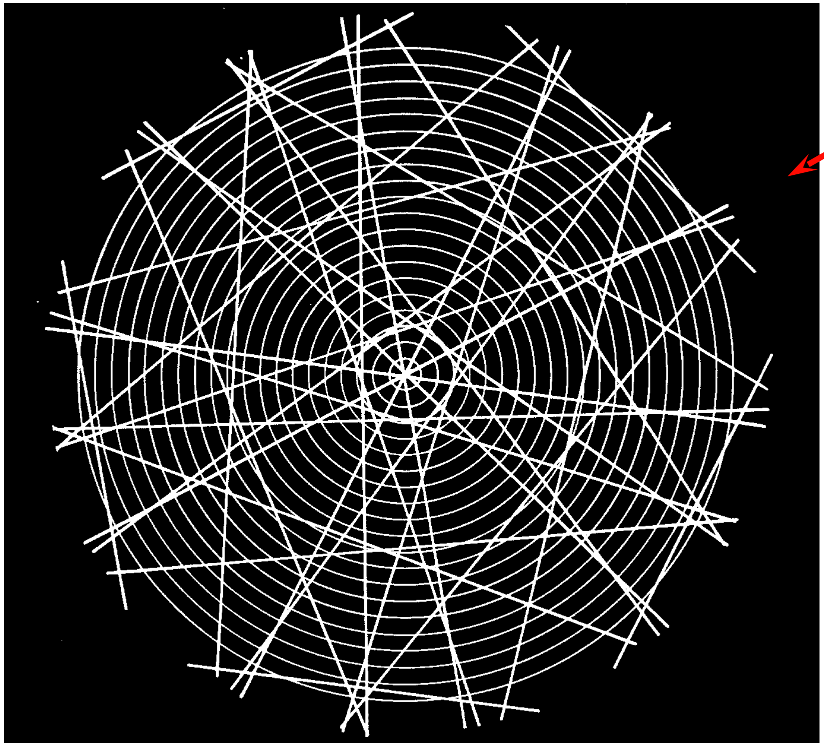
Overall scheme: $\rho \leftarrow g \leftarrow G \leftarrow F$

Icosahedral Virus 3D Reconstruction Scheme

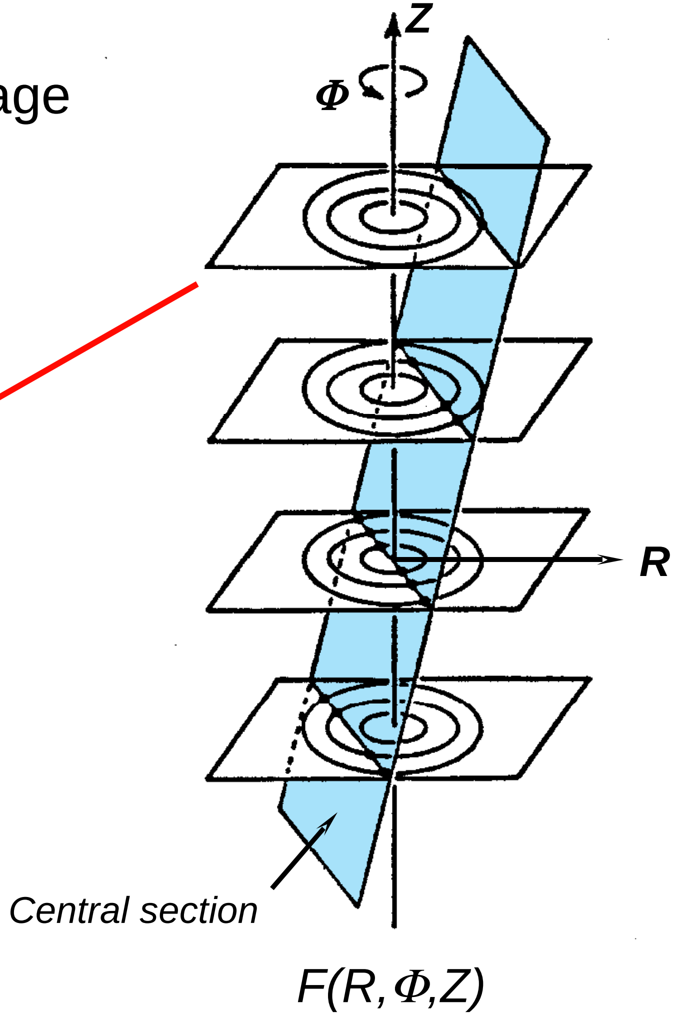
Compute 3DR

Steps:

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



Crowther, DeRosier and Klug, 1970, p.329



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

Icosahedral Virus 3D Reconstruction Scheme

Compute 3DR

ρ ← g ← G ← F

Steps:

1. Compute 2D FFT of each particle image

2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform

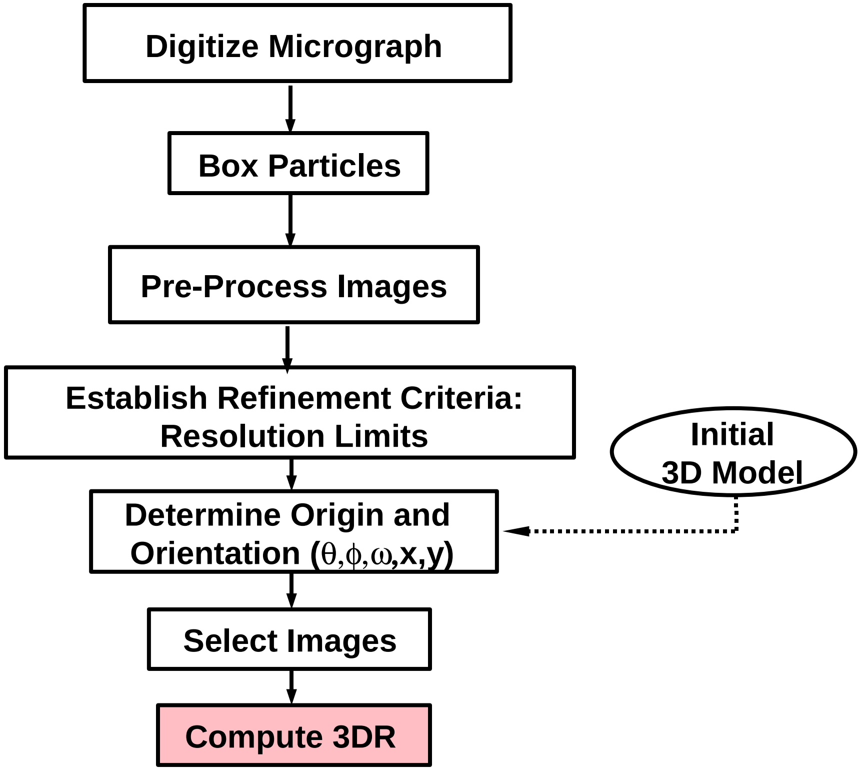
3. Compute G_n's on each annulus $G = (B\,B)^{-1} B\,F$

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

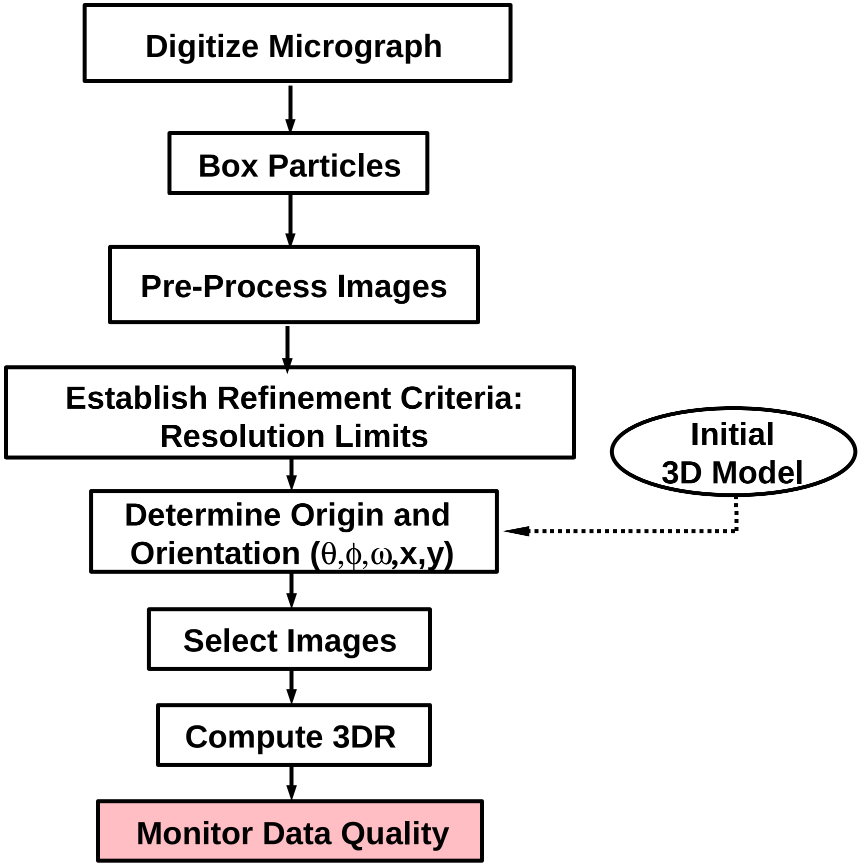
6. Convert from polar to Cartesian map ($\rho(r,\phi,z)$ --> $\rho(x,y,z)$)

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

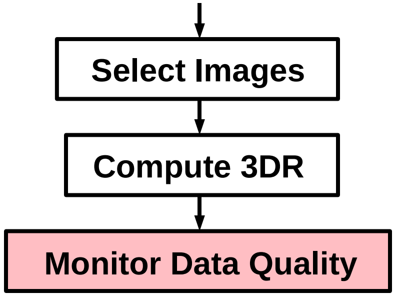


Icosahedral Virus 3D Reconstruction Scheme

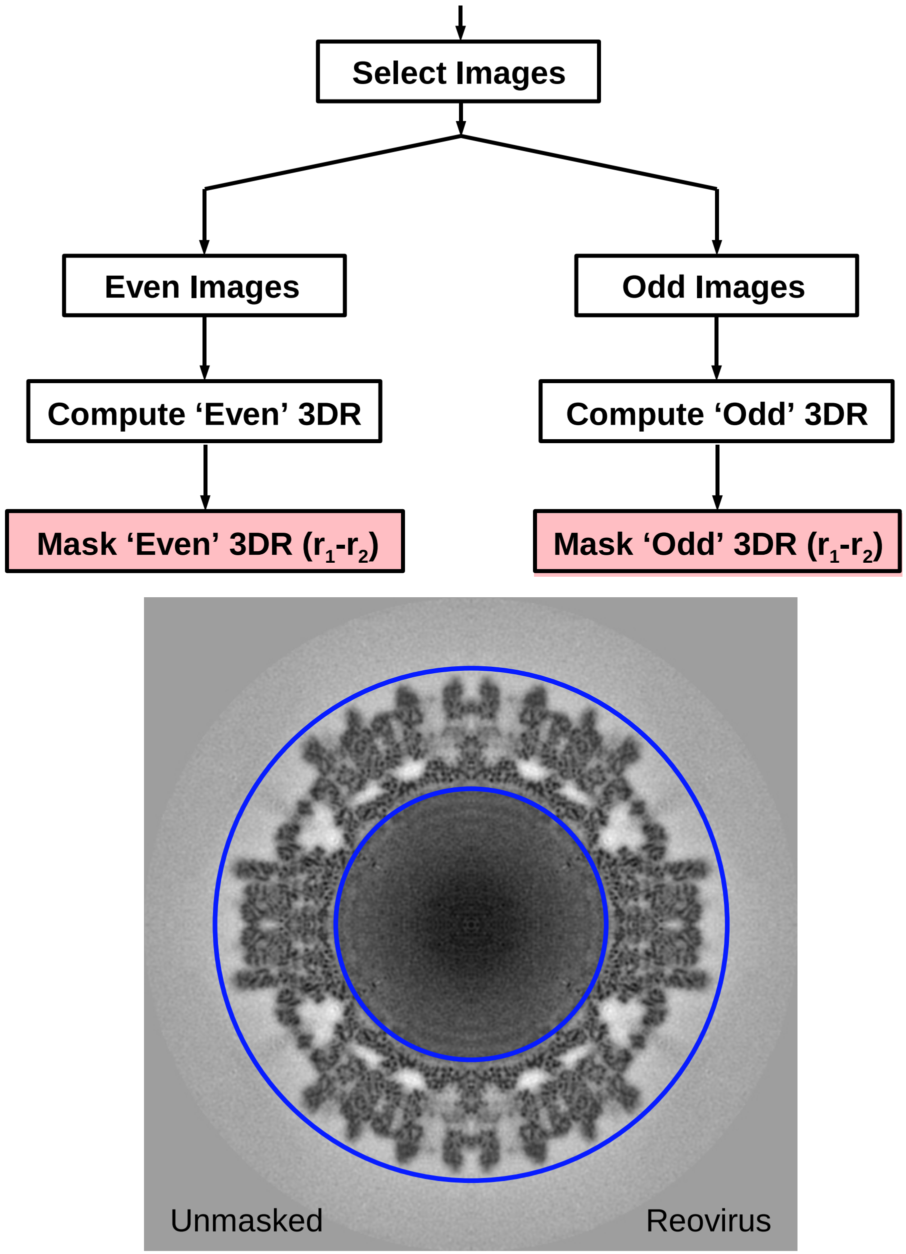
Monitor Data Quality

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

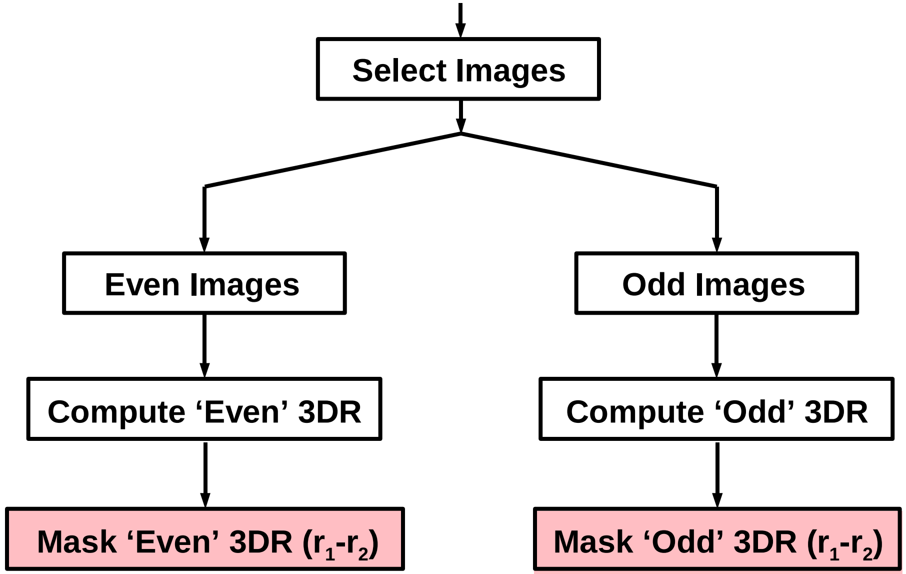
Icosahedral Virus 3D Reconstruction Scheme

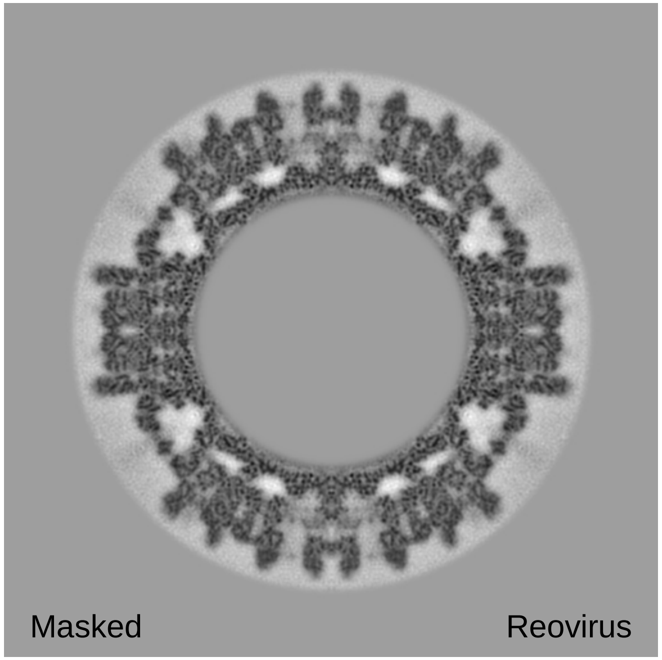


Icosahedral Virus 3D Reconstruction Scheme

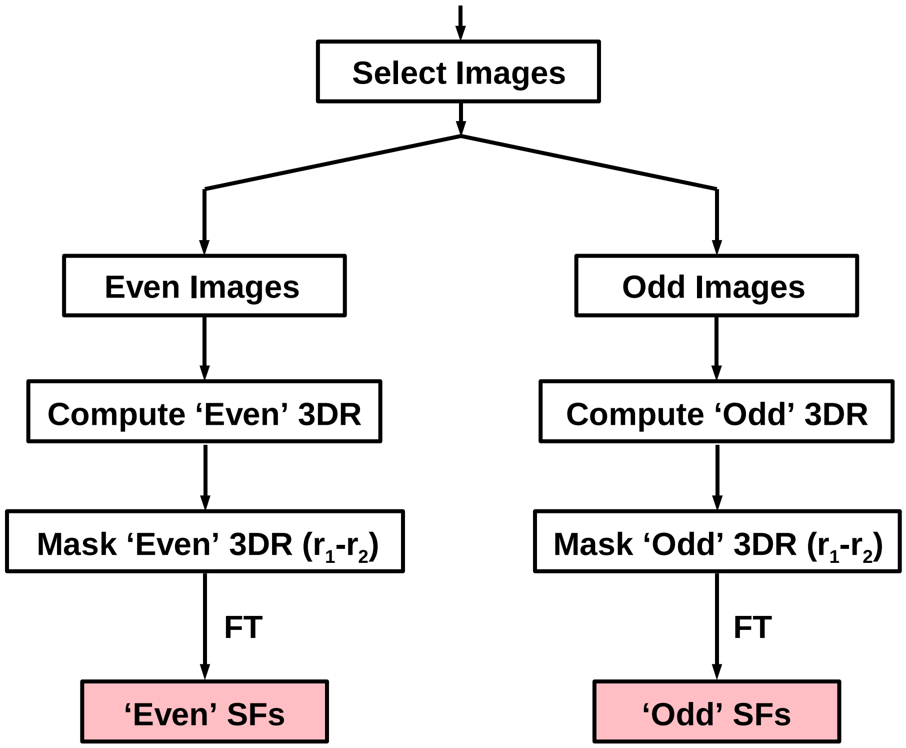


Icosahedral Virus 3D Reconstruction Scheme

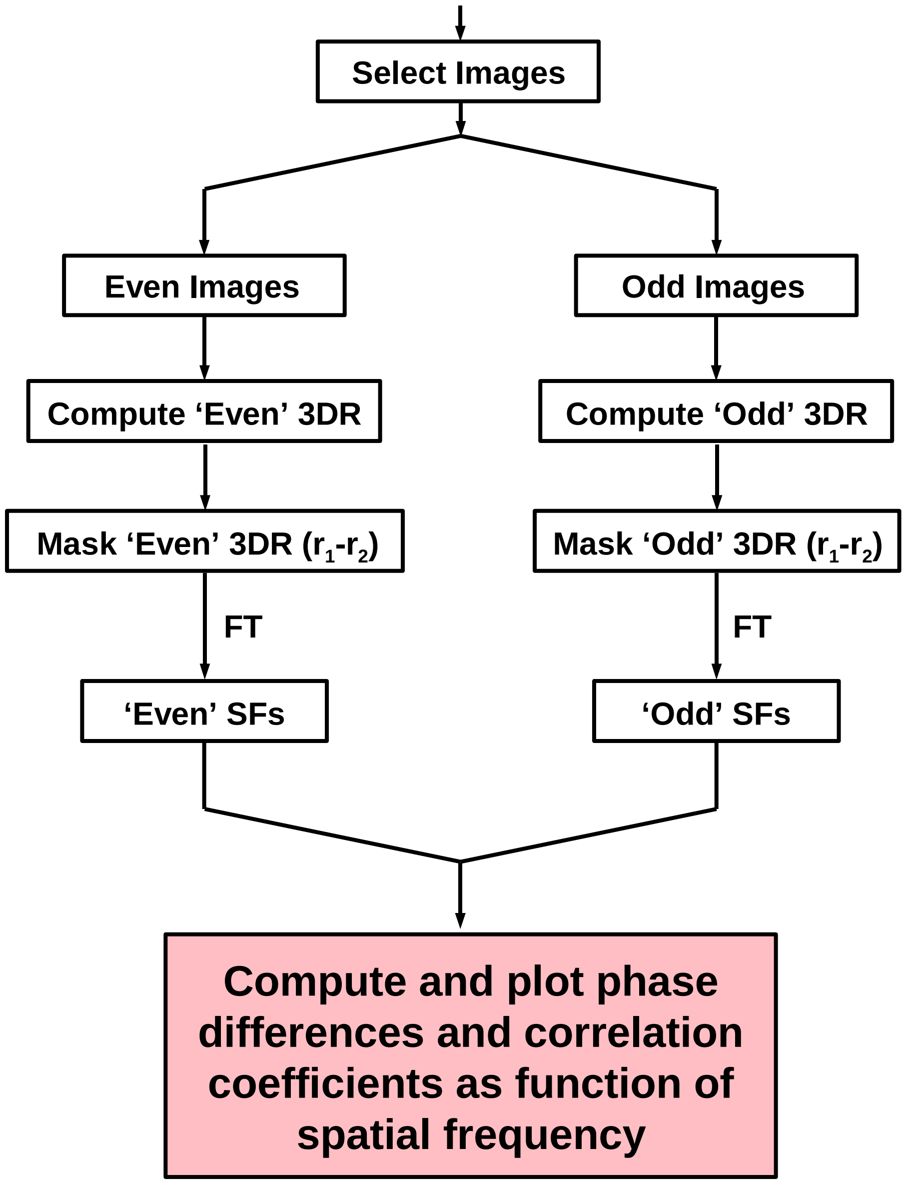




Icosahedral Virus 3D Reconstruction Scheme

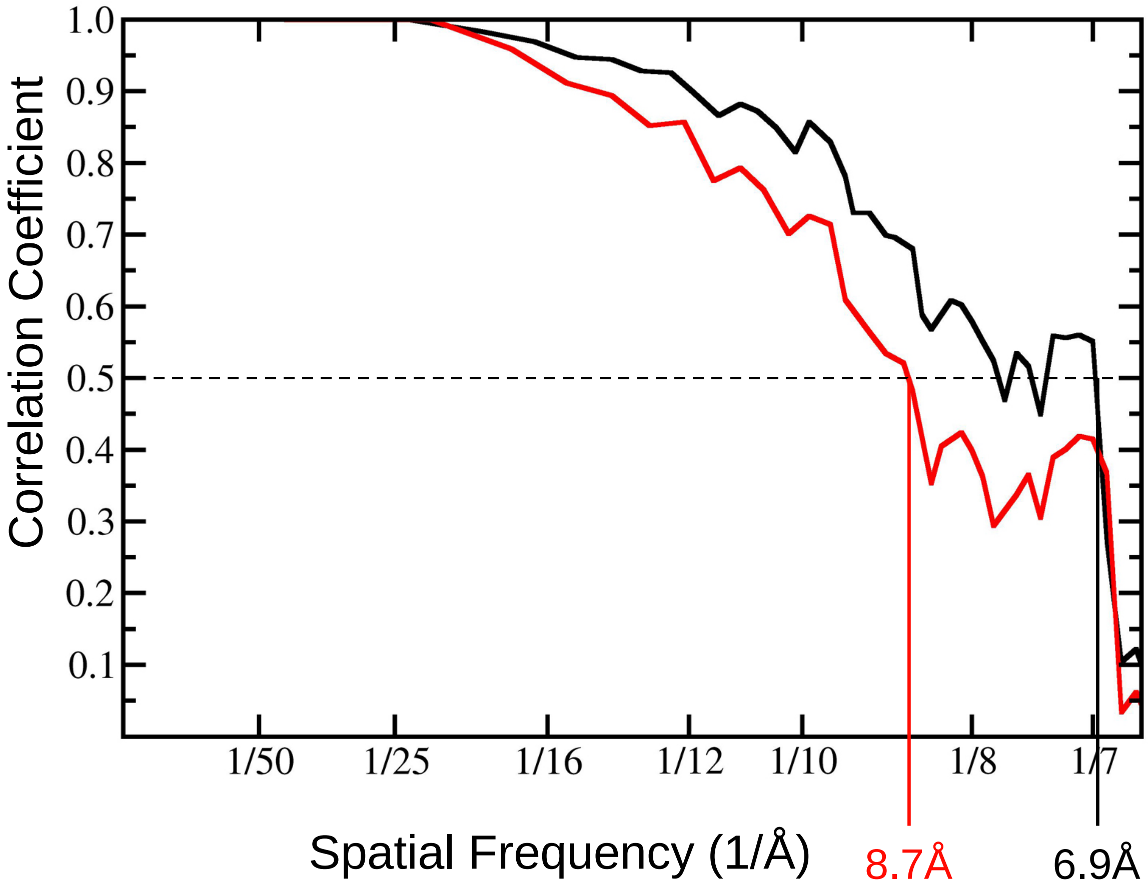


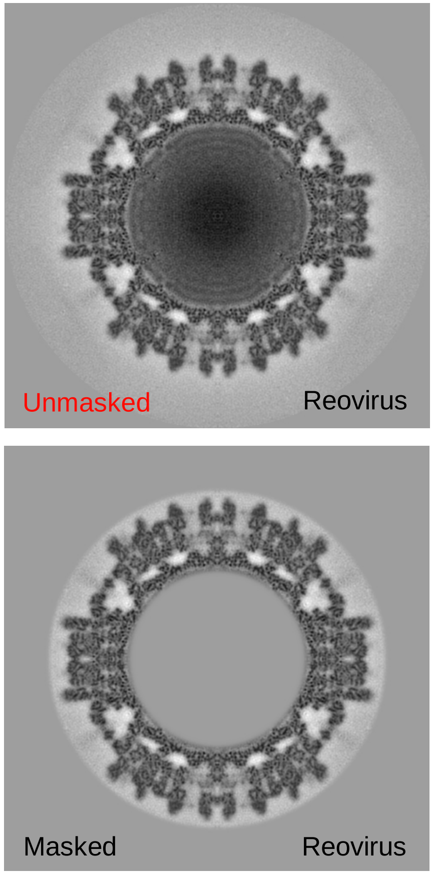
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality





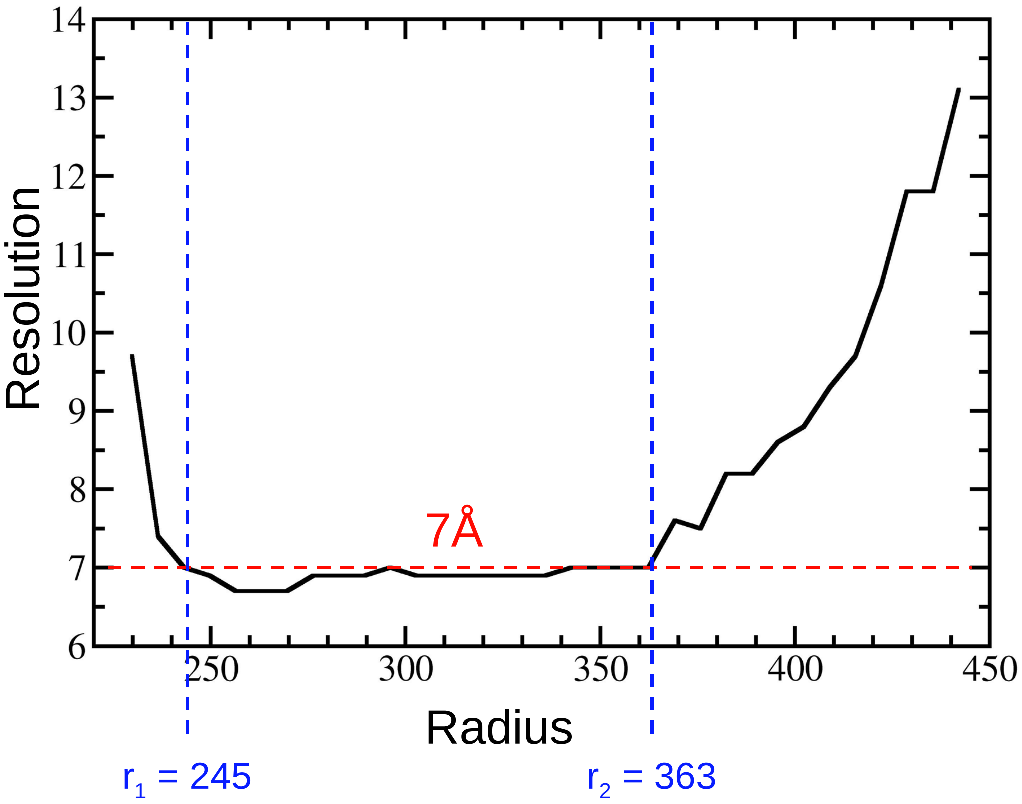
Icosahedral Virus 3D Reconstruction Scheme

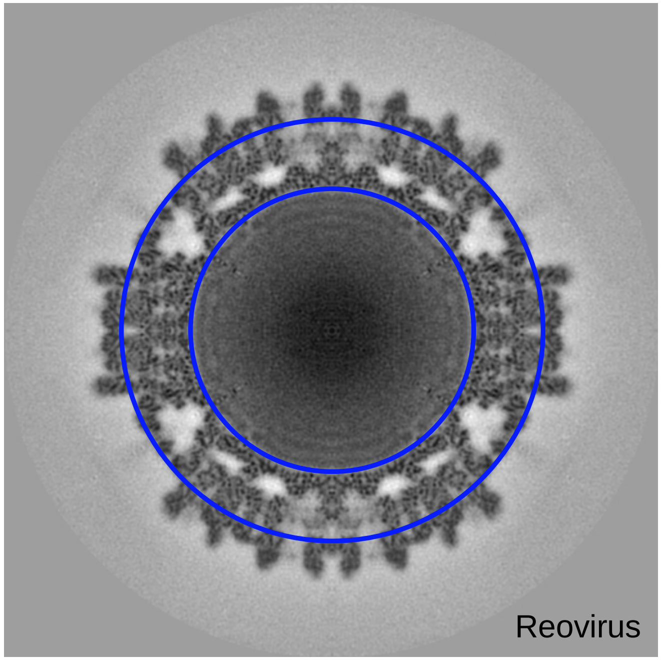
Monitor Data Quality

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

Icosahedral Virus 3D Reconstruction Scheme

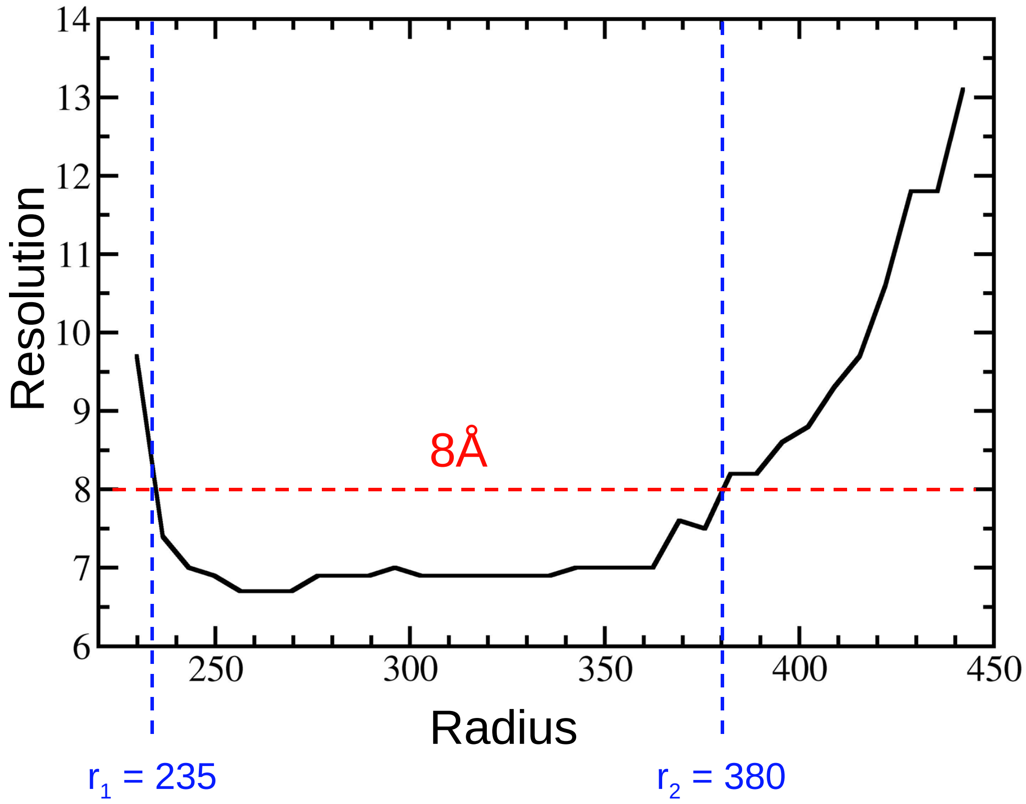
Monitor Data Quality

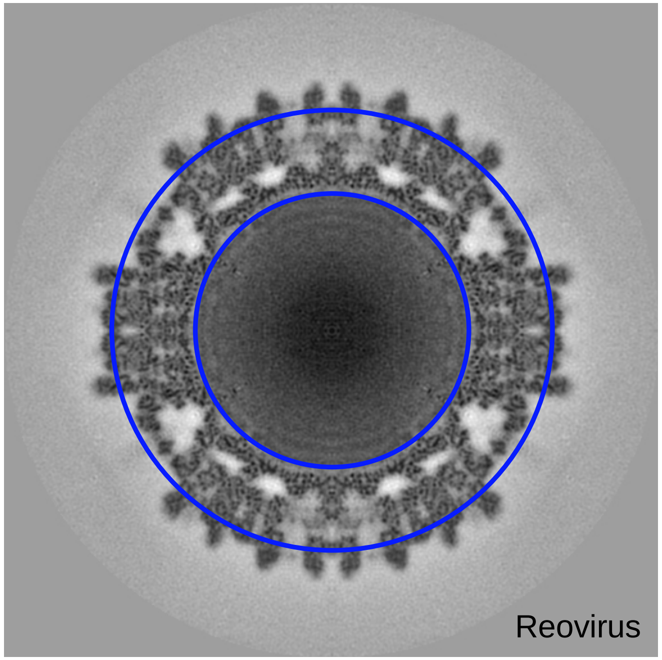




Icosahedral Virus 3D Reconstruction Scheme

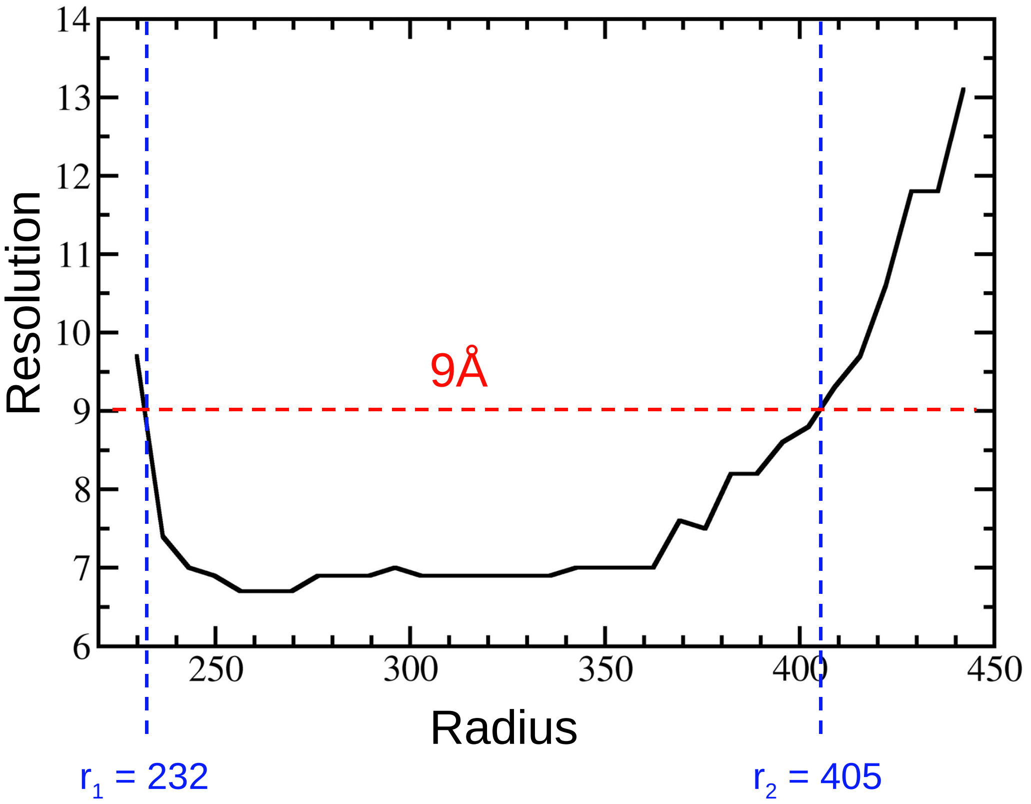
Monitor Data Quality

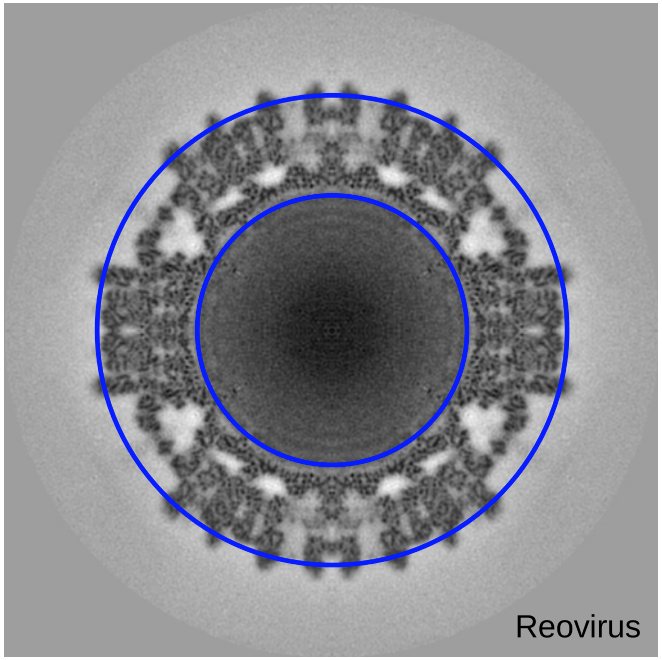




Icosahedral Virus 3D Reconstruction Scheme

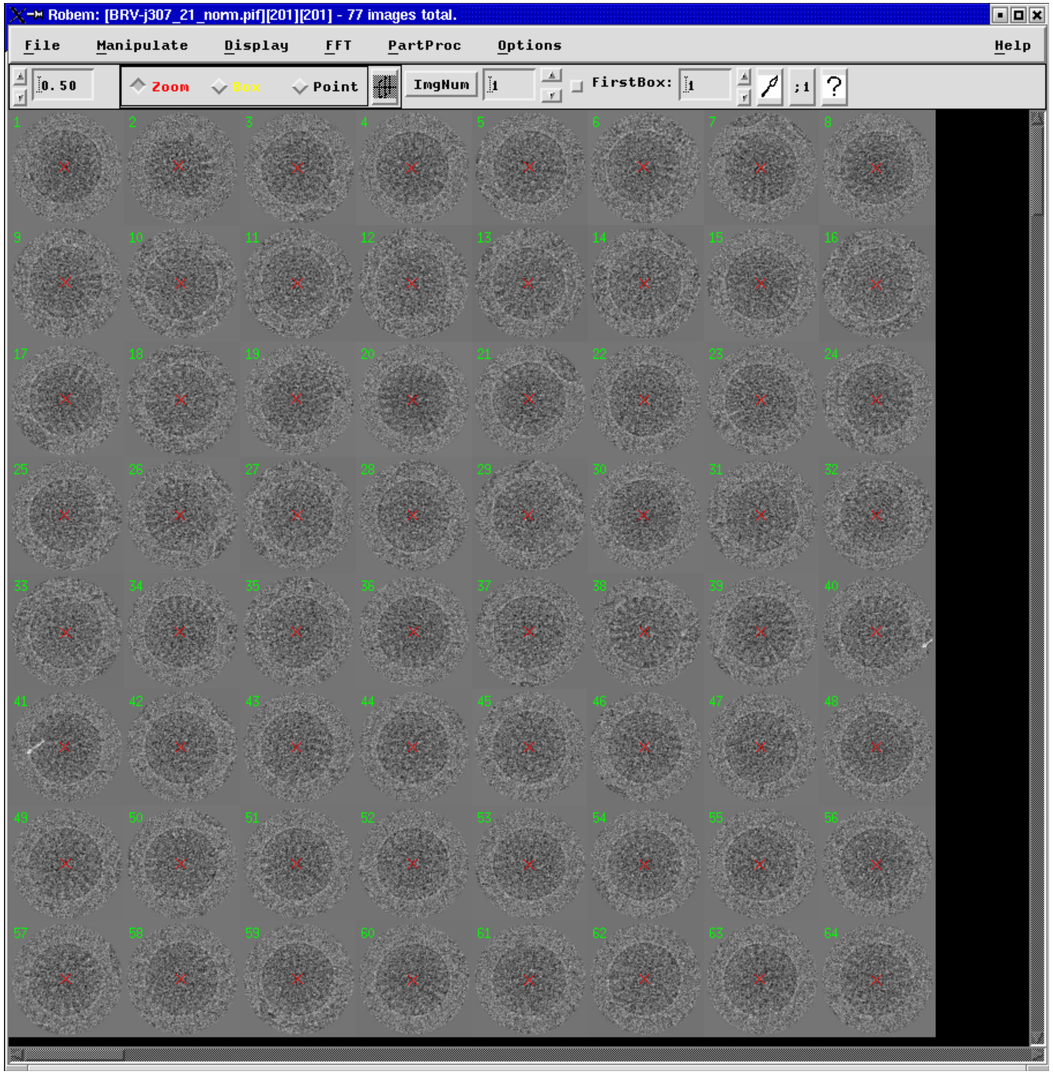
Monitor Data Quality





Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



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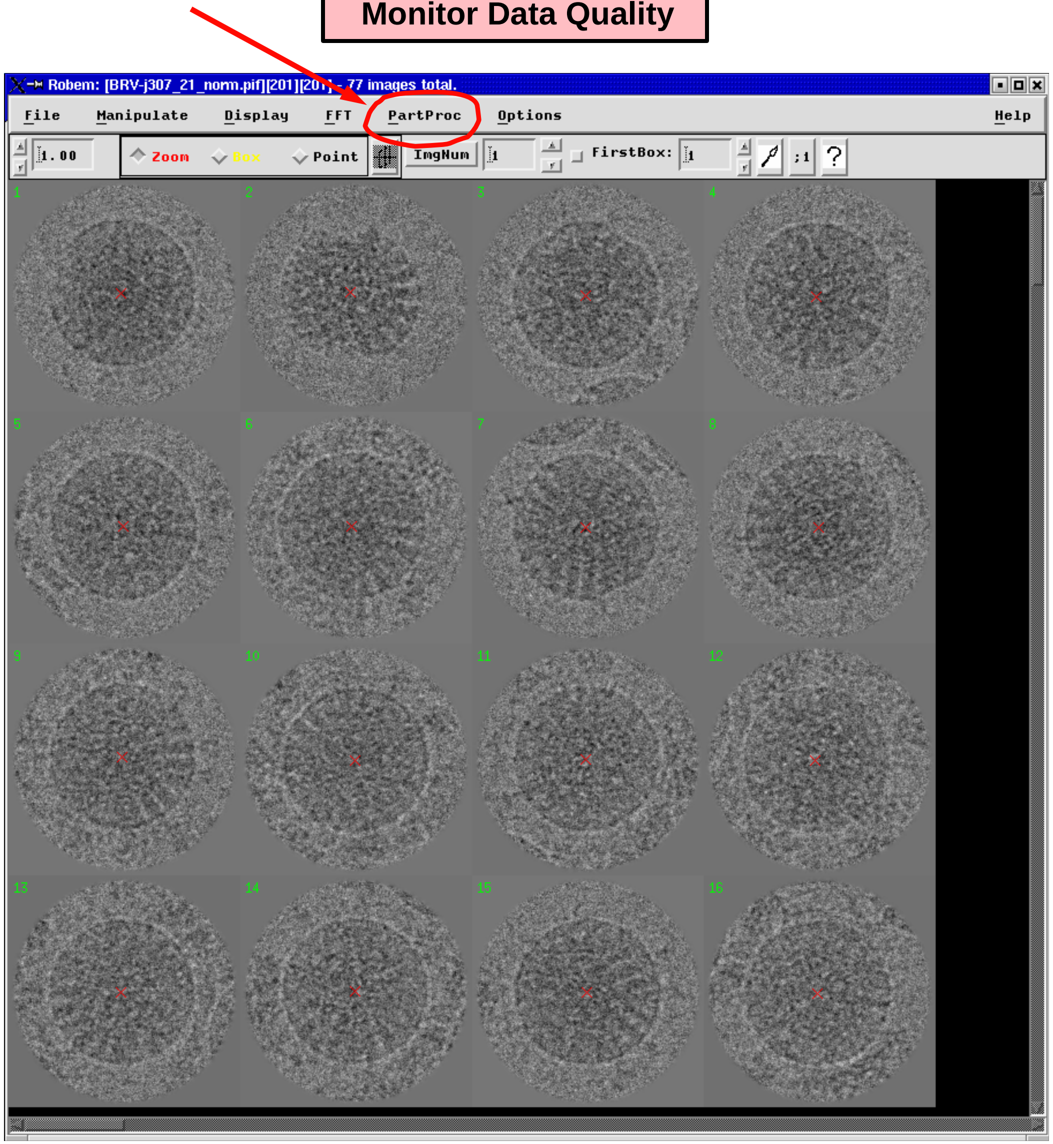

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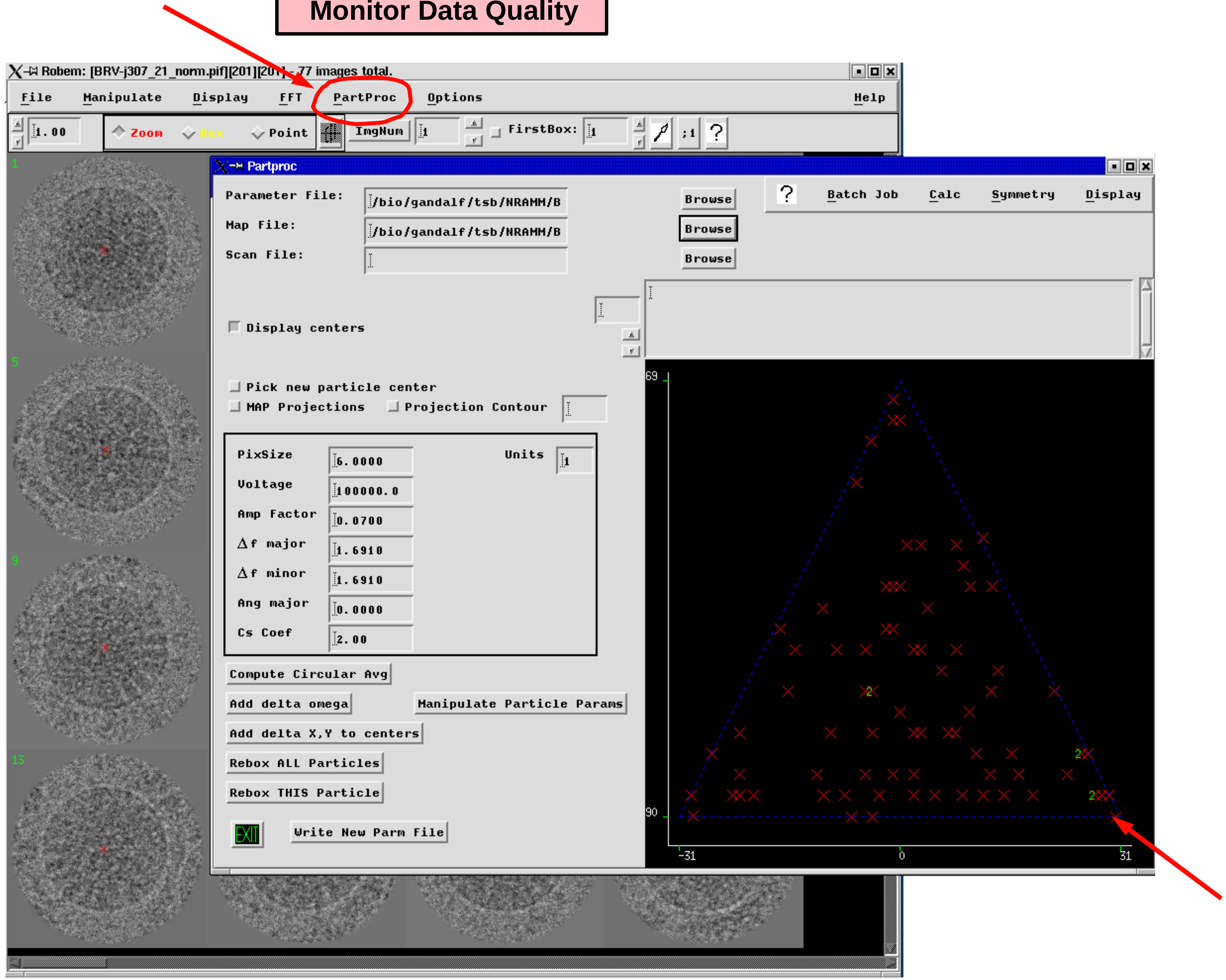

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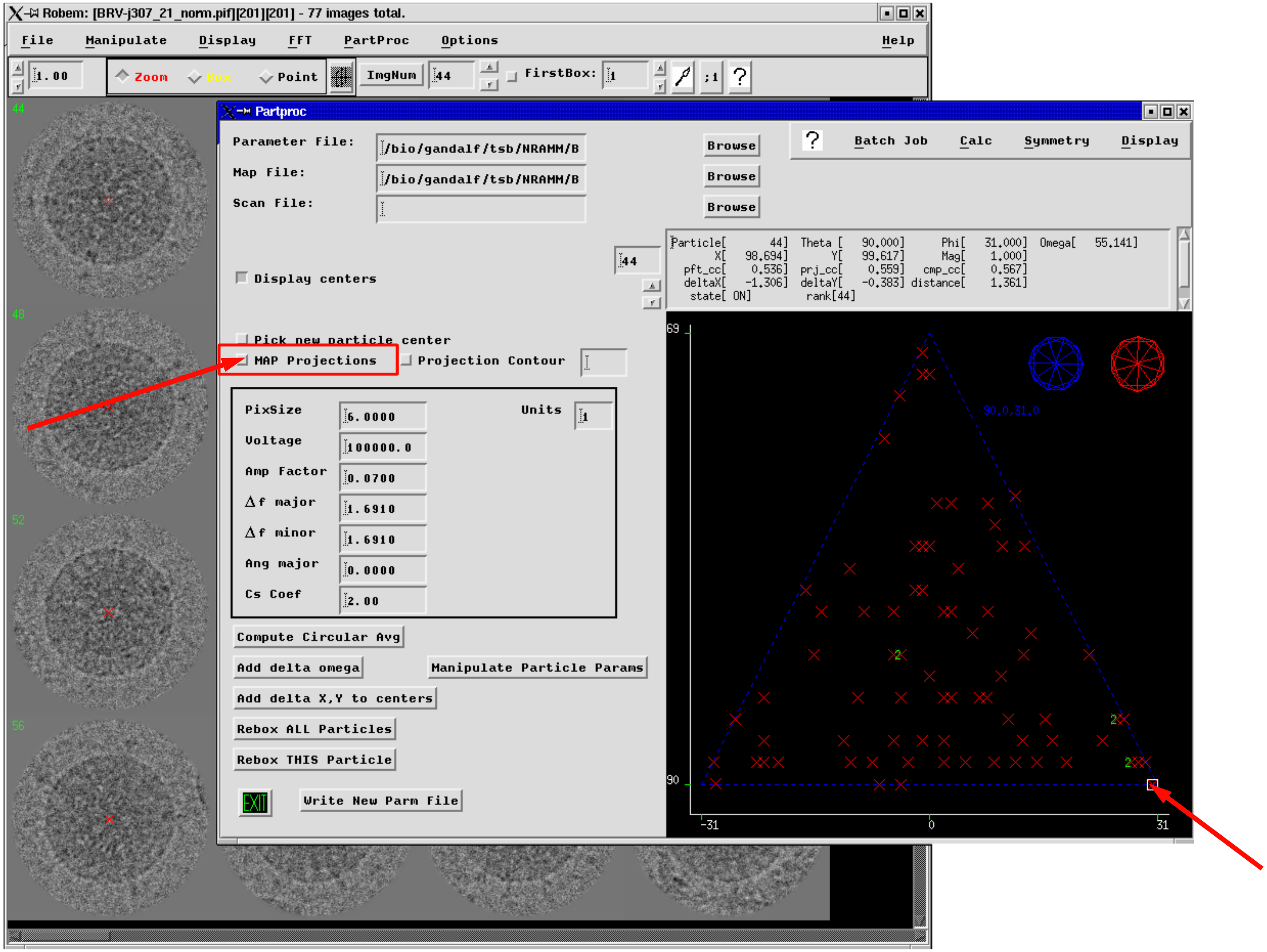

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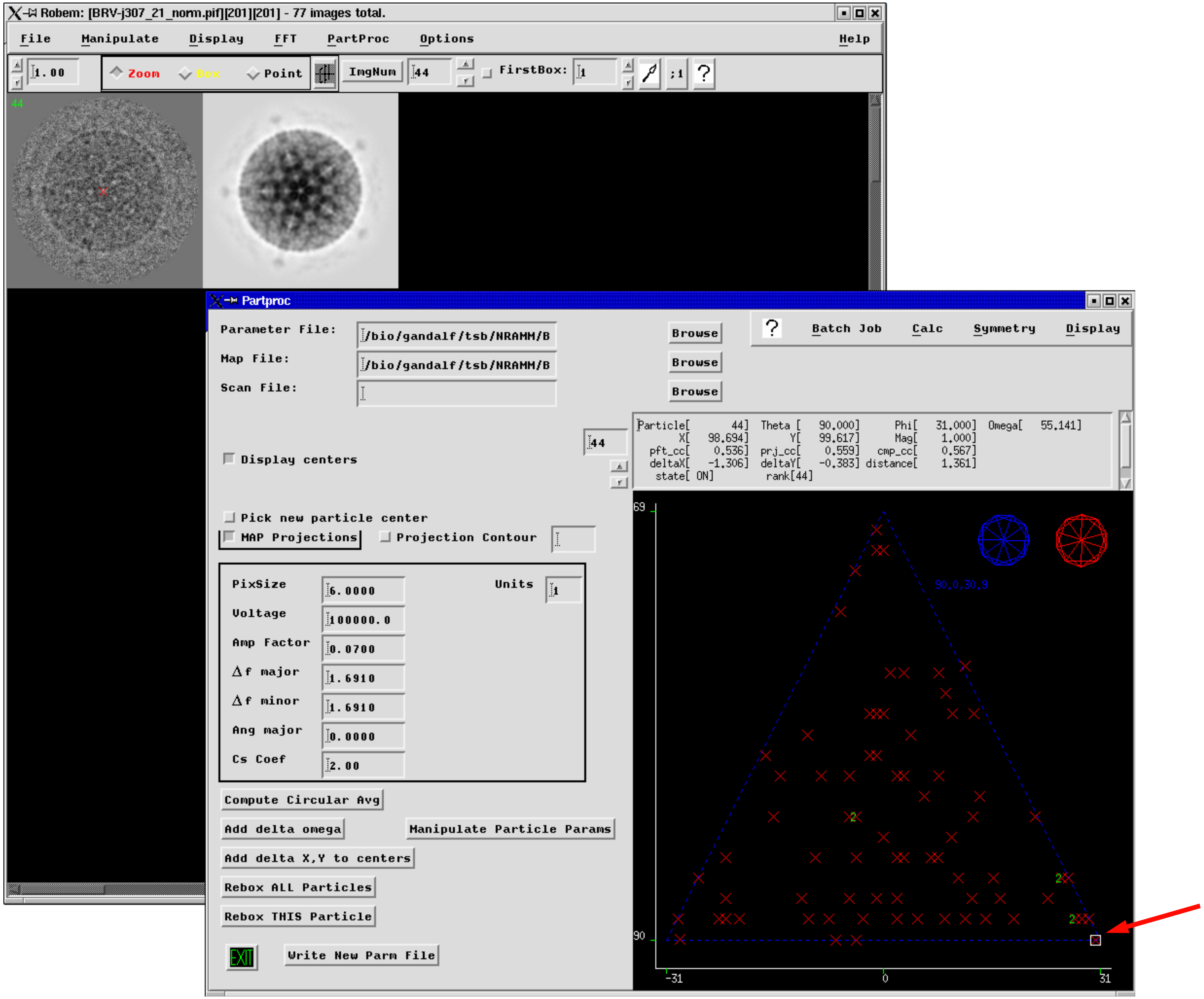

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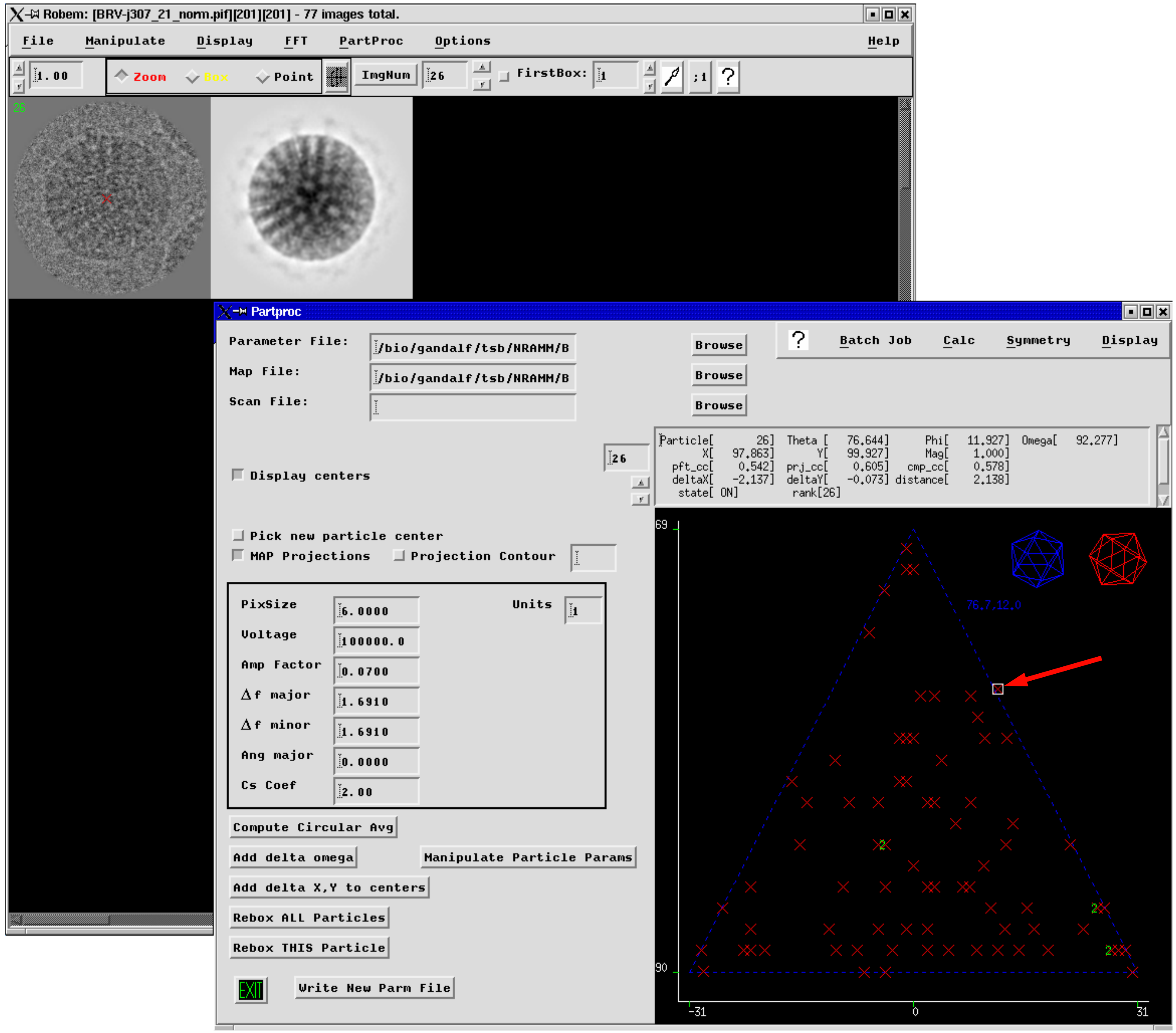

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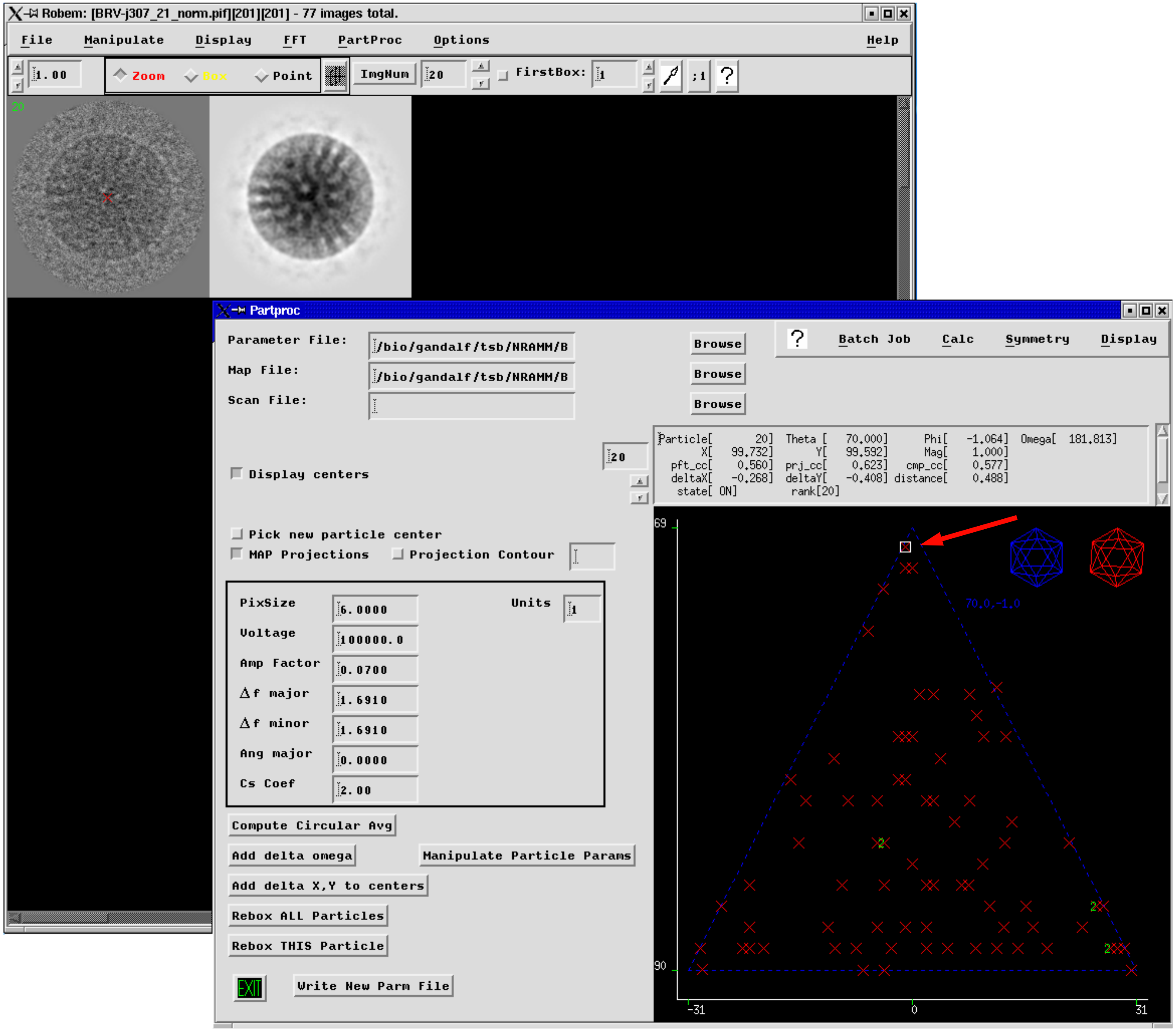

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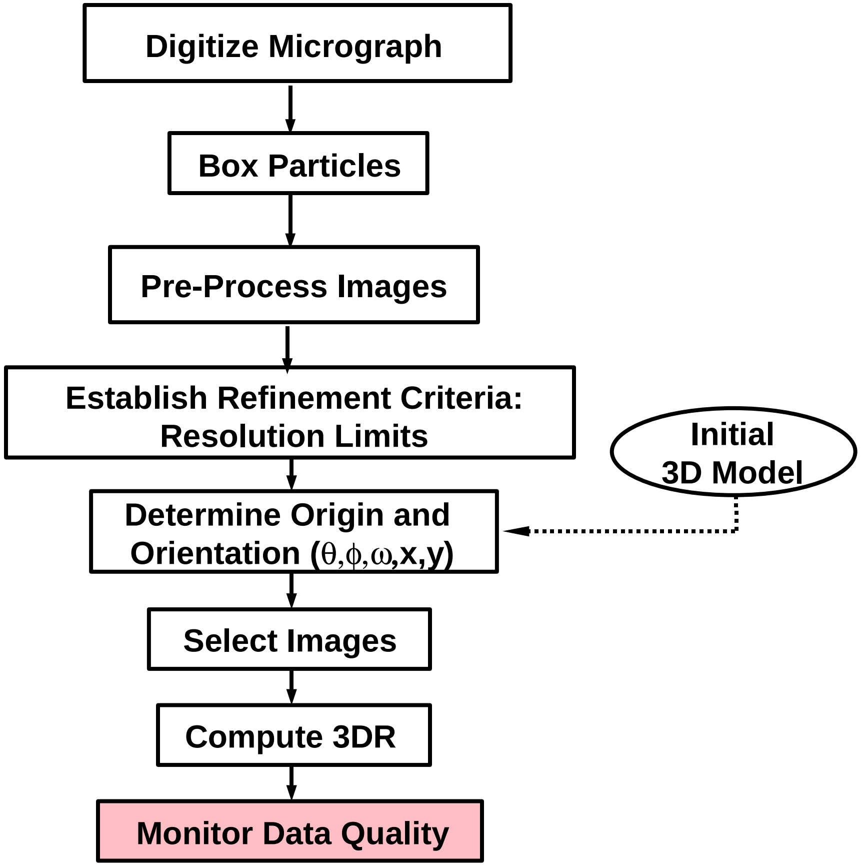

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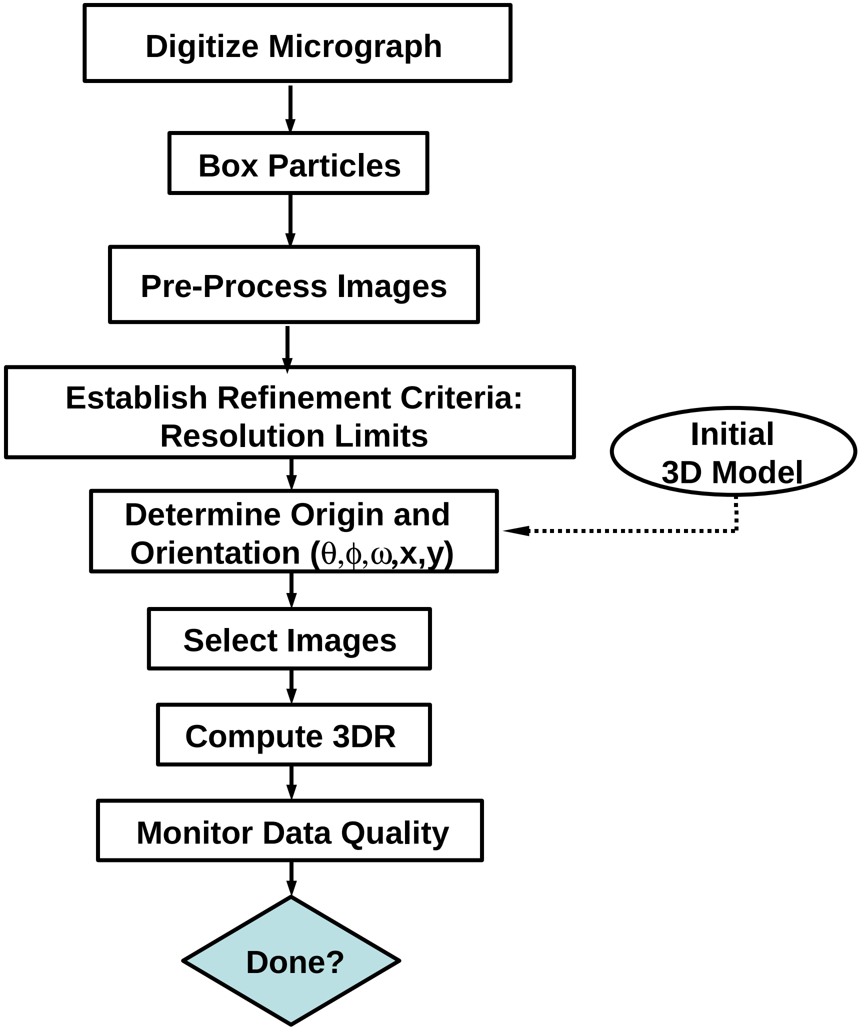

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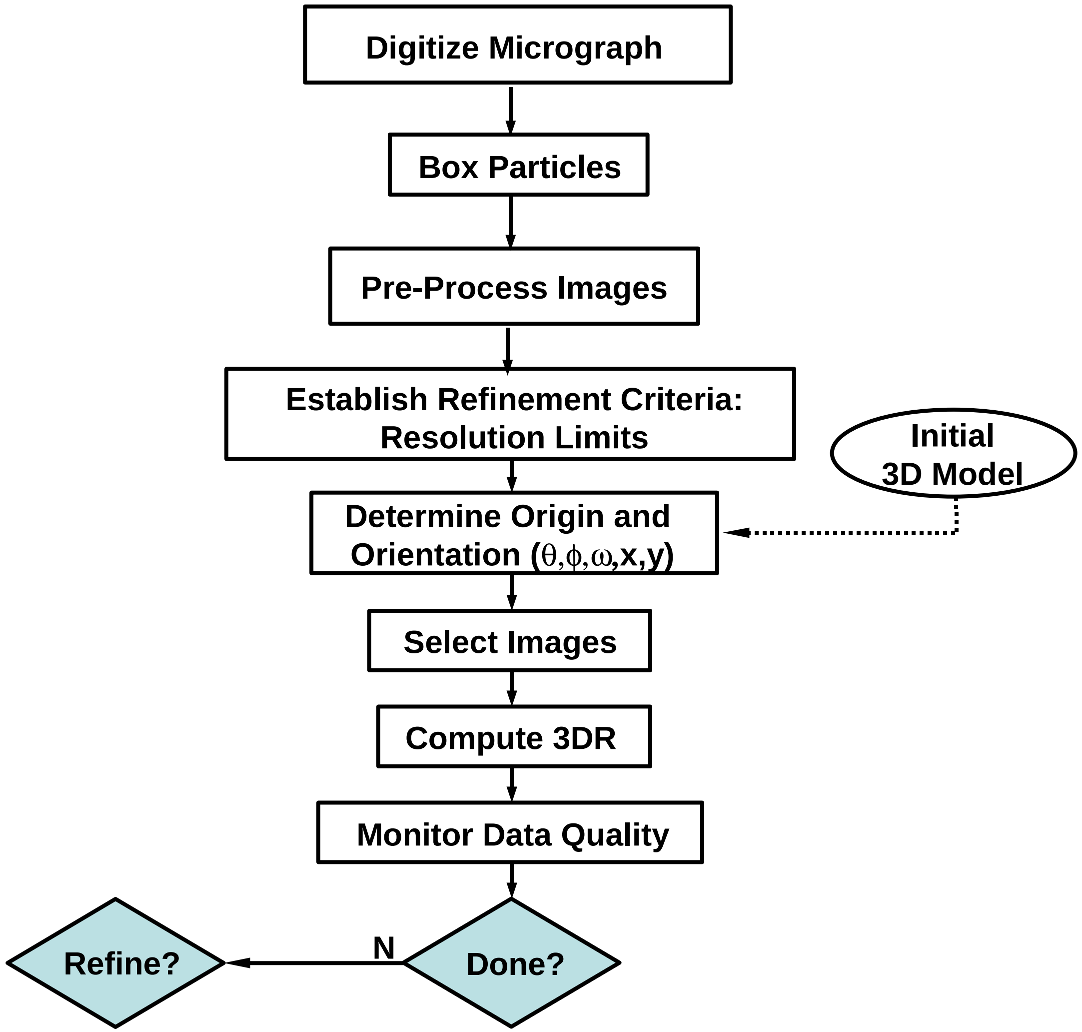

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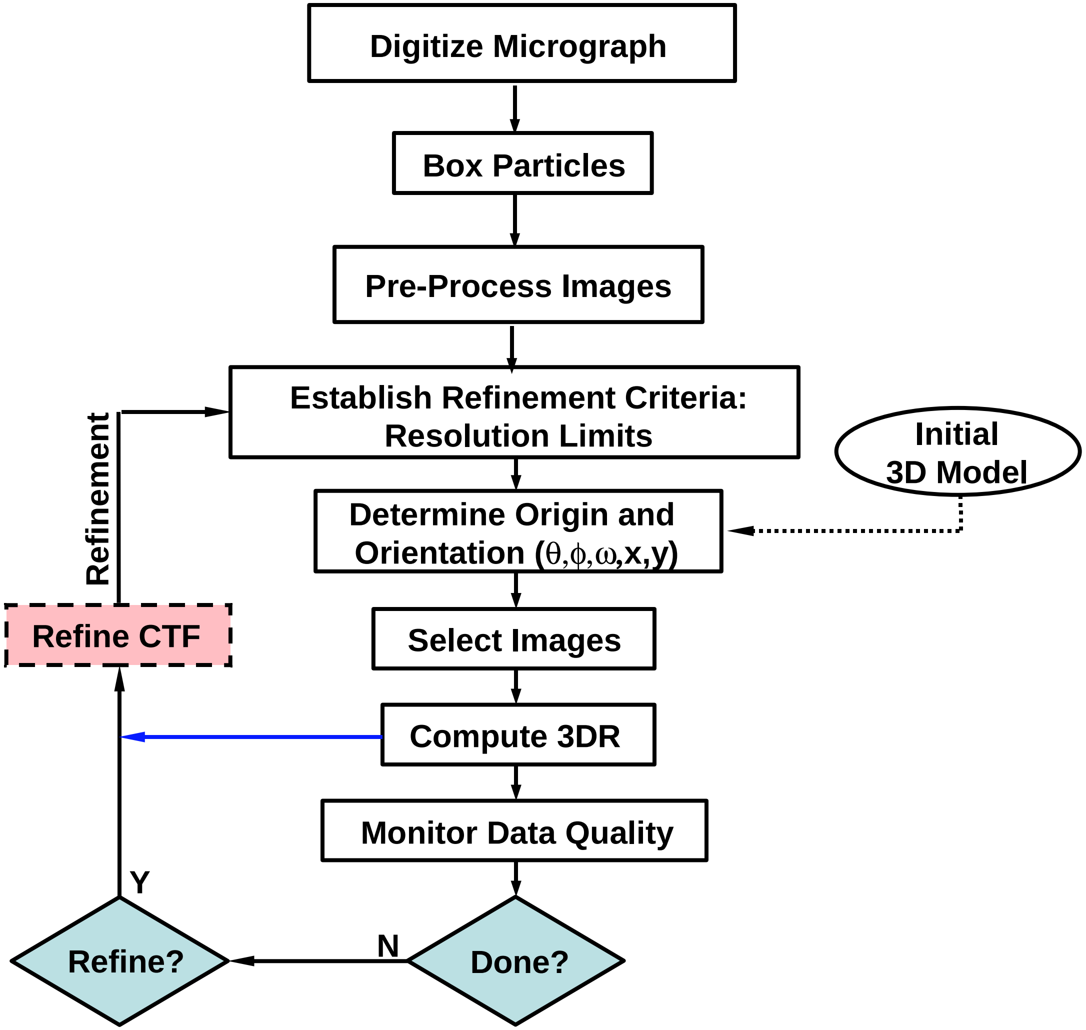

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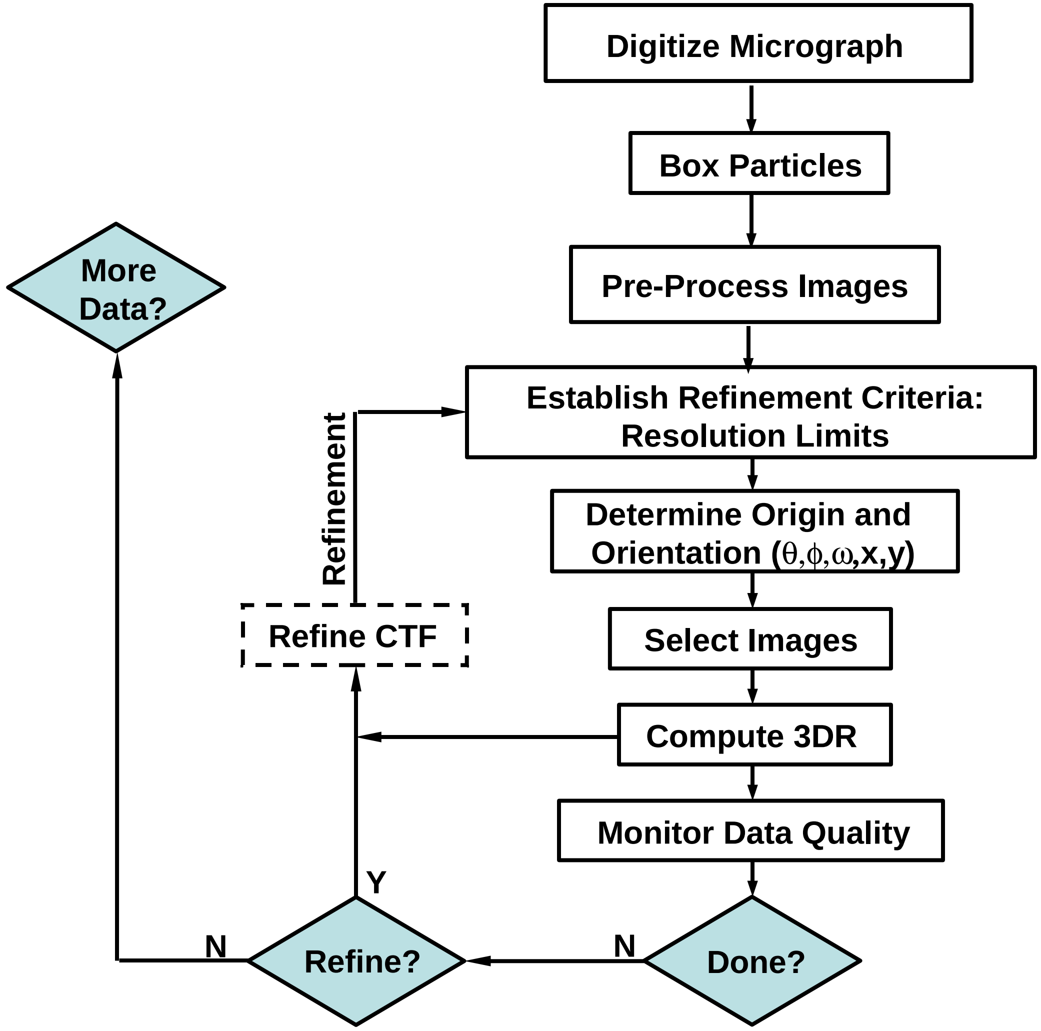

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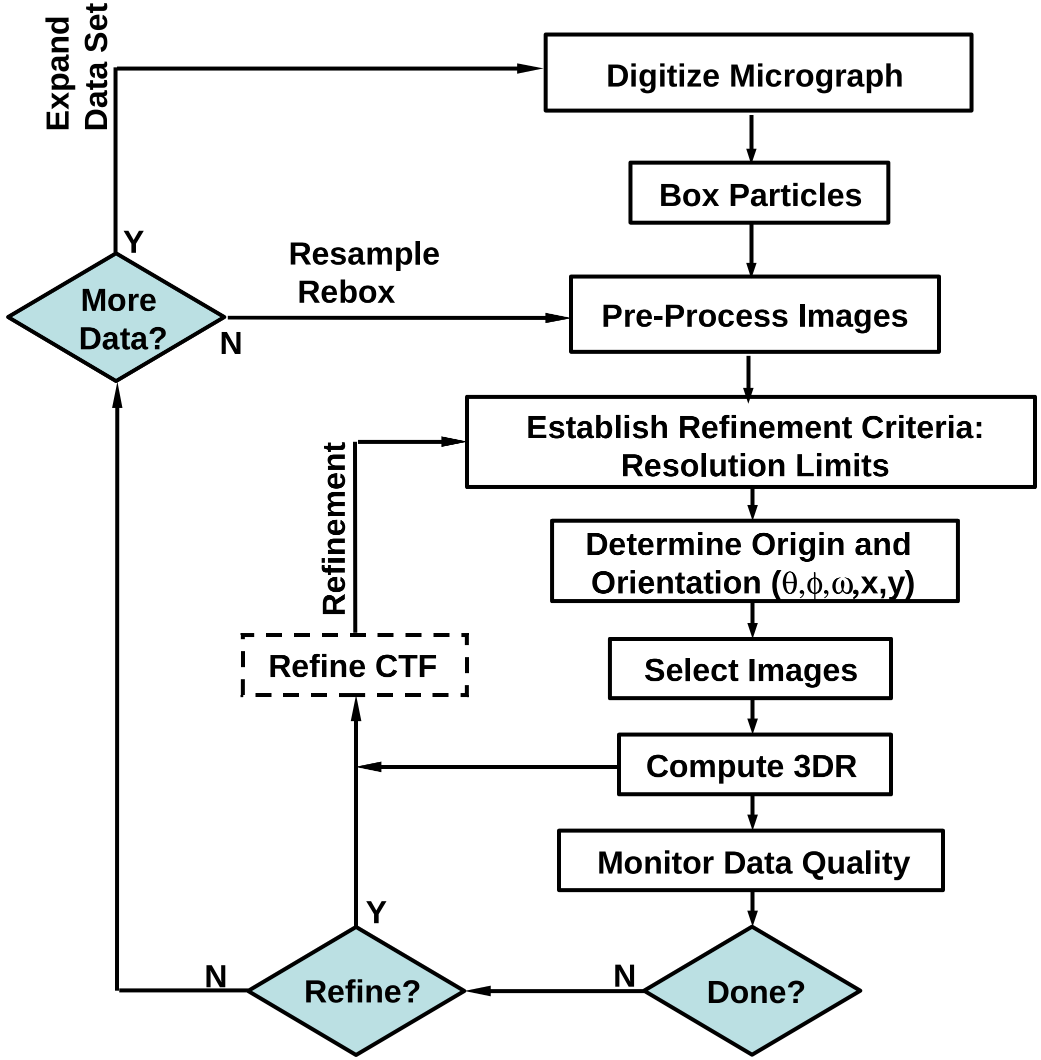

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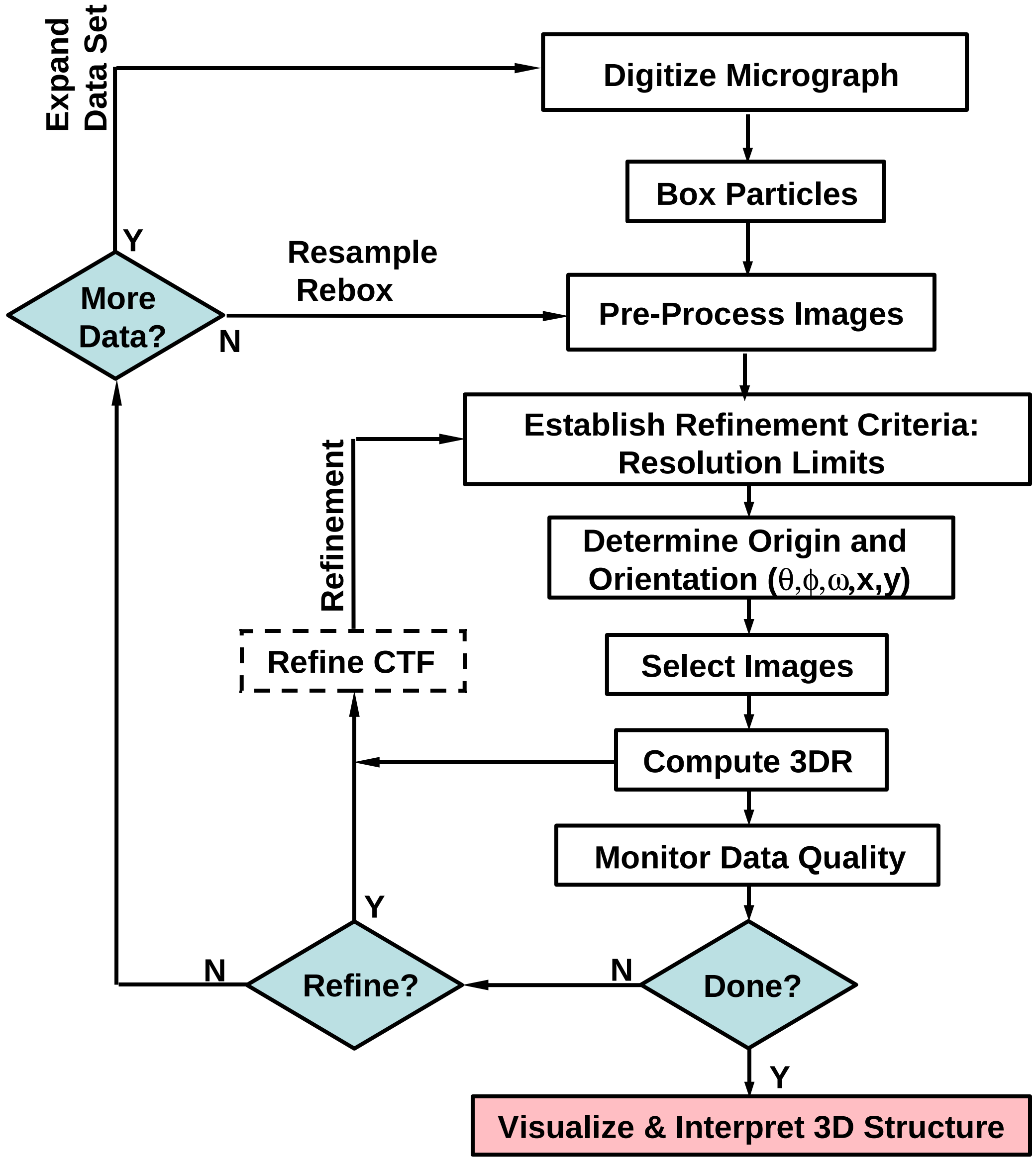

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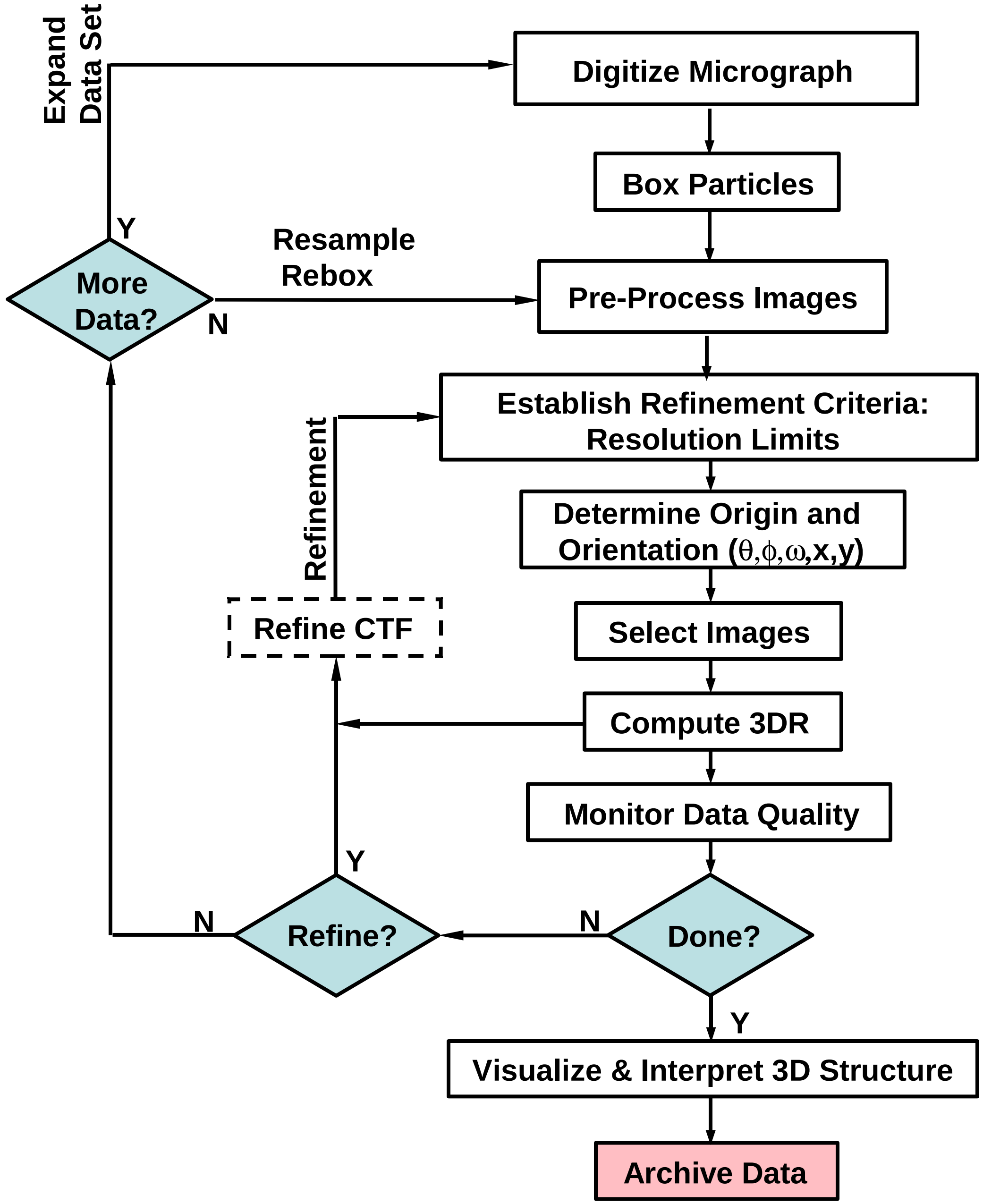
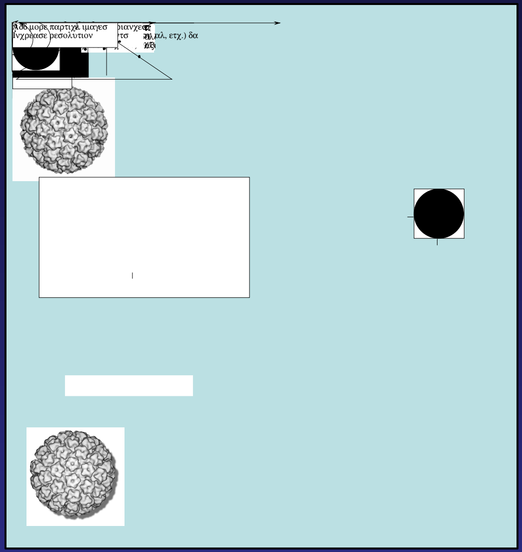


3D Reconstruction of Icosahedral Particles

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



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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 -------> PO^2R

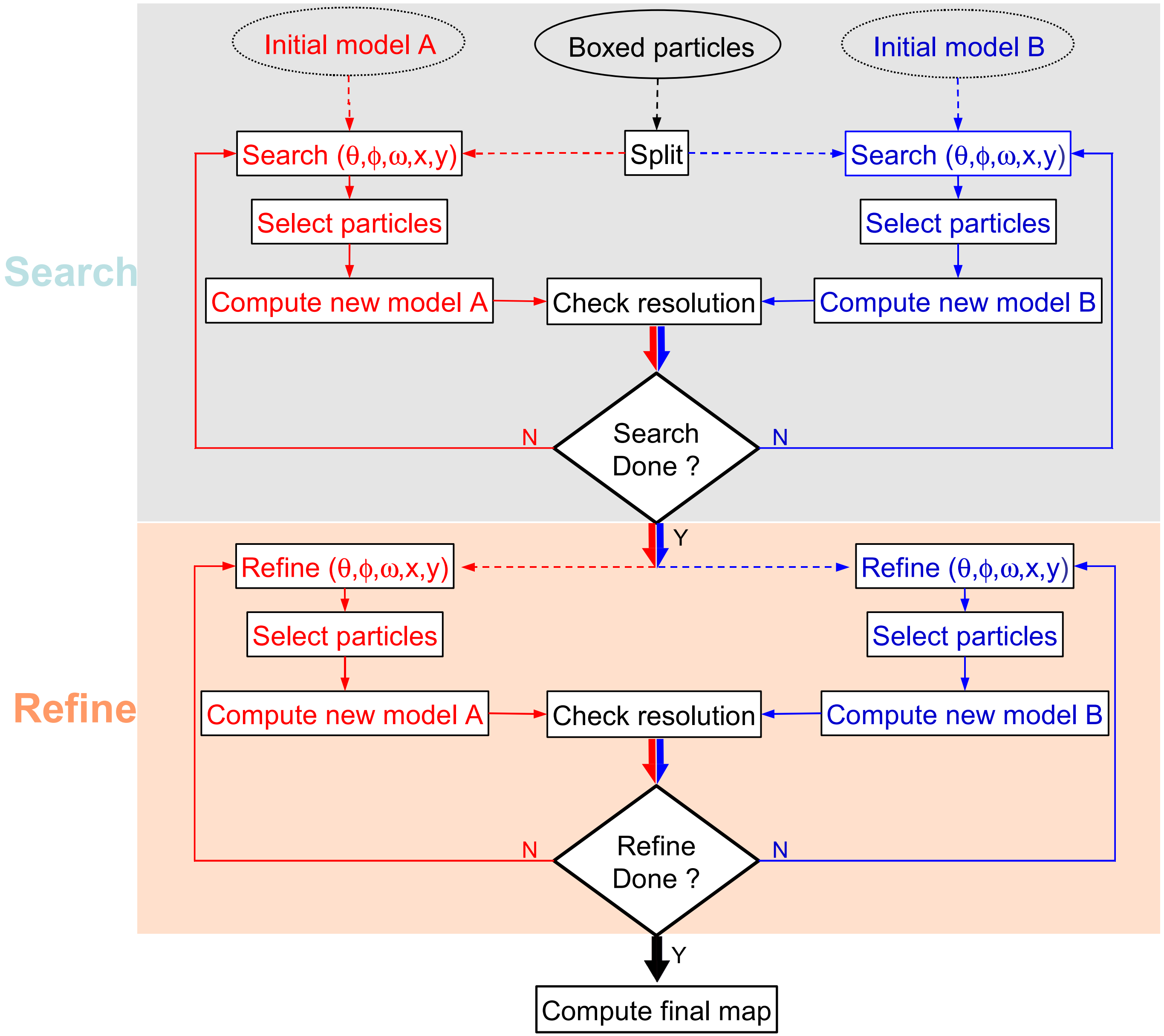
- Automation

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

- Split data set processing

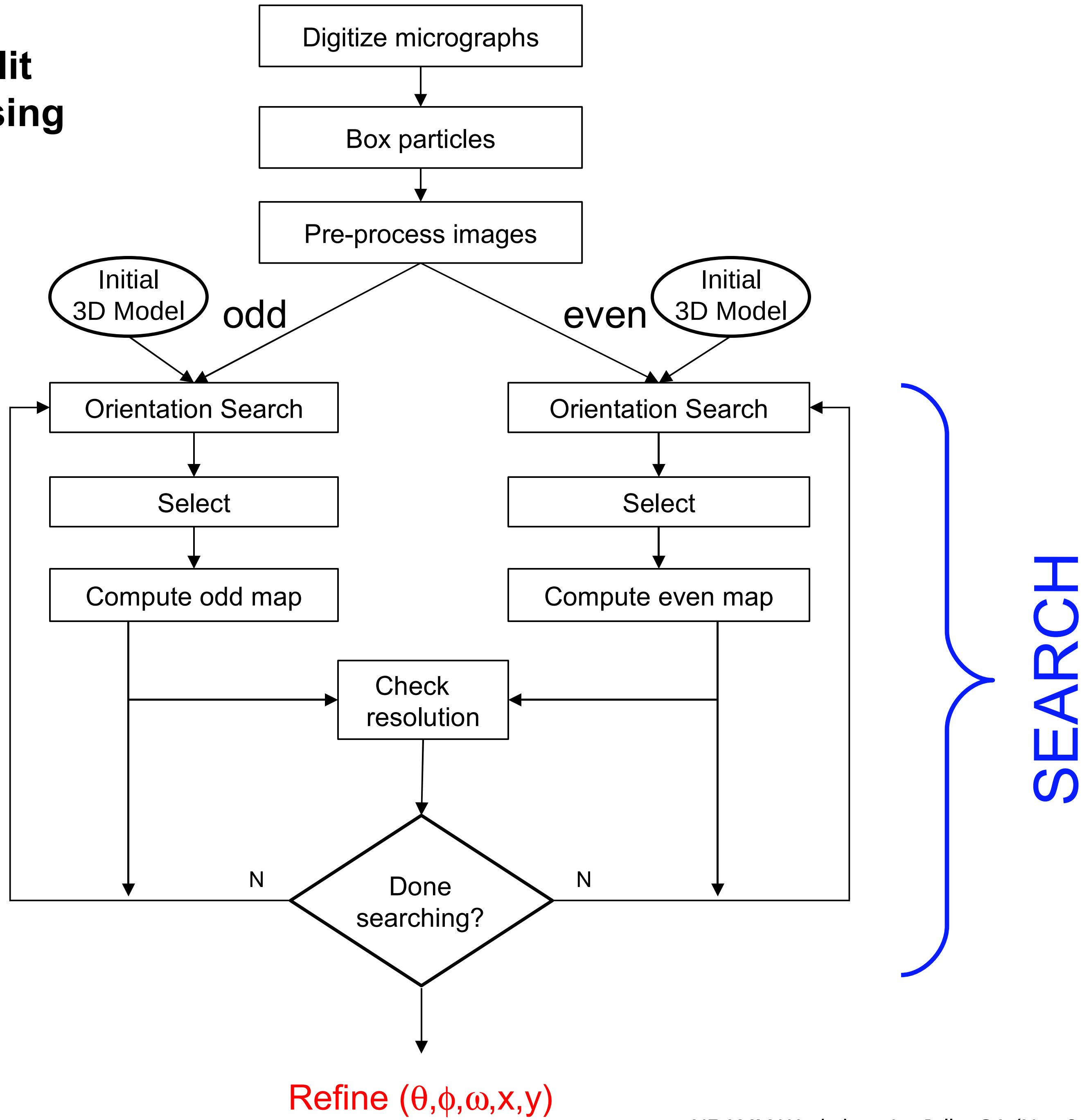
    - Divide image data at very beginning and refine ‘even’ and ‘odd’ data independently.
    - Minimizes (eliminates ?) bias in resolution assessment
    - Combine independent reconstructions to obtain ‘final’ 3DR with highest S/N

Structure Determination Flow Chart



NRAMM Workshop, La Jolla, CA (Nov 2-10, 2005)

Data Flow for Split Data Set Processing



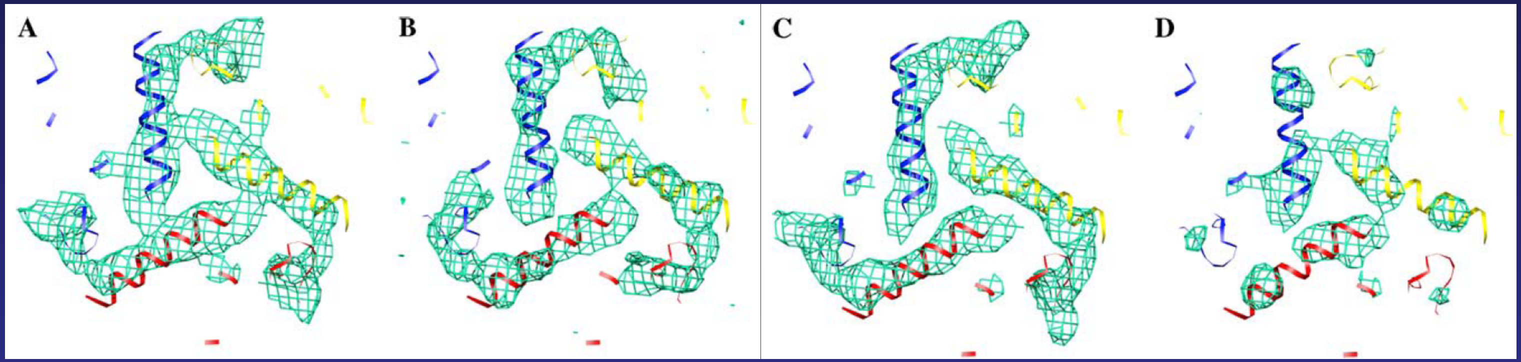
3D Reconstruction of Icosahedral Particles

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.**



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