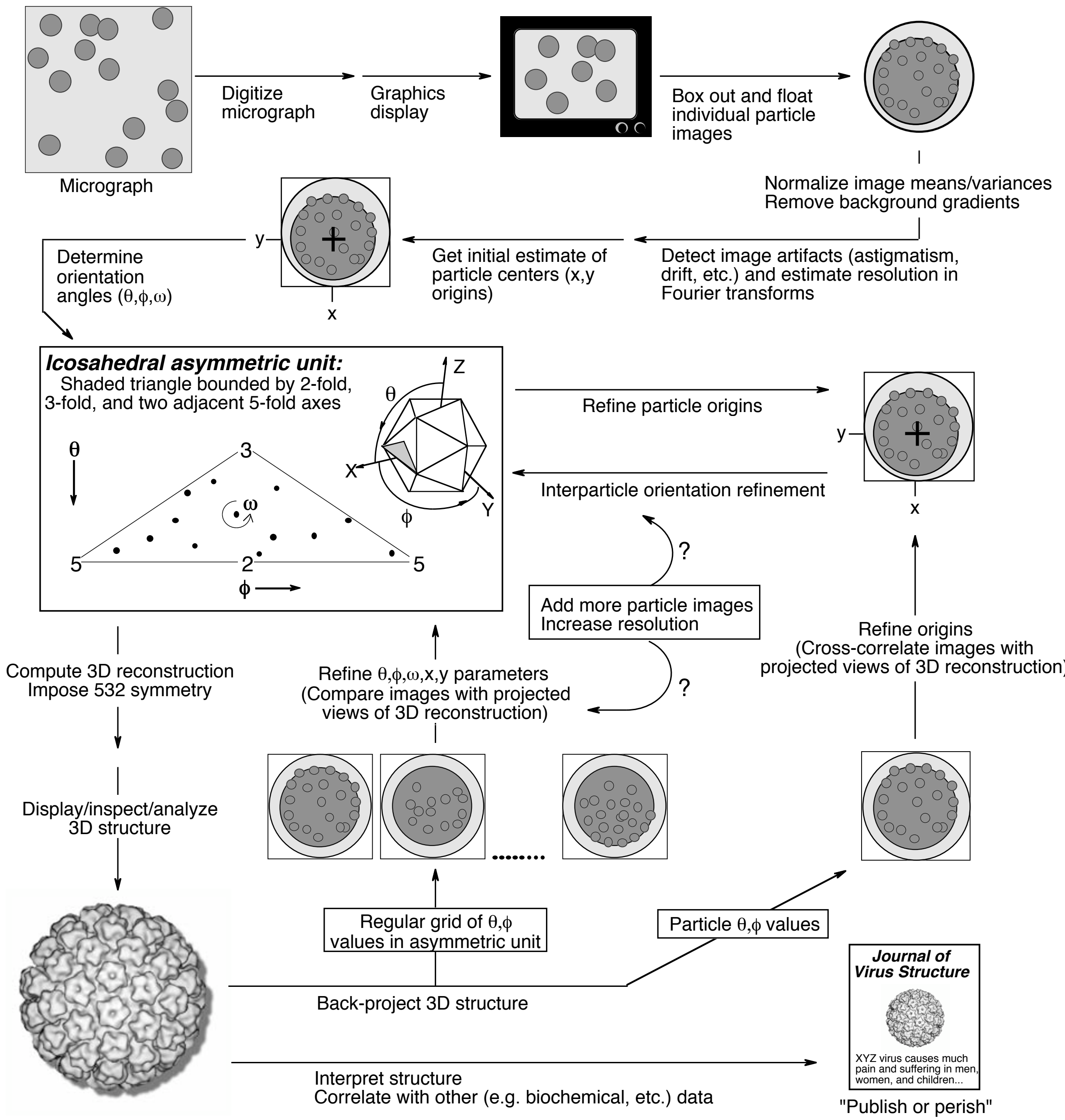
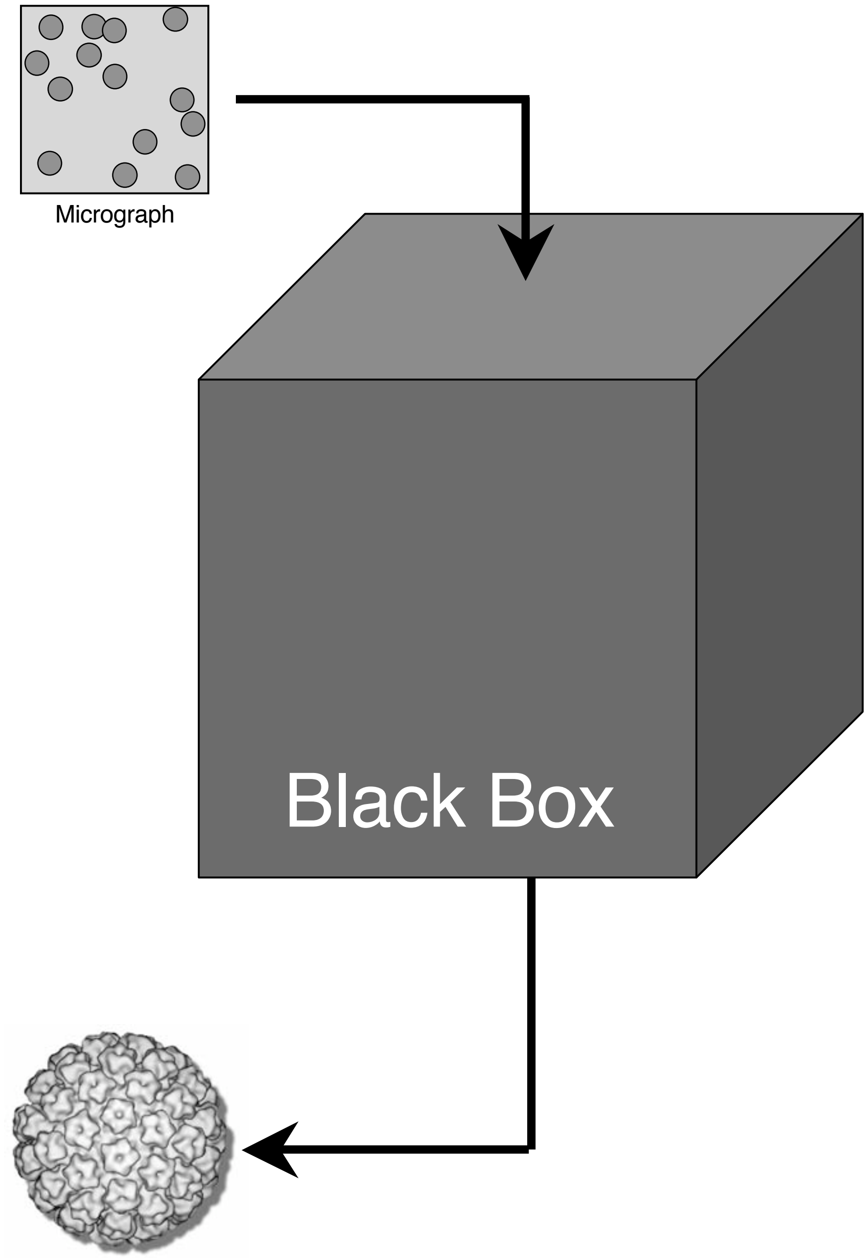
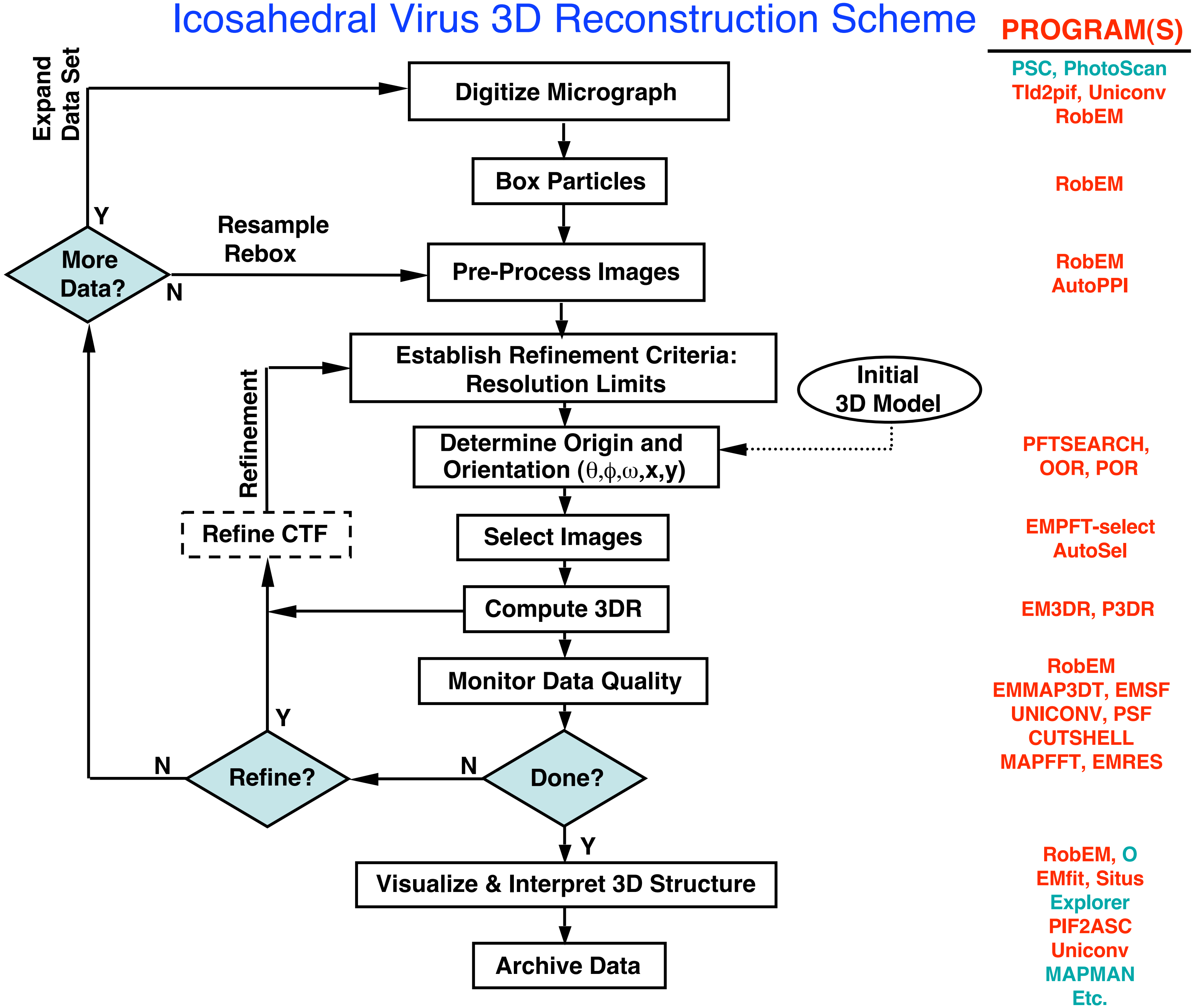
Icosahedral Particle Image Reconstruction Scheme



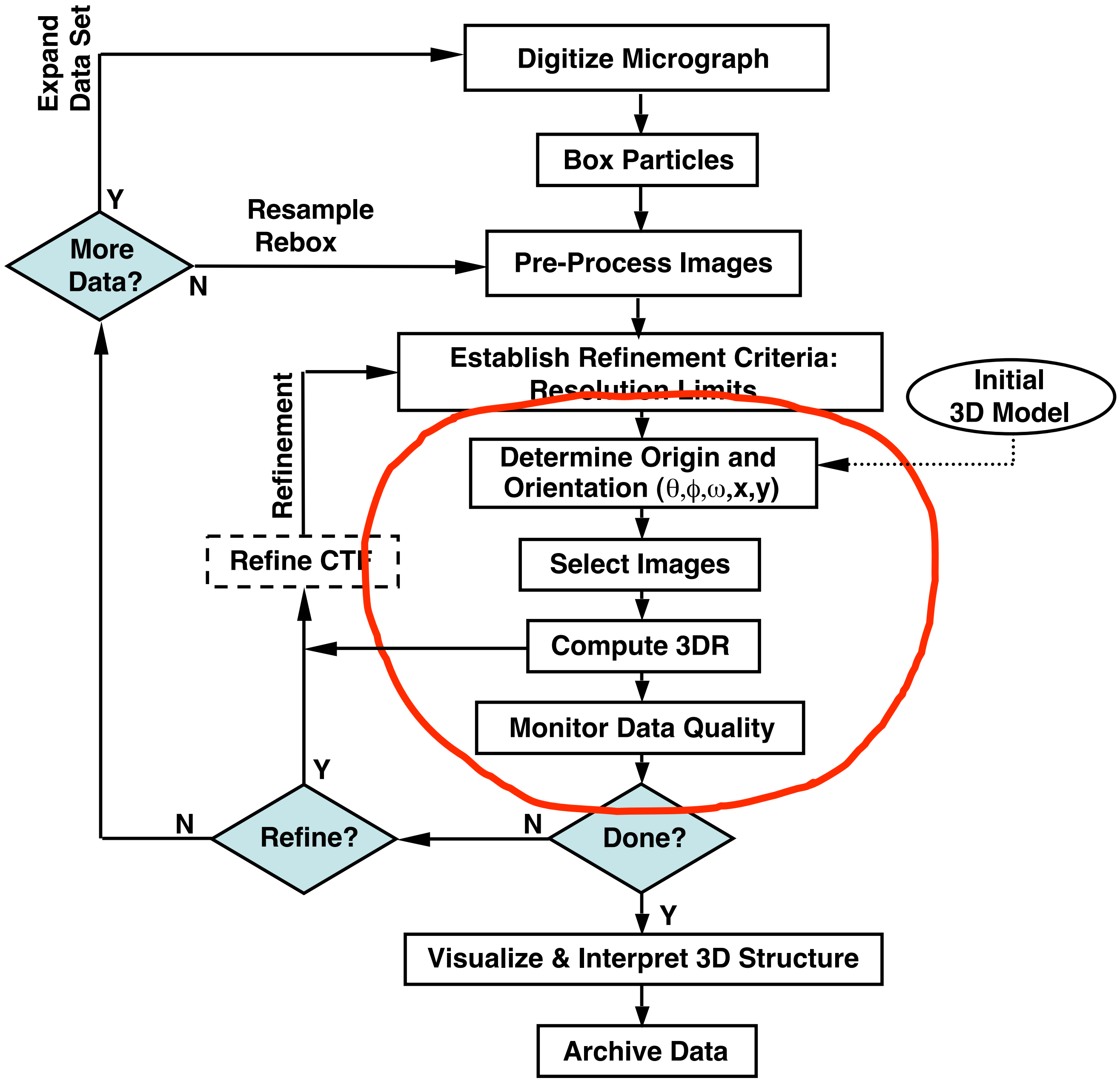
Icosahedral Particle Image Reconstruction Scheme



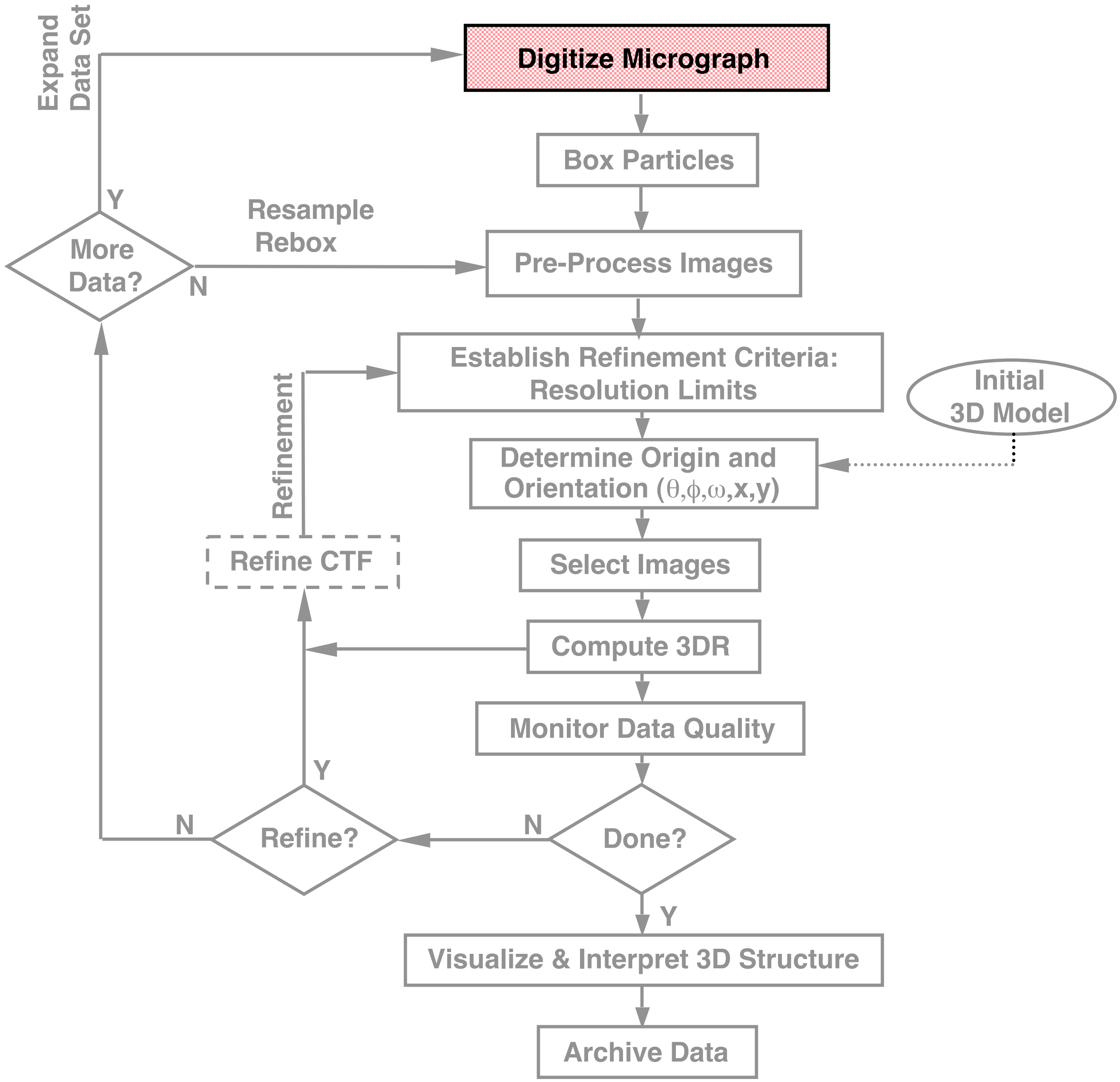
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



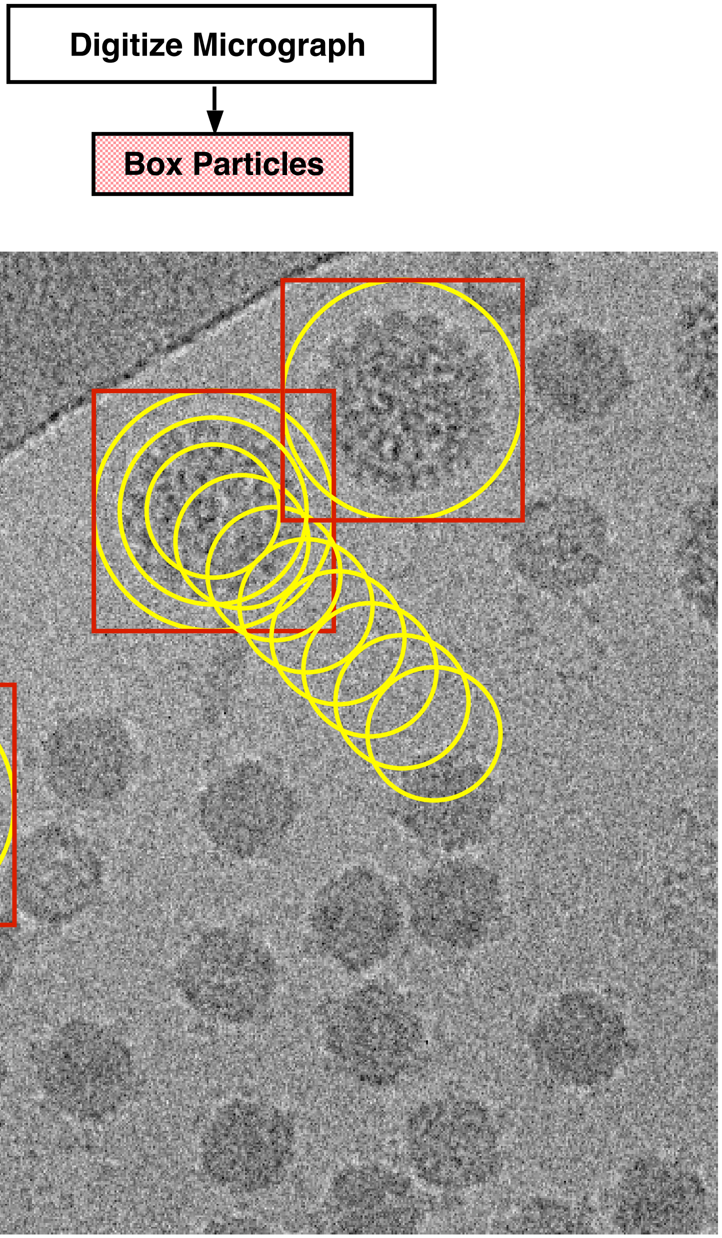
Icosahedral Virus 3D Reconstruction Scheme



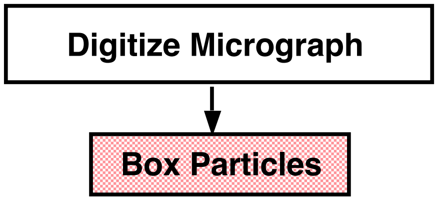
Icosahedral Virus 3D Reconstruction Scheme

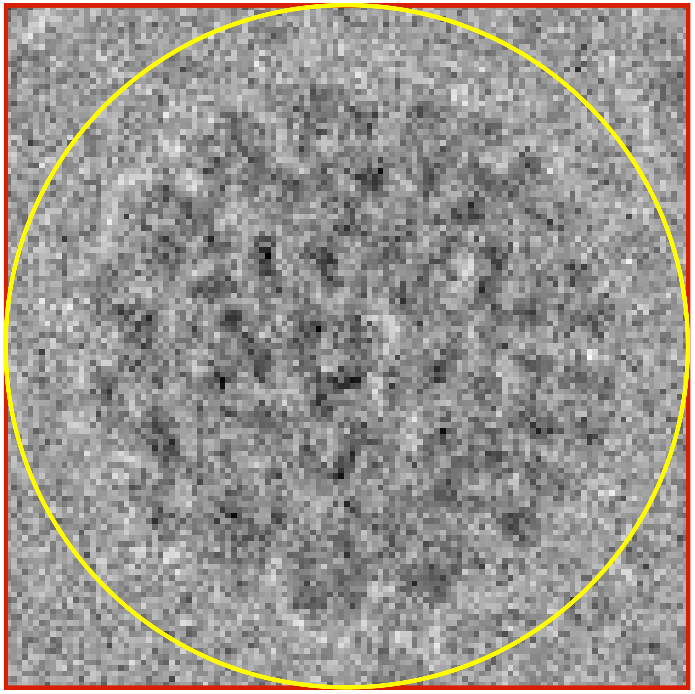
Digitize Micrograph

Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme





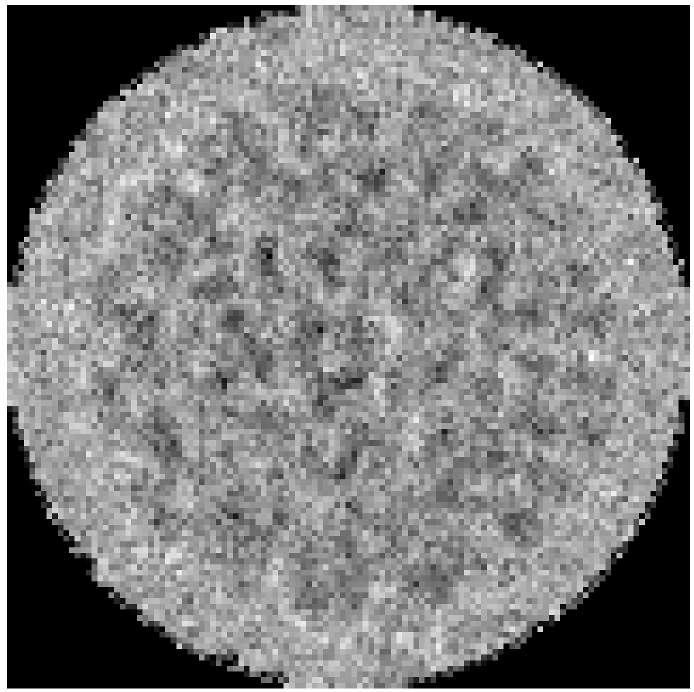
Extracted

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

↓

Box Particles



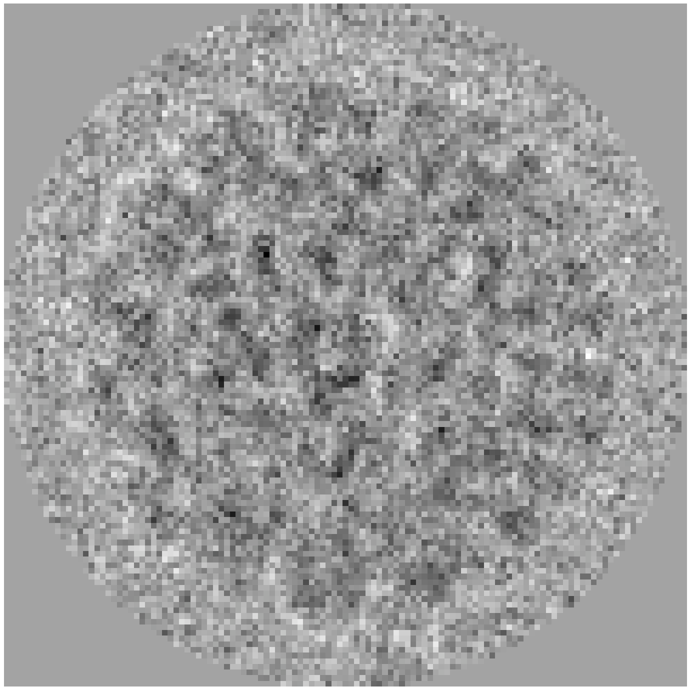
Masked

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

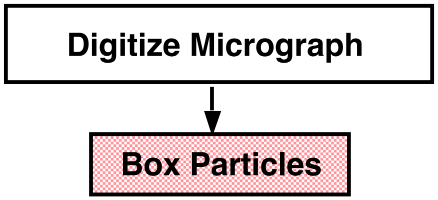
↓

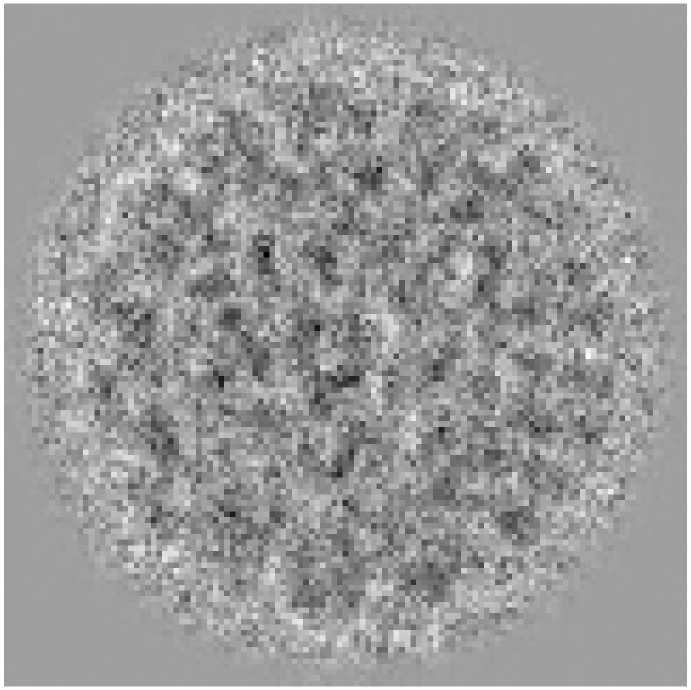
Box Particles



Floated

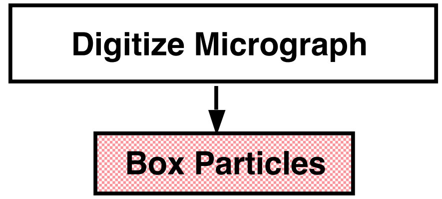
Icosahedral Virus 3D Reconstruction Scheme

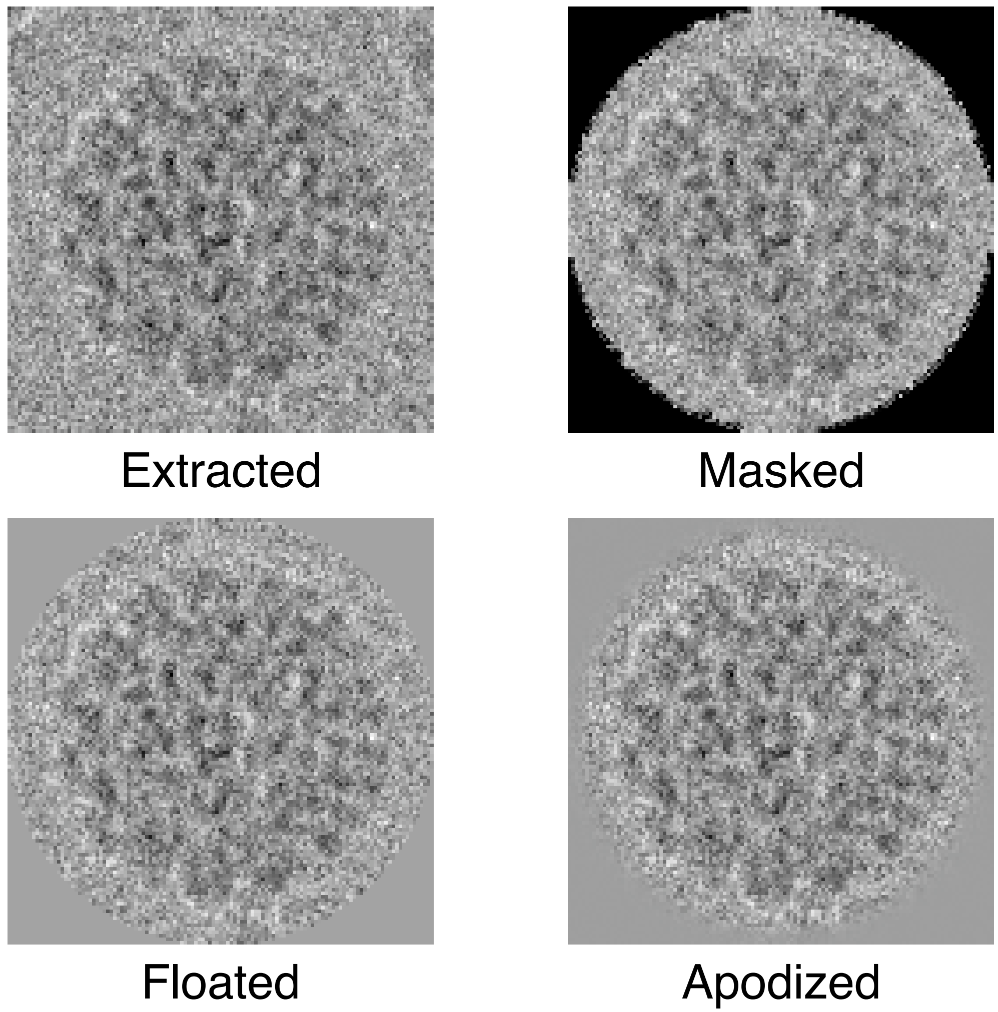




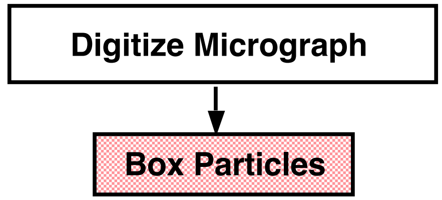
Apodized

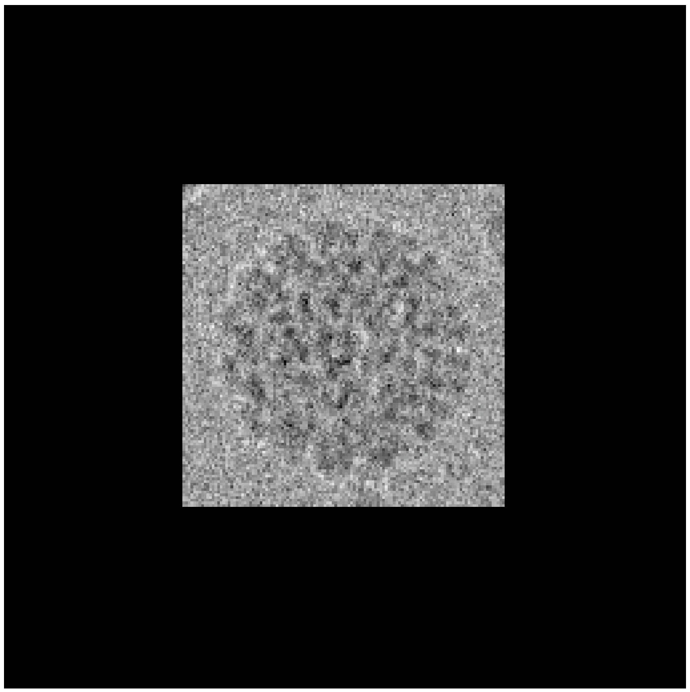
Icosahedral Virus 3D Reconstruction Scheme

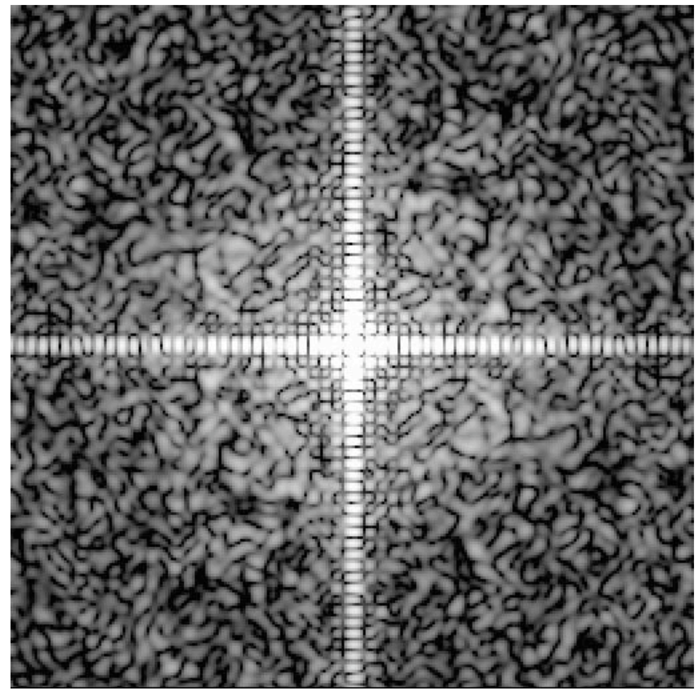




Icosahedral Virus 3D Reconstruction Scheme

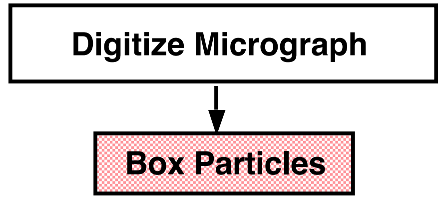


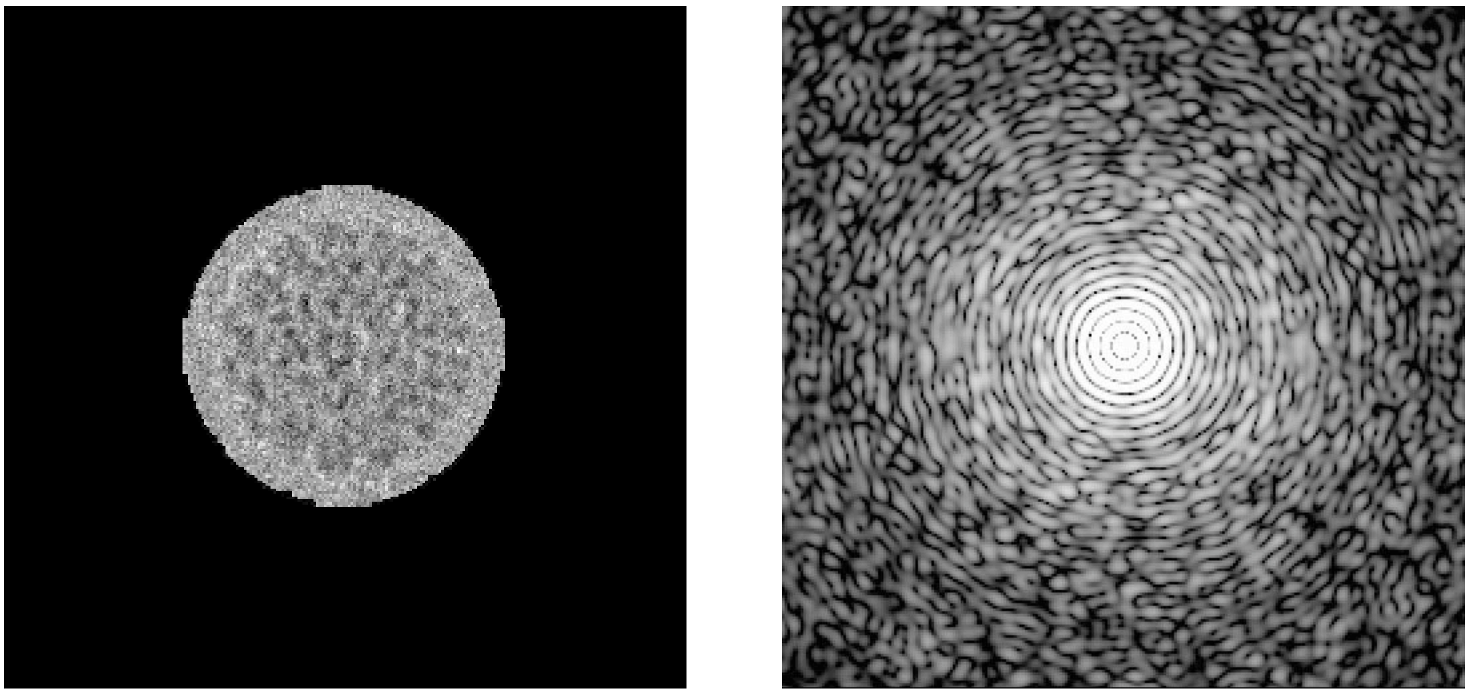




Square mask; **<u>un</u>**floated

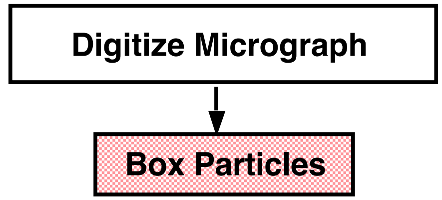
Icosahedral Virus 3D Reconstruction Scheme

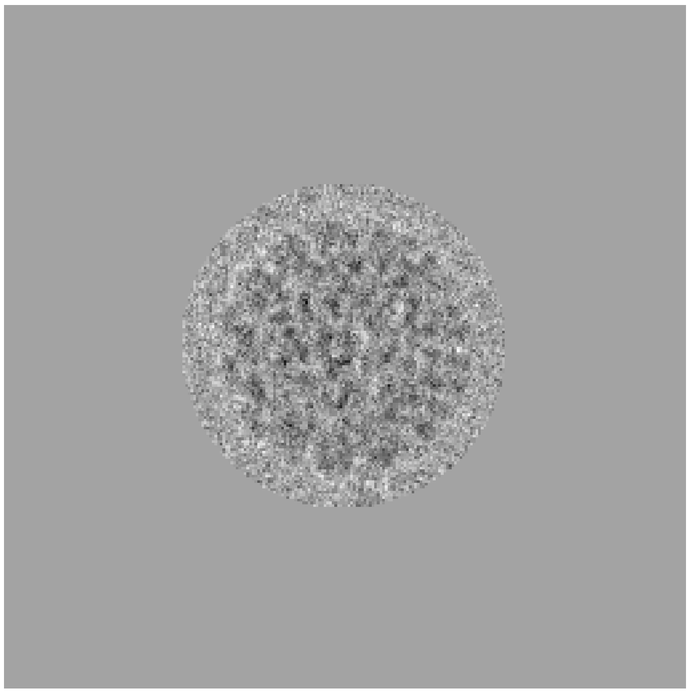


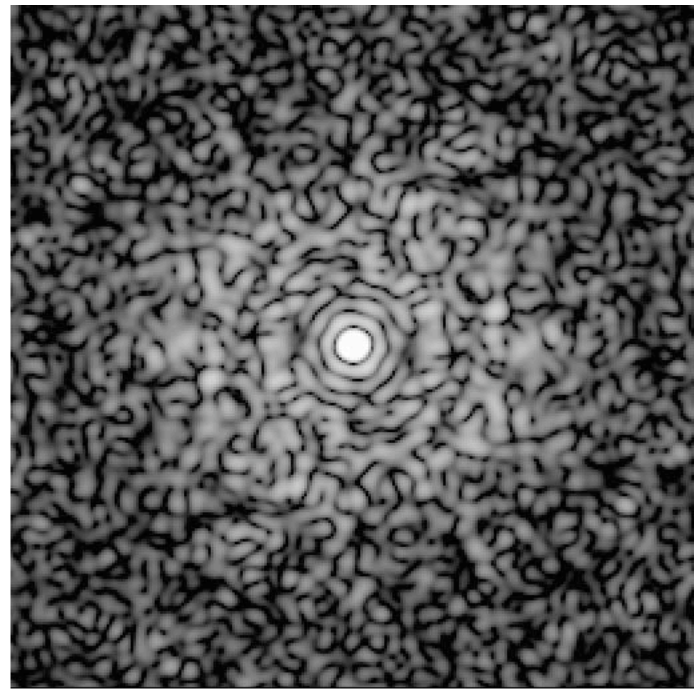


Circular mask; **un**floated

Icosahedral Virus 3D Reconstruction Scheme

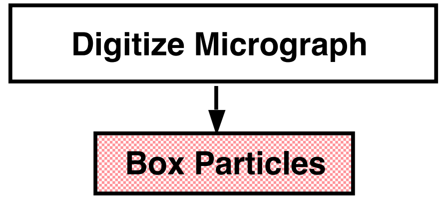


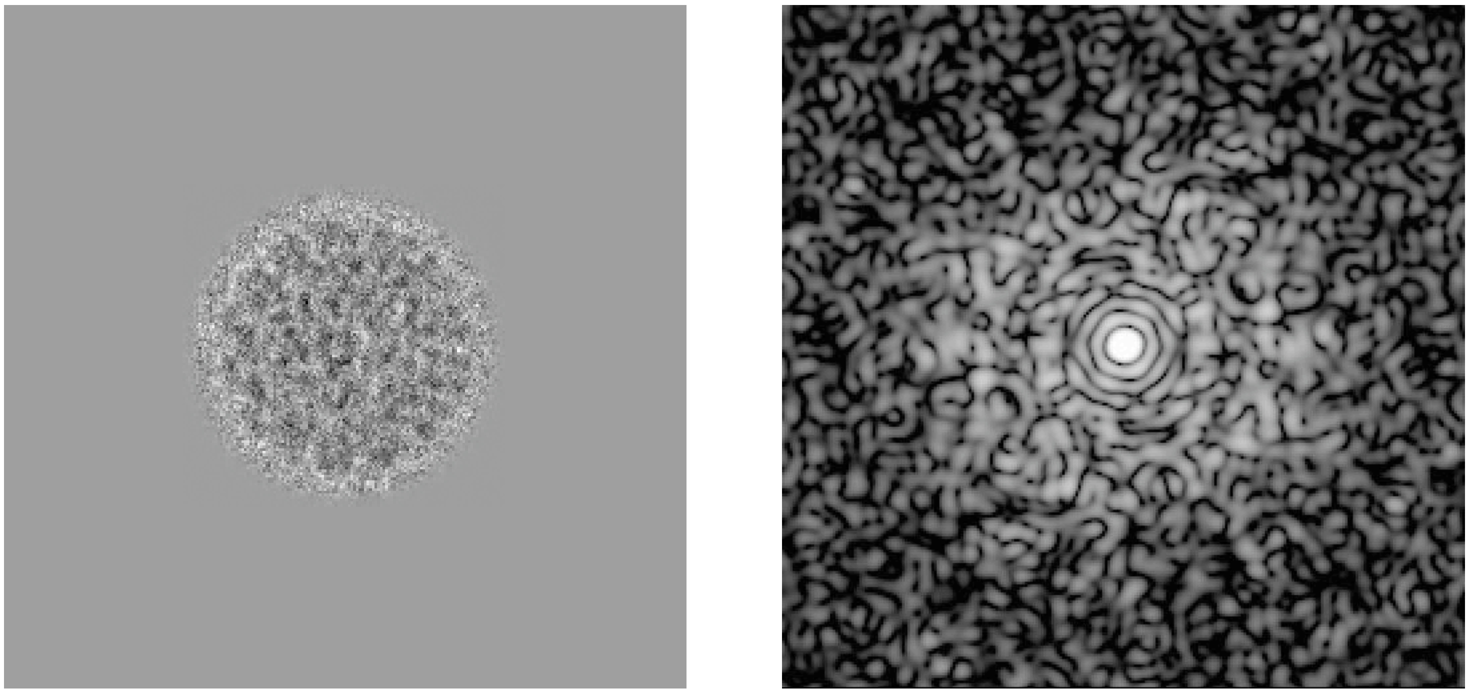




Circular mask; floated

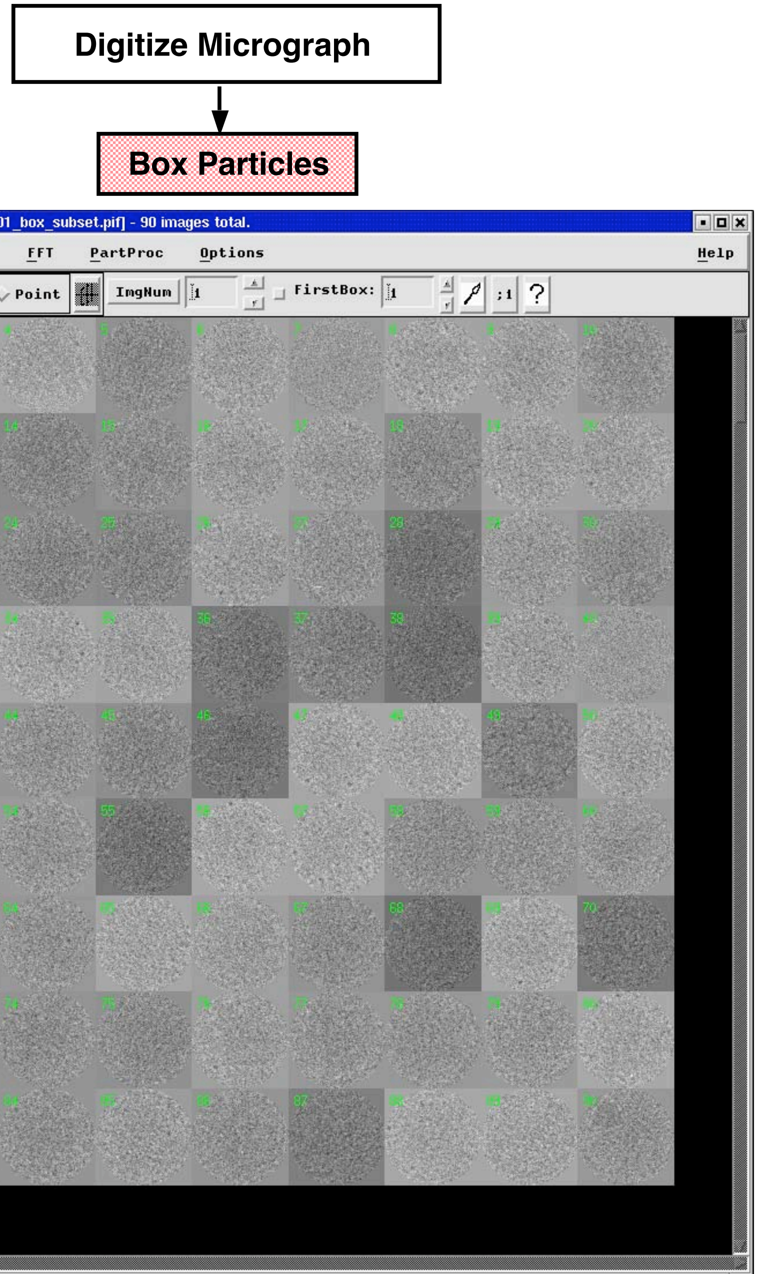
Icosahedral Virus 3D Reconstruction Scheme



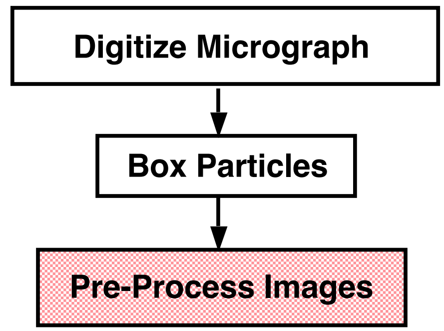


Circular mask; floated & apodized

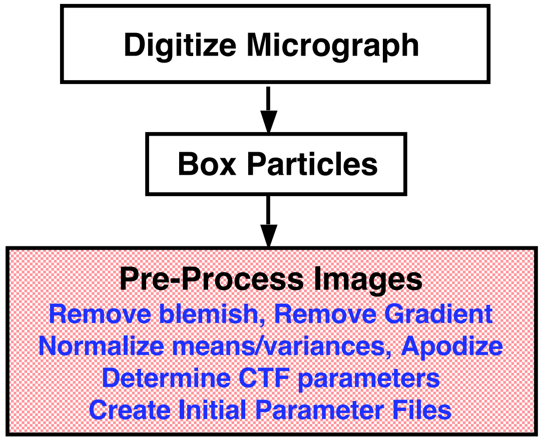
Icosahedral Virus 3D Reconstruction Scheme



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

Icosahedral Virus 3D Reconstruction Scheme

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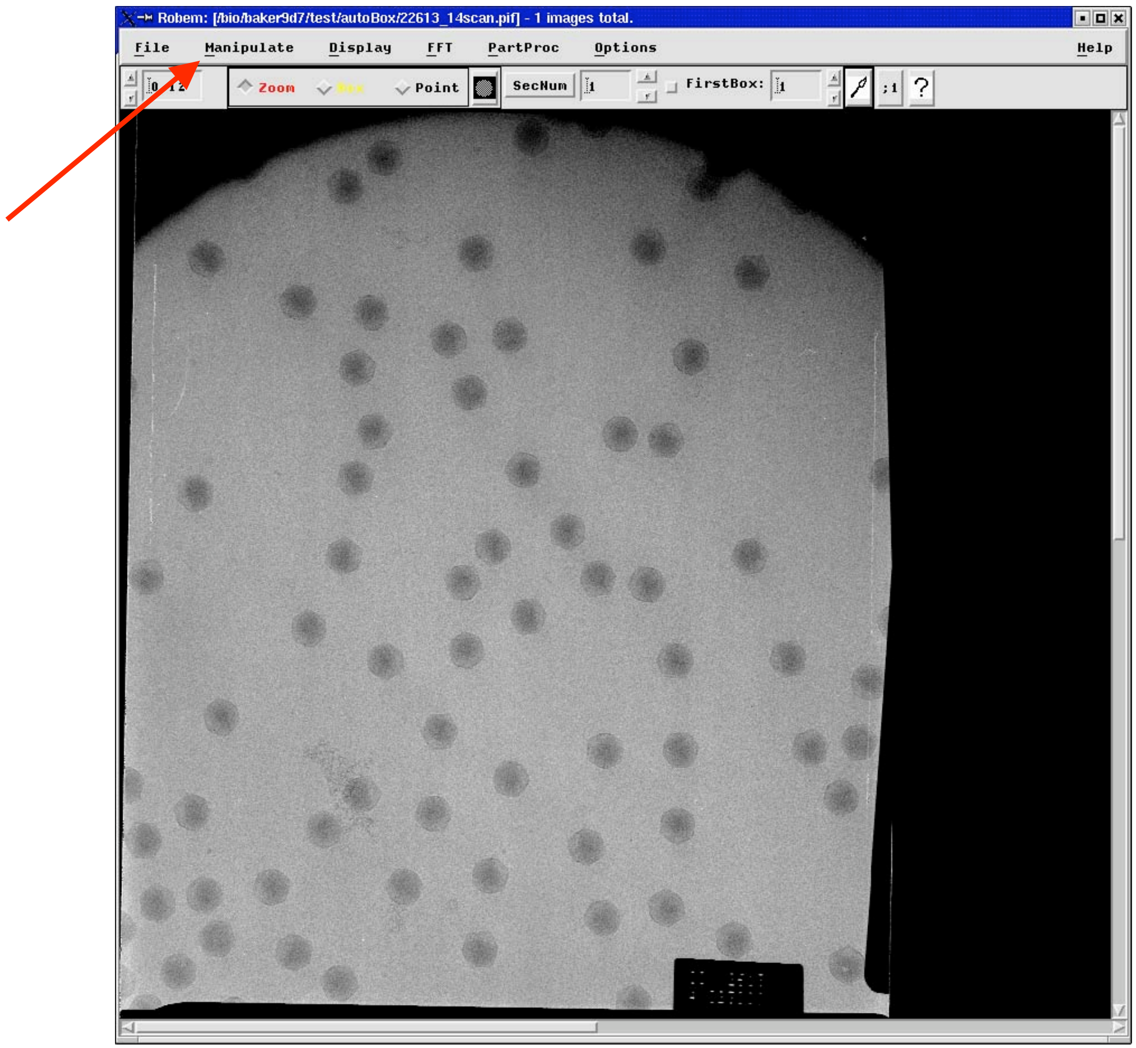

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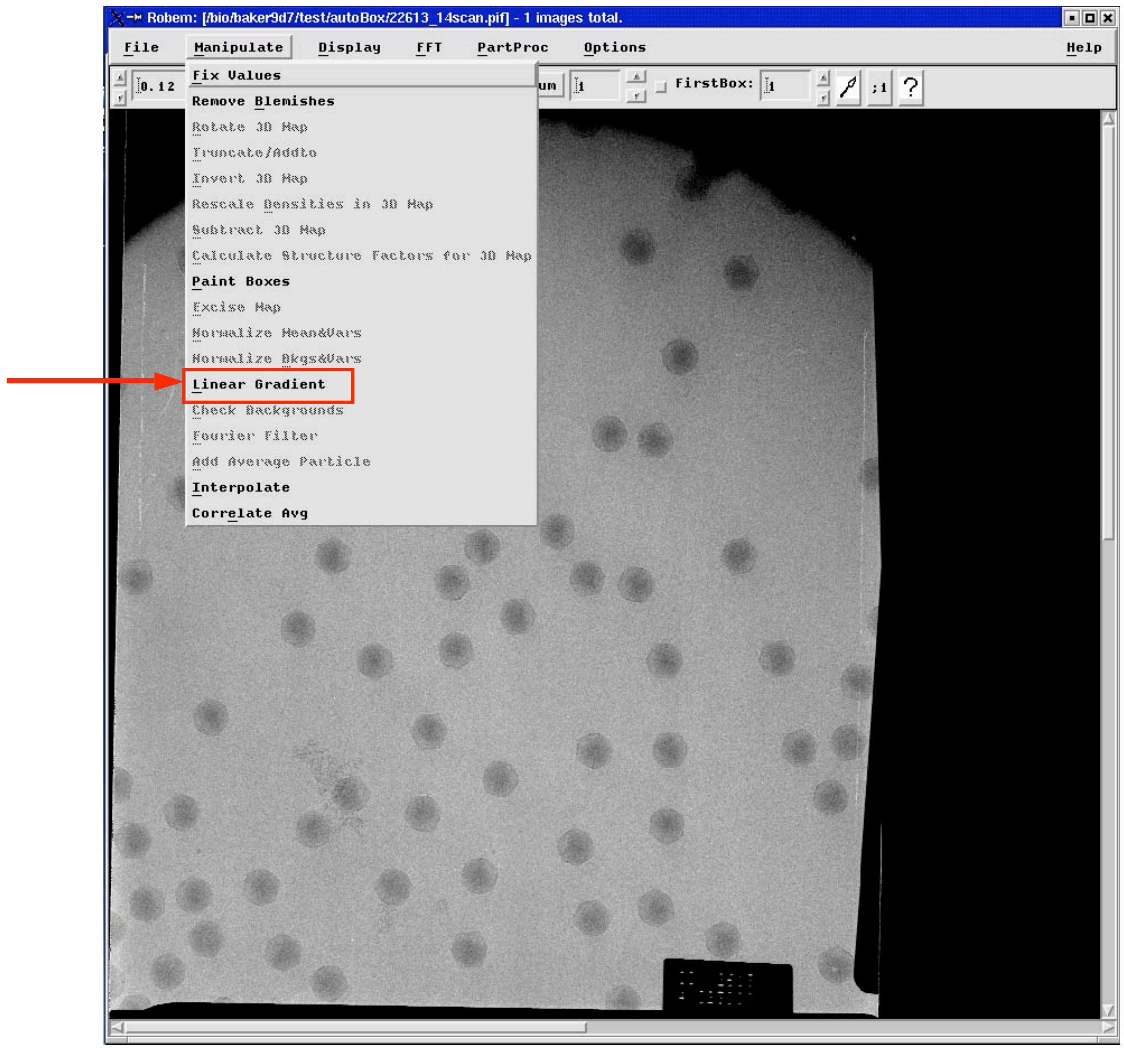

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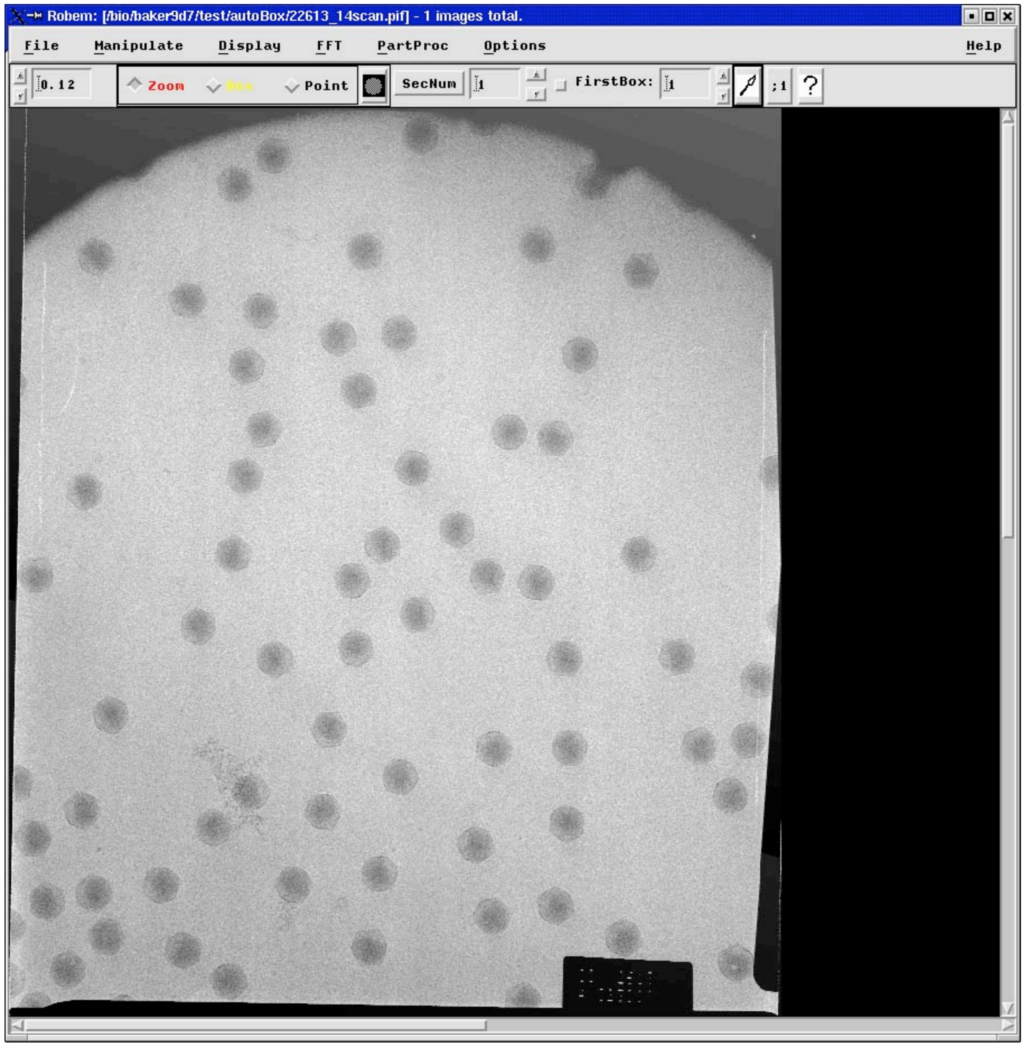

Gradient removed

Icosahedral Virus 3D Reconstruction Scheme

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Remove blemish, Remove Gradient
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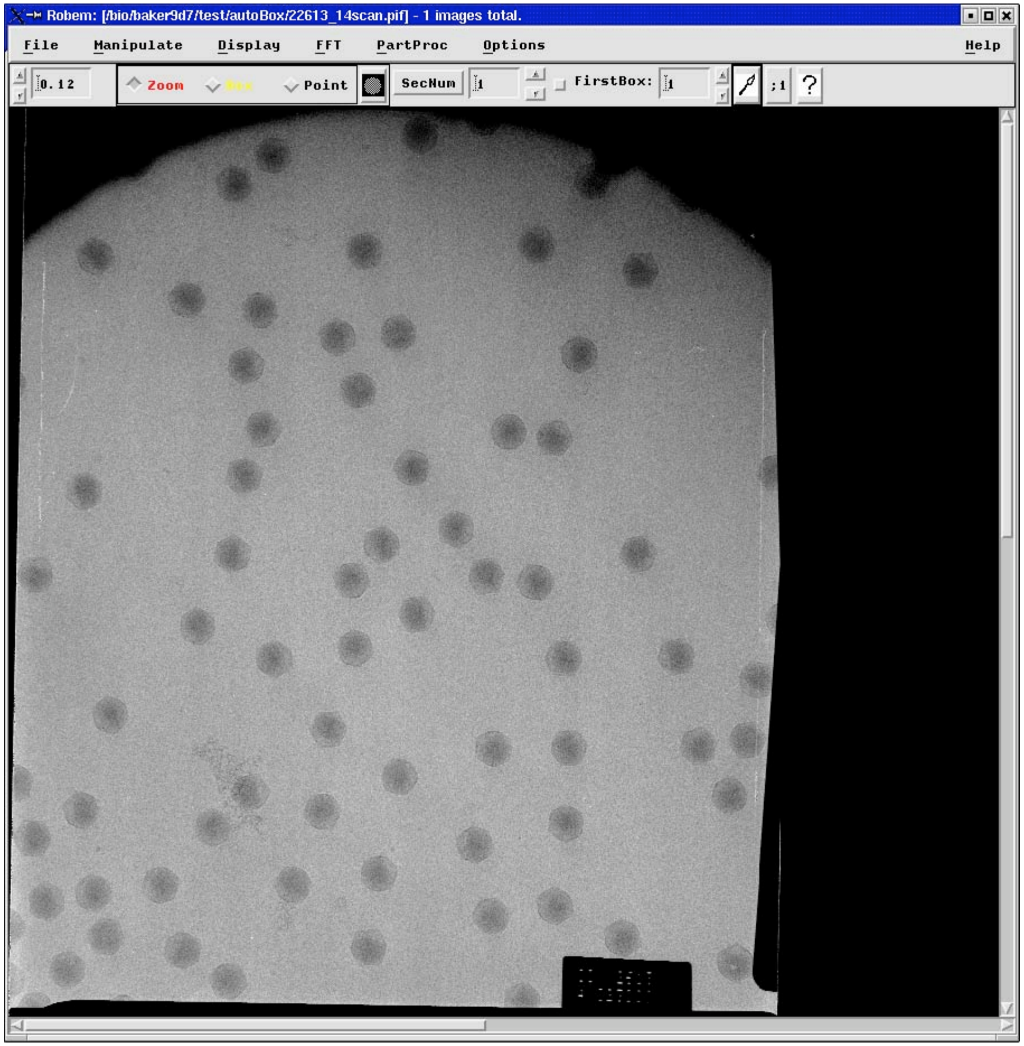

Gradient
not removed

Icosahedral Virus 3D Reconstruction Scheme

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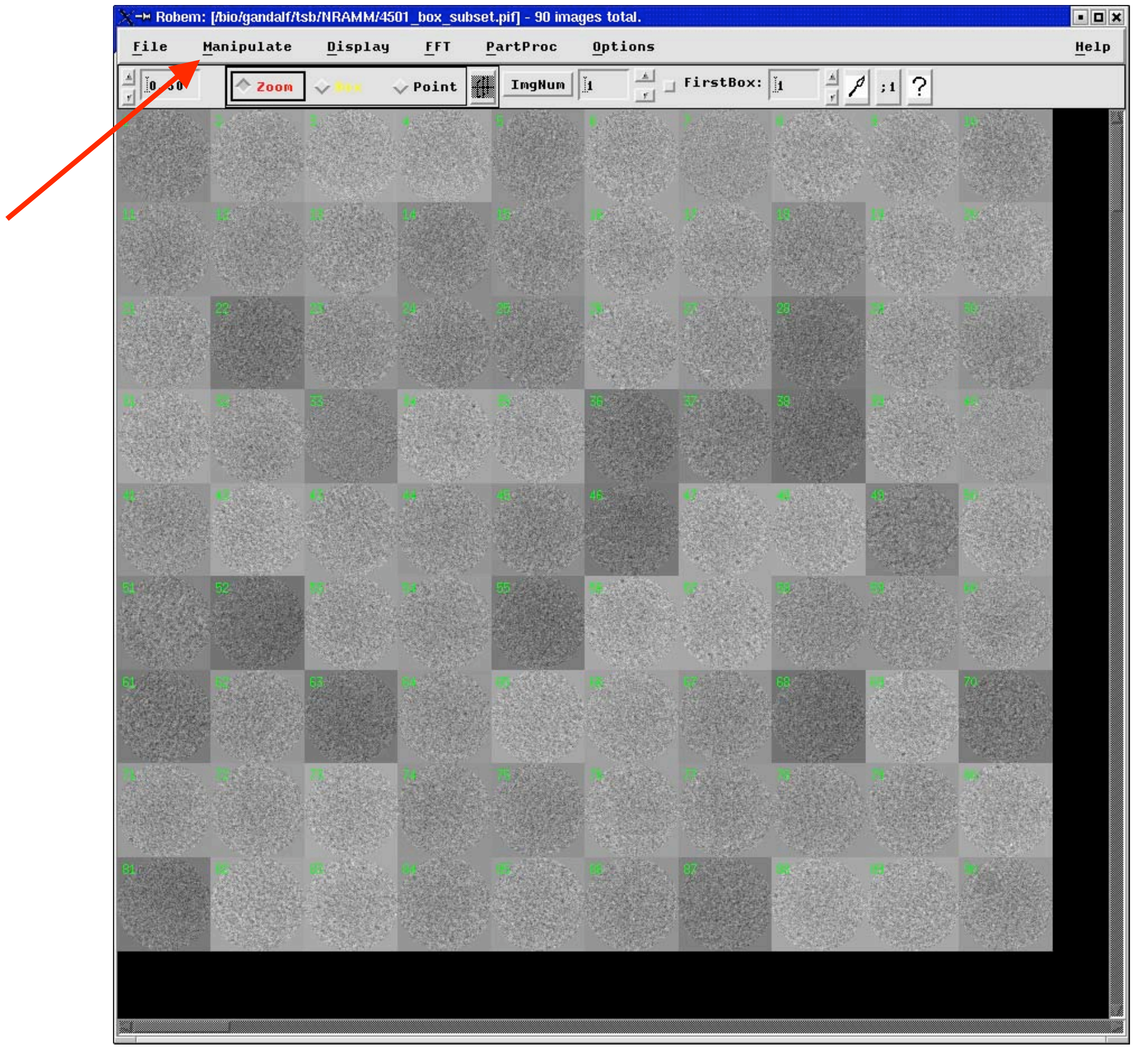

Icosahedral Virus 3D Reconstruction Scheme

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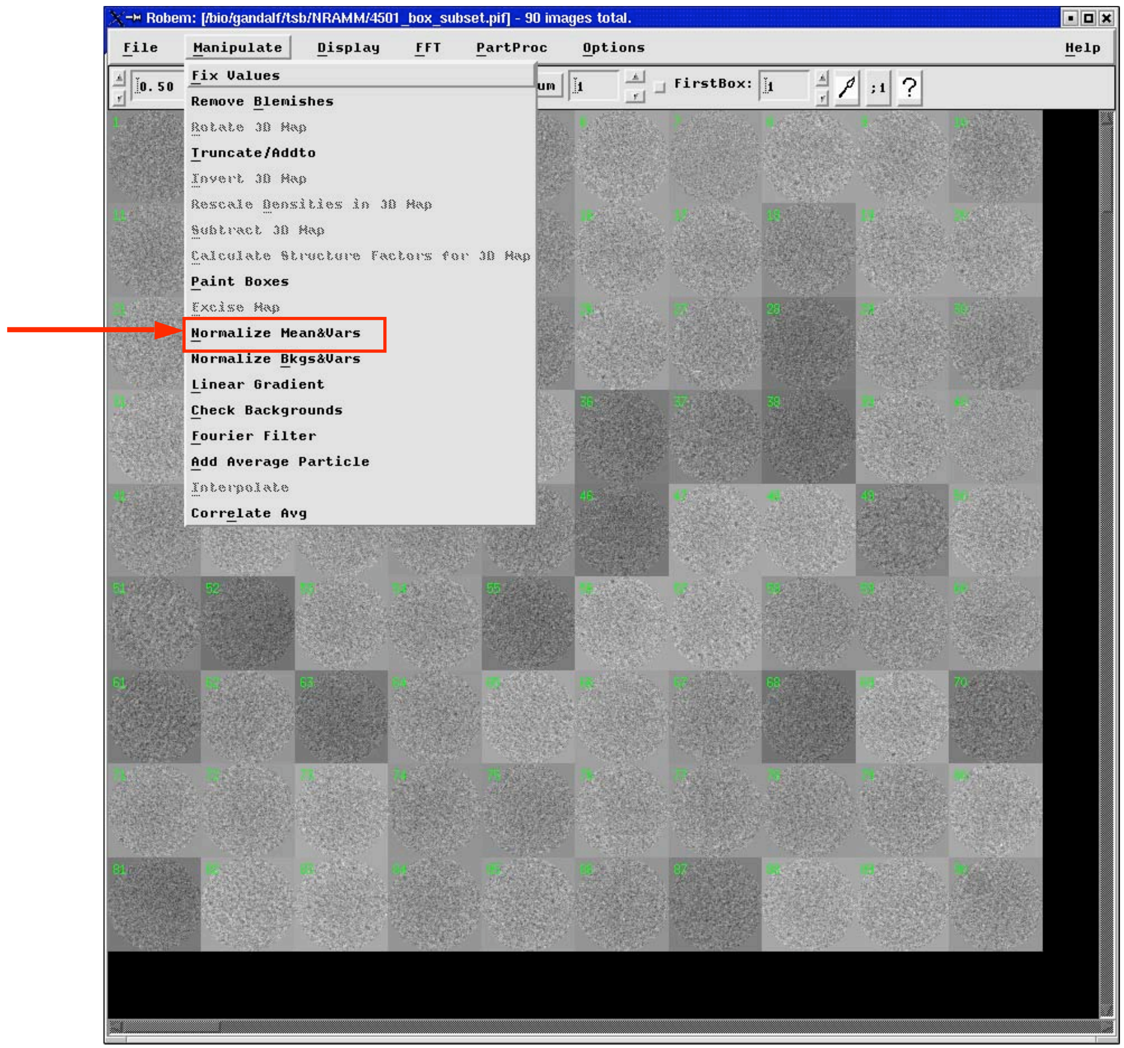

Icosahedral Virus 3D Reconstruction Scheme

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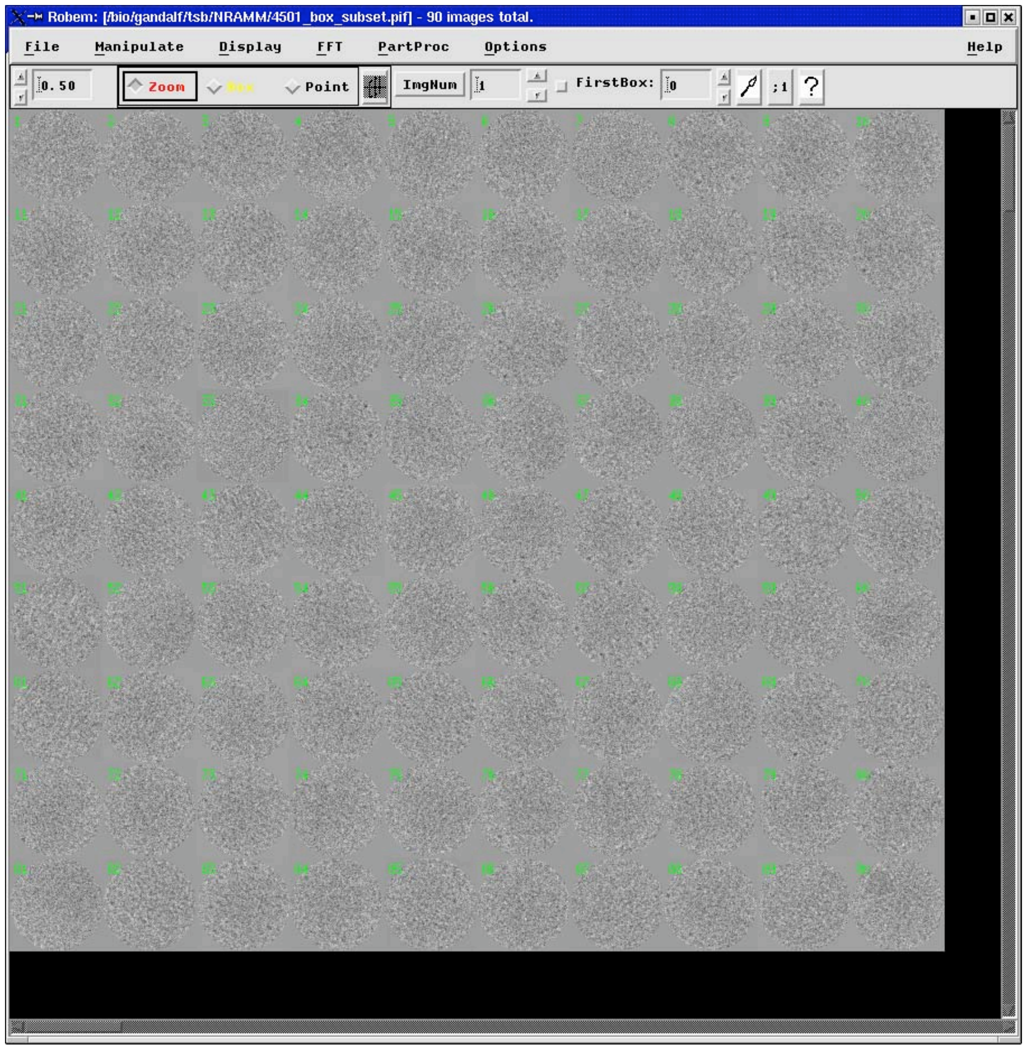

Icosahedral Virus 3D Reconstruction Scheme

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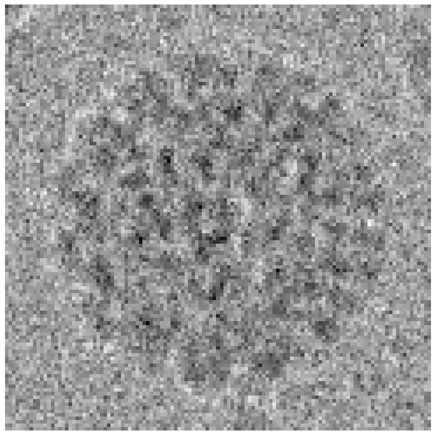
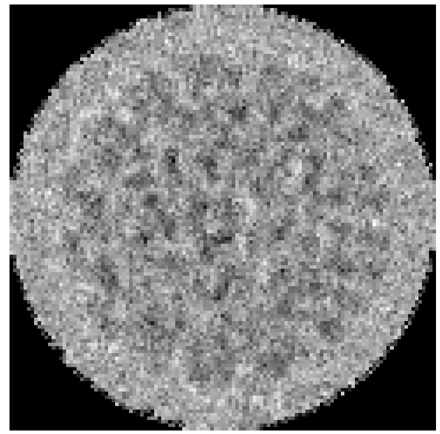
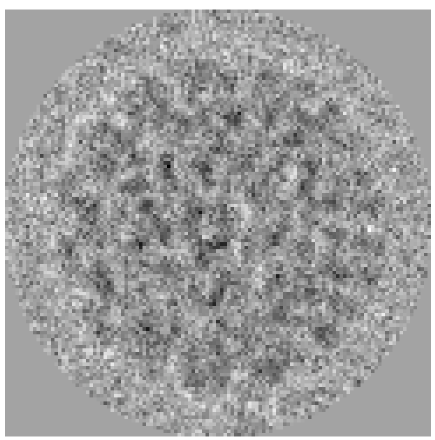


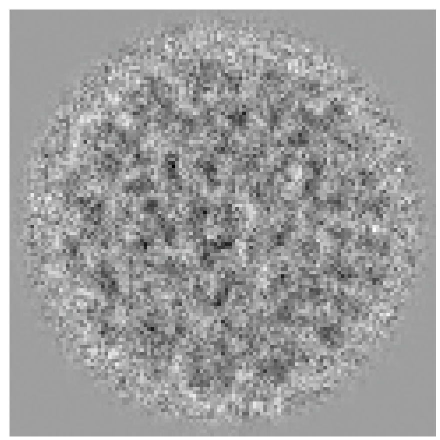
Extracted



Masked



Floated



Apodized

Icosahedral Virus 3D Reconstruction Scheme

Pre-Process Images

Remove blemish, Remove Gradient

Normalize means/variances, Apodize

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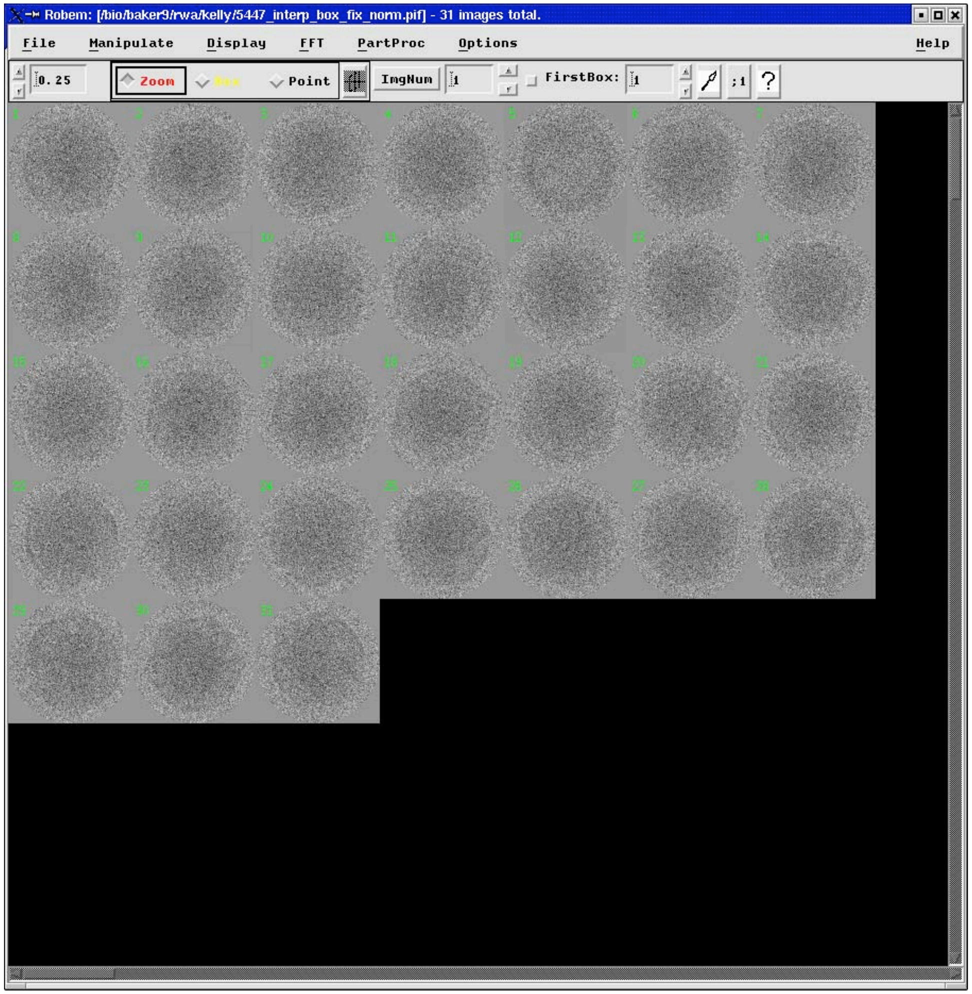

Icosahedral Virus 3D Reconstruction Scheme

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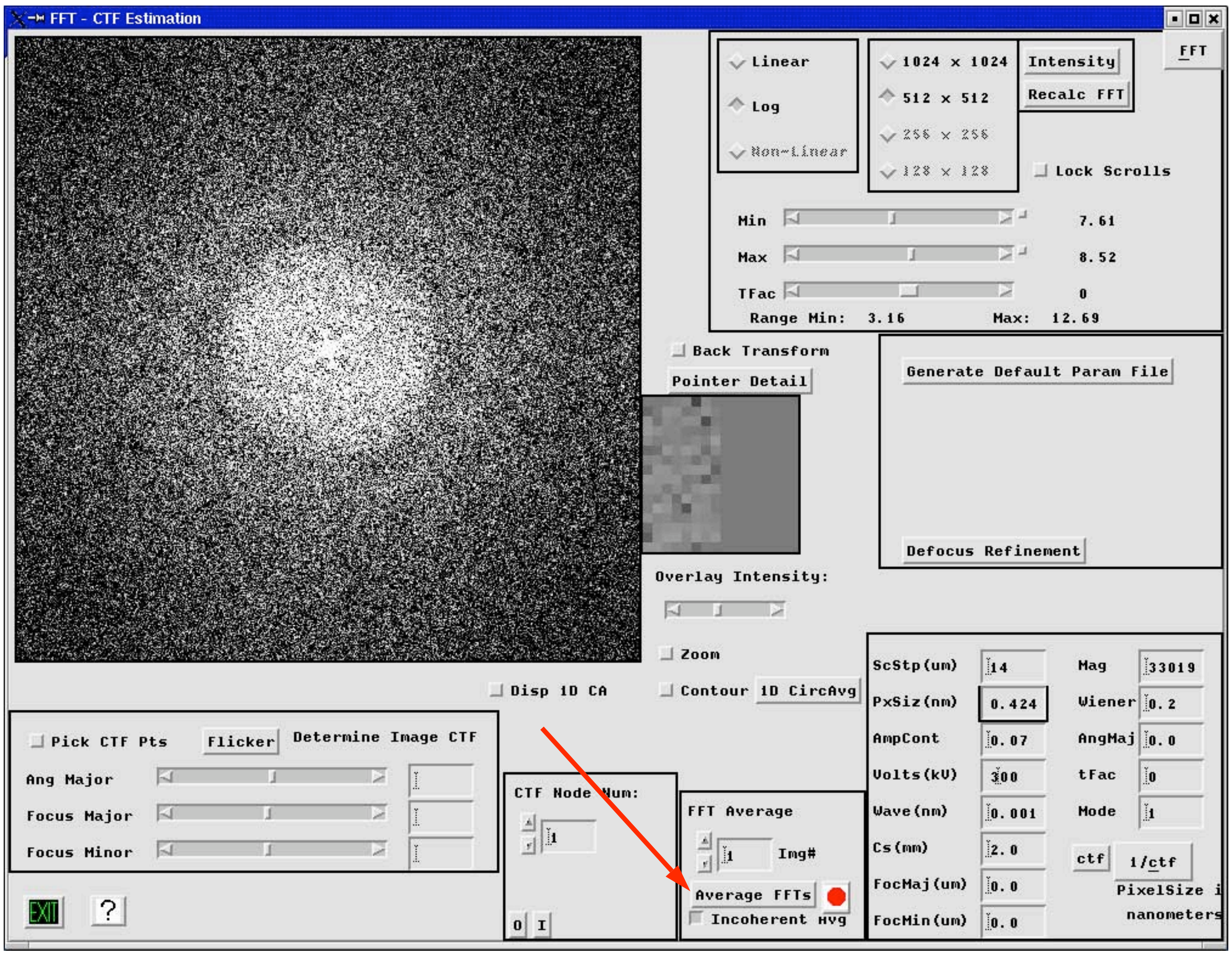

Icosahedral Virus 3D Reconstruction Scheme

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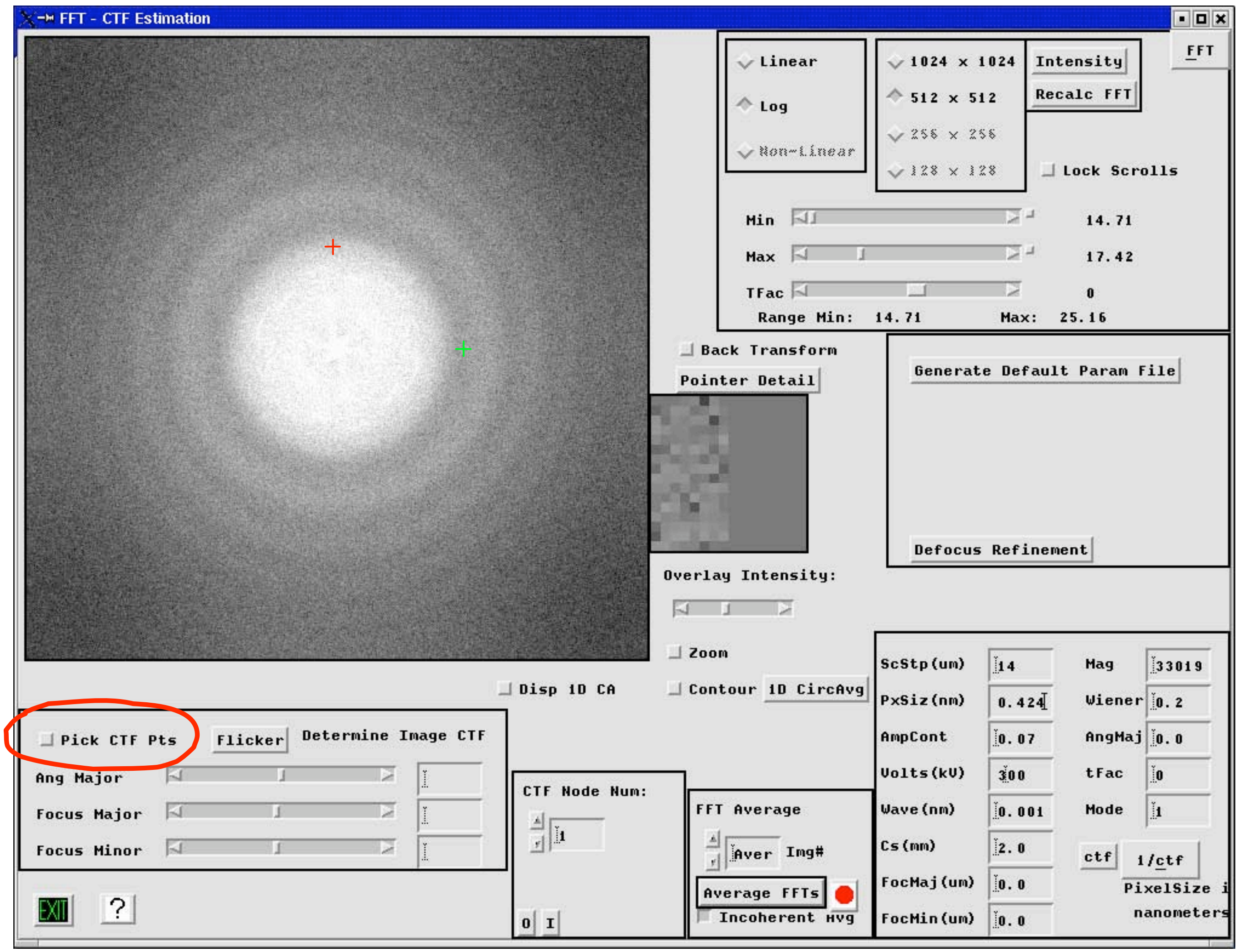

Icosahedral Virus 3D Reconstruction Scheme

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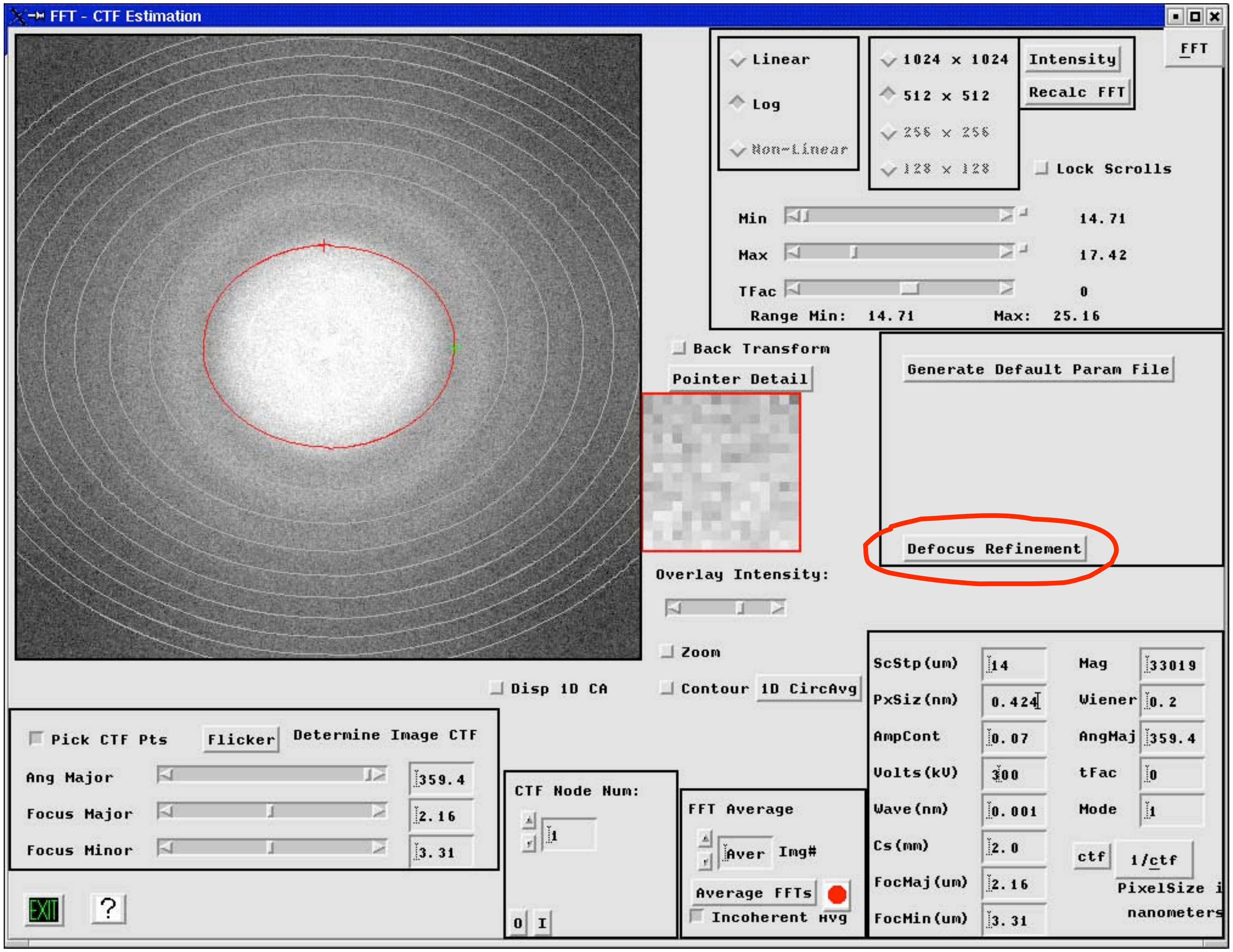

Icosahedral Virus 3D Reconstruction Scheme

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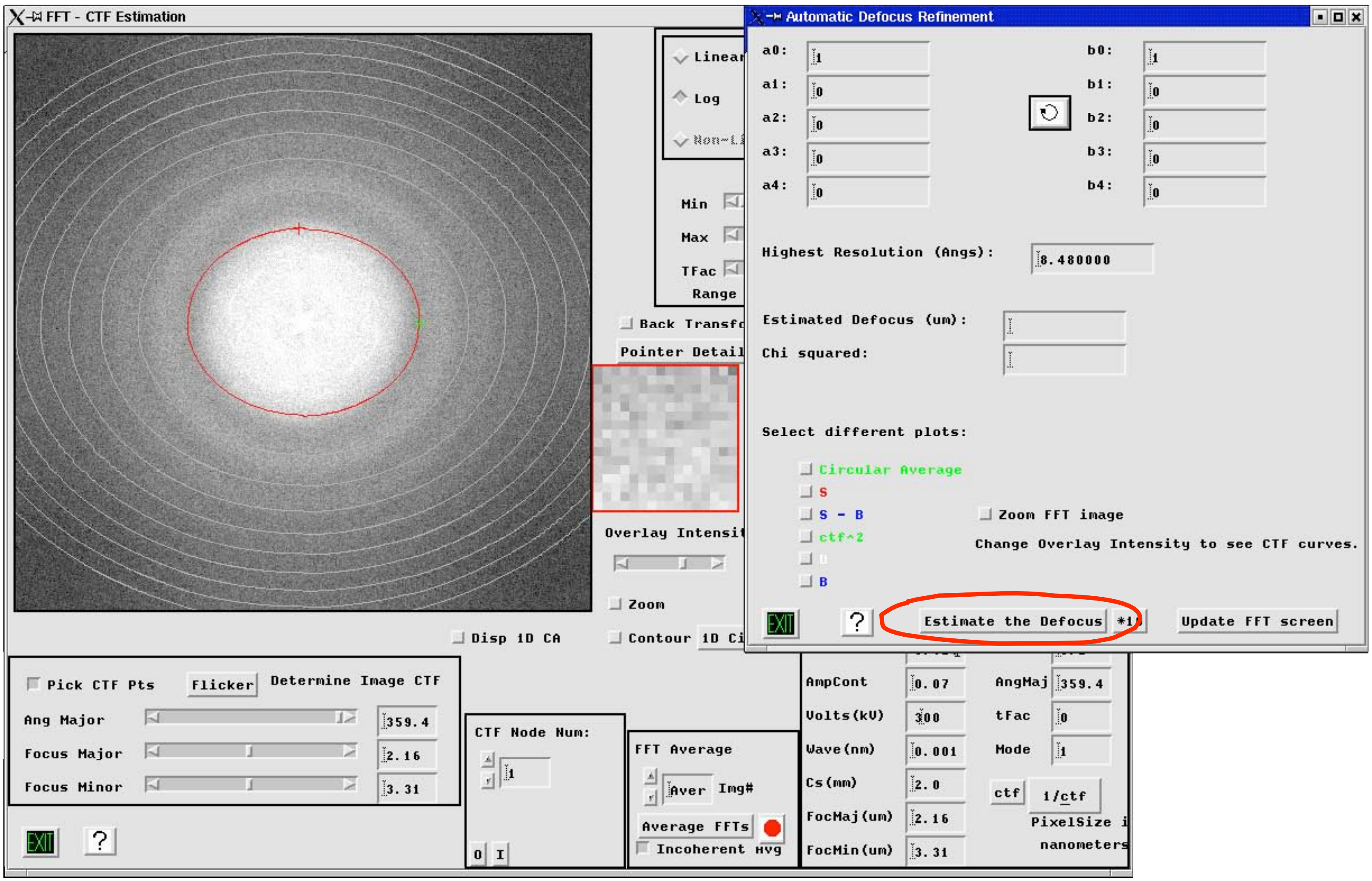

Icosahedral Virus 3D Reconstruction Scheme

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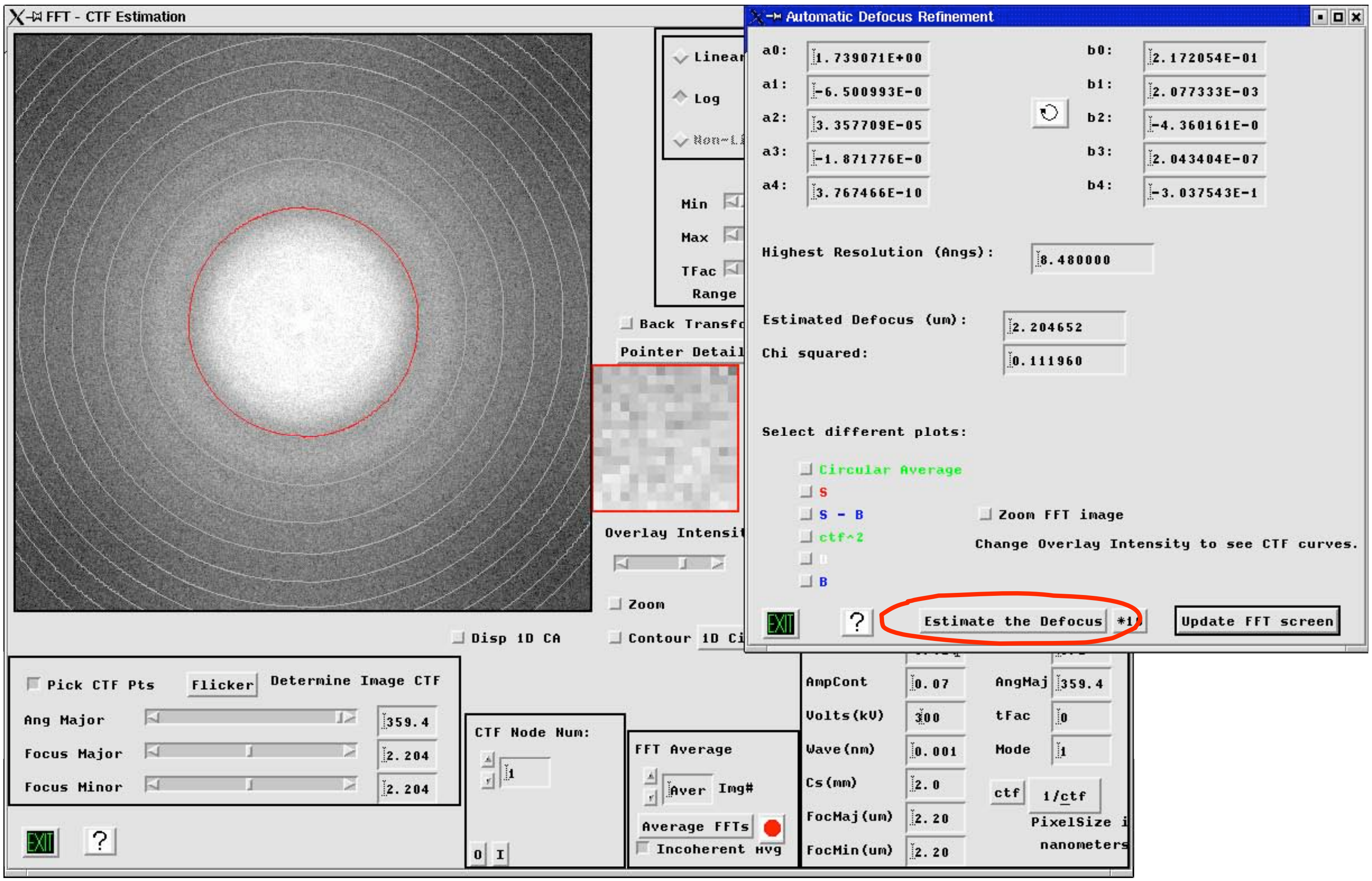

Icosahedral Virus 3D Reconstruction Scheme

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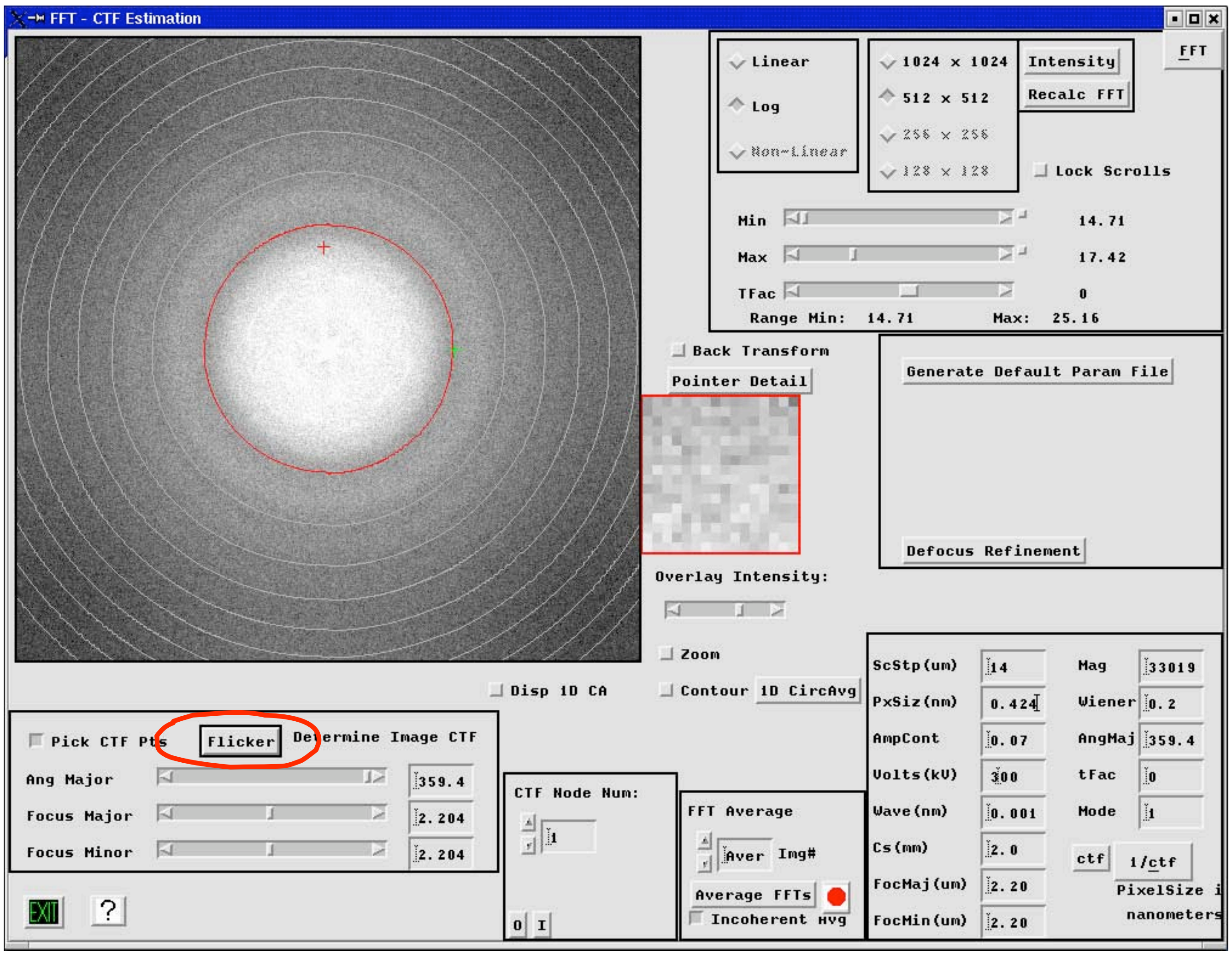

Icosahedral Virus 3D Reconstruction Scheme

Pre-Process Images

Remove blemish, Remove Gradient

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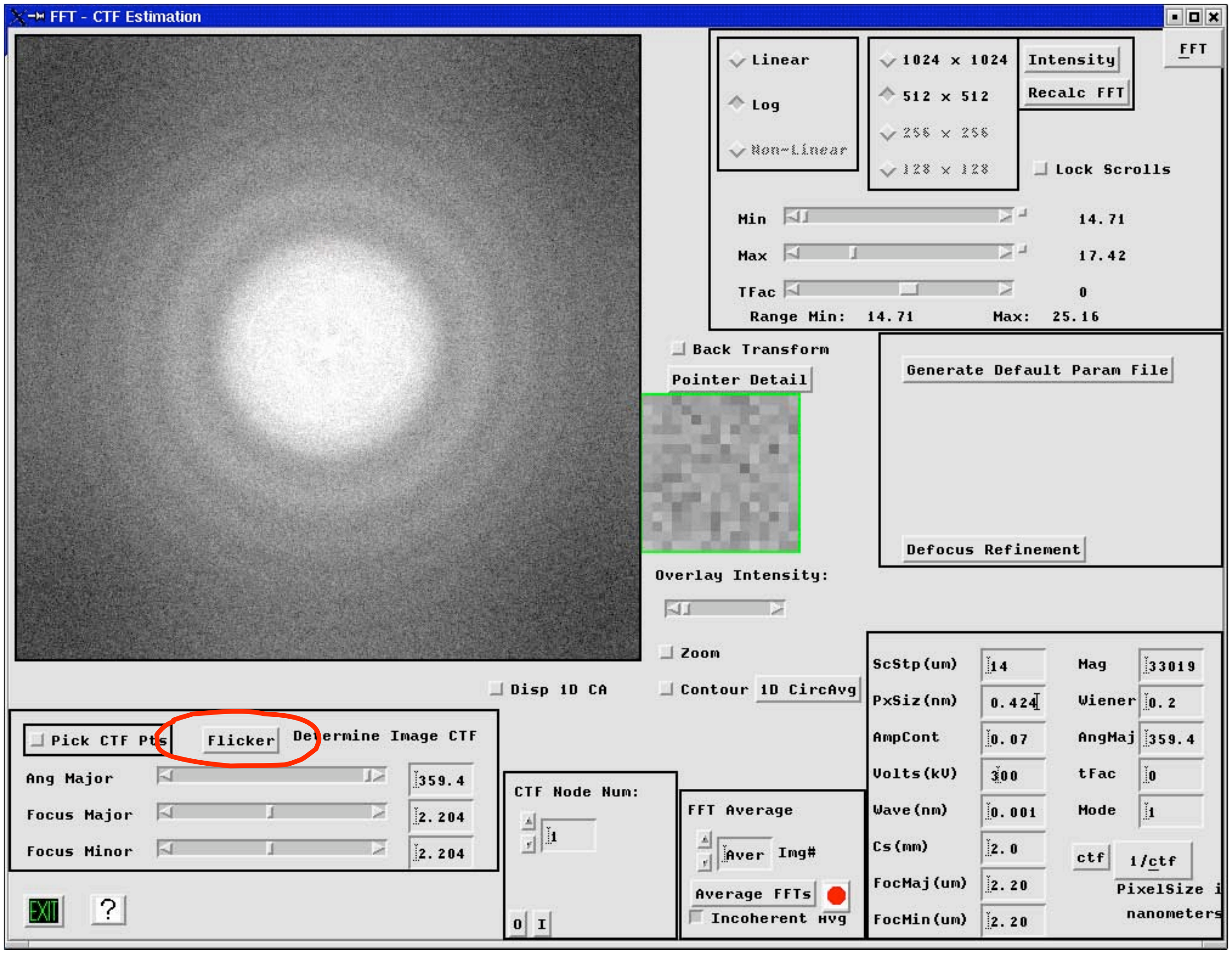
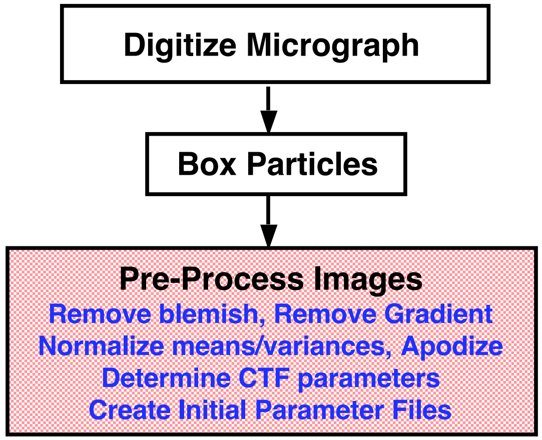
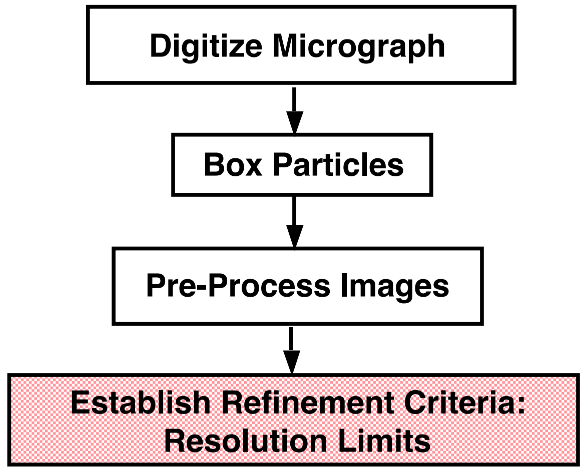


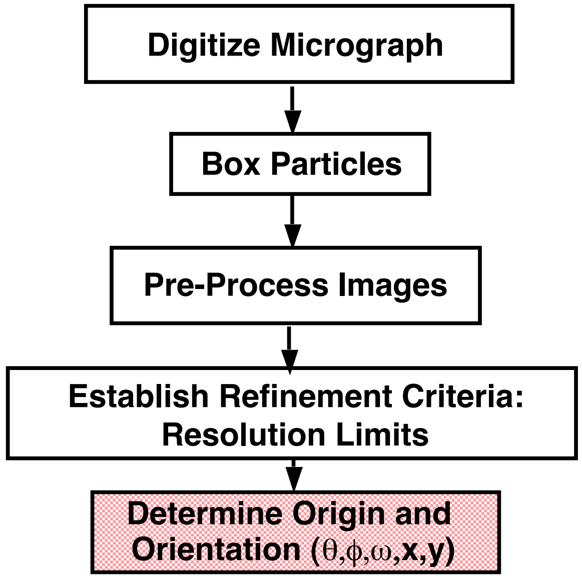
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

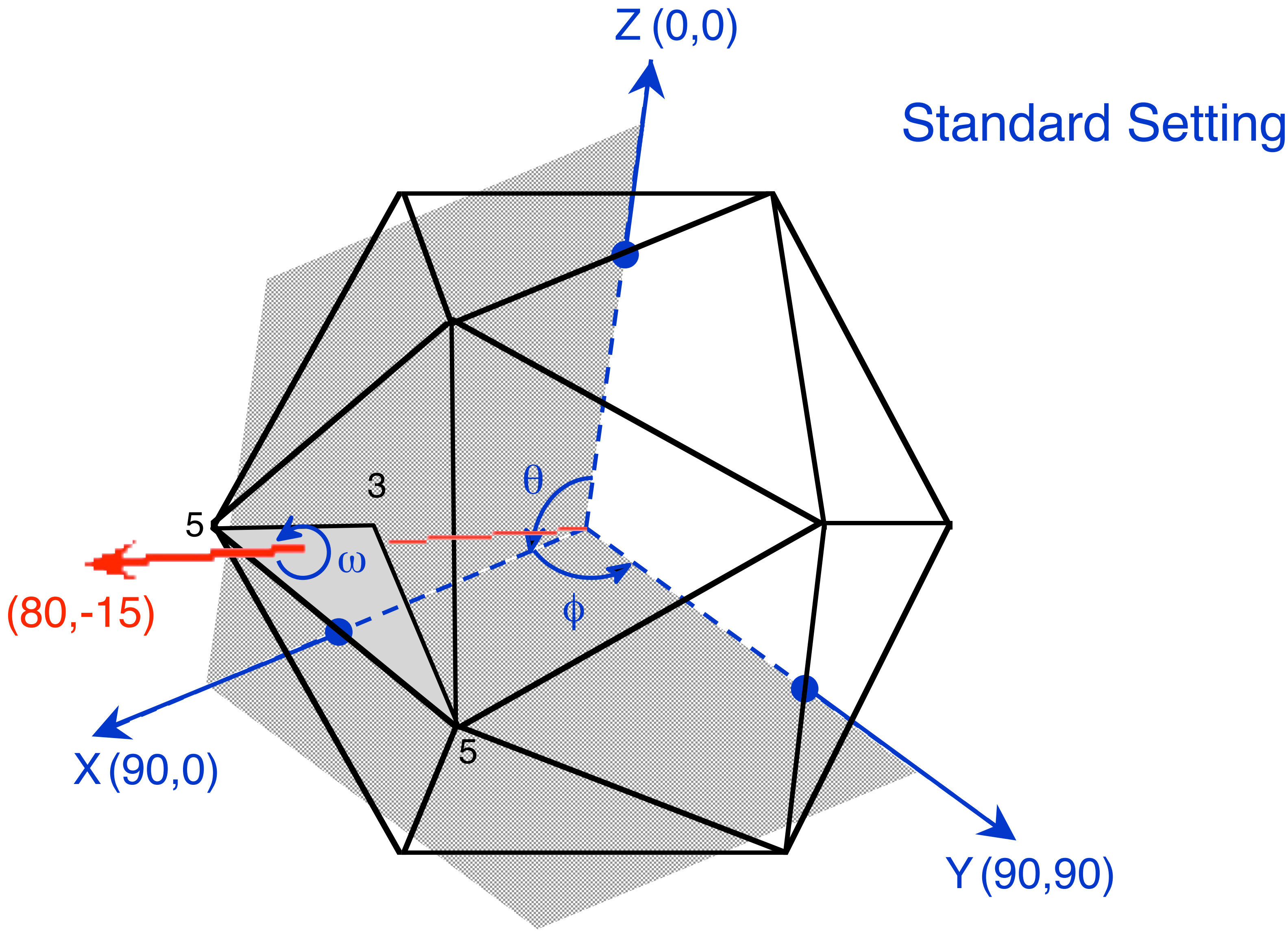
Determine Origin and Orientation (θ,ϕ,ω,x,y)

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

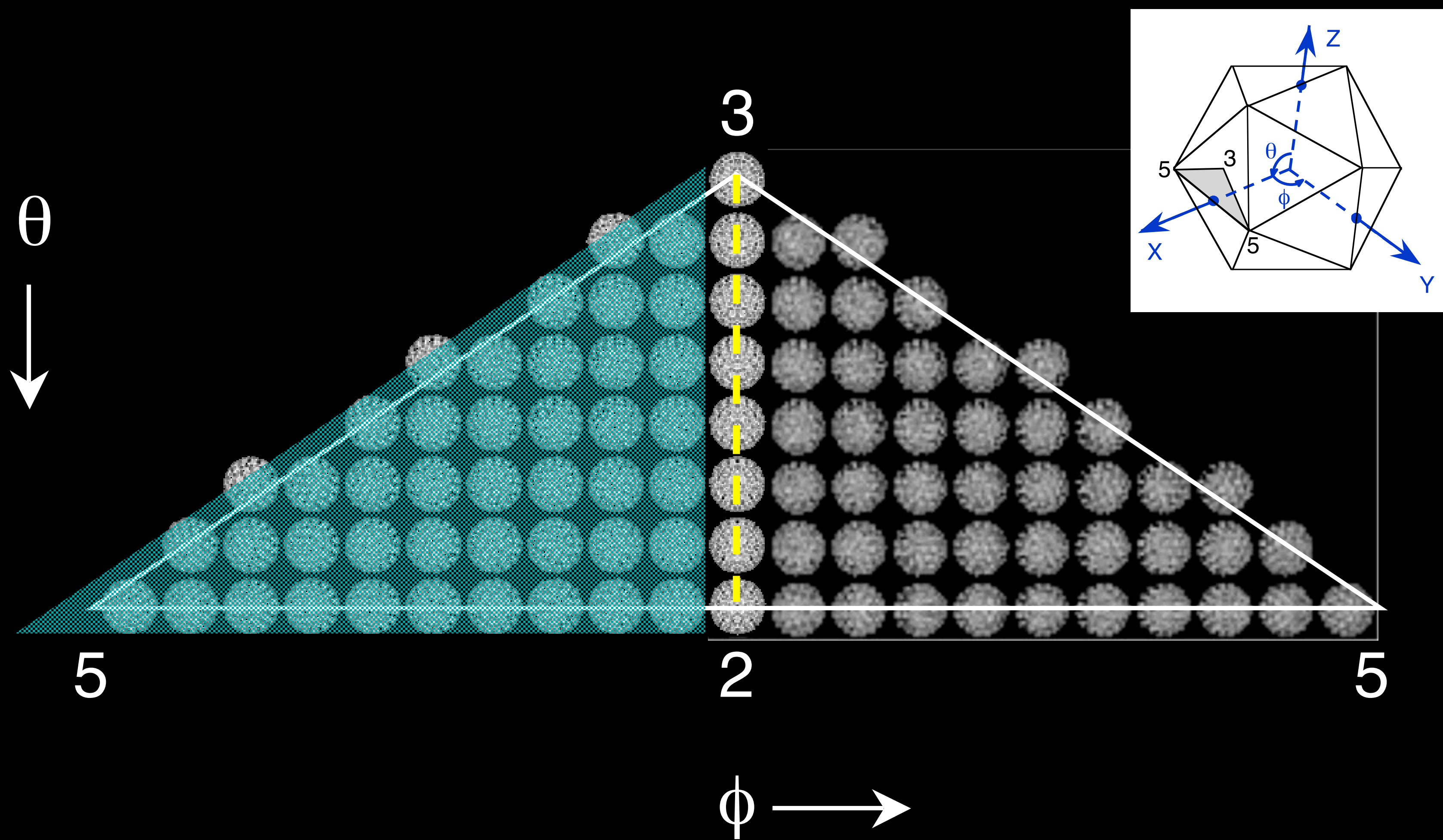
MOST IMPORTANT STEP?

Garbage in ------> garbage out

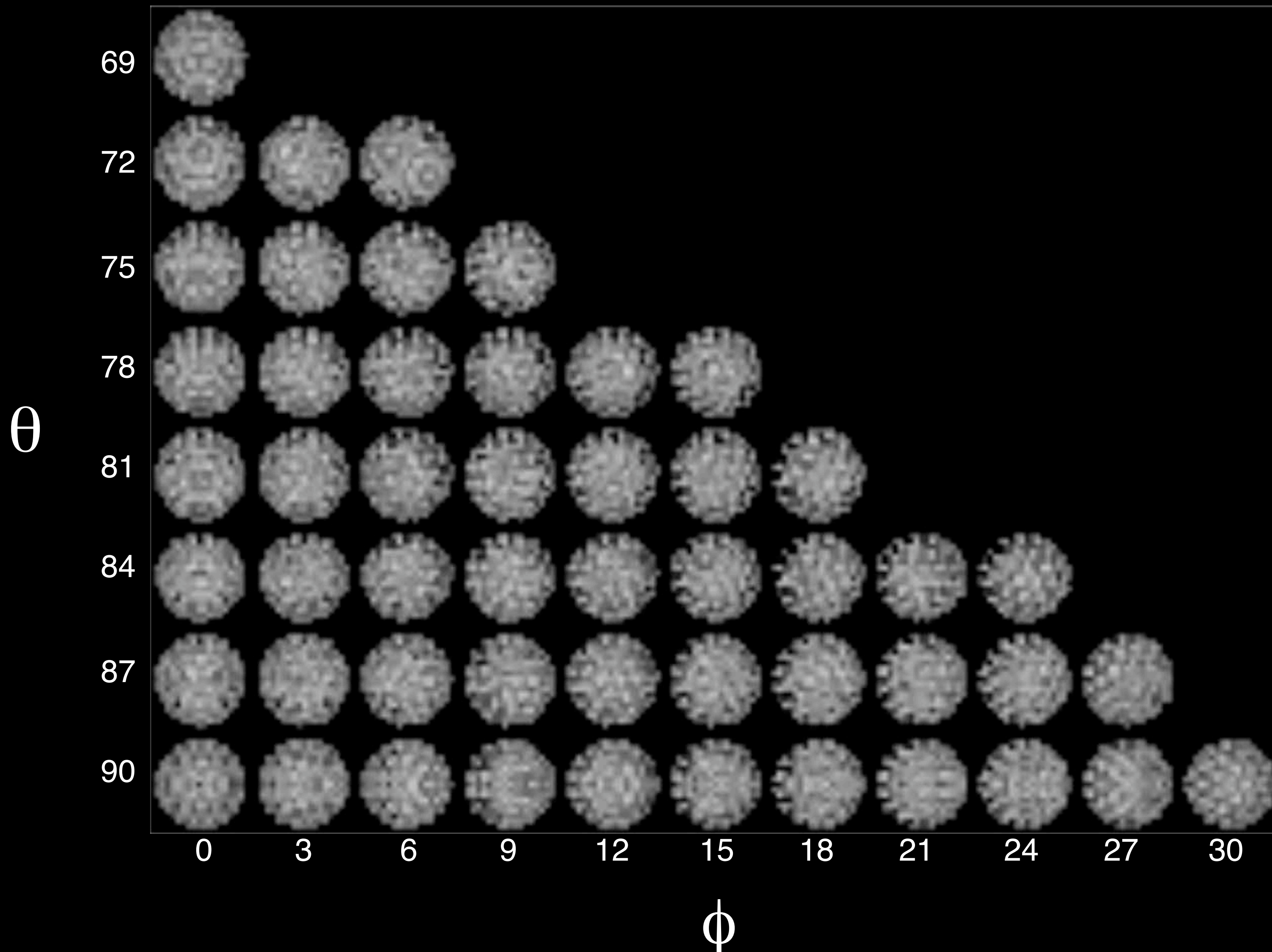
Specifying Direction of View: (θ,ϕ,ω) Orientation



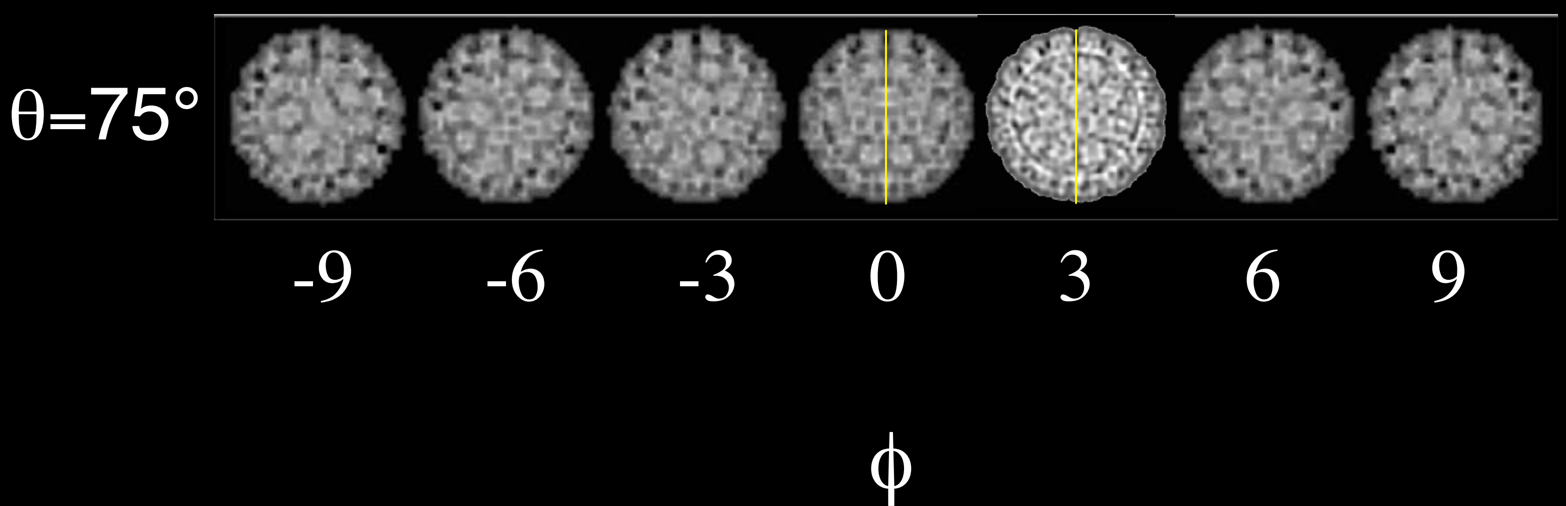
BPV Projections: Icosahedral ASU



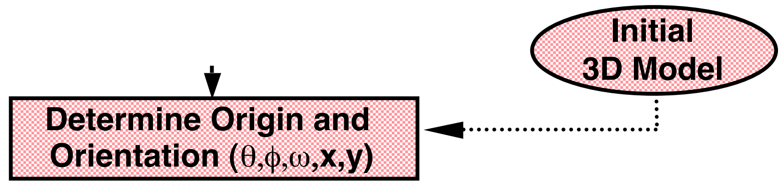
BPV Projections: 1/2 Icosahedral ASU



±ϕ Images Related by Mirror Symmetry



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

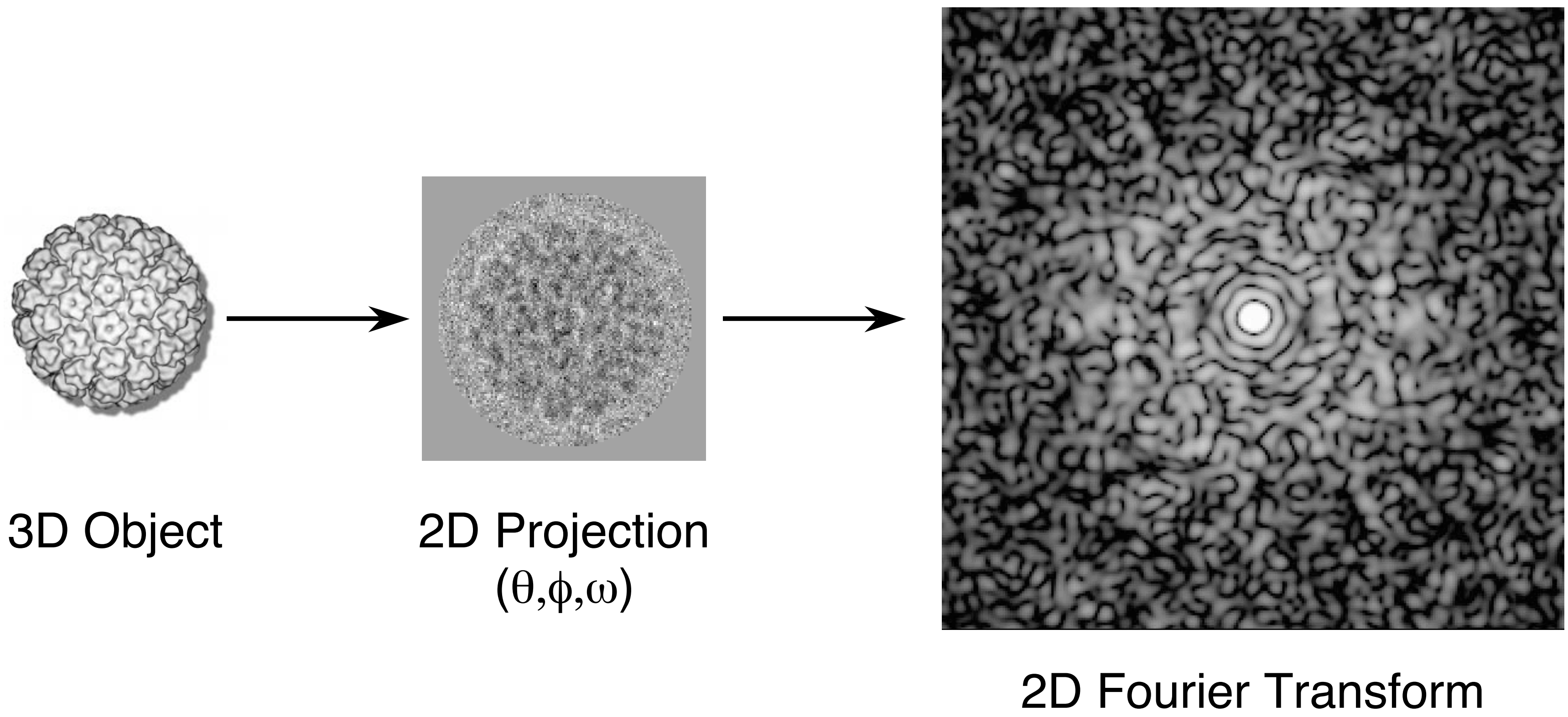
The 'gospel' continued:

"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"

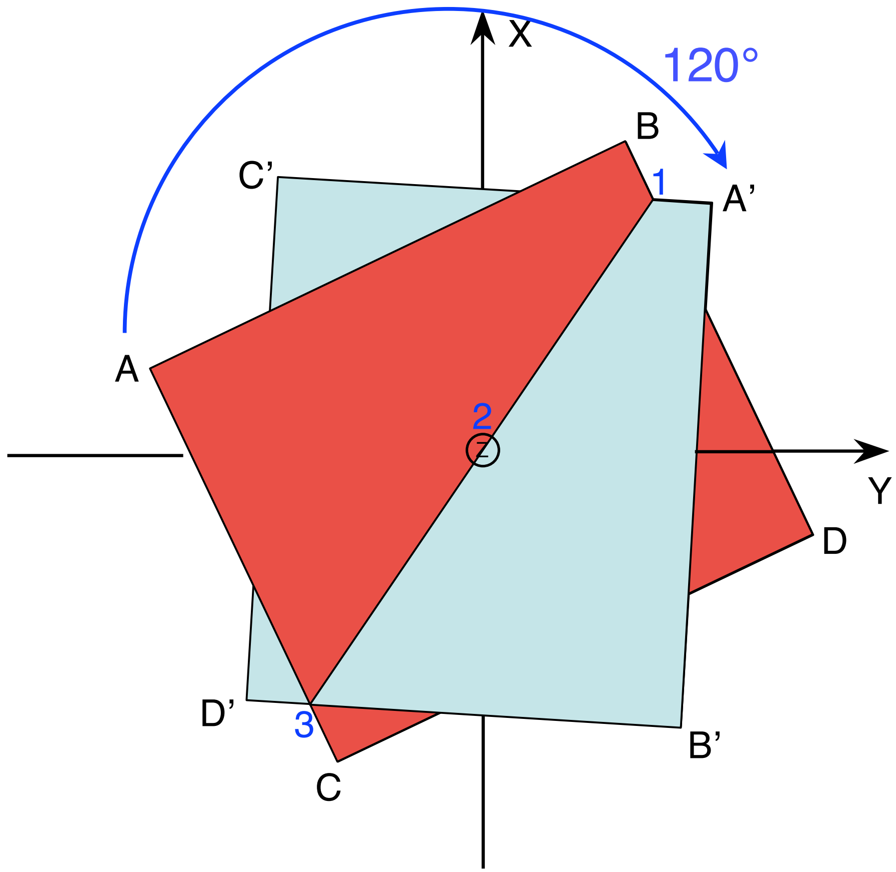
"The angular positions of these lines are dependent on the orientation of the particle."

Orientation Determination by Common Lines



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

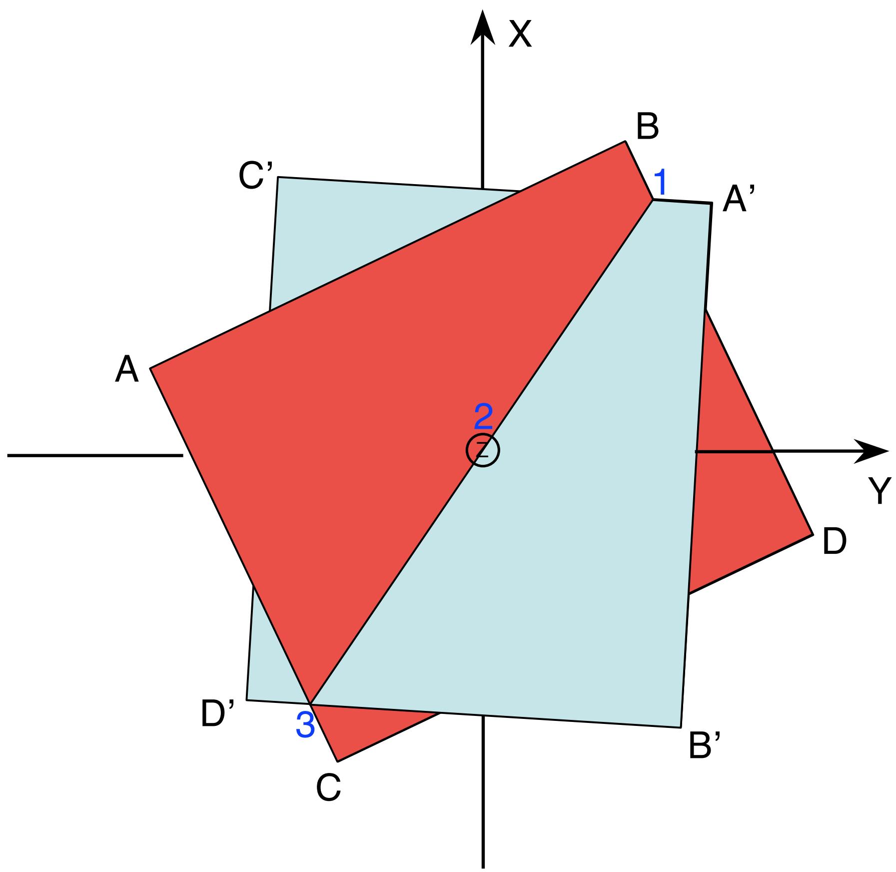
Let z-direction coincide with **3-fold** axis of symmetry

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

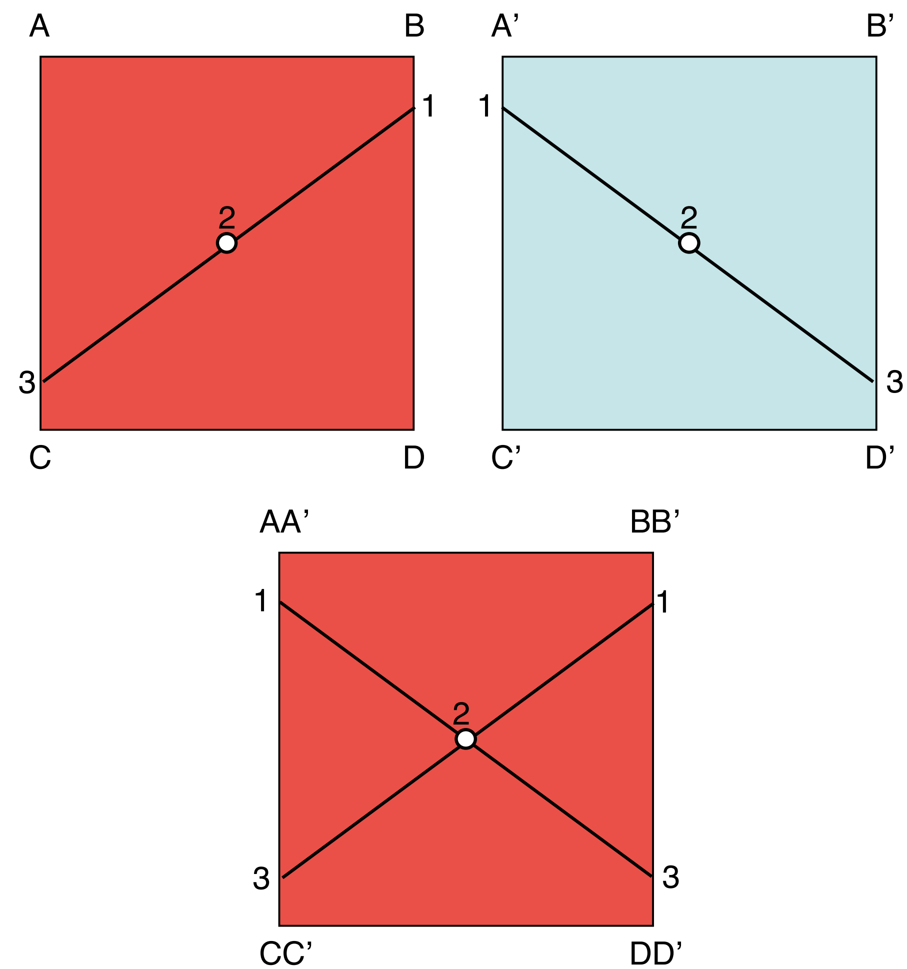
Both planes have **common values** along the **line** (1,2,3) of their intersection

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

Orientation Determination by Common Lines

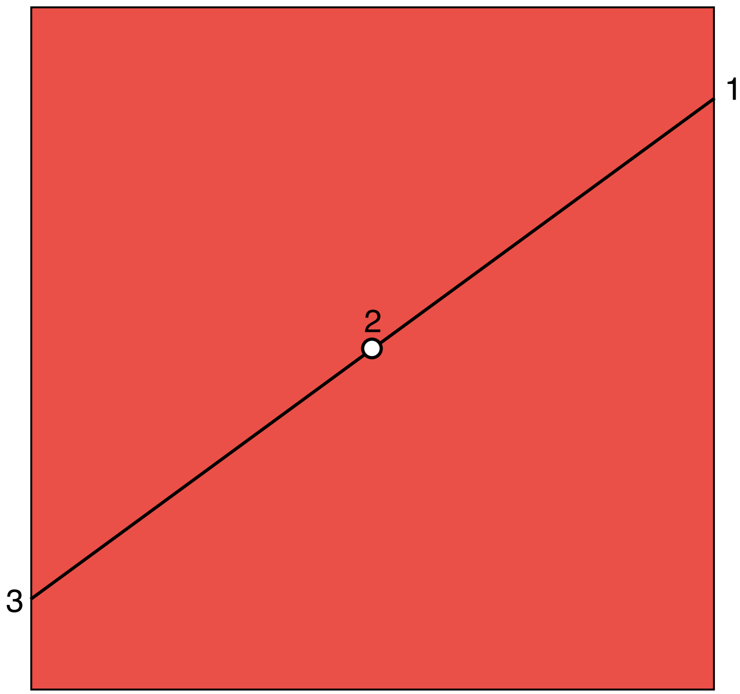


Adapted from Moody (1990) Fig. 7.68, p.245

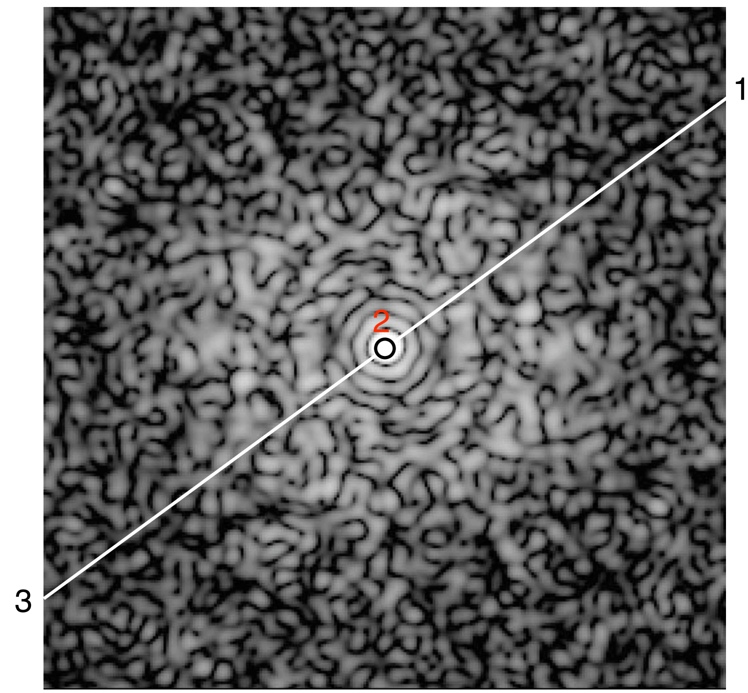


Adapted from Moody (1990) Fig. 7.69, p.246

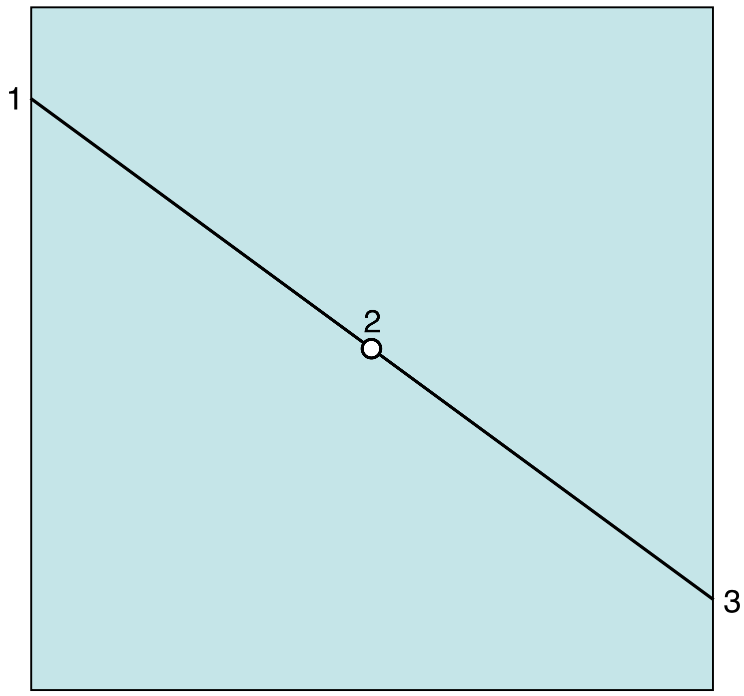
Orientation Determination by Common Lines



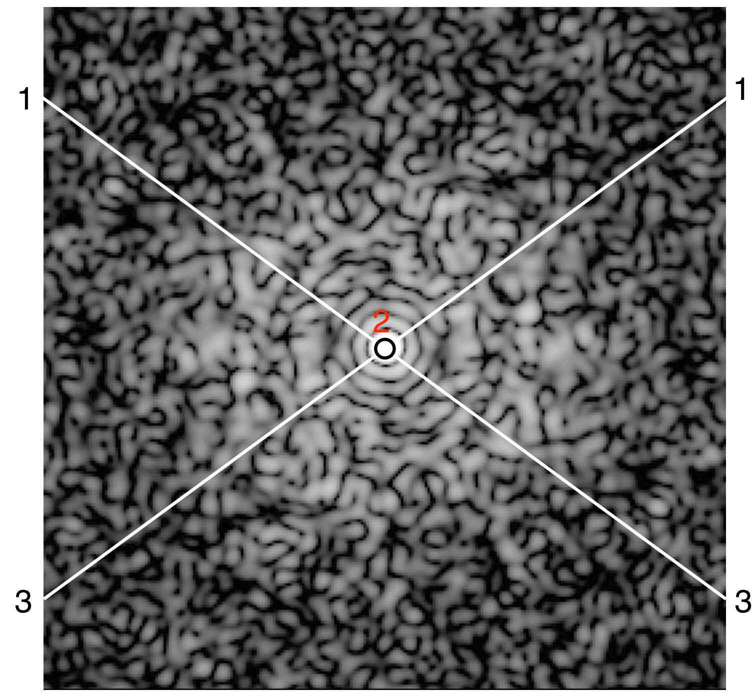
Original Transform Plane



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

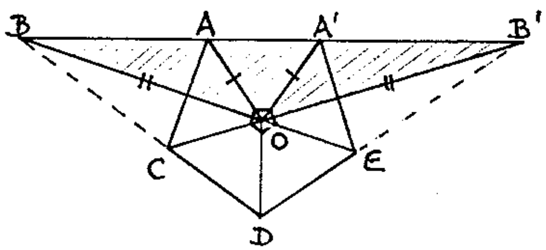
2-folds: $\frac{30}{2}$ x 1 = 15 real lines

37 common lines

III.D.5 3D Fourier Reconstruction Methods

III.D.5.a 3D Reconstruction of Objects with Icosahedral Symmetry

Orientation Determination by Common Lines

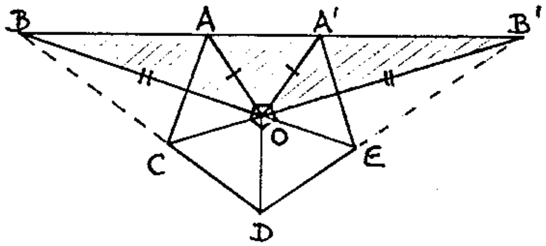


Each **5-fold** generates **2 pairs** of common lines (OA,OA') and (OB, OB')

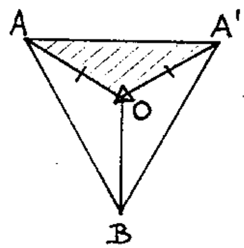
III.D.5 3D Fourier Reconstruction Methods

III.D.5.a 3D Reconstruction of Objects with Icosahedral Symmetry

Orientation Determination by Common Lines



Each **5-fold** generates **2 pairs** of common lines (OA,OA') and (OB, OB')

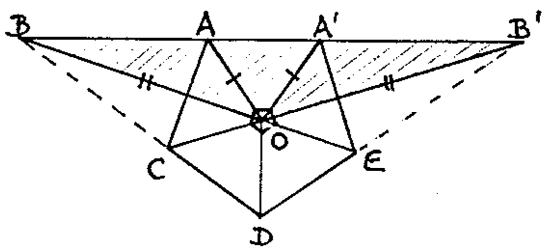


Each **3-fold** generates **1 pair** of common lines (OA,OA')

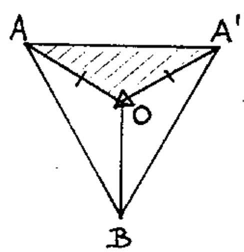
III.D.5 3D Fourier Reconstruction Methods

III.D.5.a 3D Reconstruction of Objects with Icosahedral Symmetry

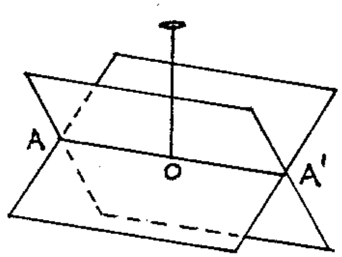
Orientation Determination by Common Lines



Each **5-fold** generates **2 pairs** of common lines (OA,OA') and (OB, OB')



Each **3-fold** generates **1 pair** of common lines (OA,OA')

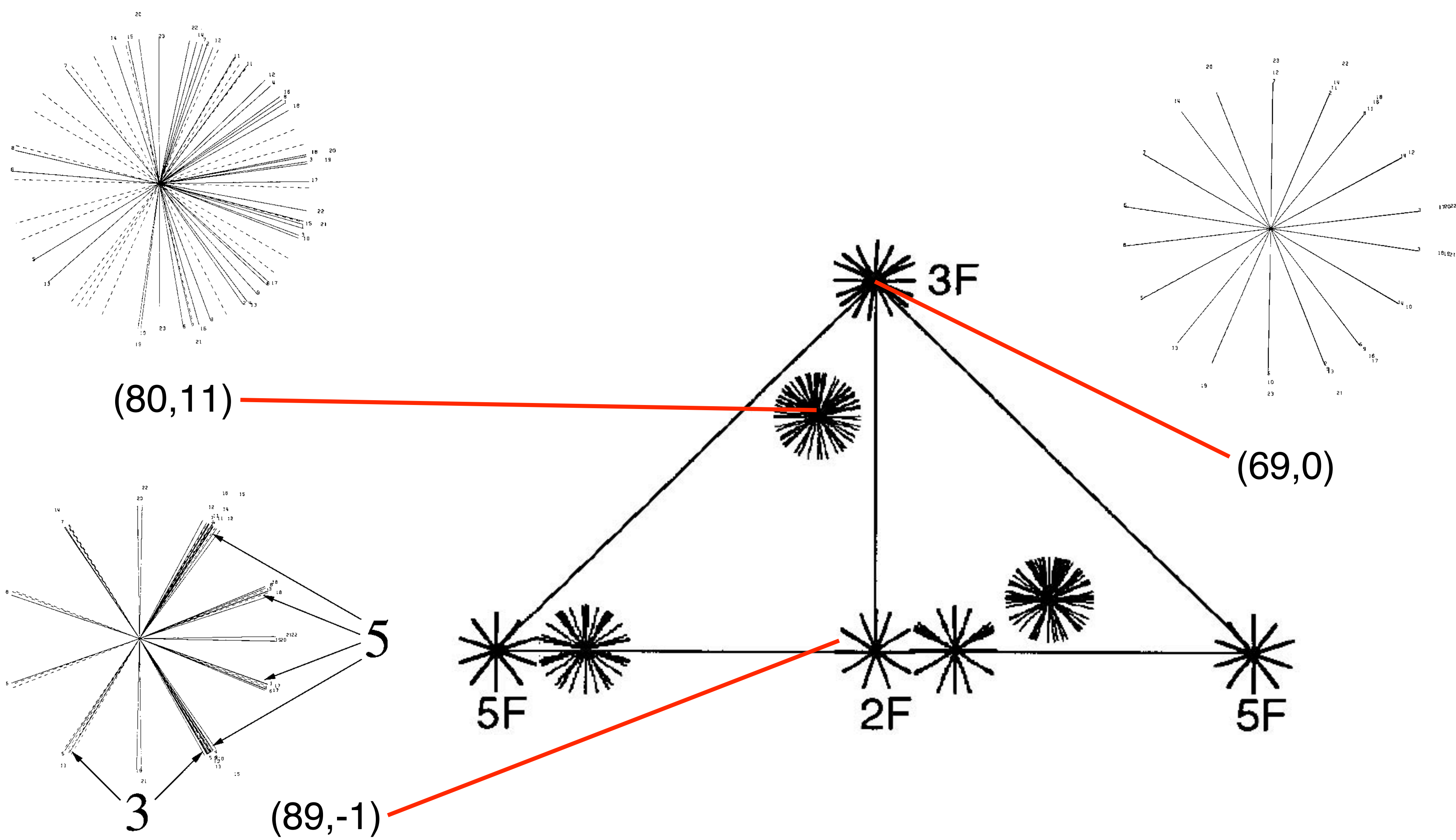


Each **2-fold** generates **1 real line**

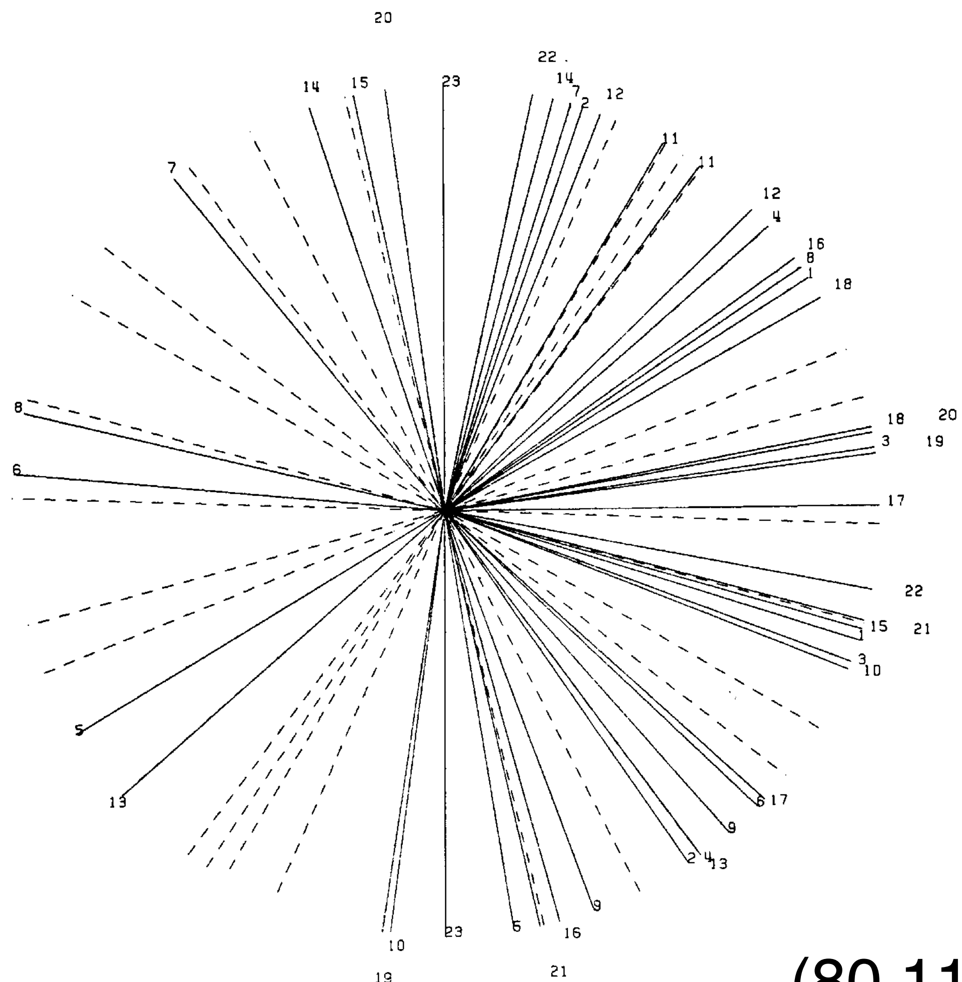
F(OA) = F(OA') = F*(OA)

2-fold Friedel

Orientation Determination by Common Lines

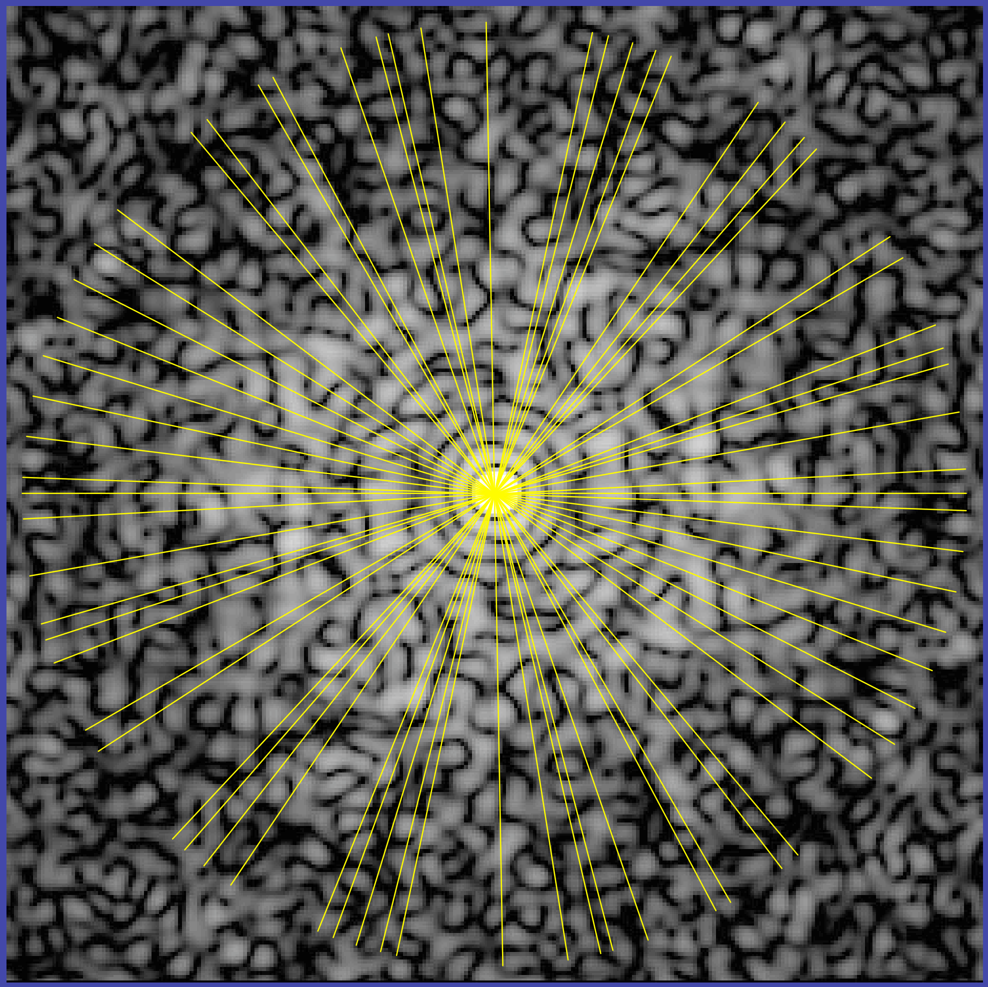


Orientation Determination by Common Lines



(80,11)

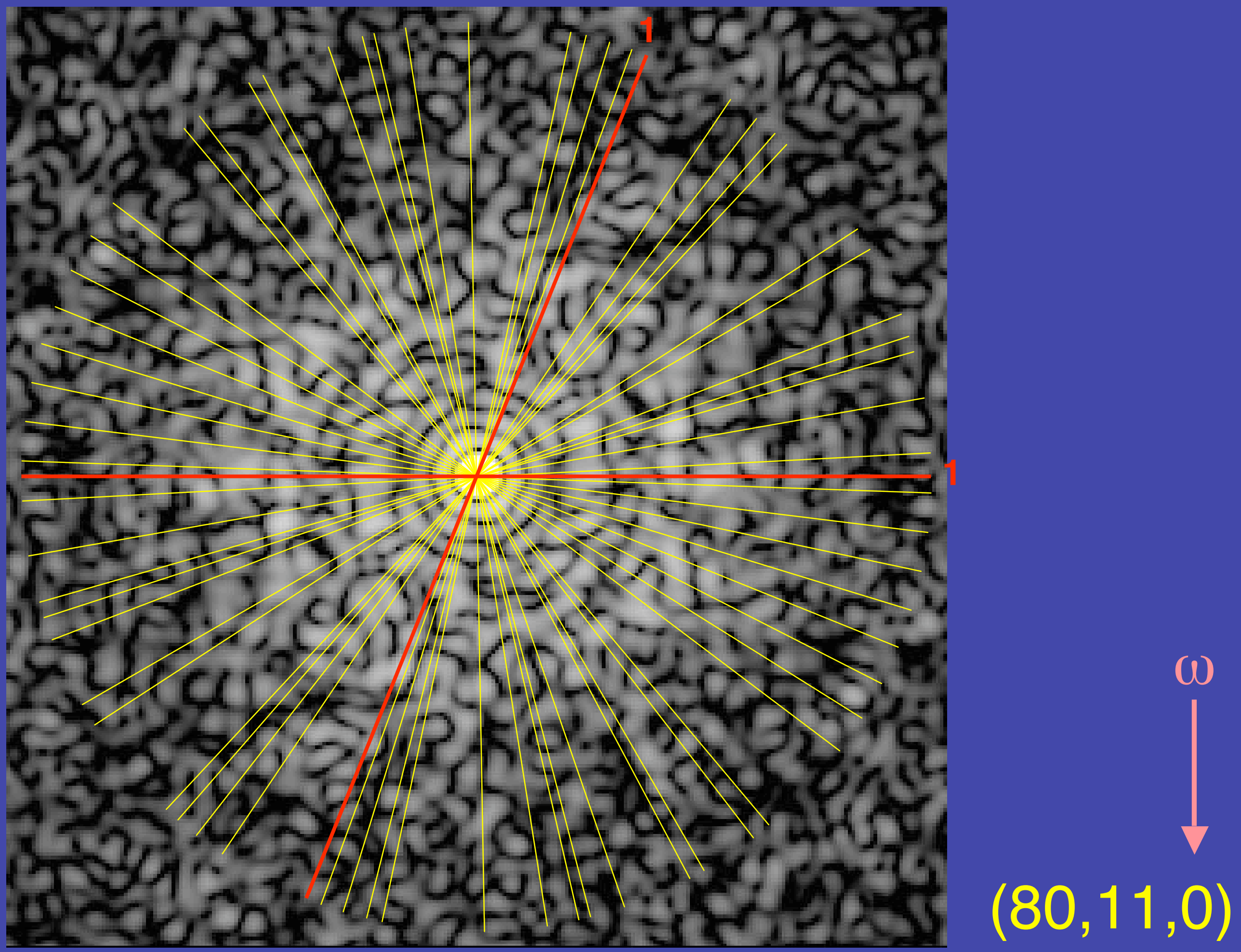
Orientation Determination by Common Lines



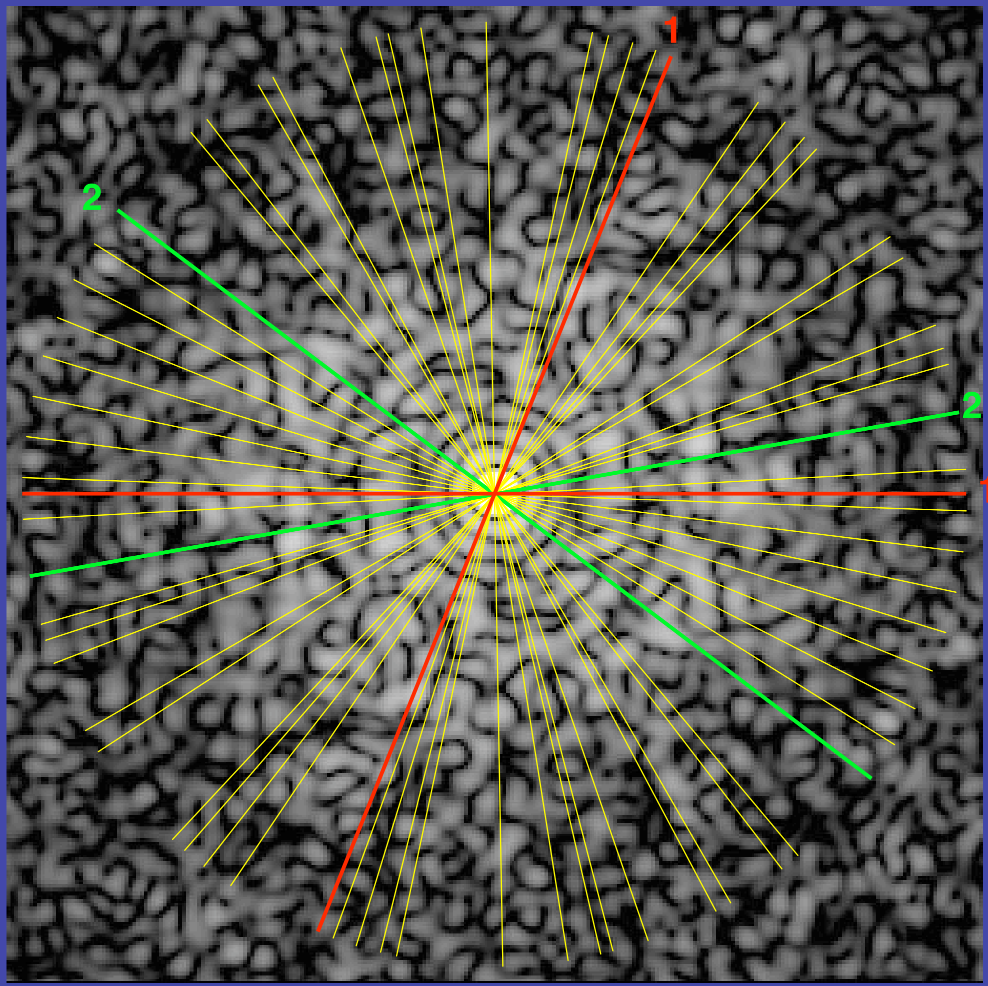
(80,11)

What is (θ,ϕ,ω) for this particle?

Orientation Determination by Common Lines



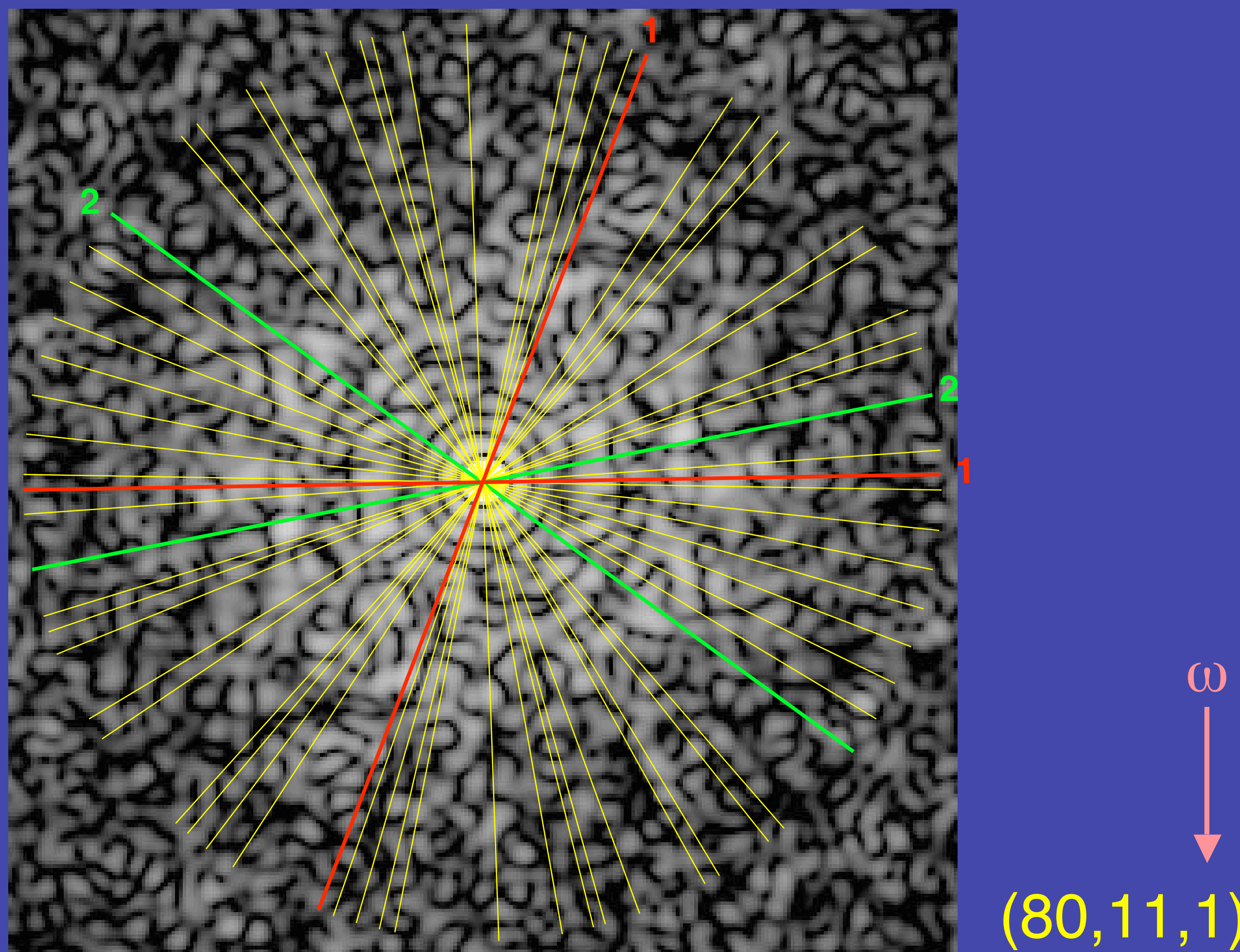
Orientation Determination by Common Lines



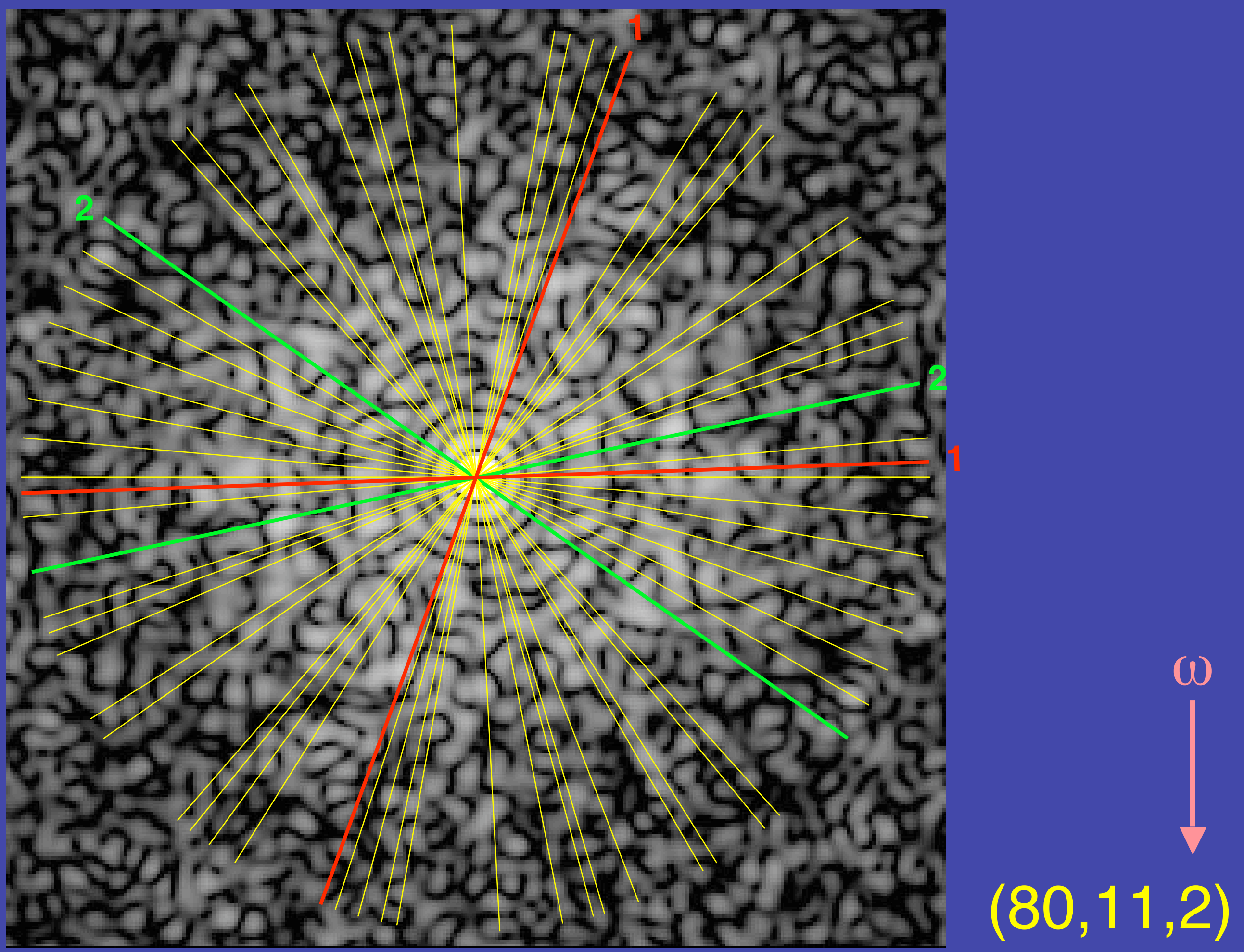
ω

(80,11,0)

Orientation Determination by Common Lines



Orientation Determination by Common Lines



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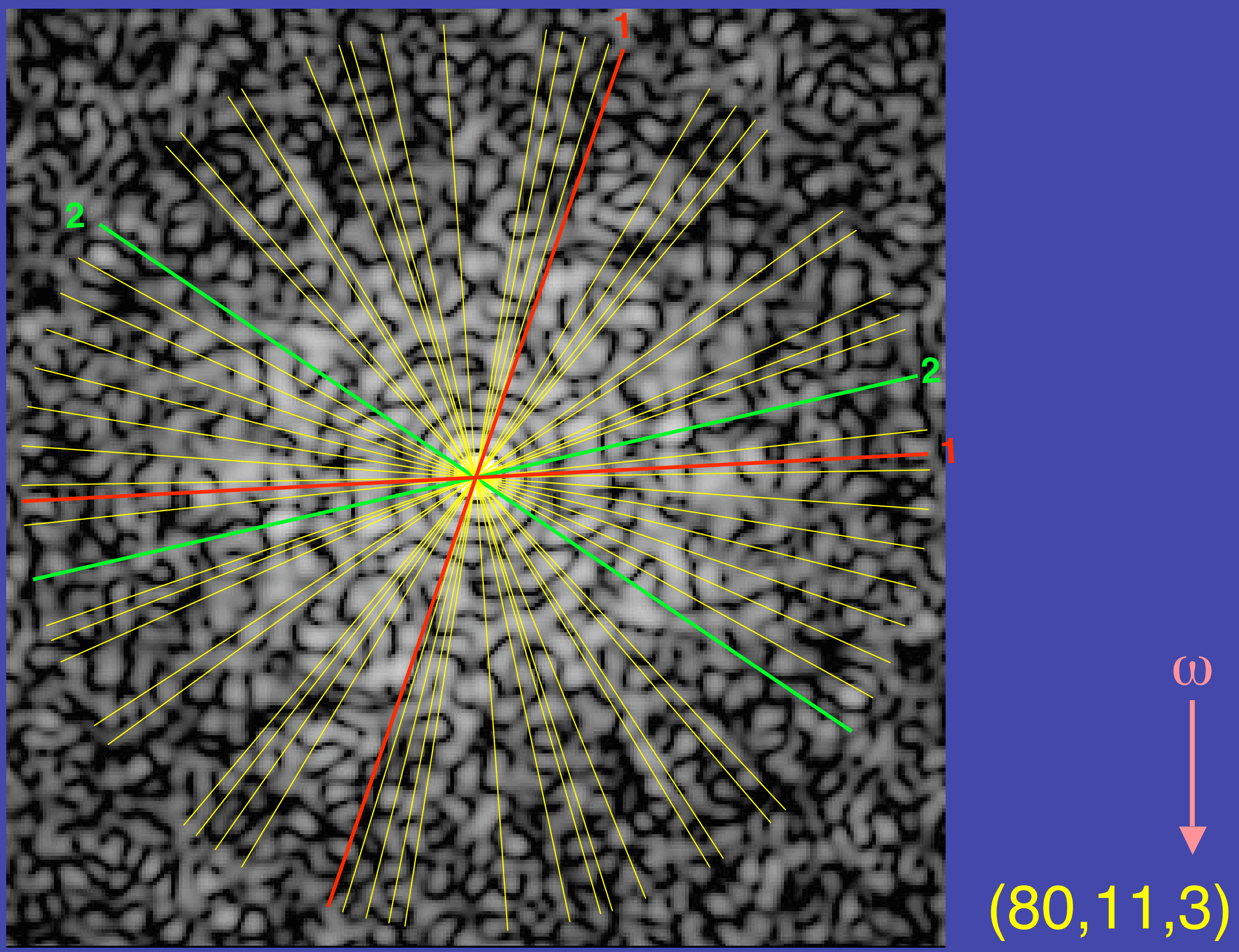

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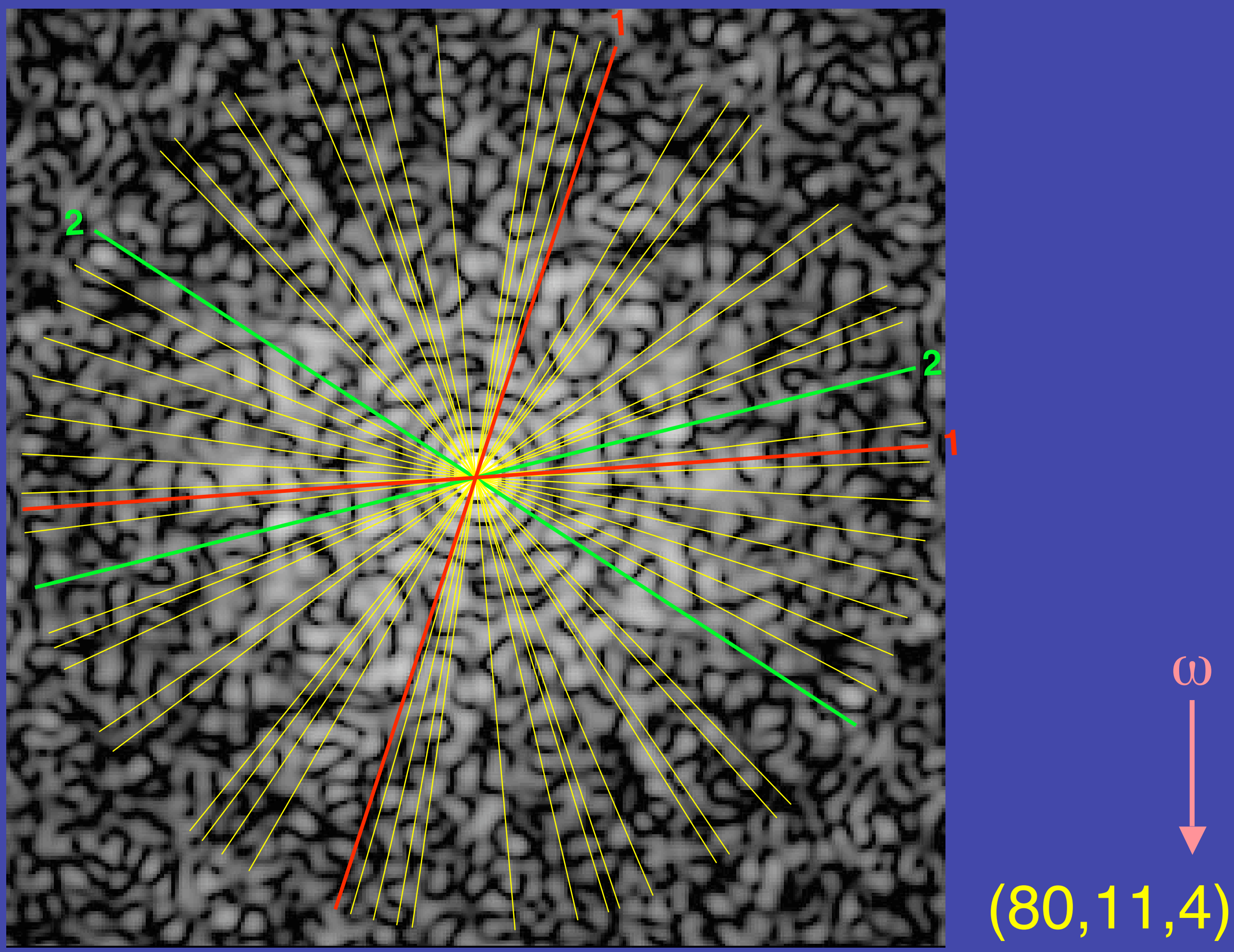
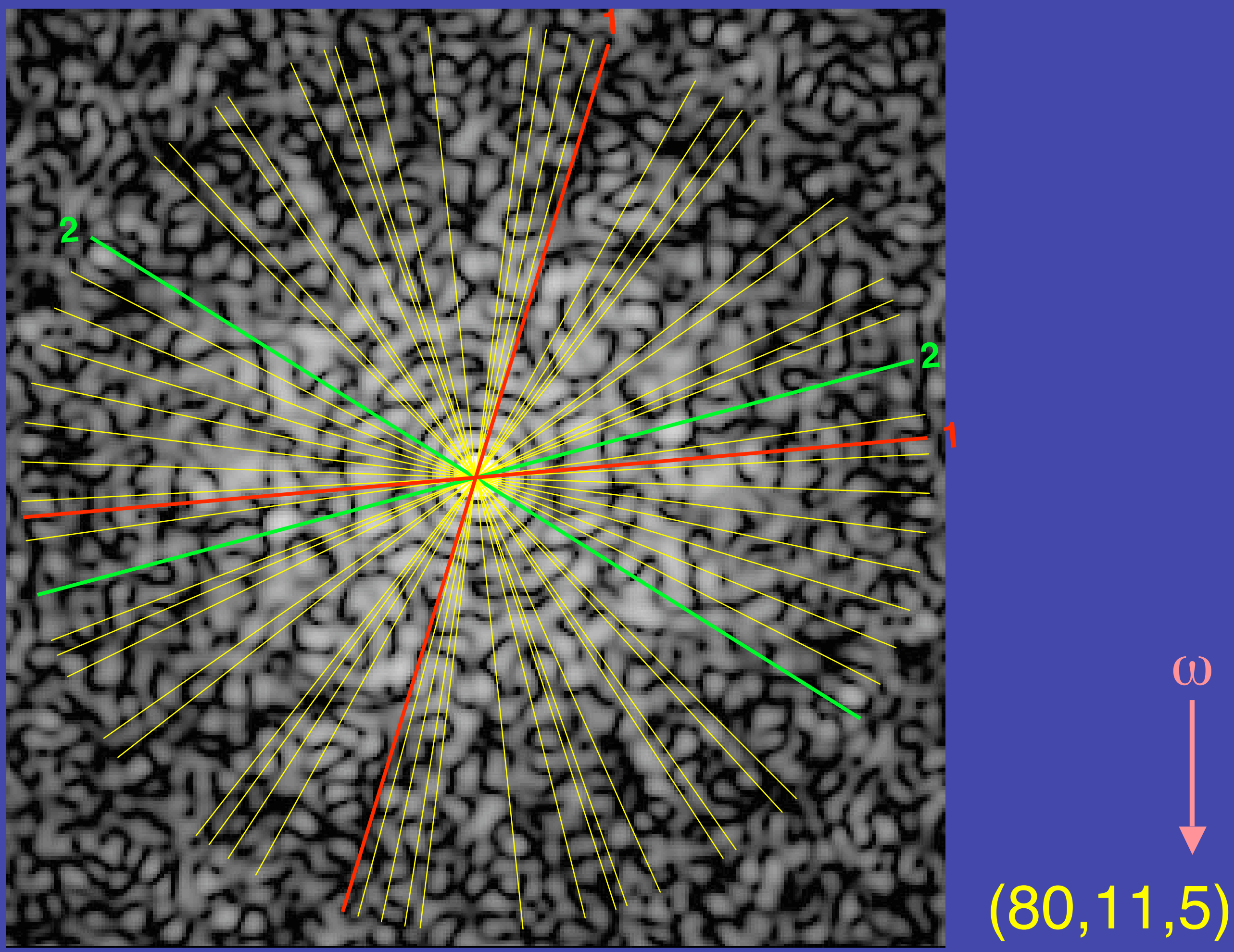


Orientation Determination by Common Lines



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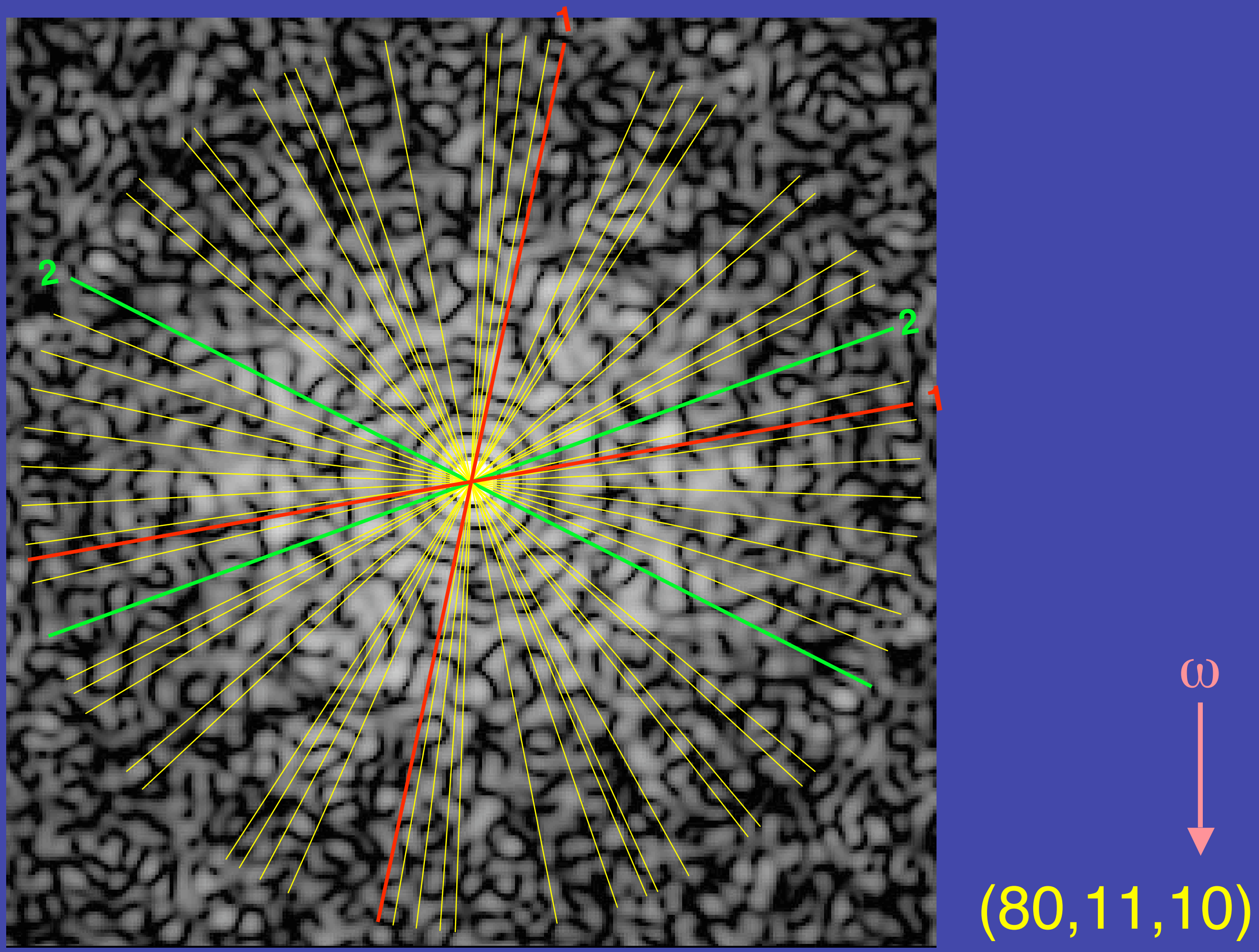
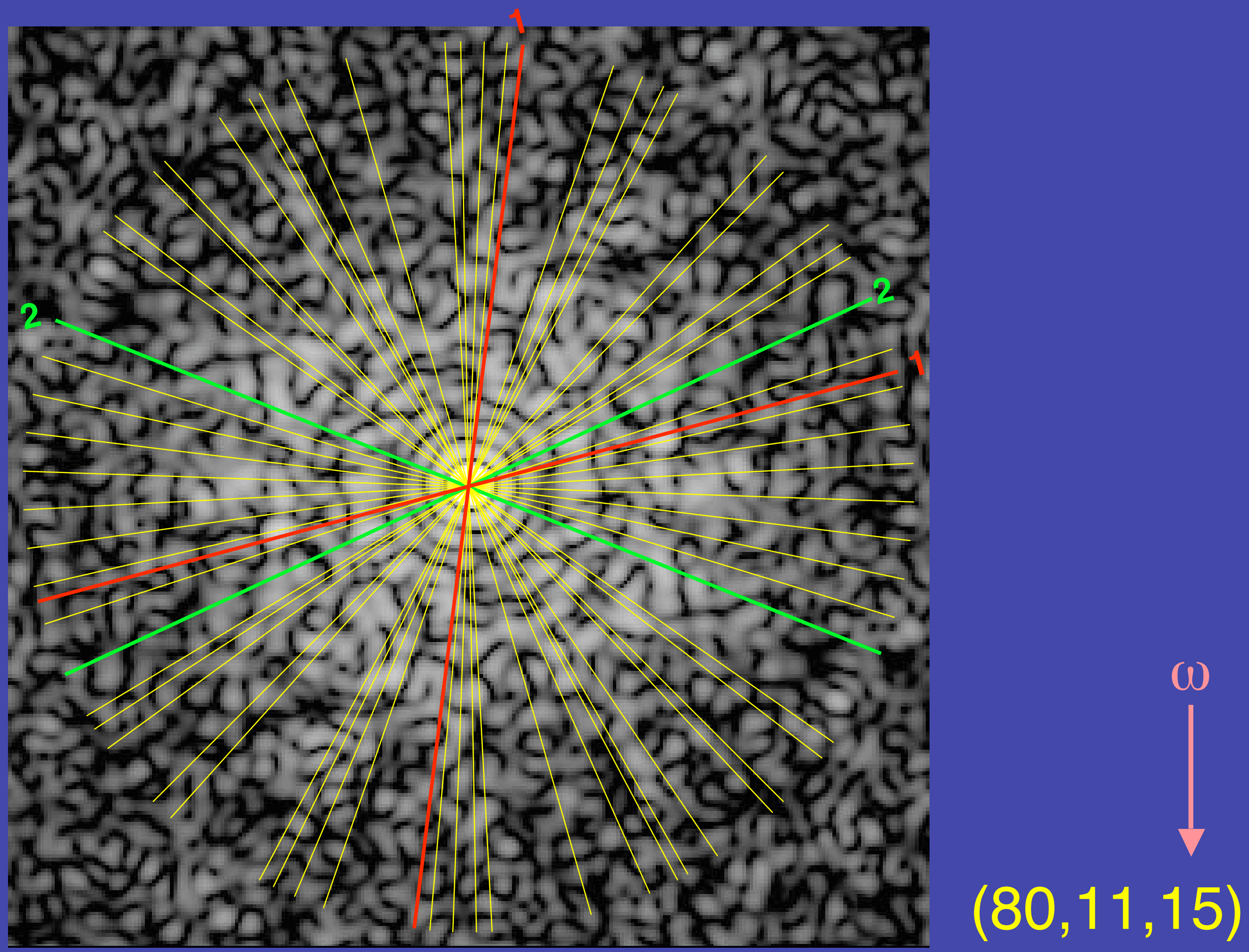


Orientation Determination by Common Lines



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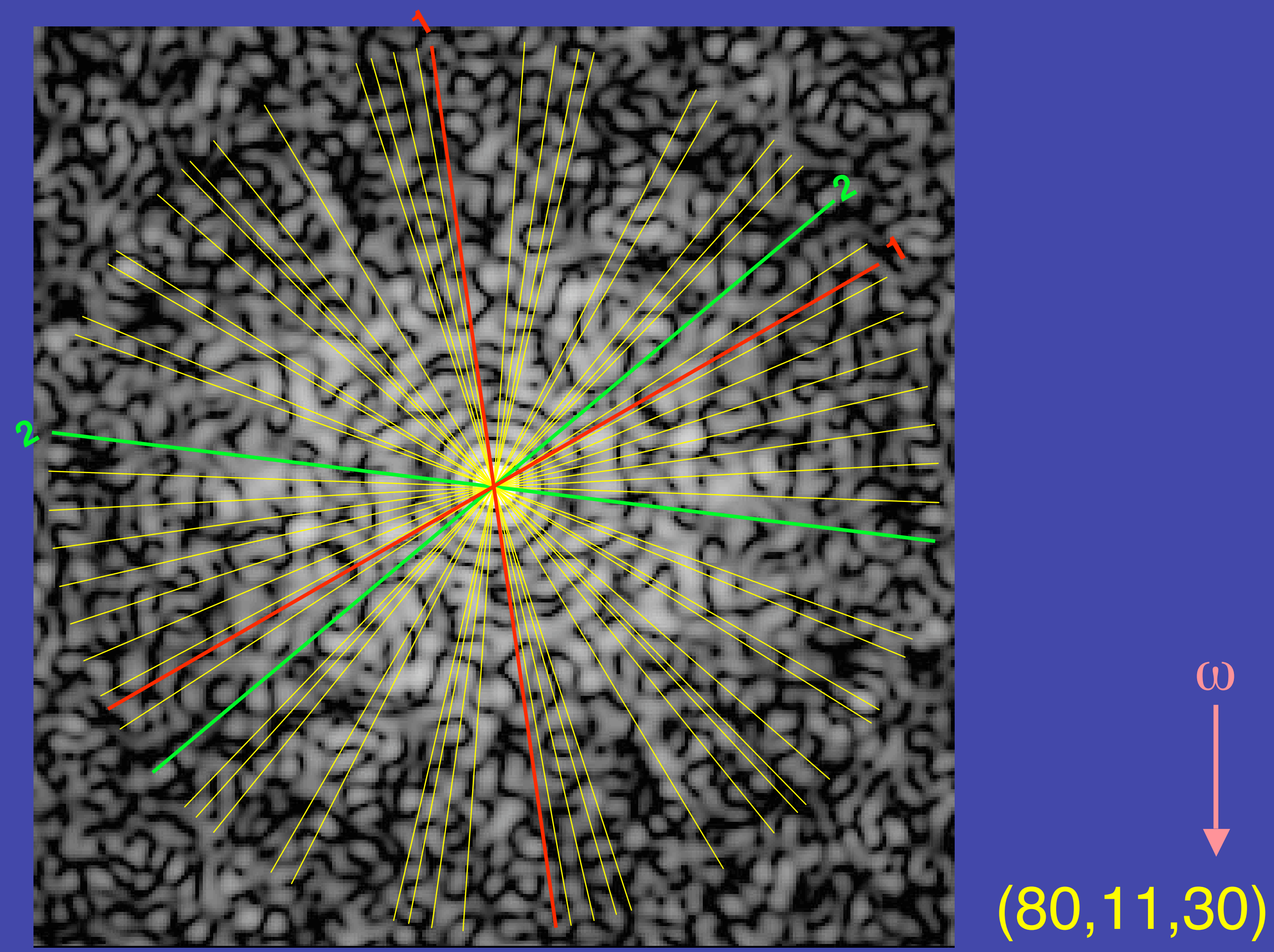
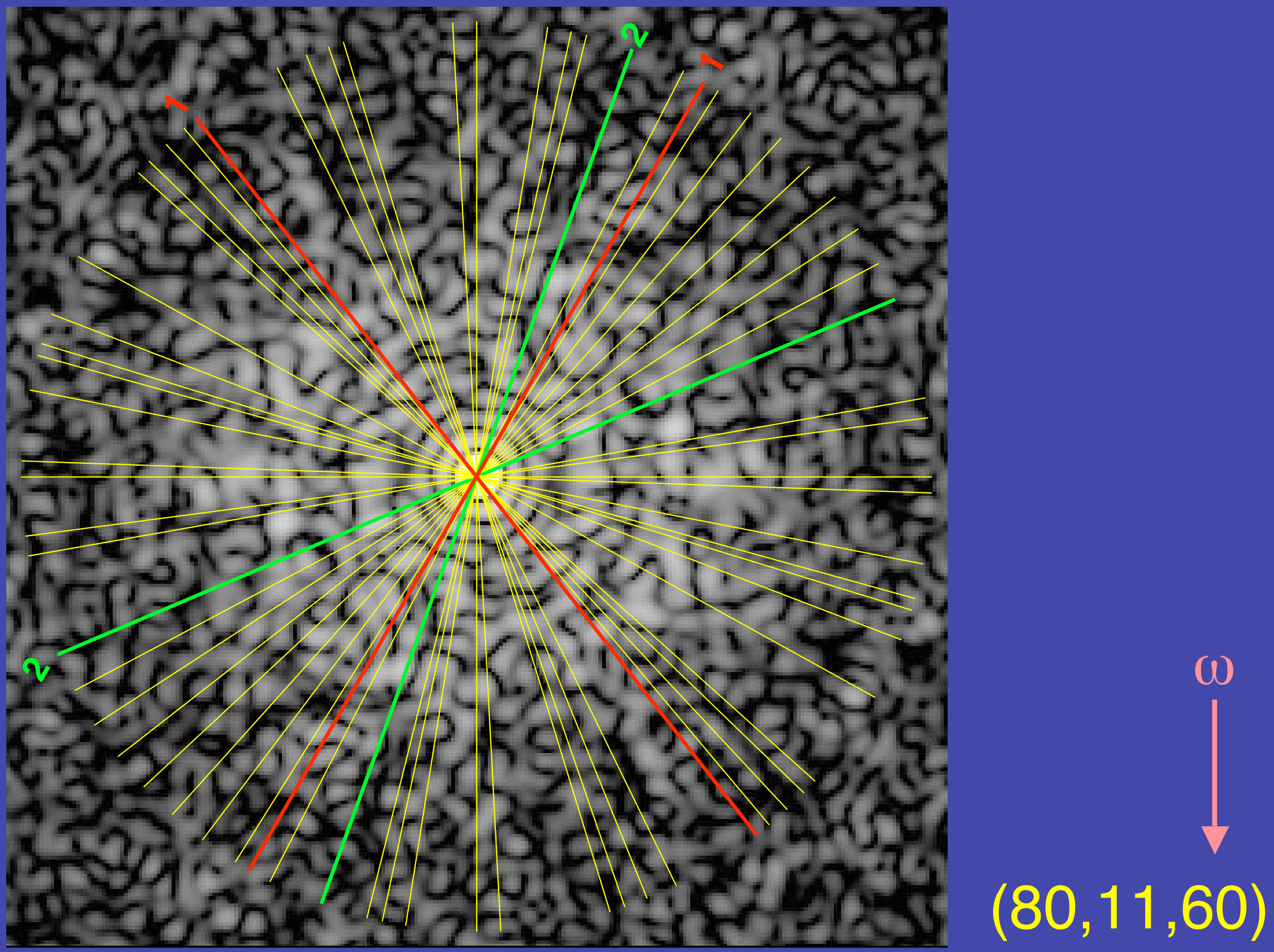
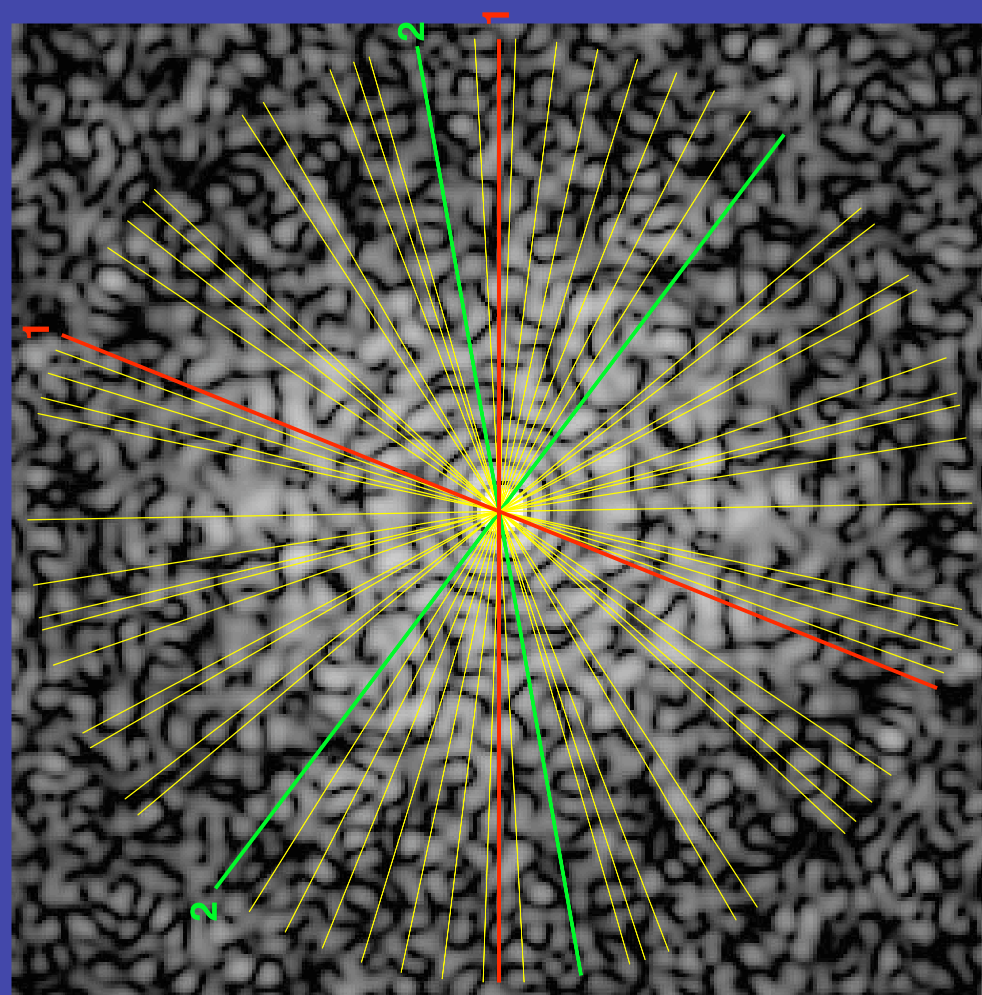


Orientation Determination by Common Lines



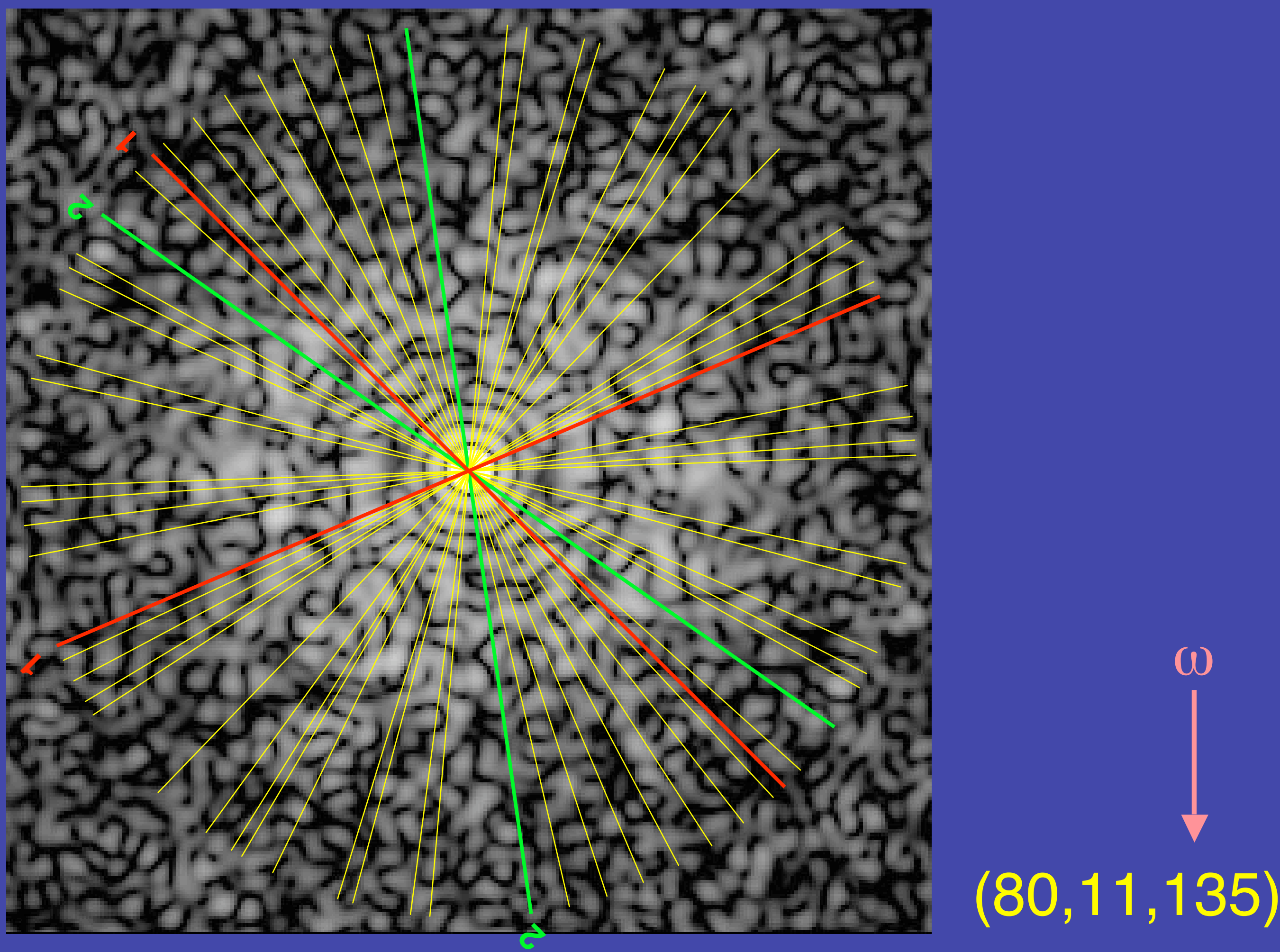
Orientation Determination by Common Lines



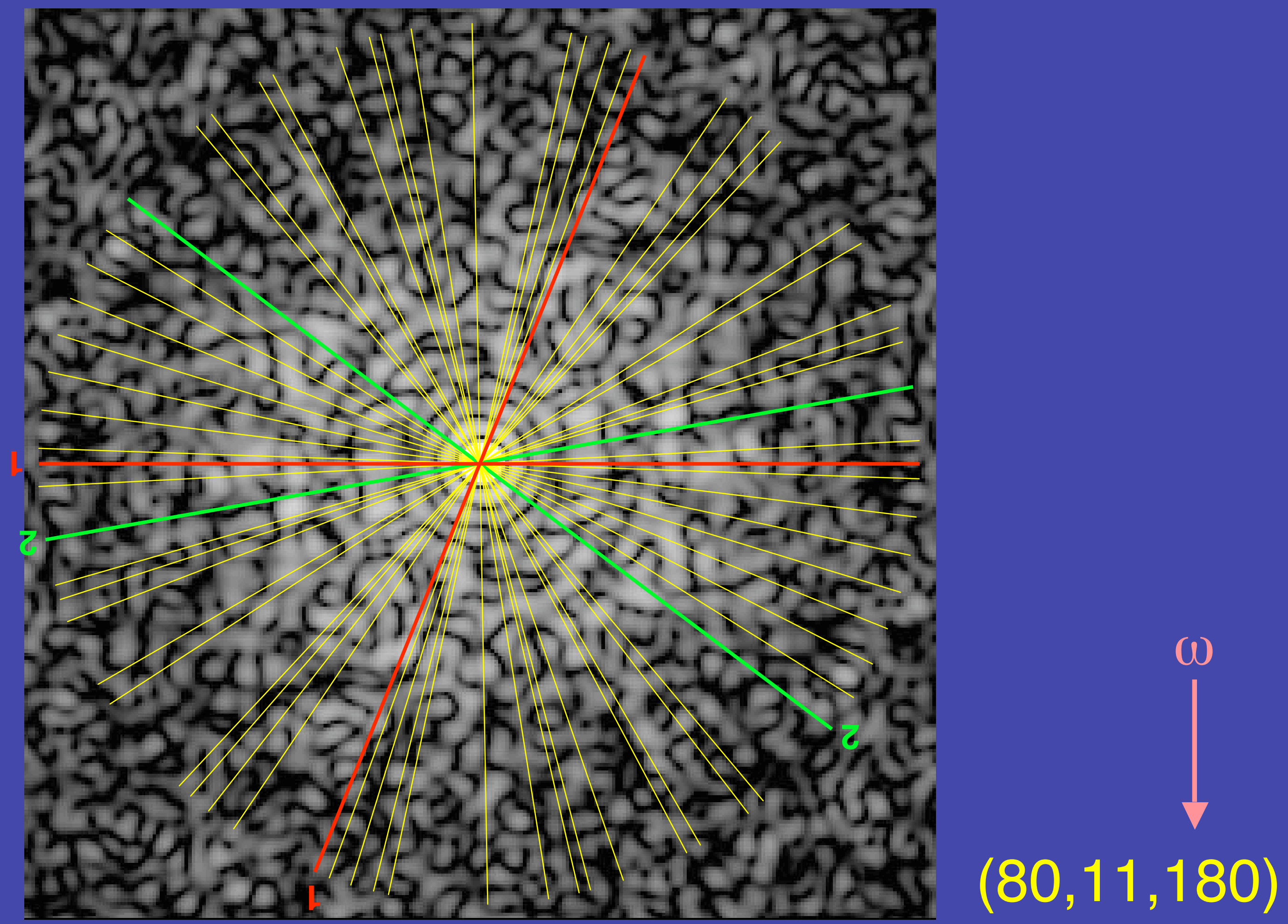
ω

(80,11,90)

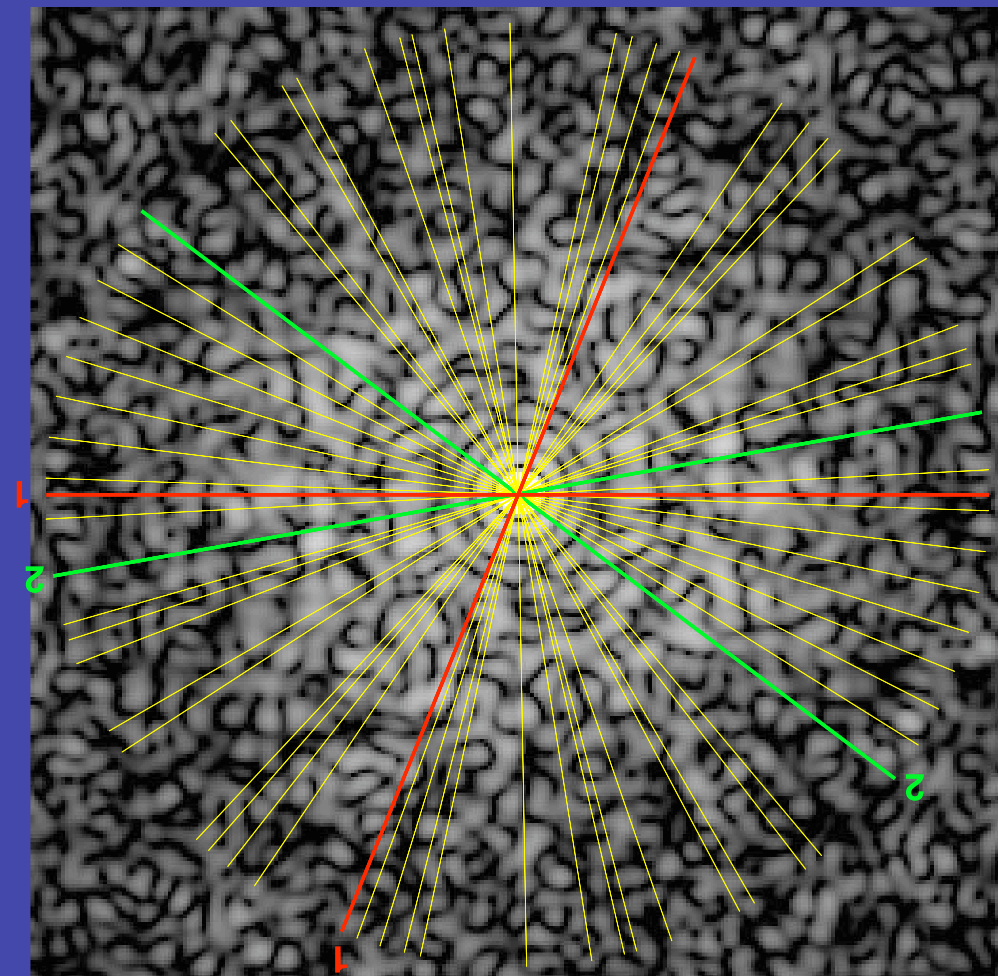
Orientation Determination by Common Lines



Orientation Determination by Common Lines



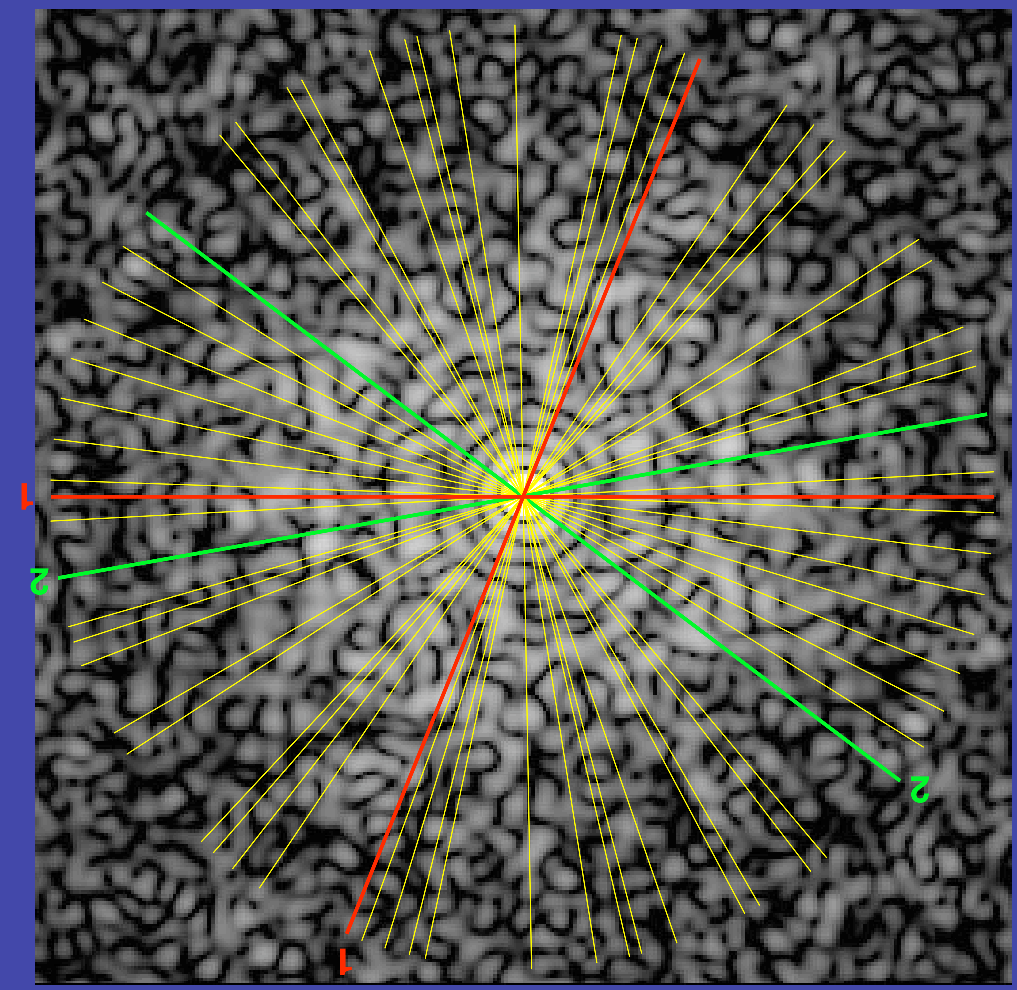
Orientation Determination by Common Lines



(80,11,ω)

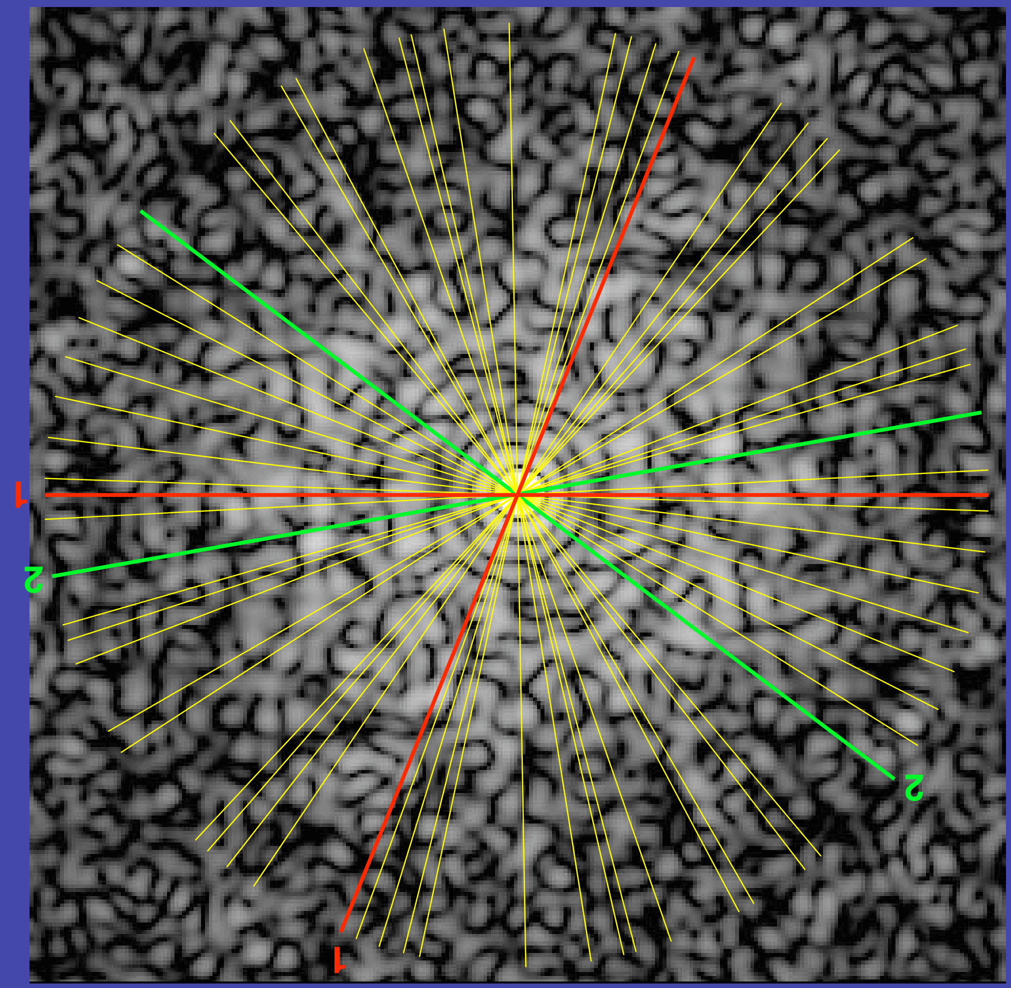
Metric: Identify ω that gives lowest phase residual

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)

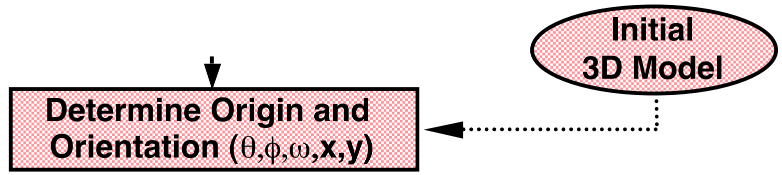
Common Lines

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*):

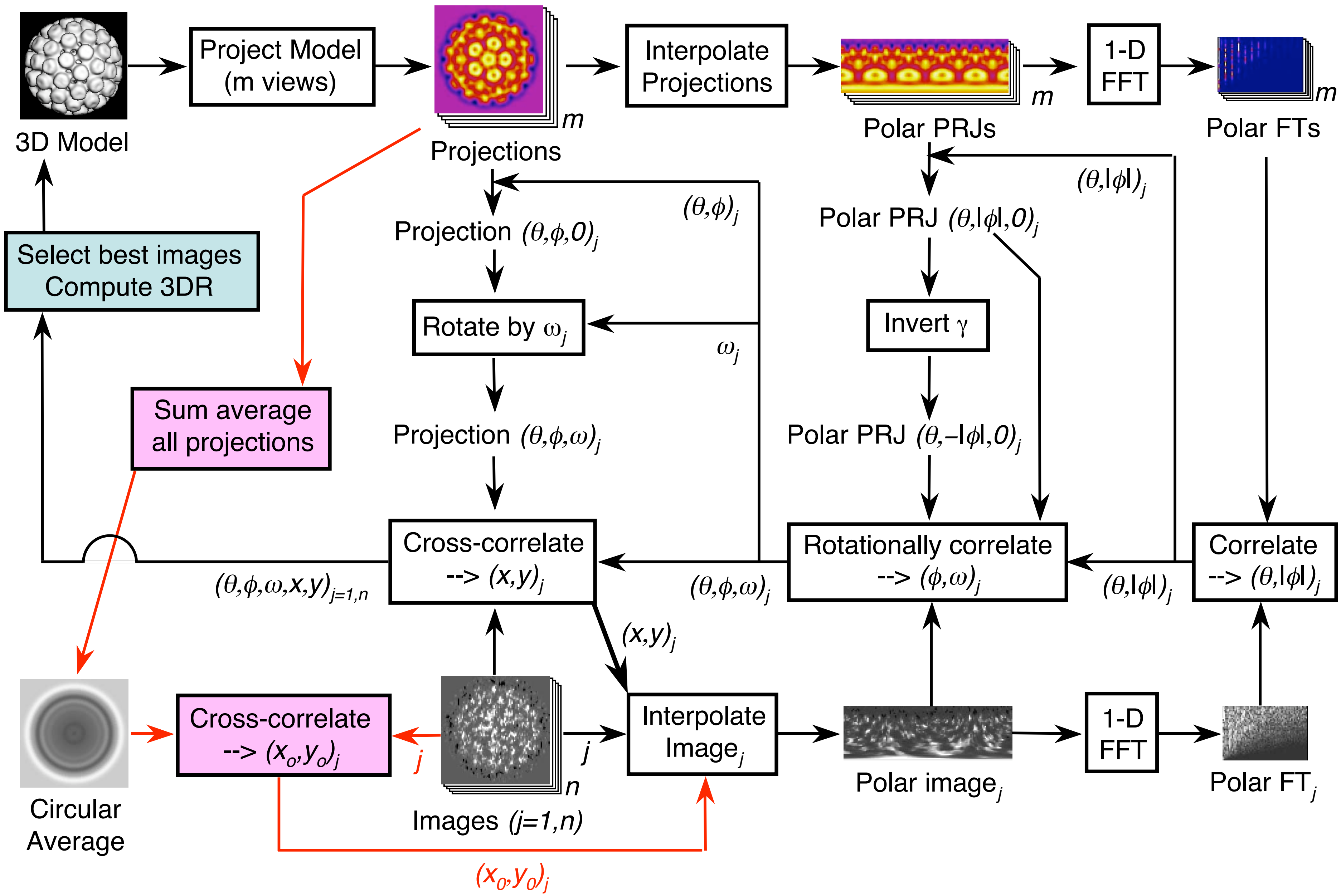
1. Common lines

2. Model-based (template) matching

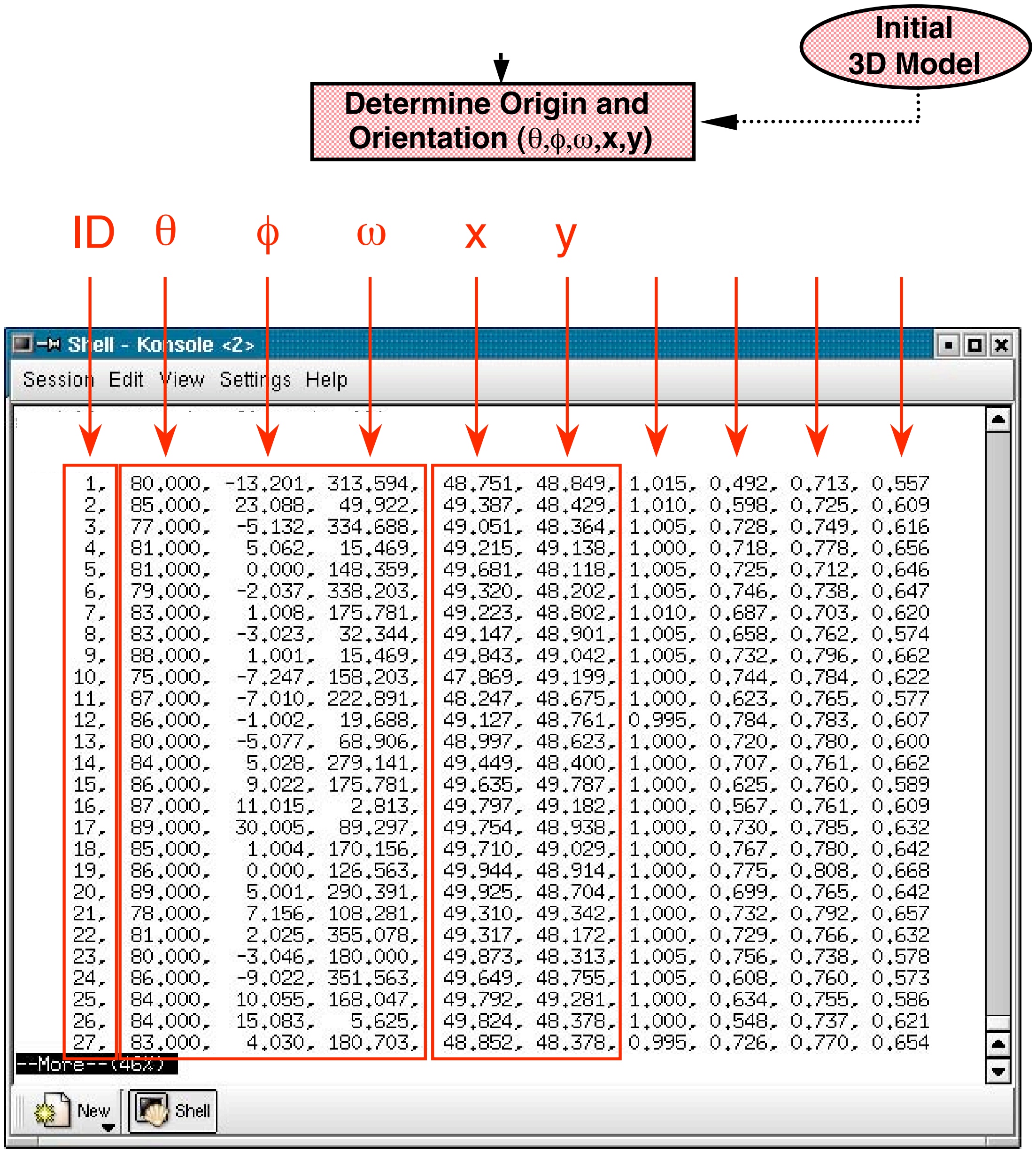
Bulk of structures now solved this way

Details discussed later if you like!

PFT Program Flow Chart

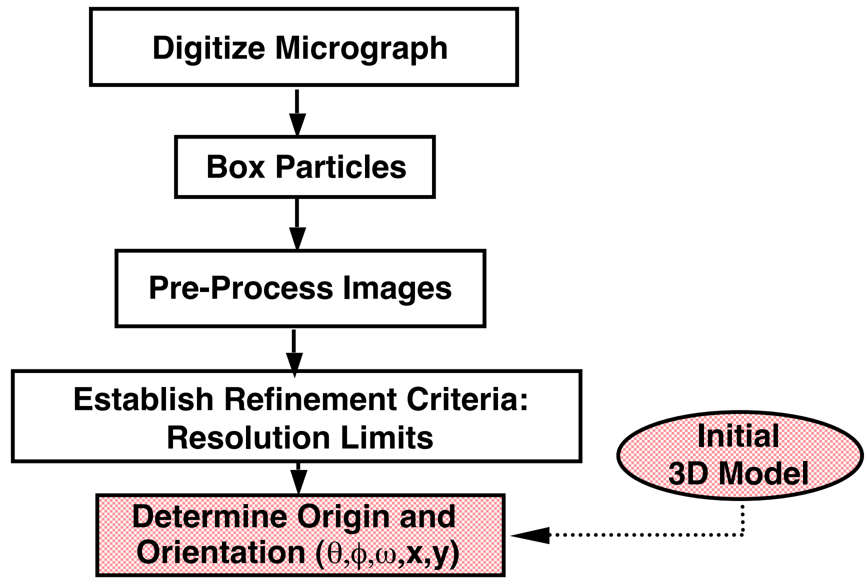


Icosahedral Virus 3D Reconstruction Scheme



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



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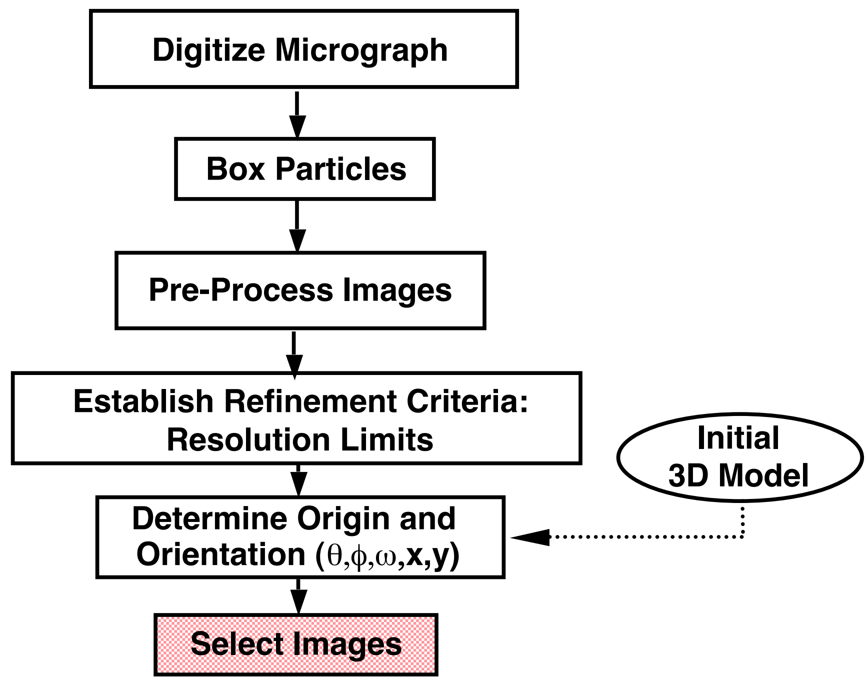
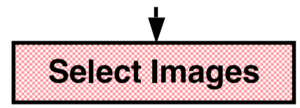


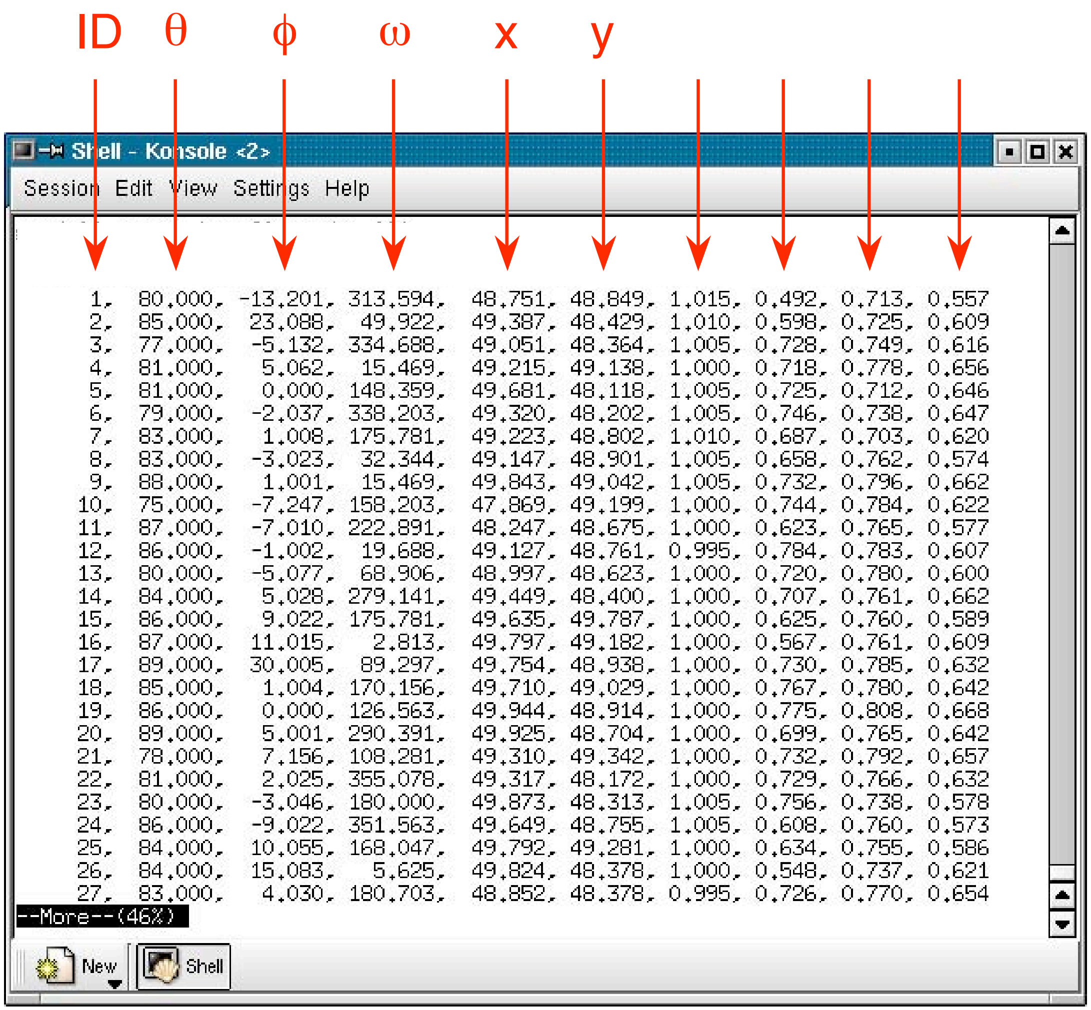
Icosahedral Virus 3D Reconstruction Scheme



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

Icosahedral Virus 3D Reconstruction Scheme

Select Images



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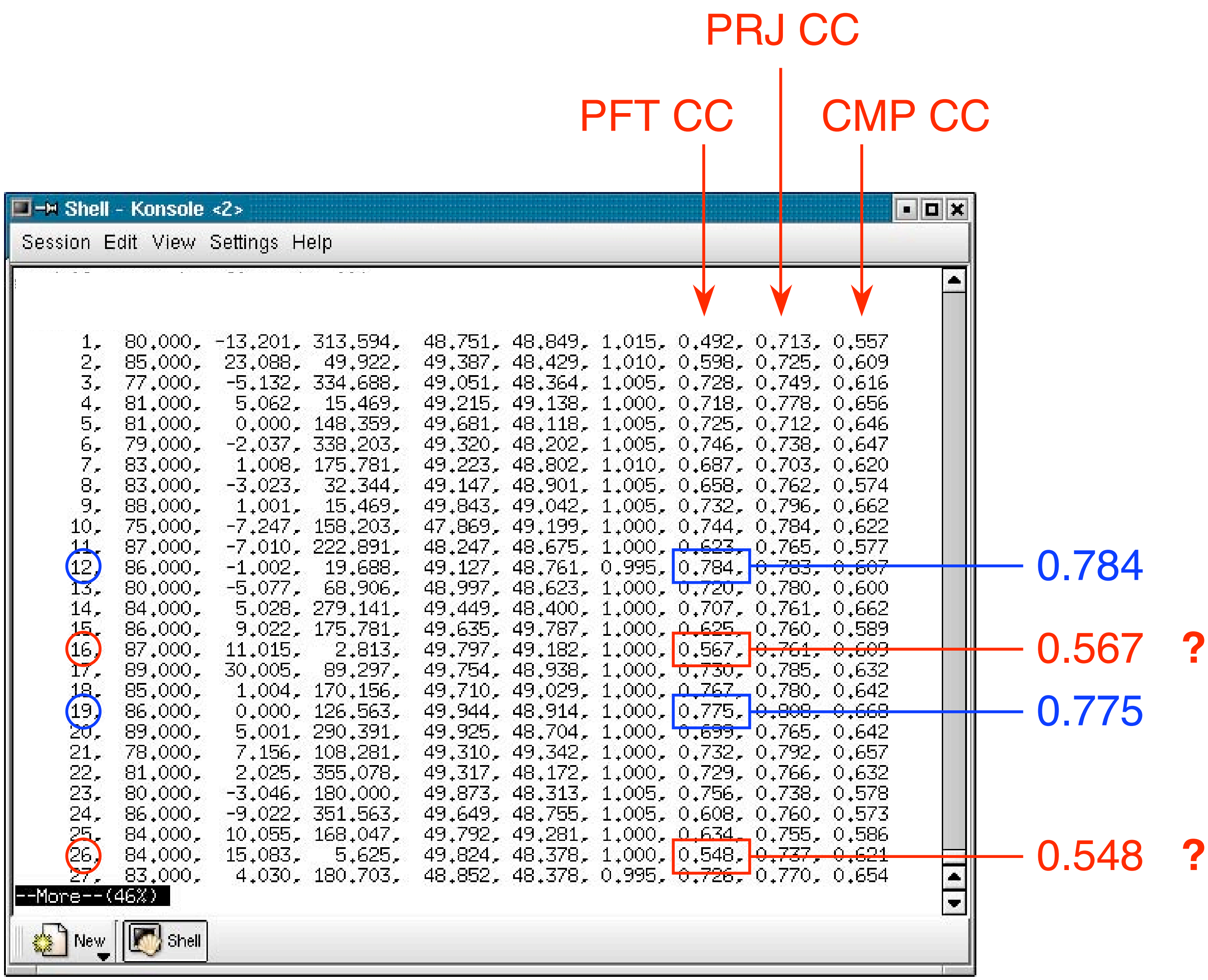
Icosahedral Virus 3D Reconstruction Scheme

Select Images



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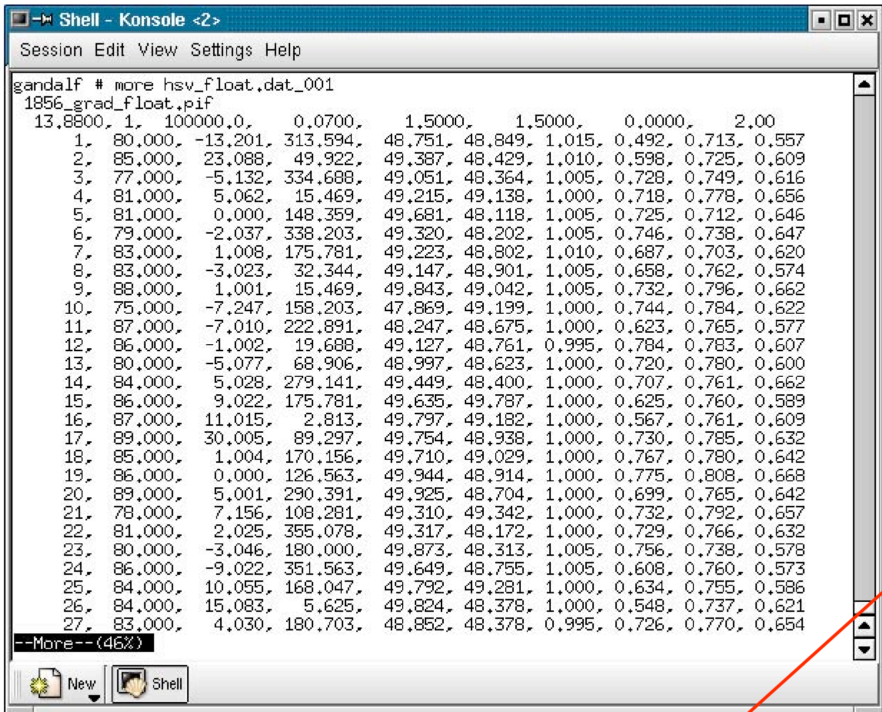
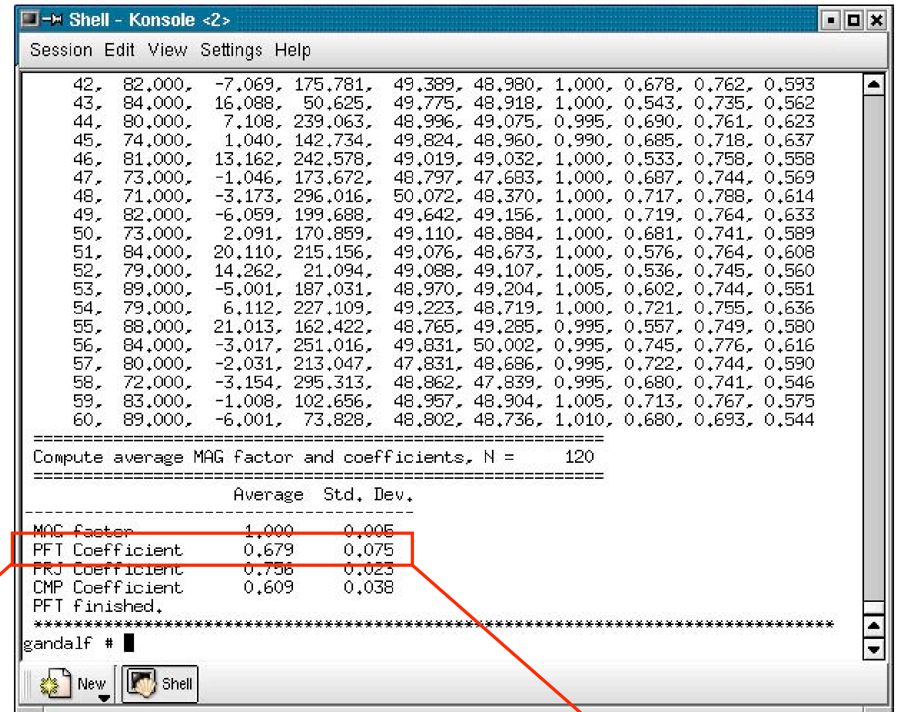
15.	86.000	9.022	175.781	49.635	49.787	1.000	0.625	0.760	0.589
16.	87.000	11.015	2.813	49.797	49.182	1.000	0.567	0.761	0.609
17.	89.000	30.005	89.297	49.754	48.938	1.000	0.730	0.785	0.632
18.	85.000	1.004	170.156	49.710	49.029	1.000	0.767	0.780	0.642
19.	86.000	0.000	126.563	49.944	48.914	1.000	0.775	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

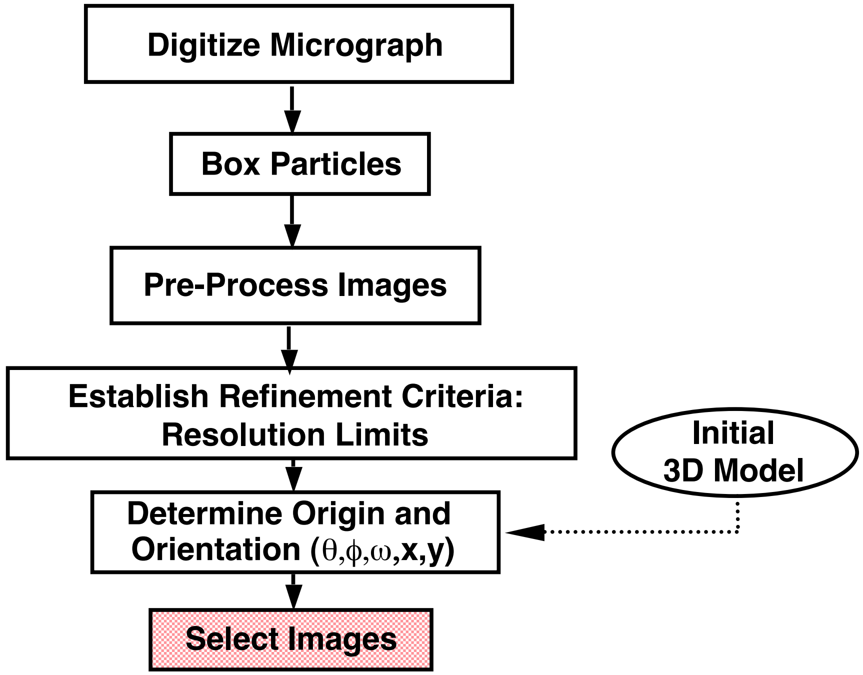
Select Images



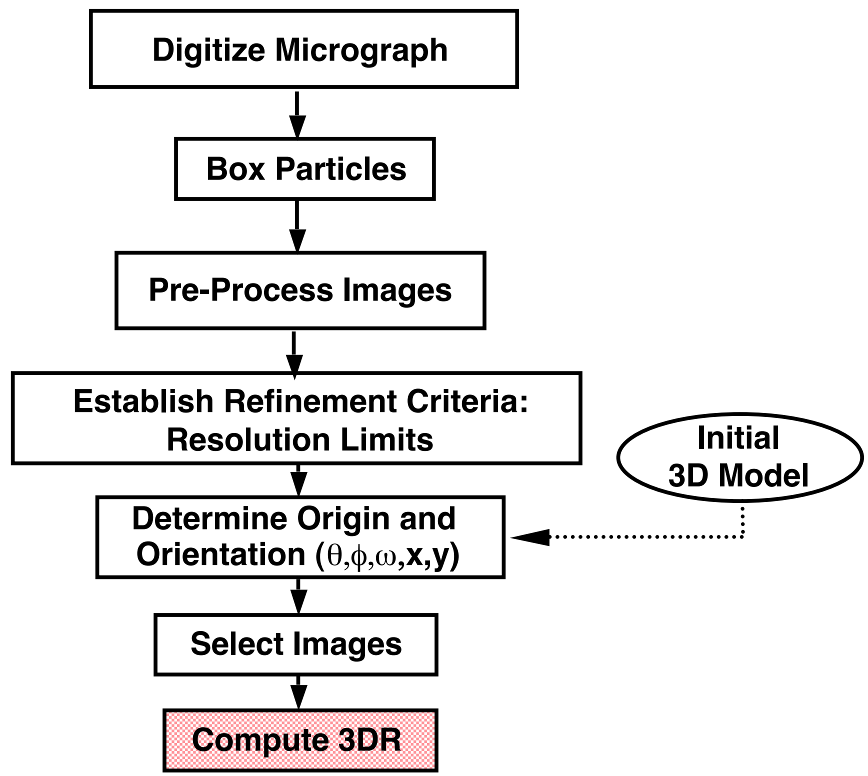


PFT Coefficient 0.679 ± 0.075

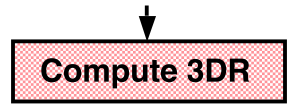
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

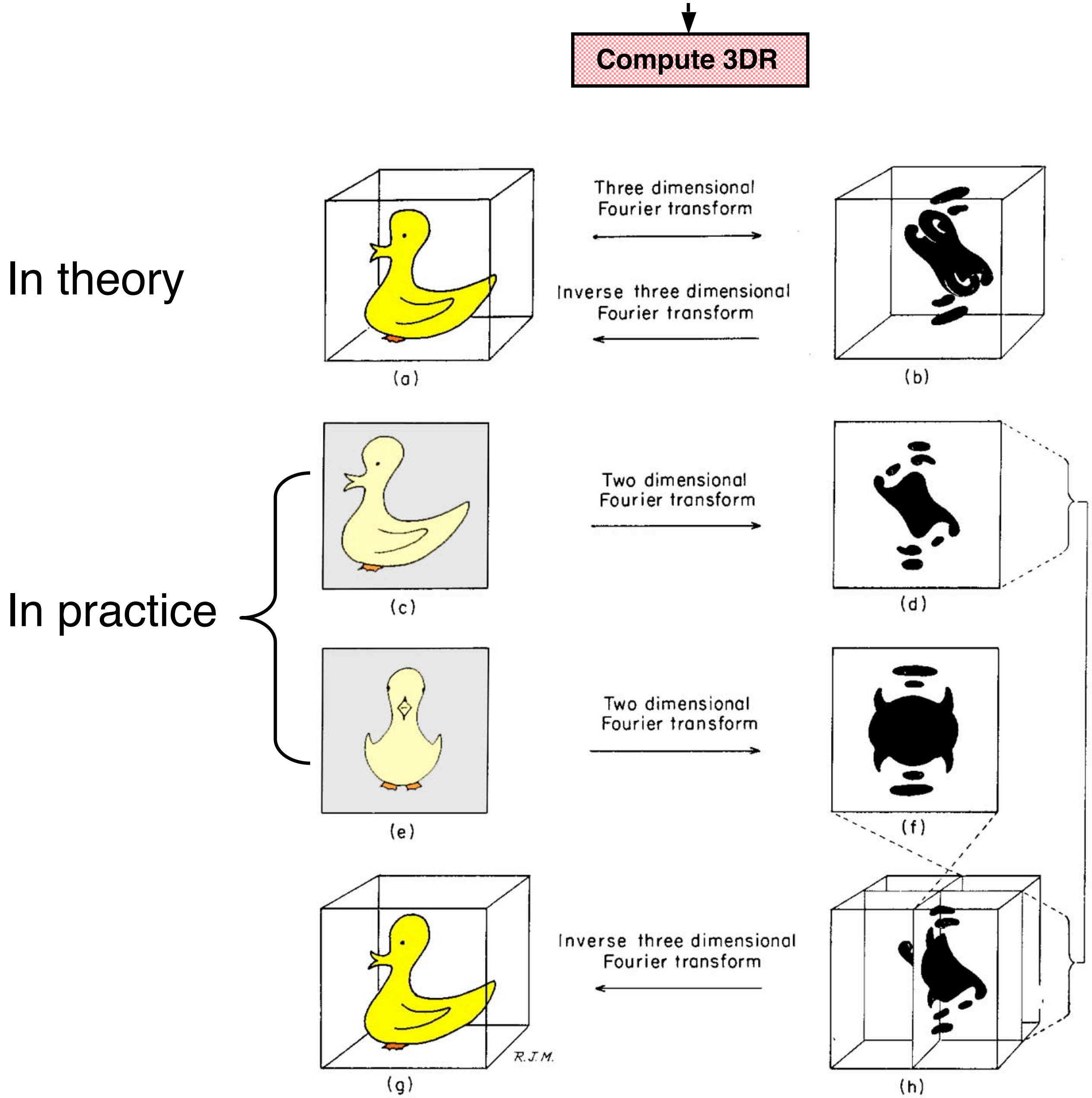


Icosahedral Virus 3D Reconstruction Scheme



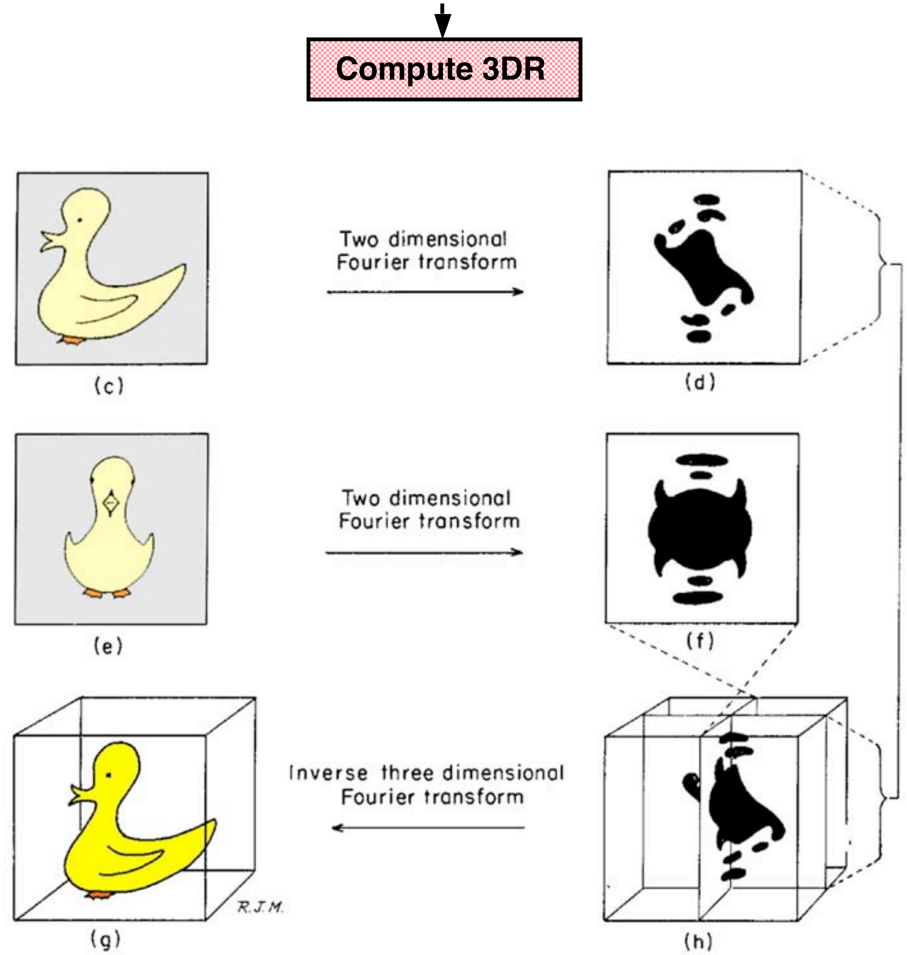
Goal: combine "good" particle images to compute a 3D density map

Icosahedral Virus 3D Reconstruction Scheme



From Lake (1972), p.174

Icosahedral Virus 3D Reconstruction Scheme



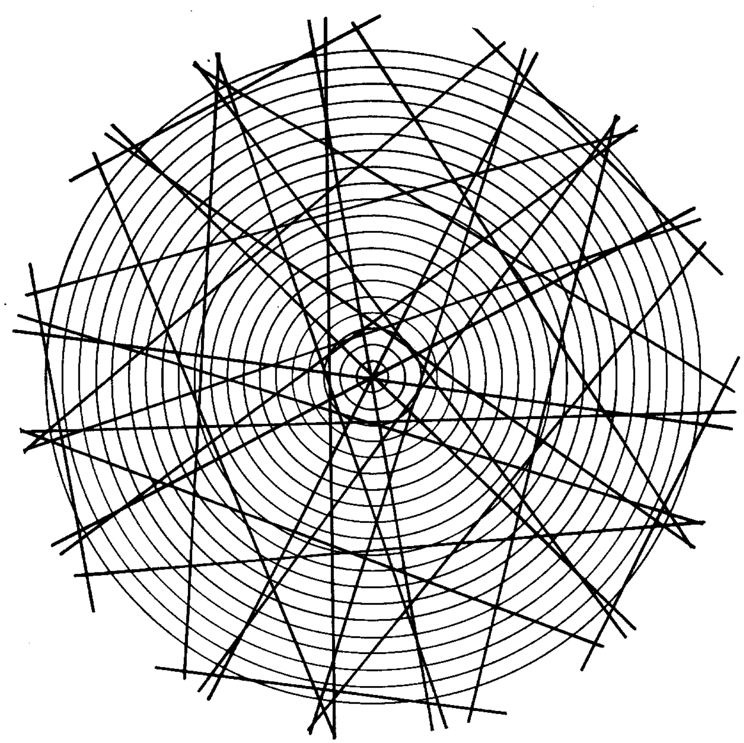
Overall scheme: $\rho \leftarrow g \leftarrow G \leftarrow F$

Icosahedral Virus 3D Reconstruction Scheme

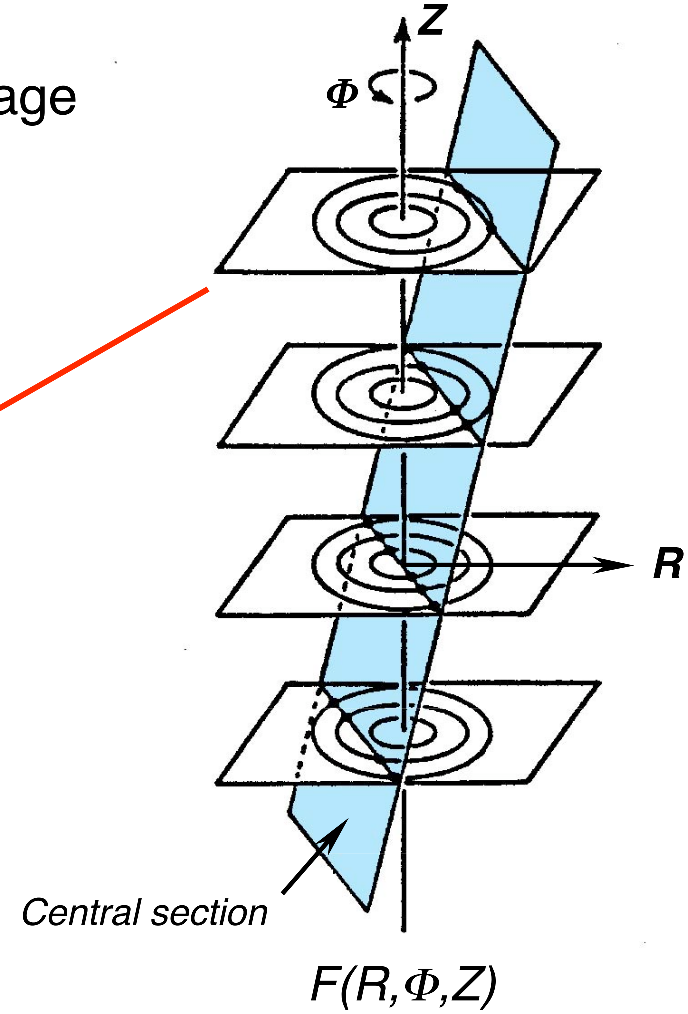
Compute 3DR

Steps:

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



Crowther, DeRosier and Klug, 1970, p.329



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

Icosahedral Virus 3D Reconstruction Scheme

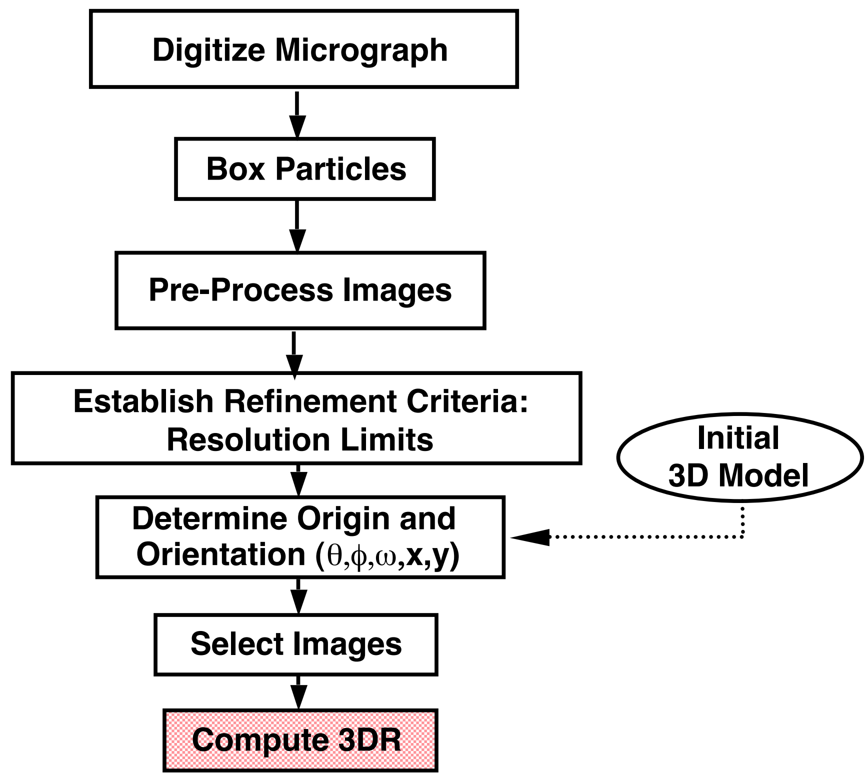
Compute 3DR

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

Steps:

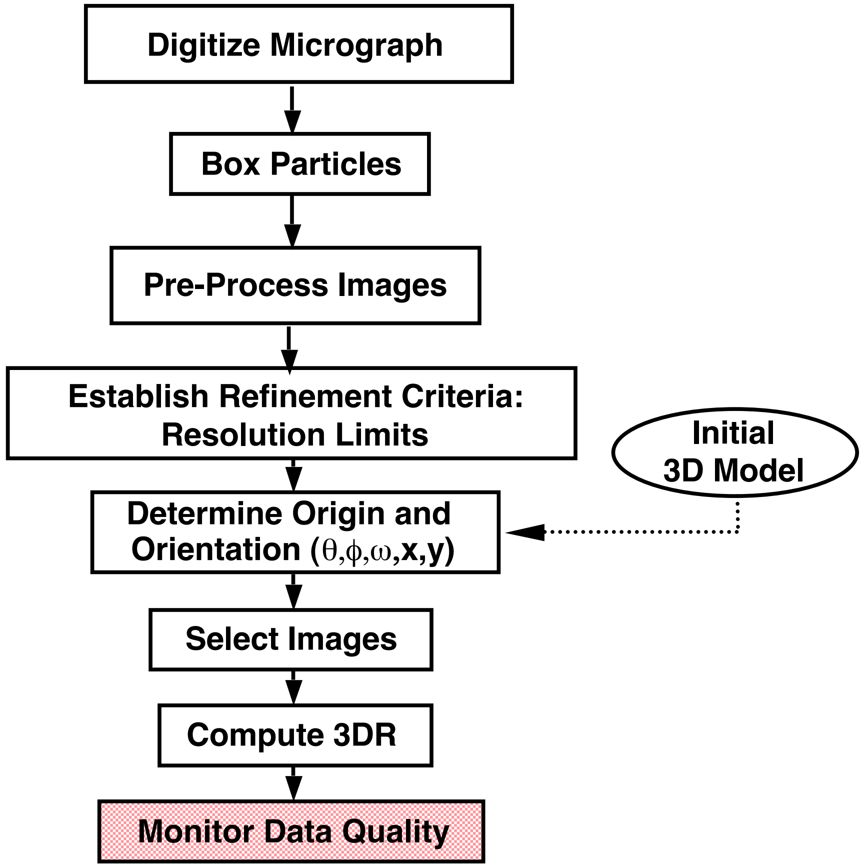
1. Compute 2D FFT of each particle image
2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform
3. Compute G_n's on each annulus $G = \left(B^{\dagger}B\right)^{-1} B^{\dagger}F$
4. Compute g_n's from G_n's (Fourier-Bessel transform)
5. Compute polar density map ($\rho(r,\phi,z)$) from g_n's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

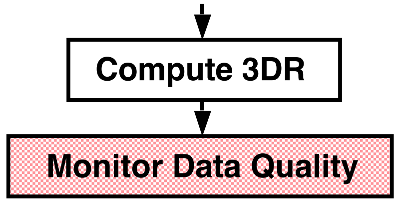


Icosahedral Virus 3D Reconstruction Scheme

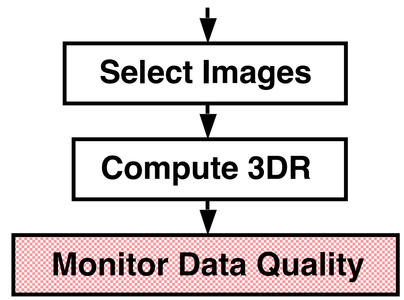
Monitor Data Quality

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

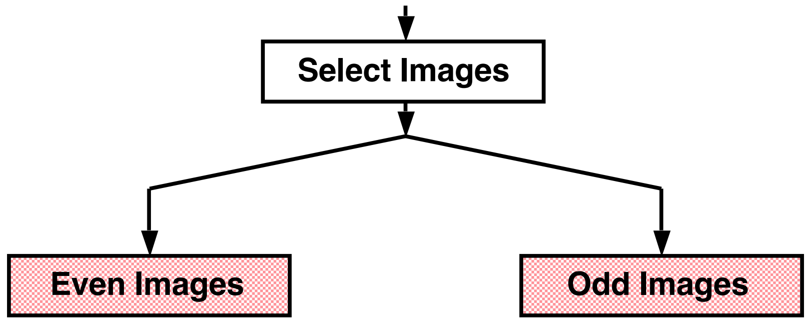
Icosahedral Virus 3D Reconstruction Scheme



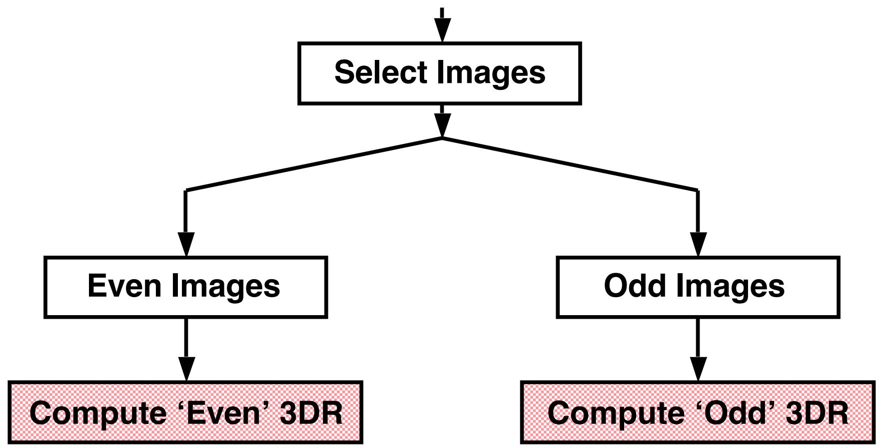
Icosahedral Virus 3D Reconstruction Scheme



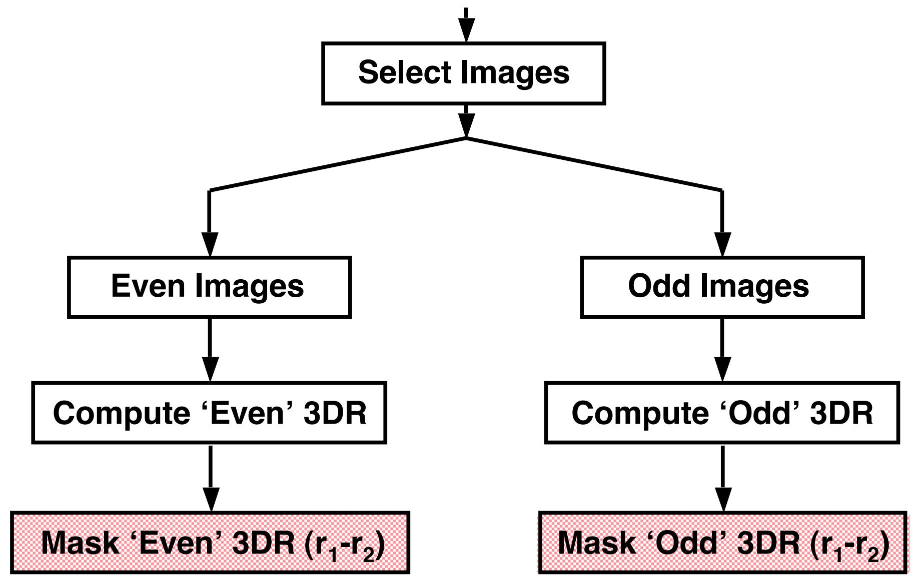
Icosahedral Virus 3D Reconstruction Scheme

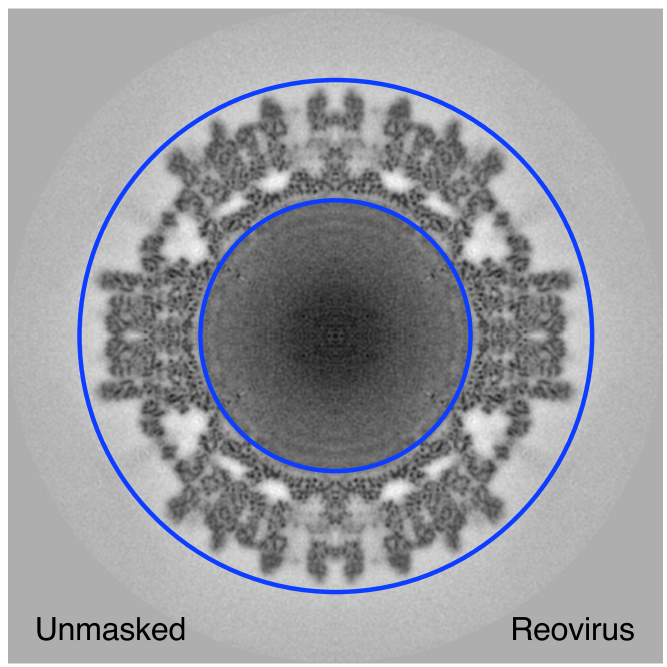


Icosahedral Virus 3D Reconstruction Scheme

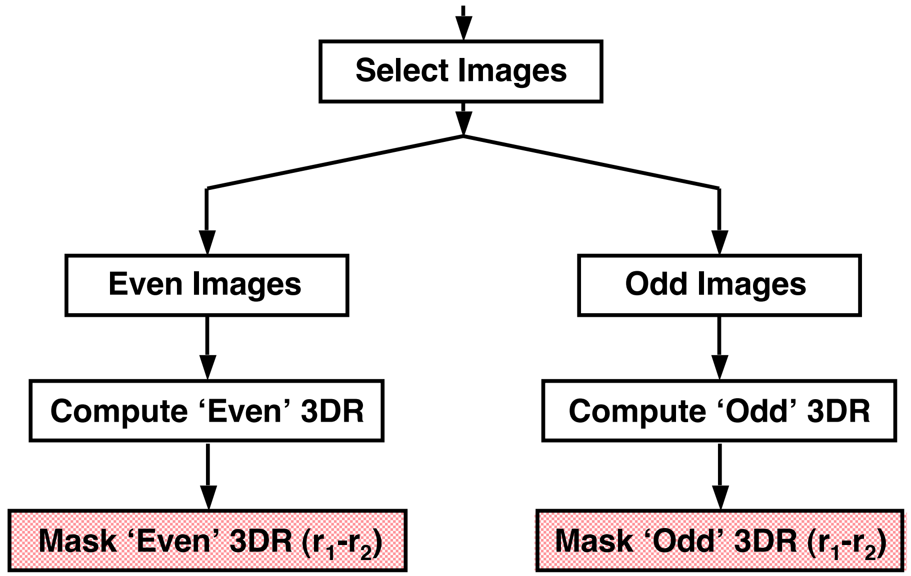


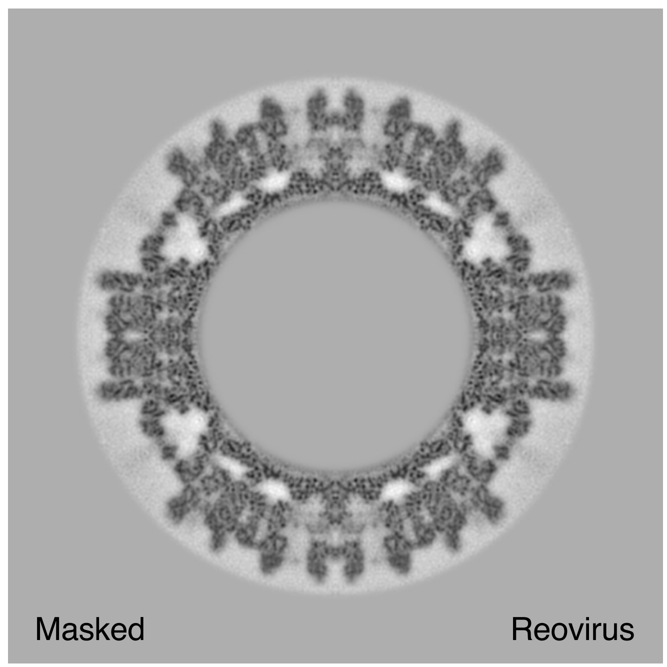
Icosahedral Virus 3D Reconstruction Scheme



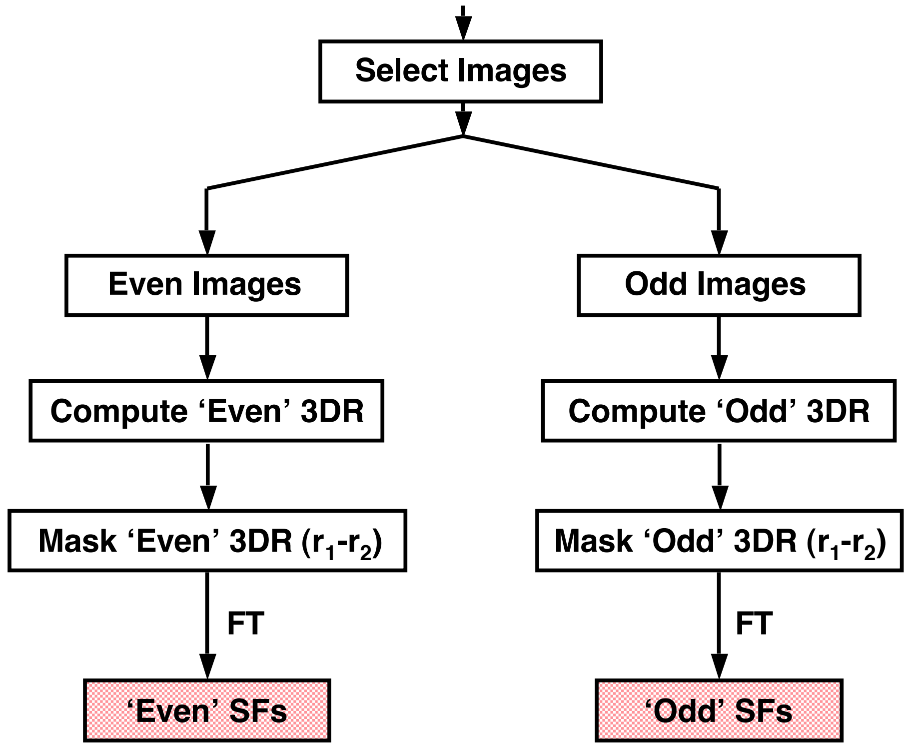


Icosahedral Virus 3D Reconstruction Scheme

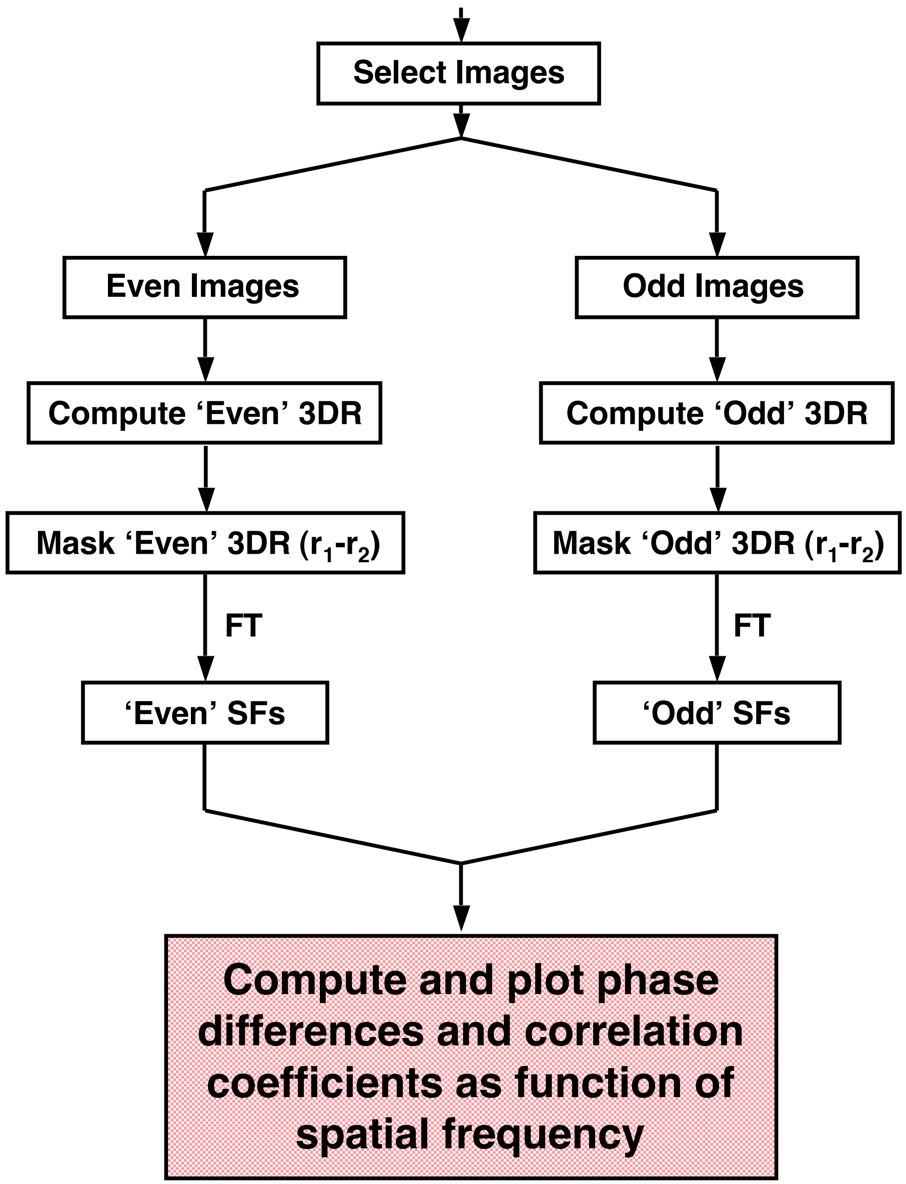




Icosahedral Virus 3D Reconstruction Scheme

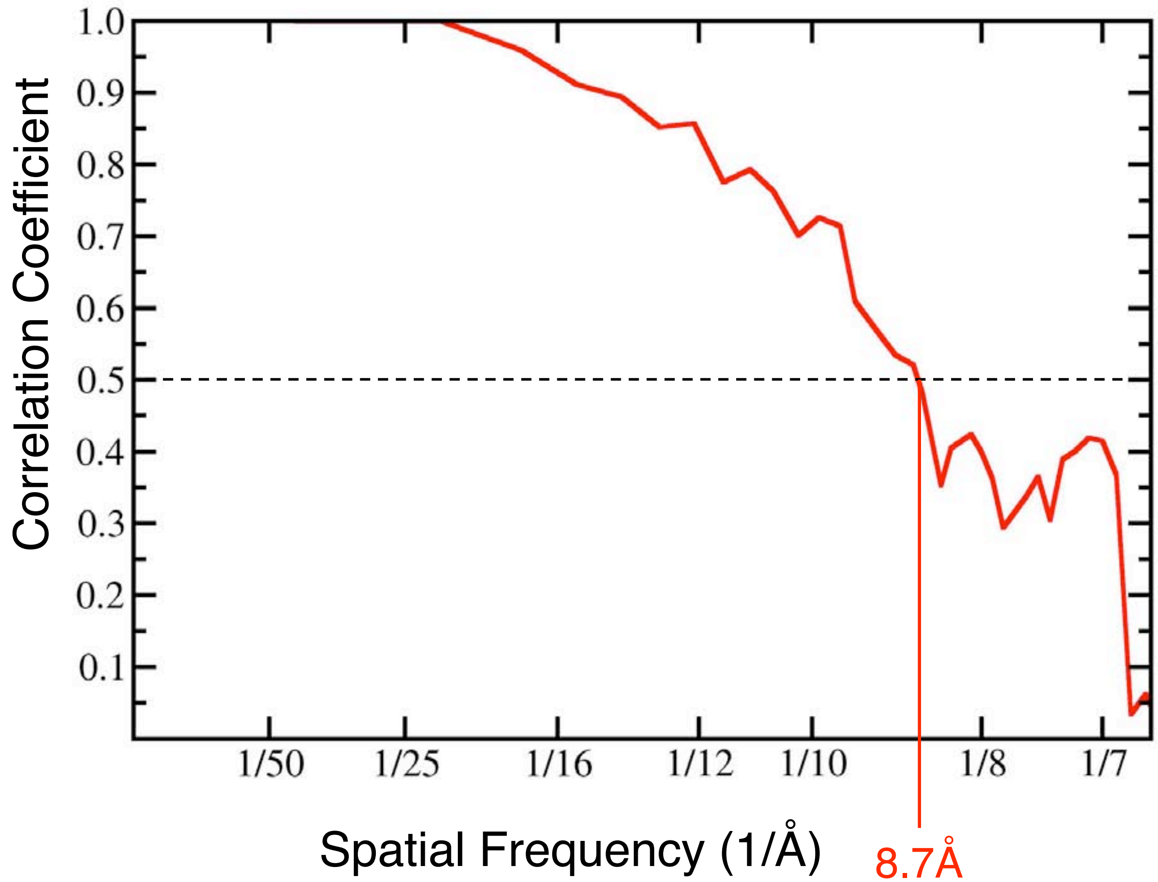


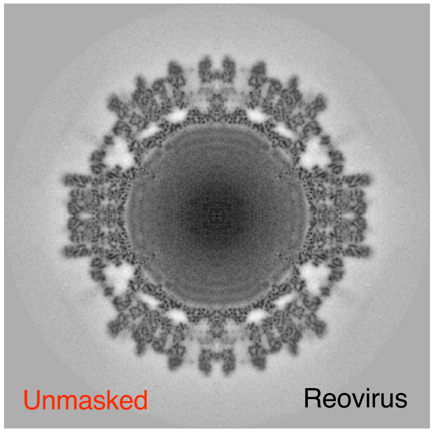
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

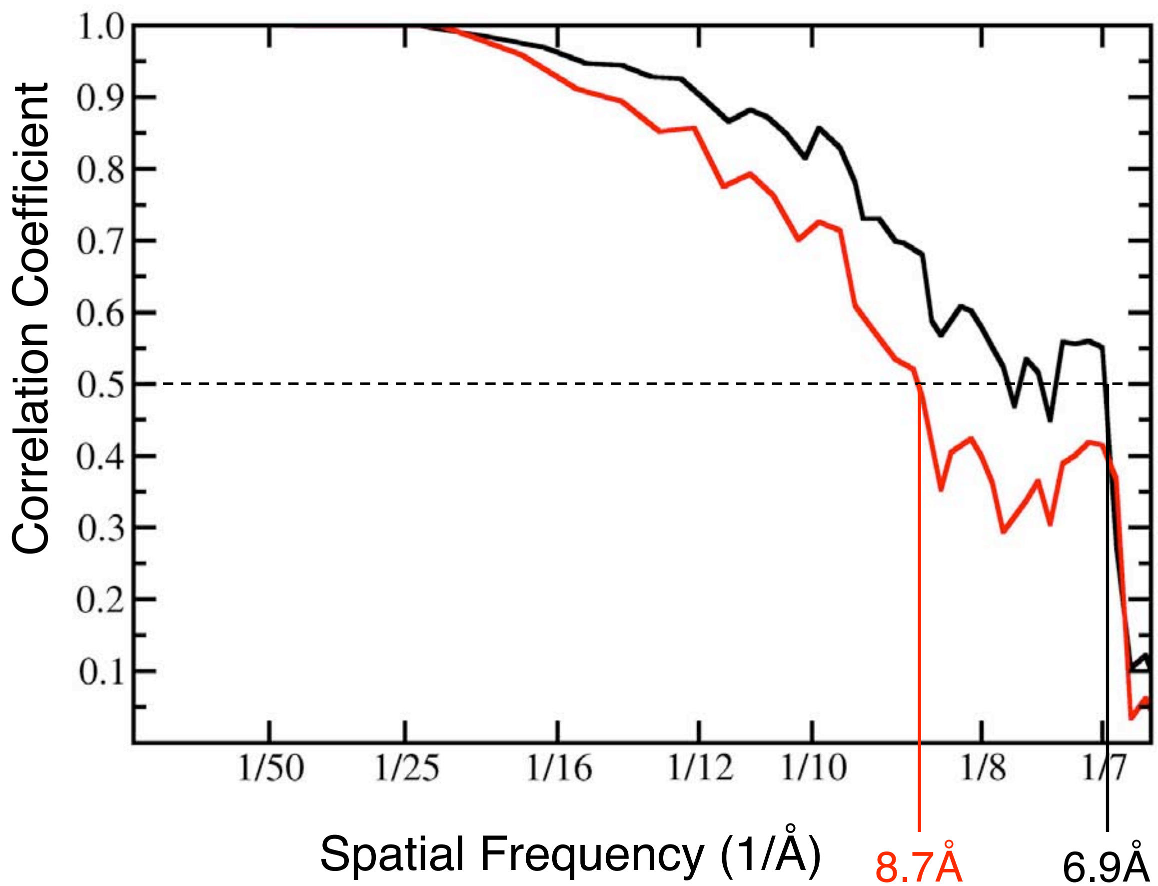
Monitor Data Quality

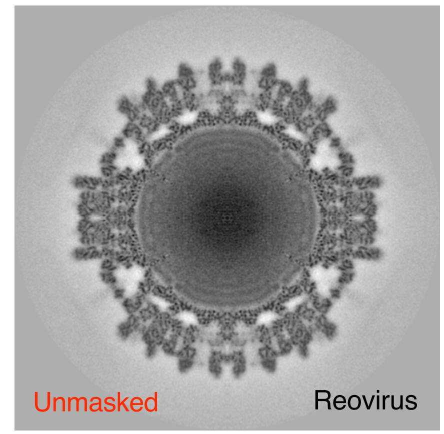


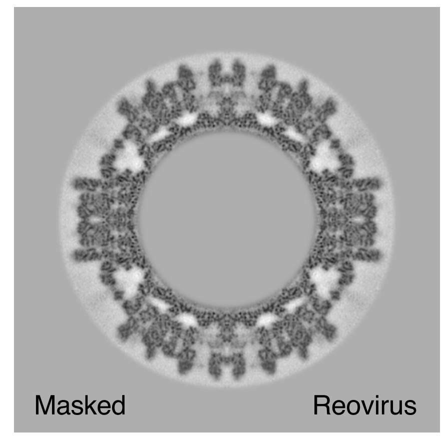


Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality





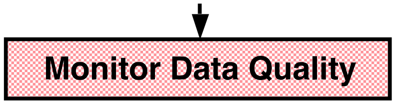


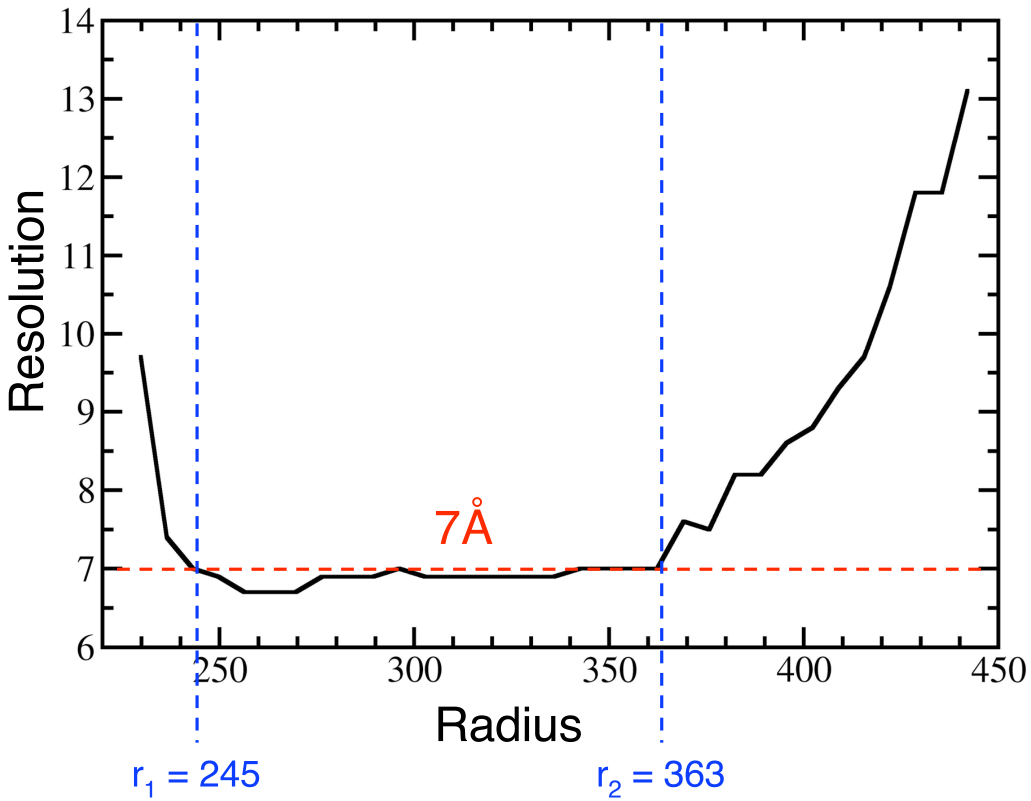
Icosahedral Virus 3D Reconstruction Scheme

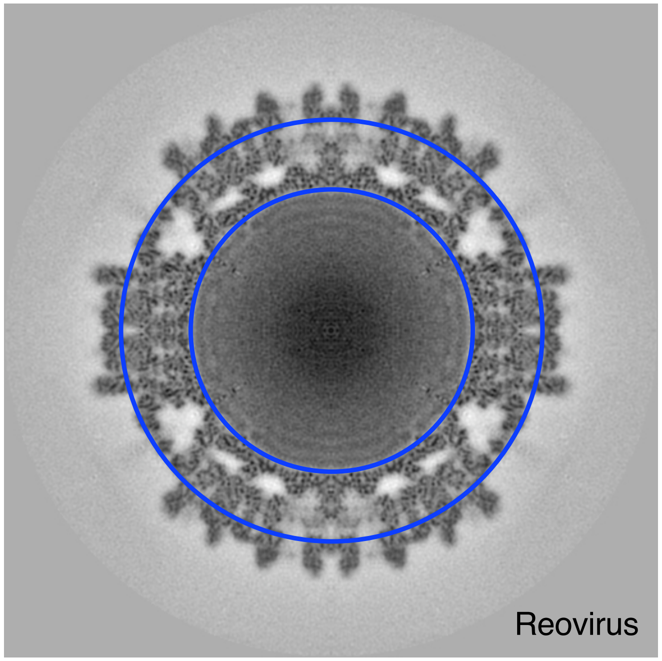
Monitor Data Quality

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

Icosahedral Virus 3D Reconstruction Scheme

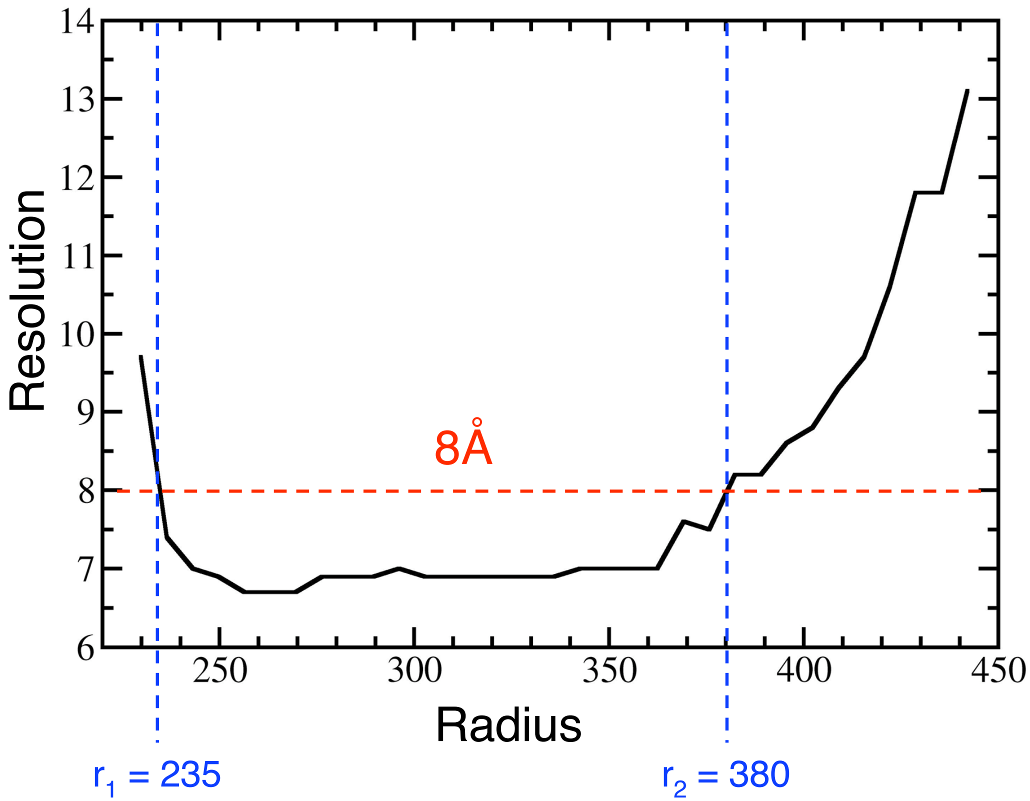


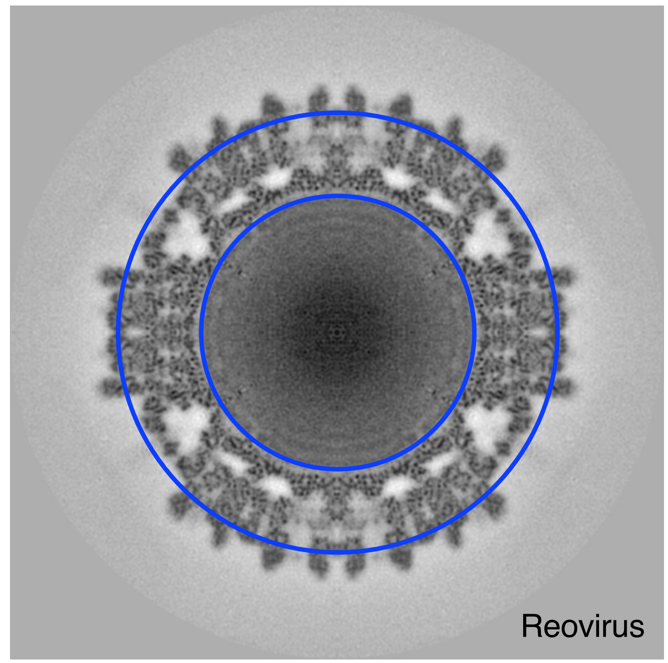




Icosahedral Virus 3D Reconstruction Scheme

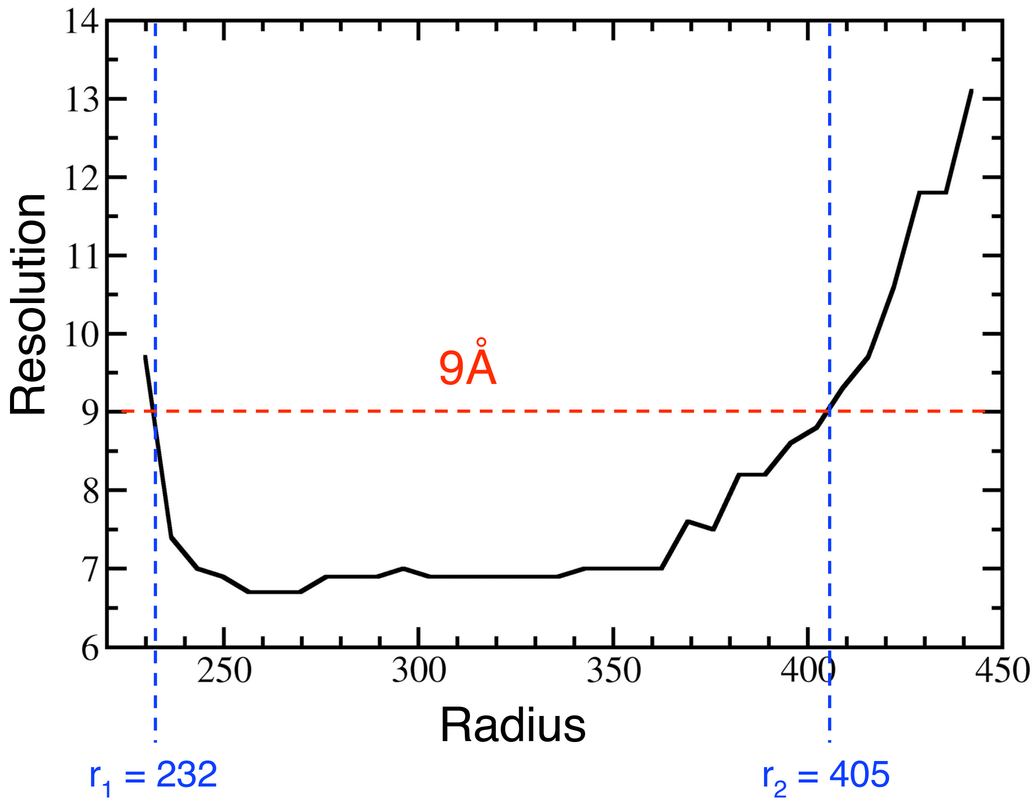
Monitor Data Quality

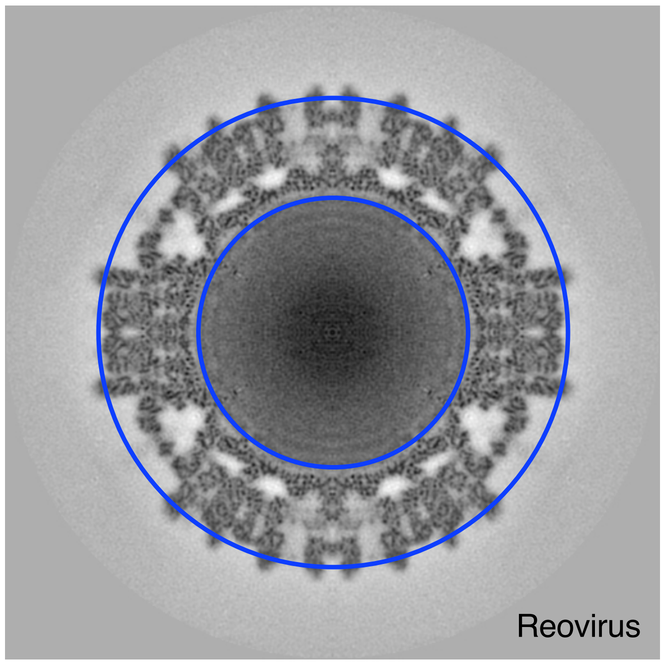




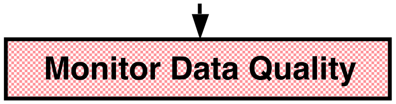
Icosahedral Virus 3D Reconstruction Scheme

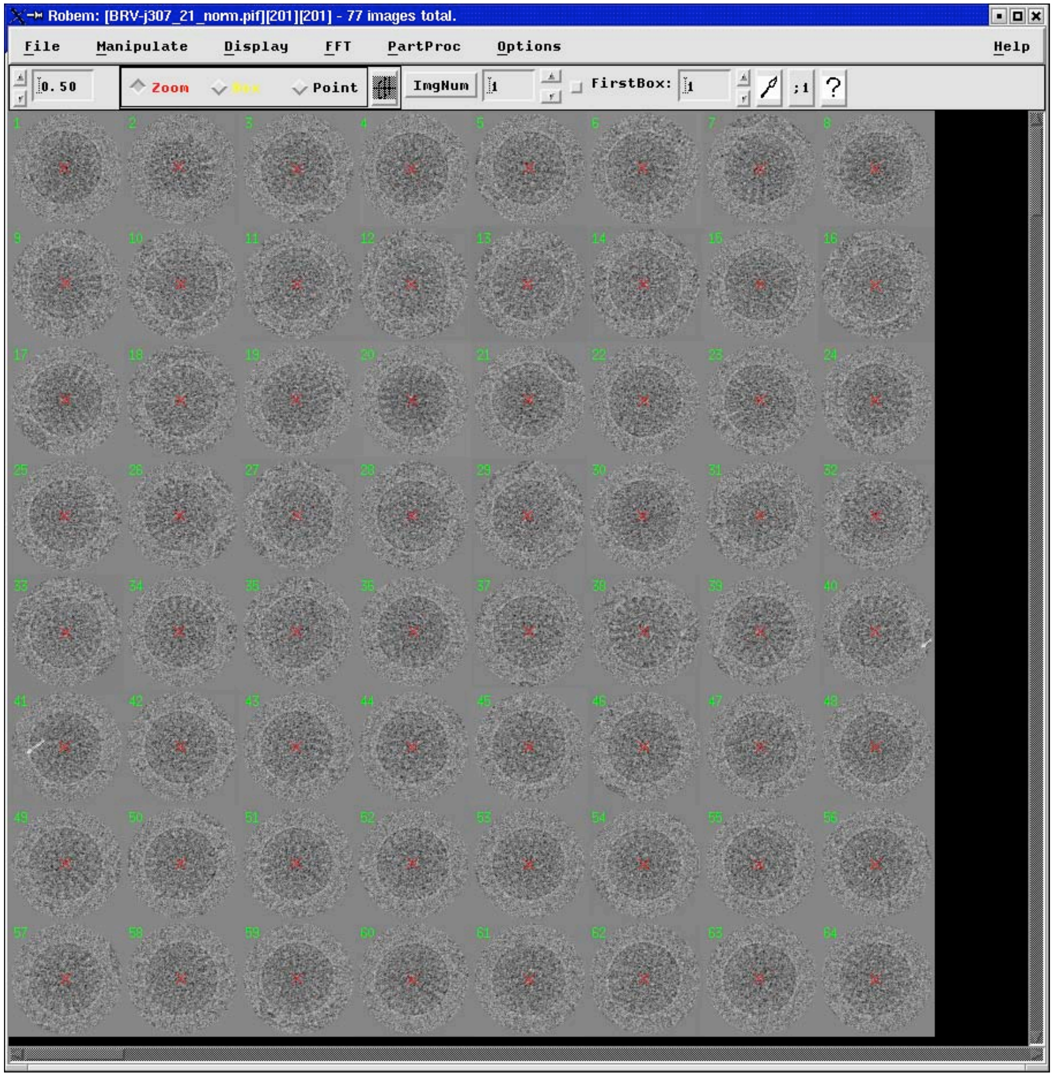
Monitor Data Quality





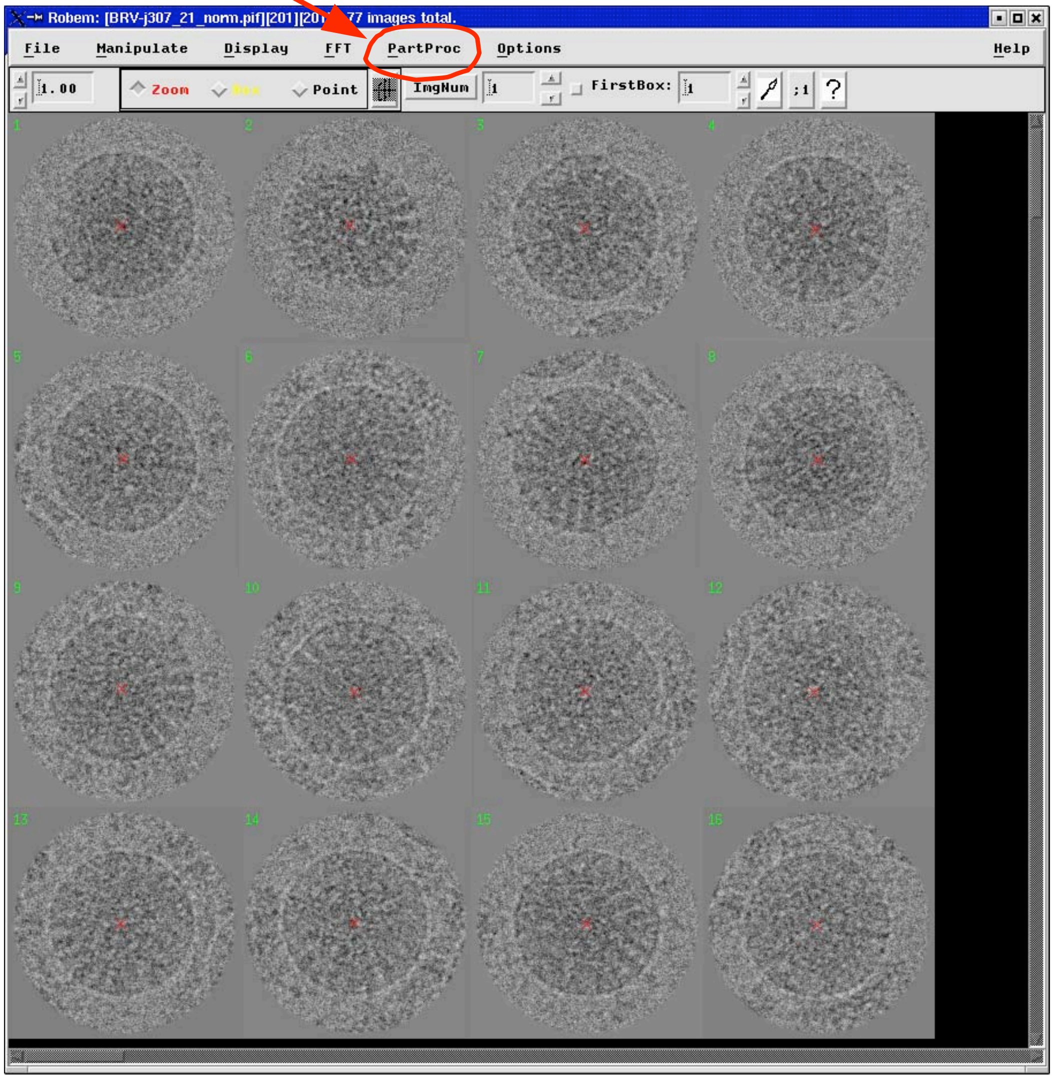
Icosahedral Virus 3D Reconstruction Scheme





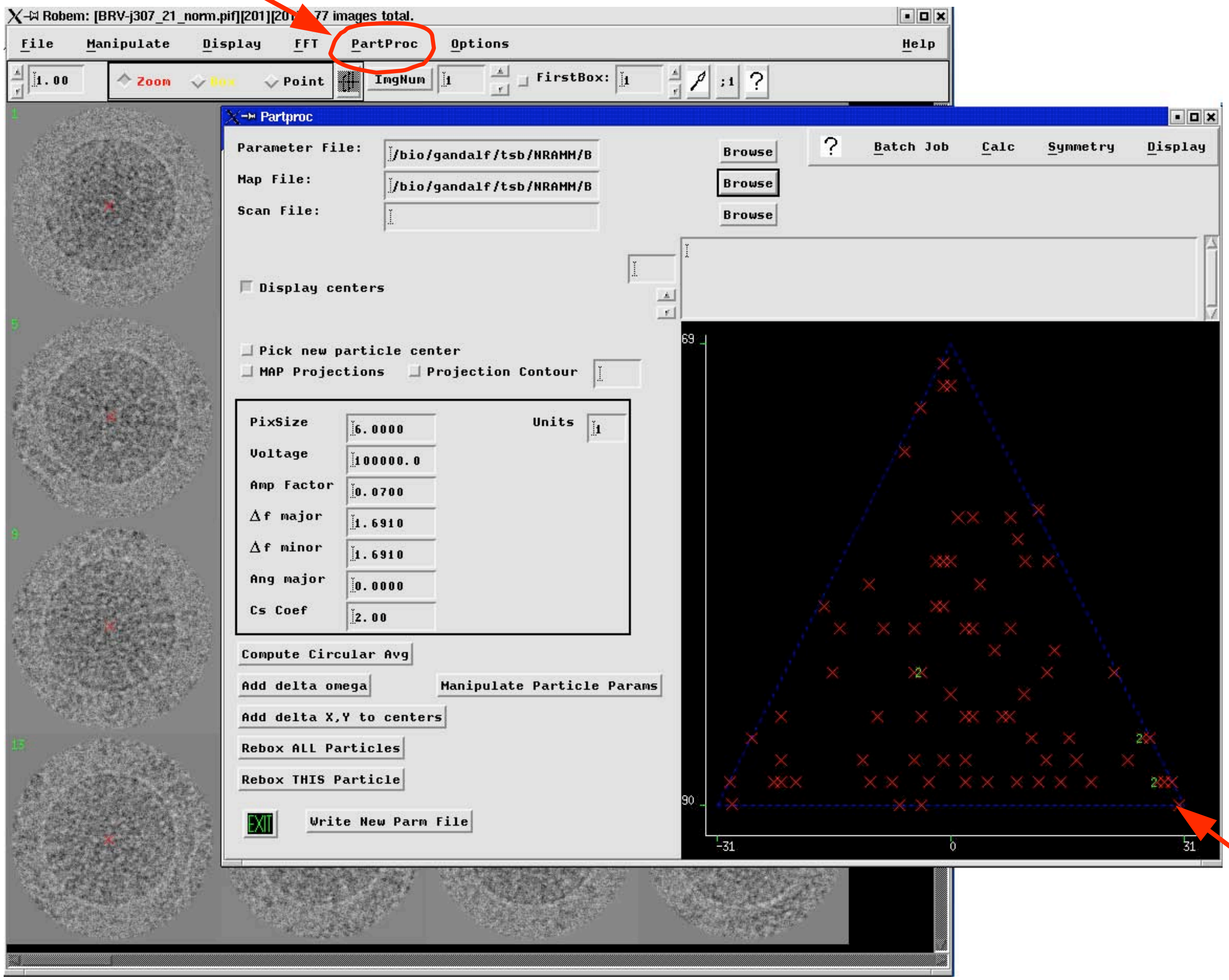
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



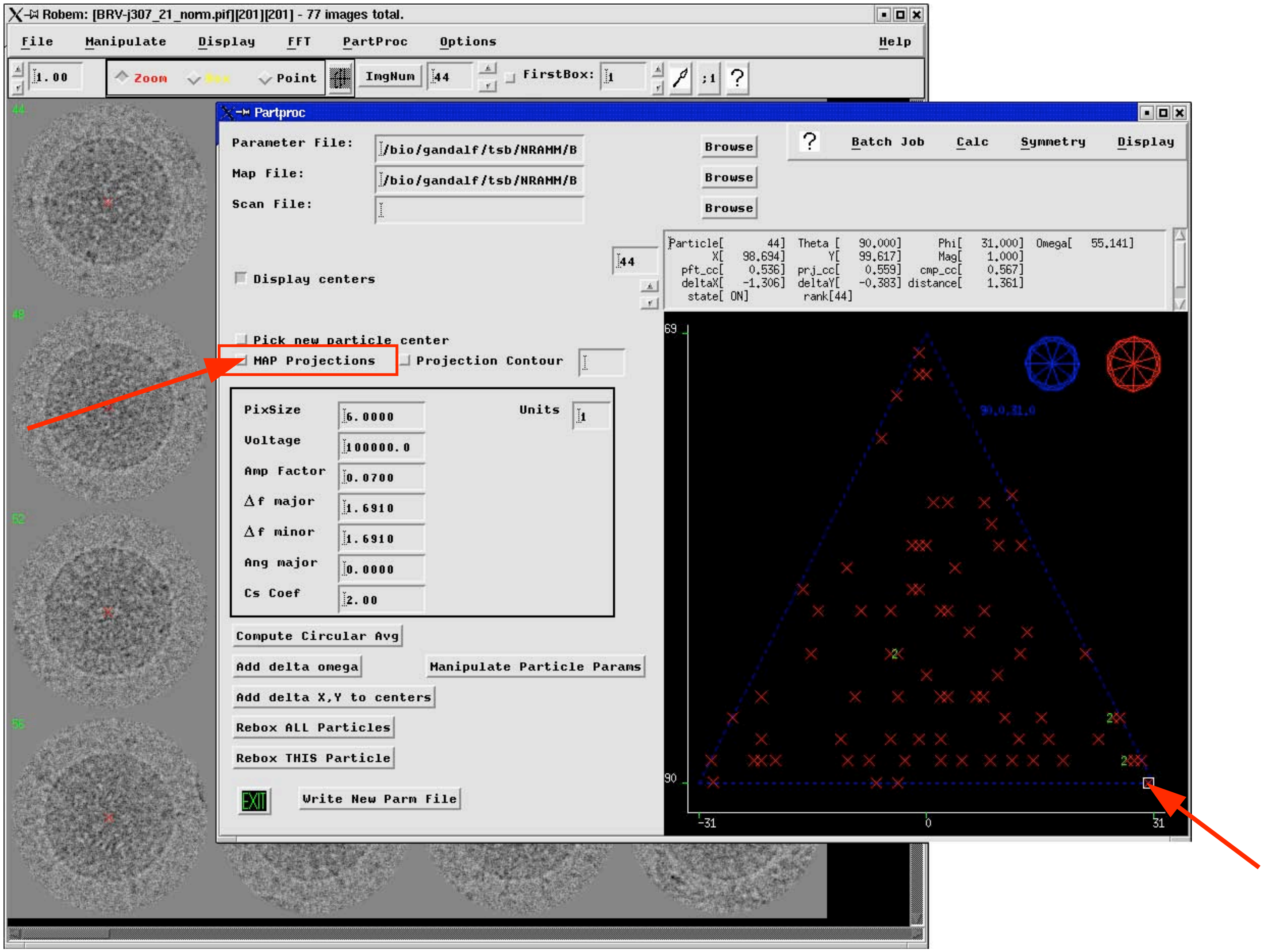
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



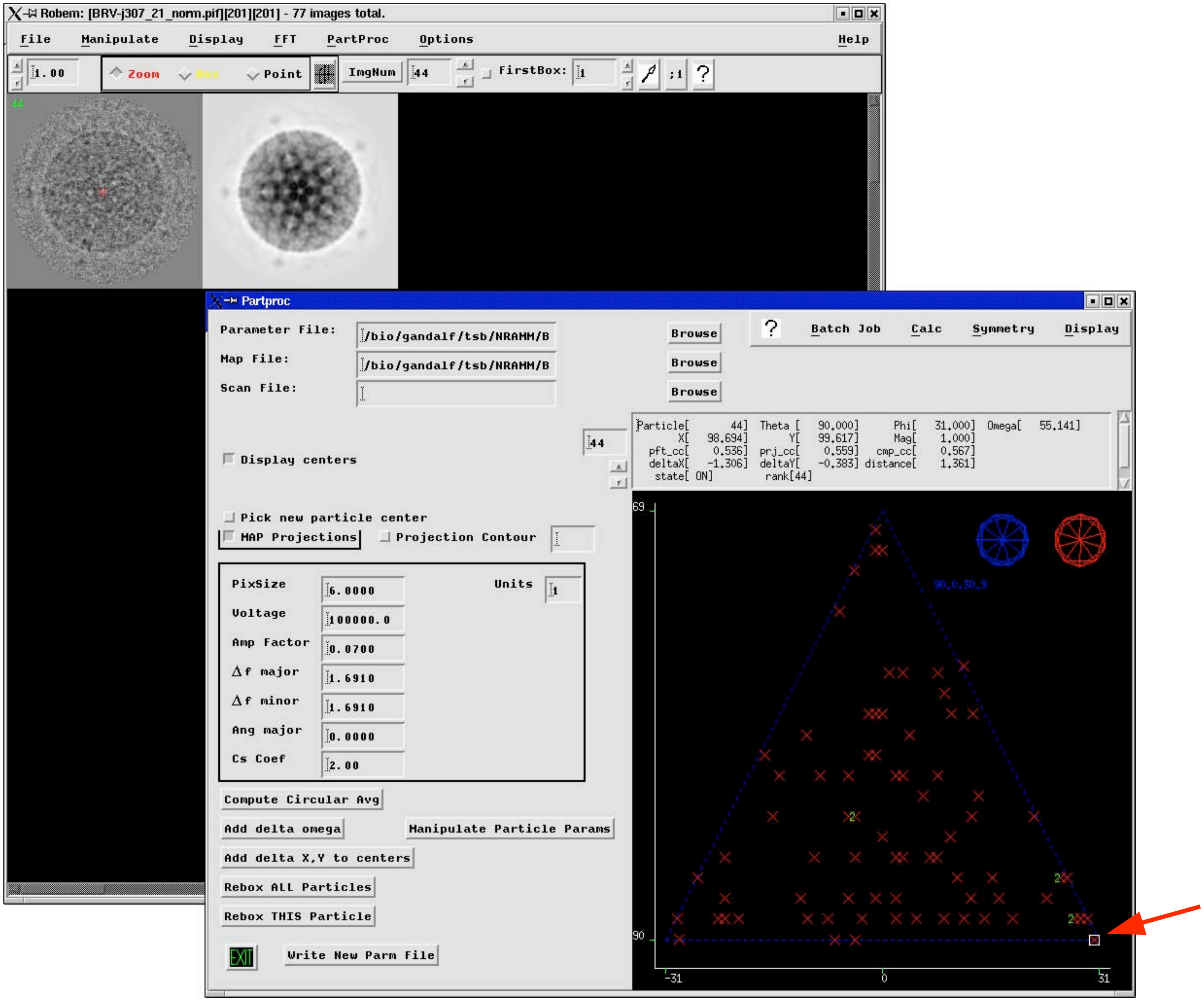
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



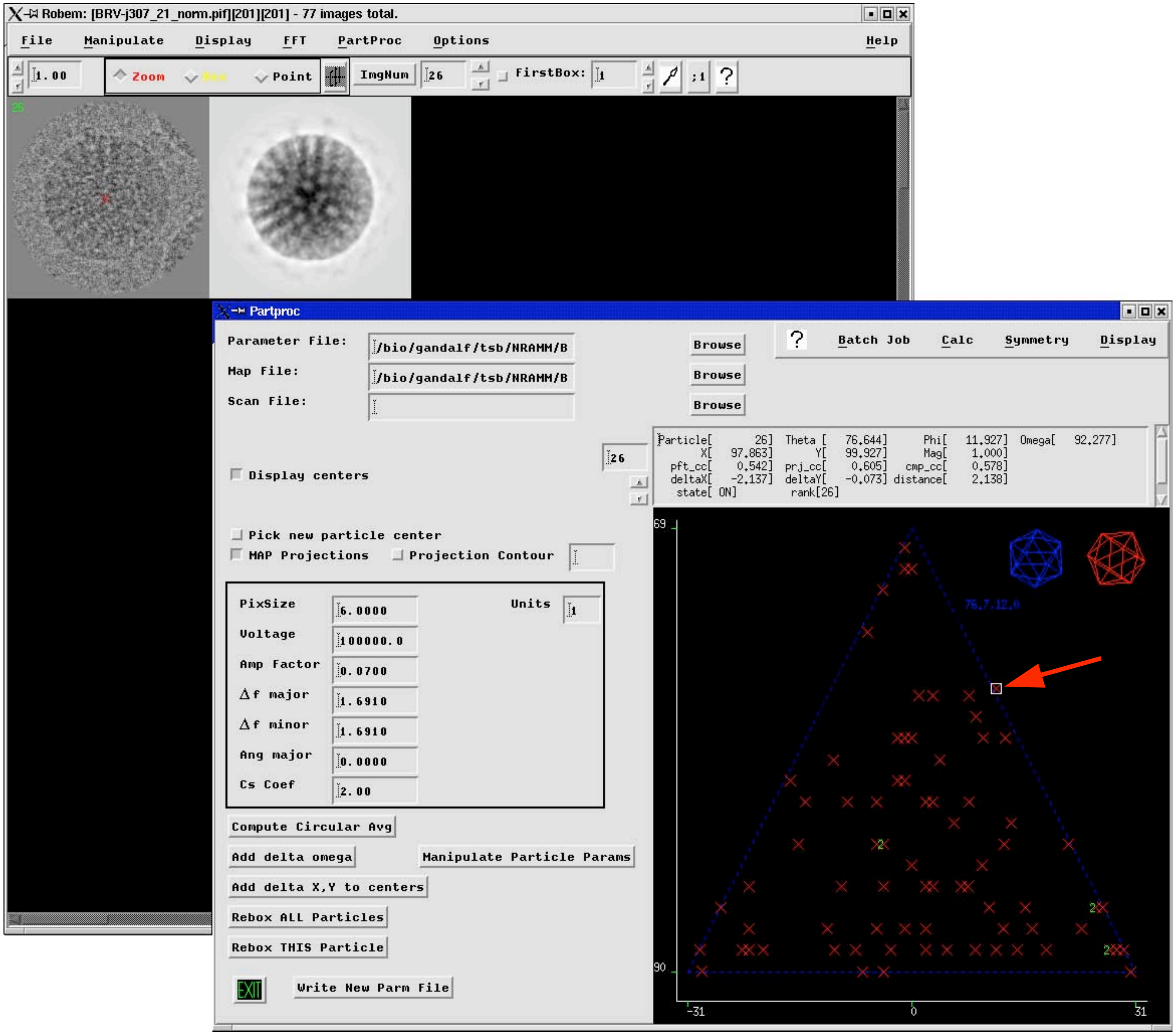
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



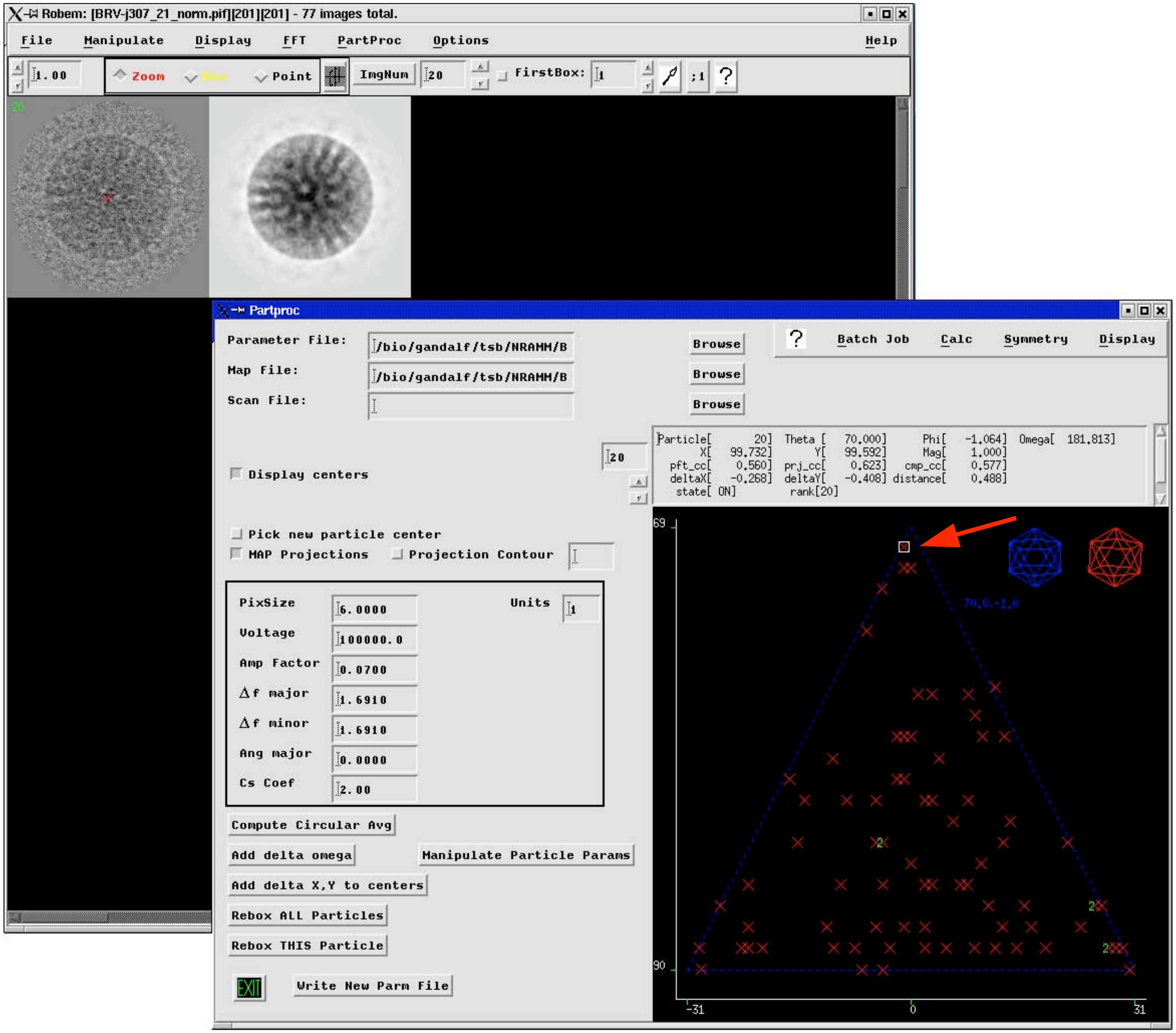
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality

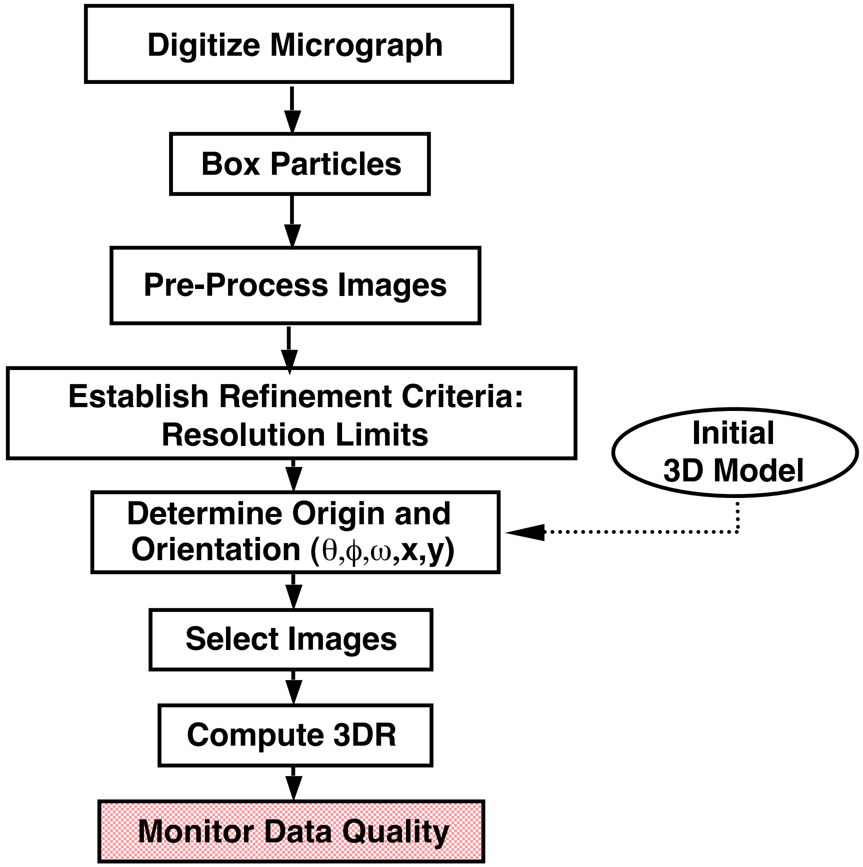


Icosahedral Virus 3D Reconstruction Scheme

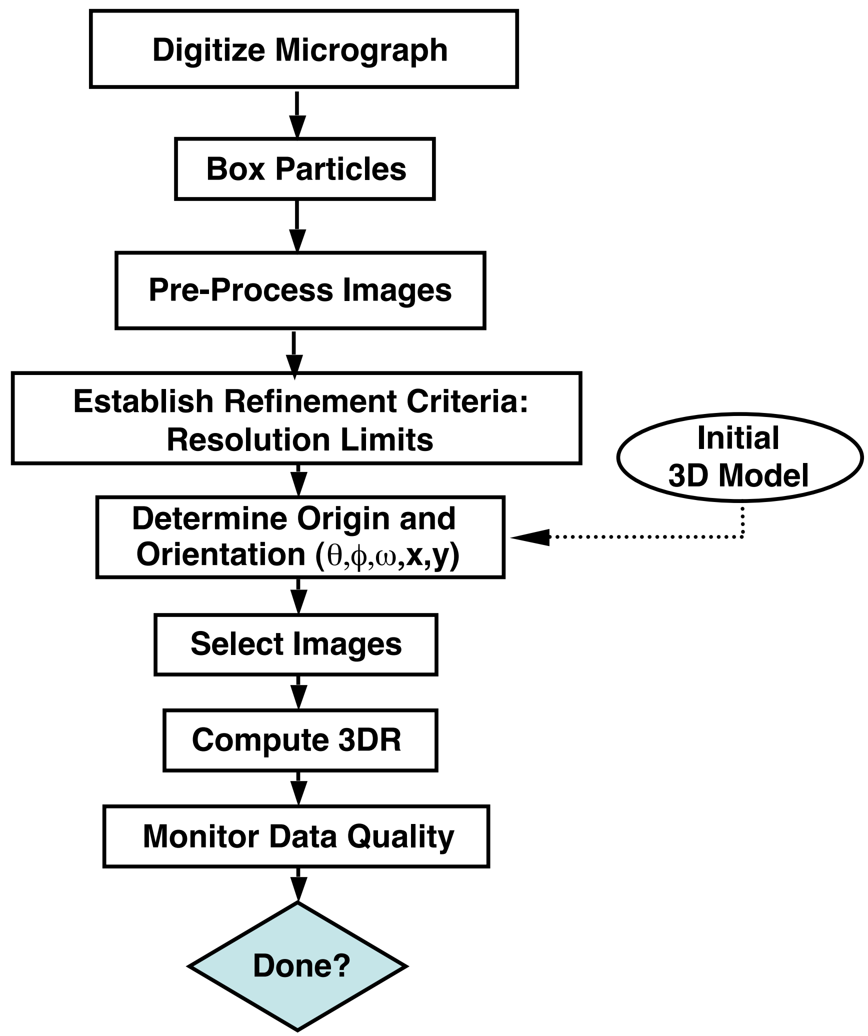
Monitor Data Quality



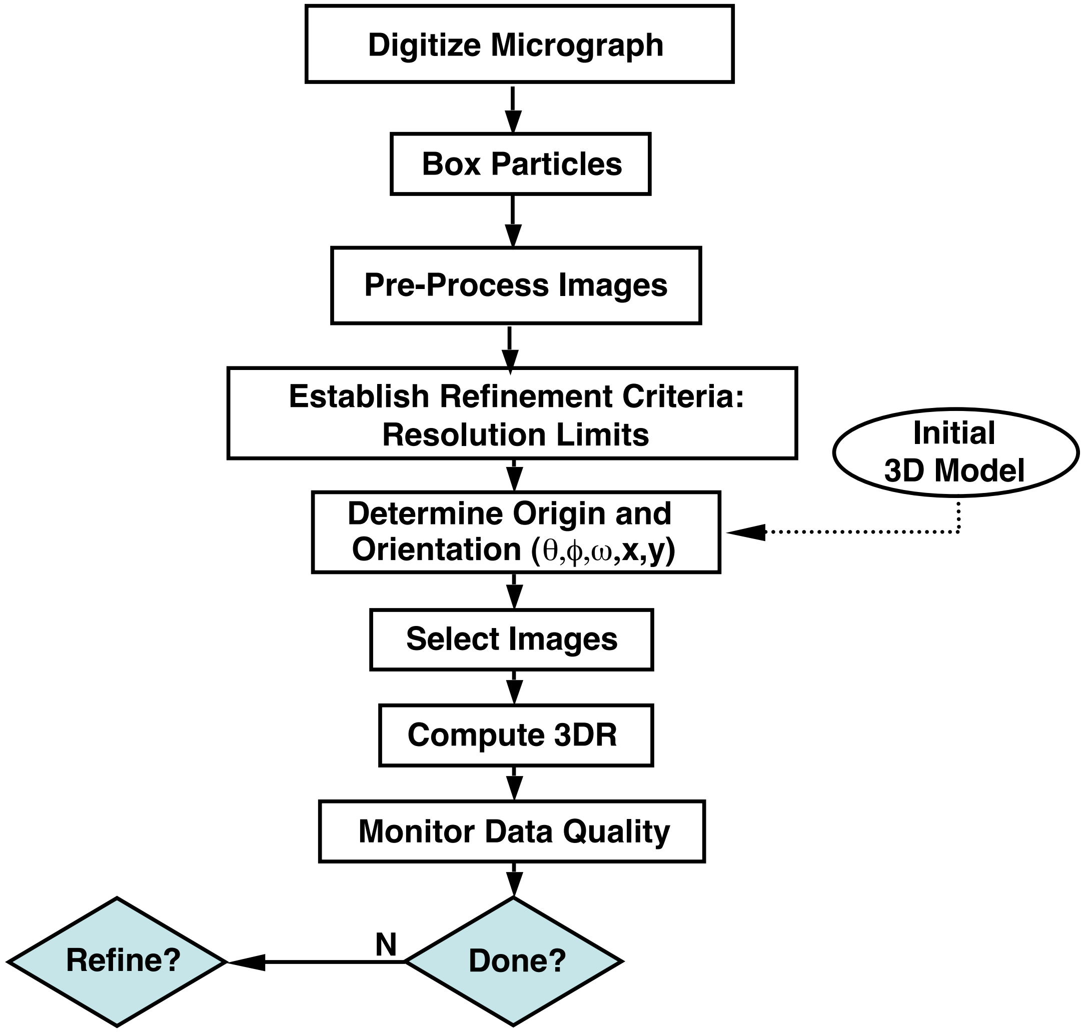
Icosahedral Virus 3D Reconstruction Scheme



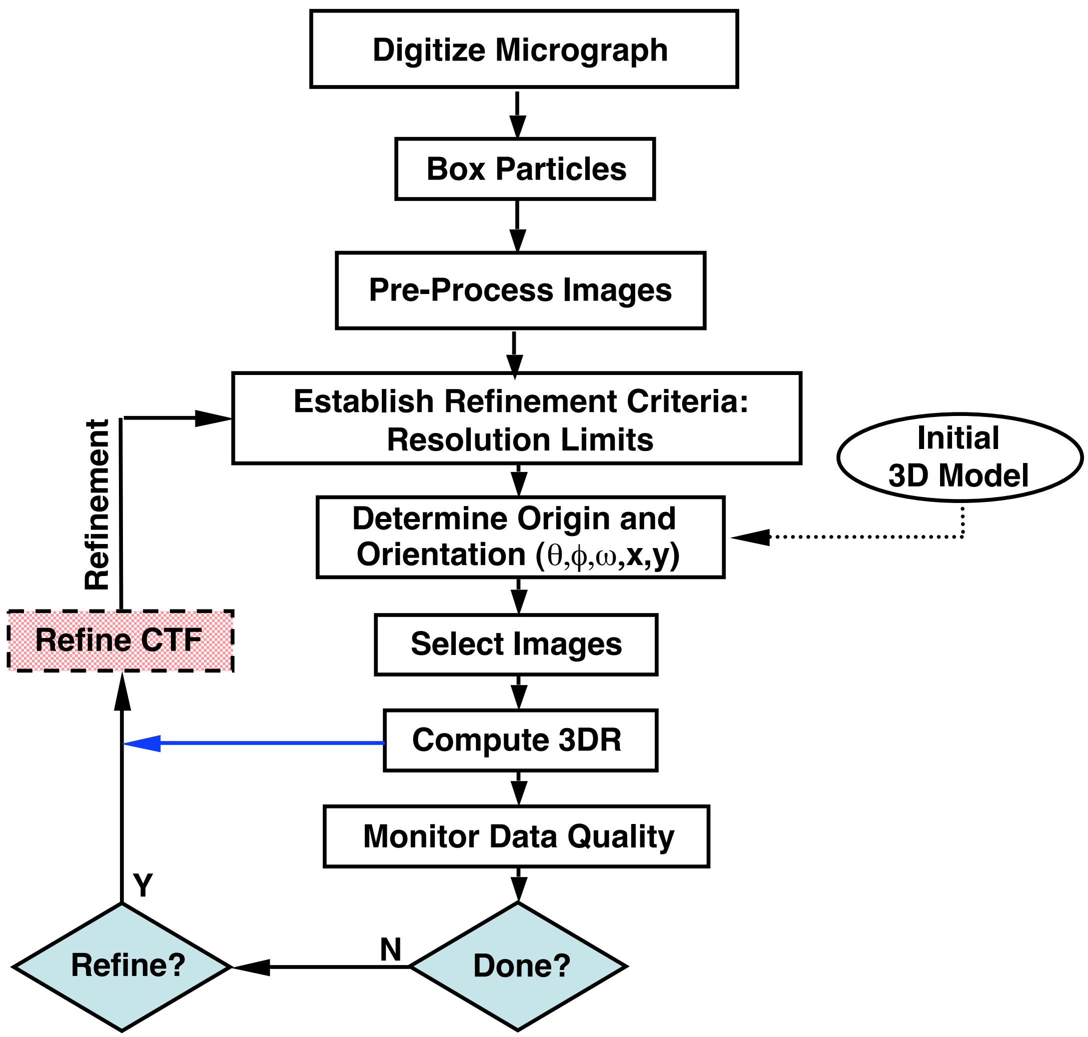
Icosahedral Virus 3D Reconstruction Scheme



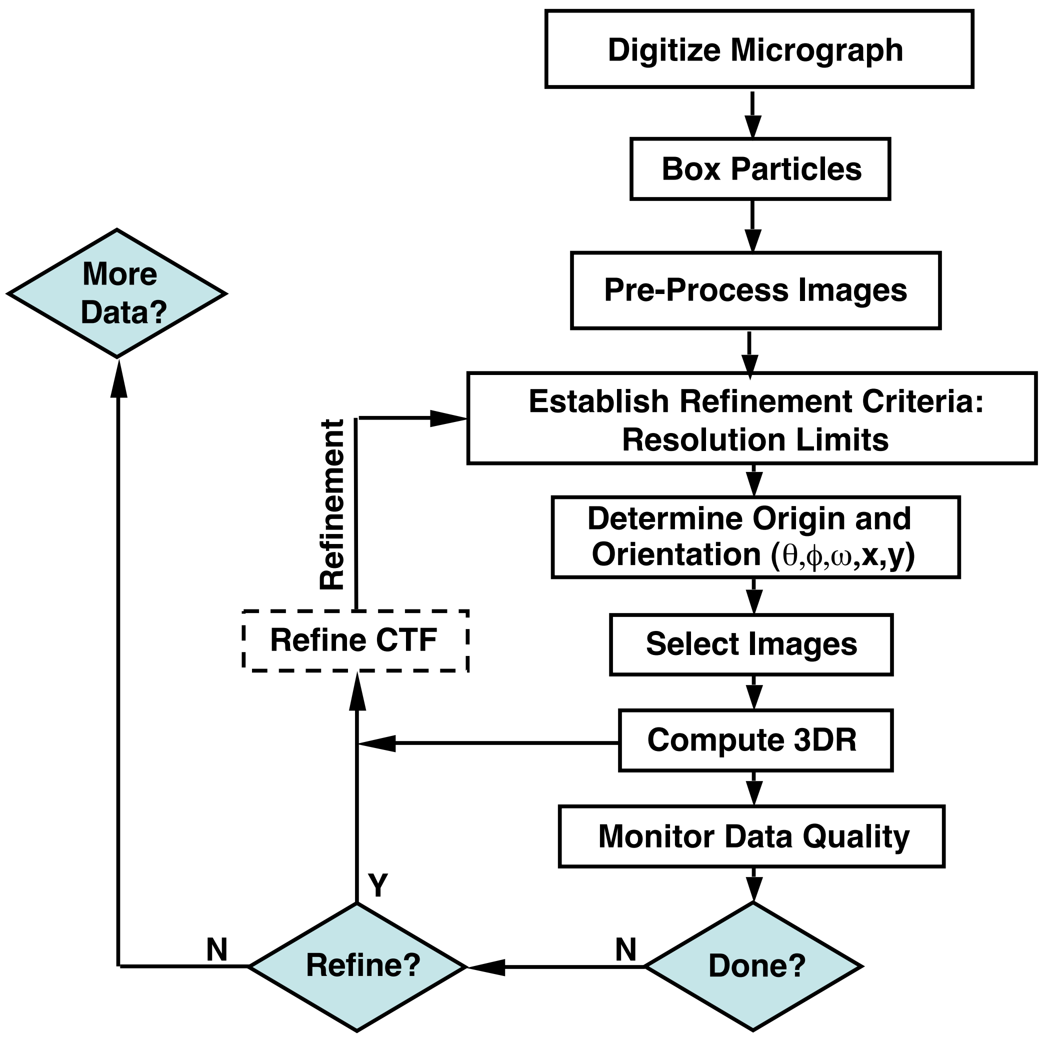
Icosahedral Virus 3D Reconstruction Scheme



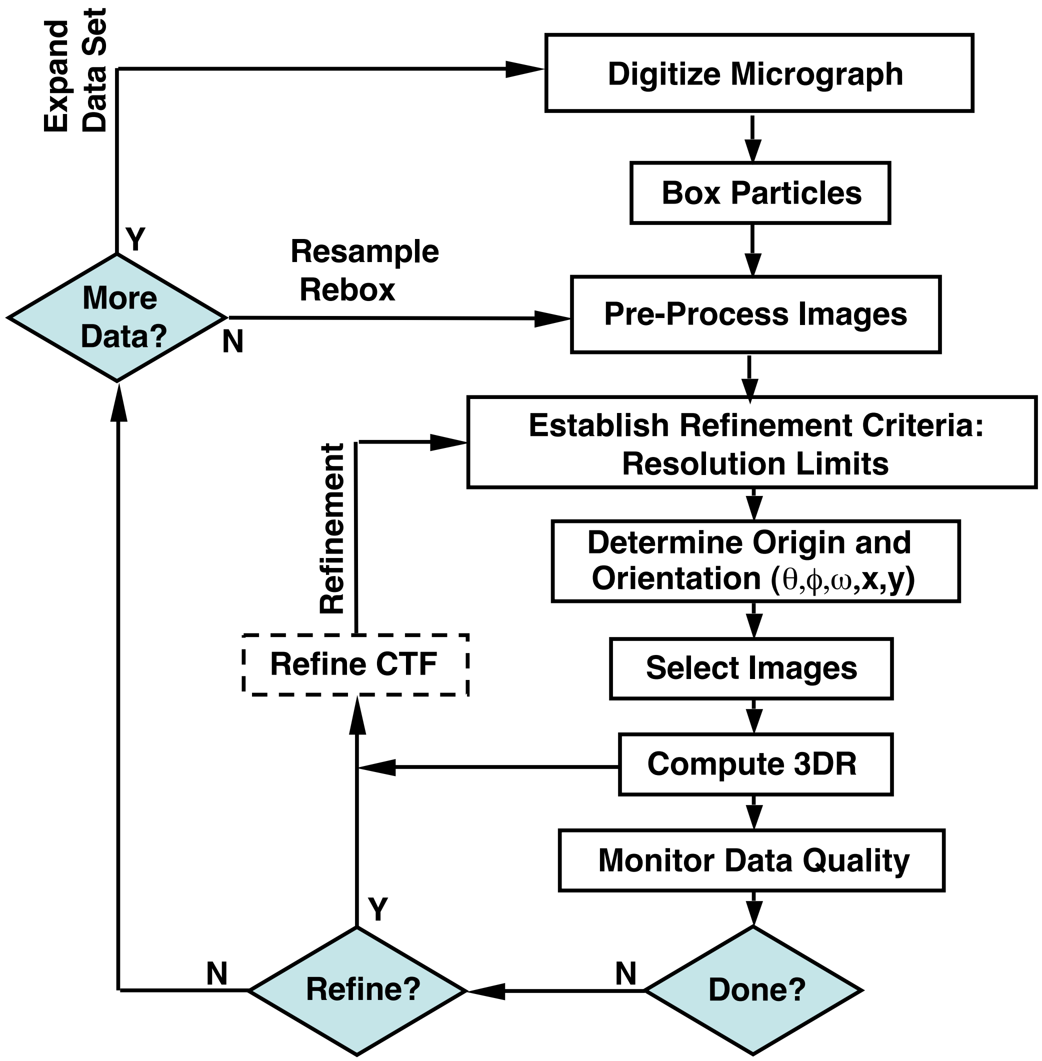
Icosahedral Virus 3D Reconstruction Scheme



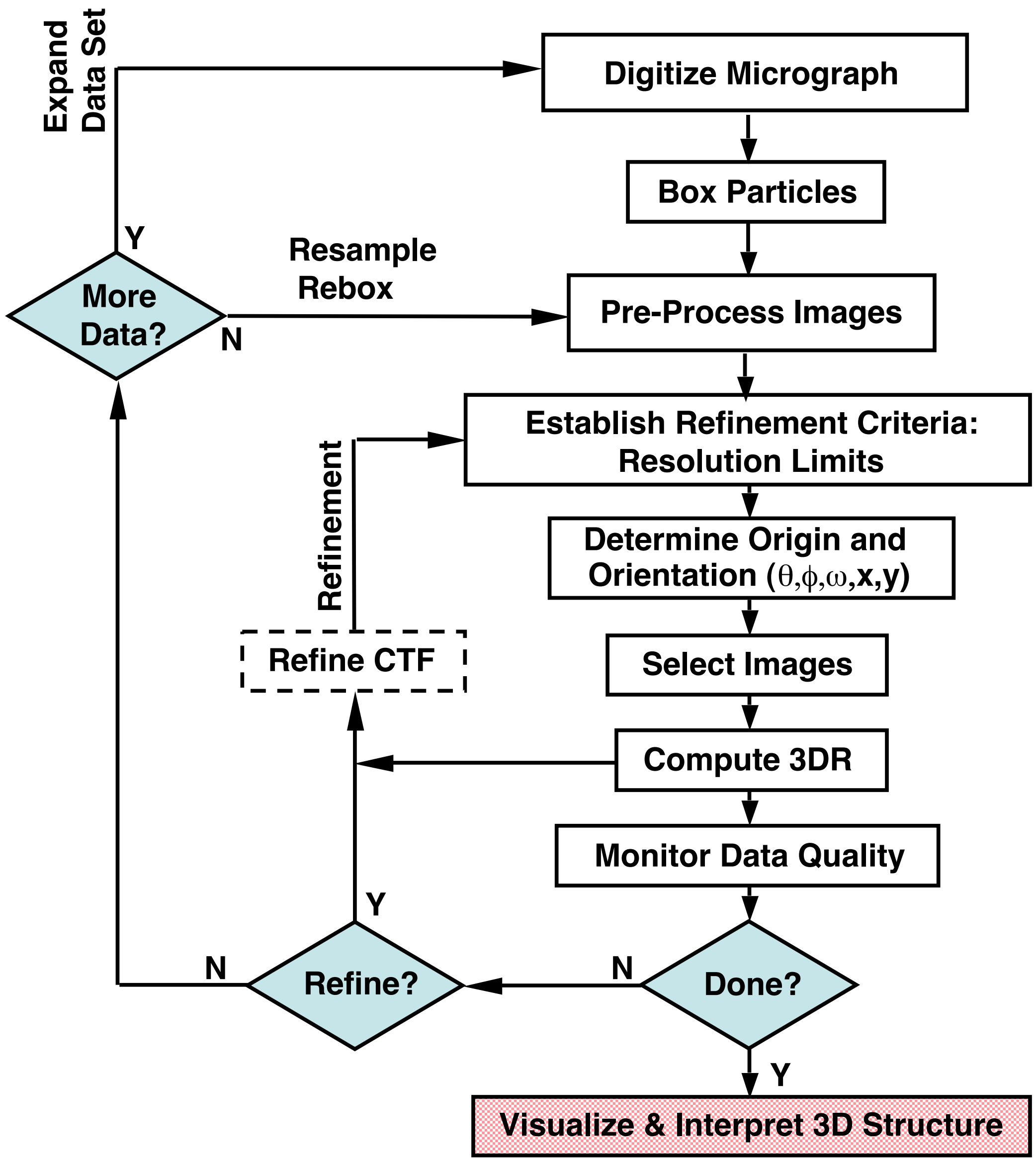
Icosahedral Virus 3D Reconstruction Scheme



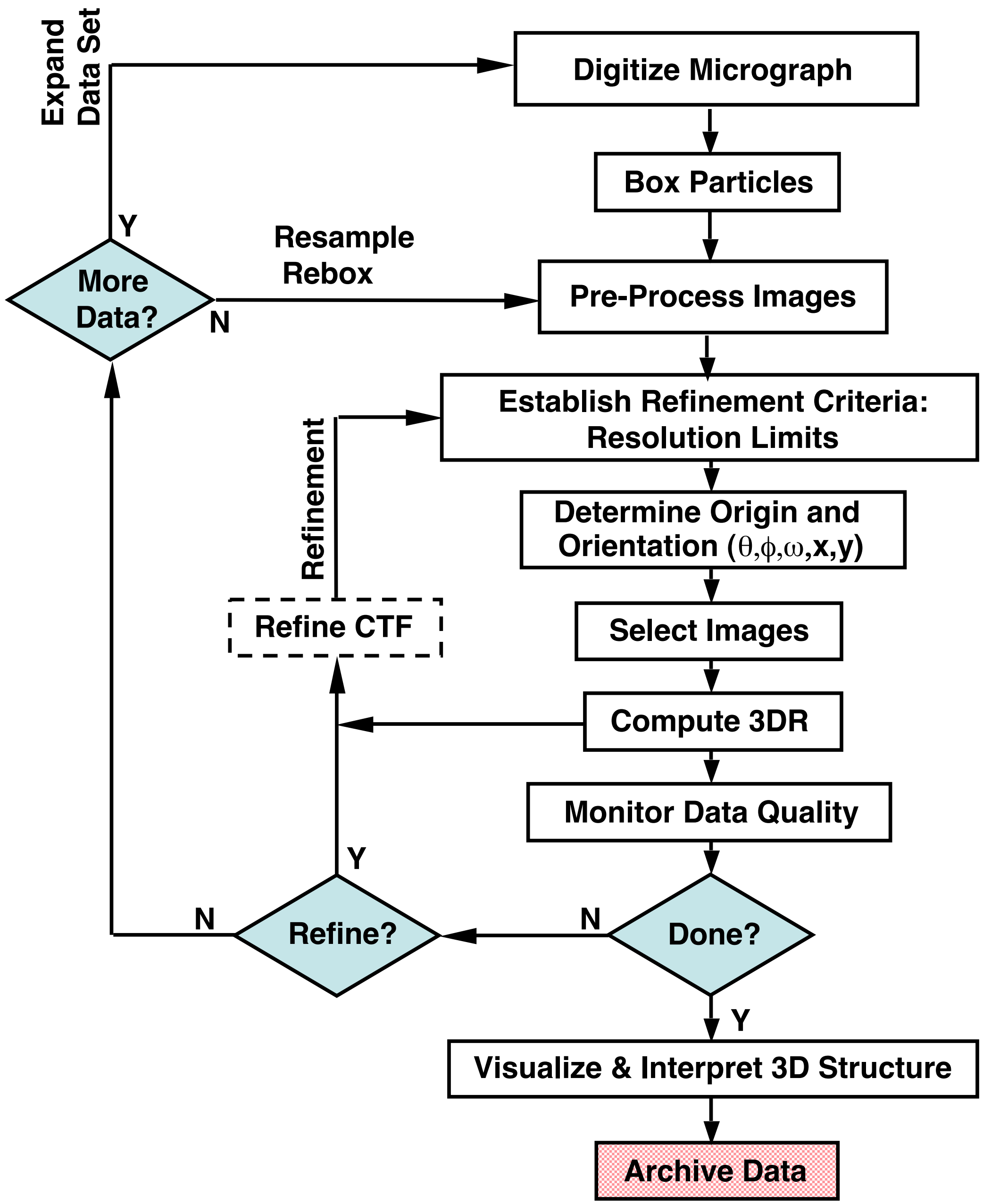
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



3D Reconstruction of Icosahedral Particles

Outline

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

3D Reconstruction of Icosahedral Particles

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

End of Sec.III.D.5