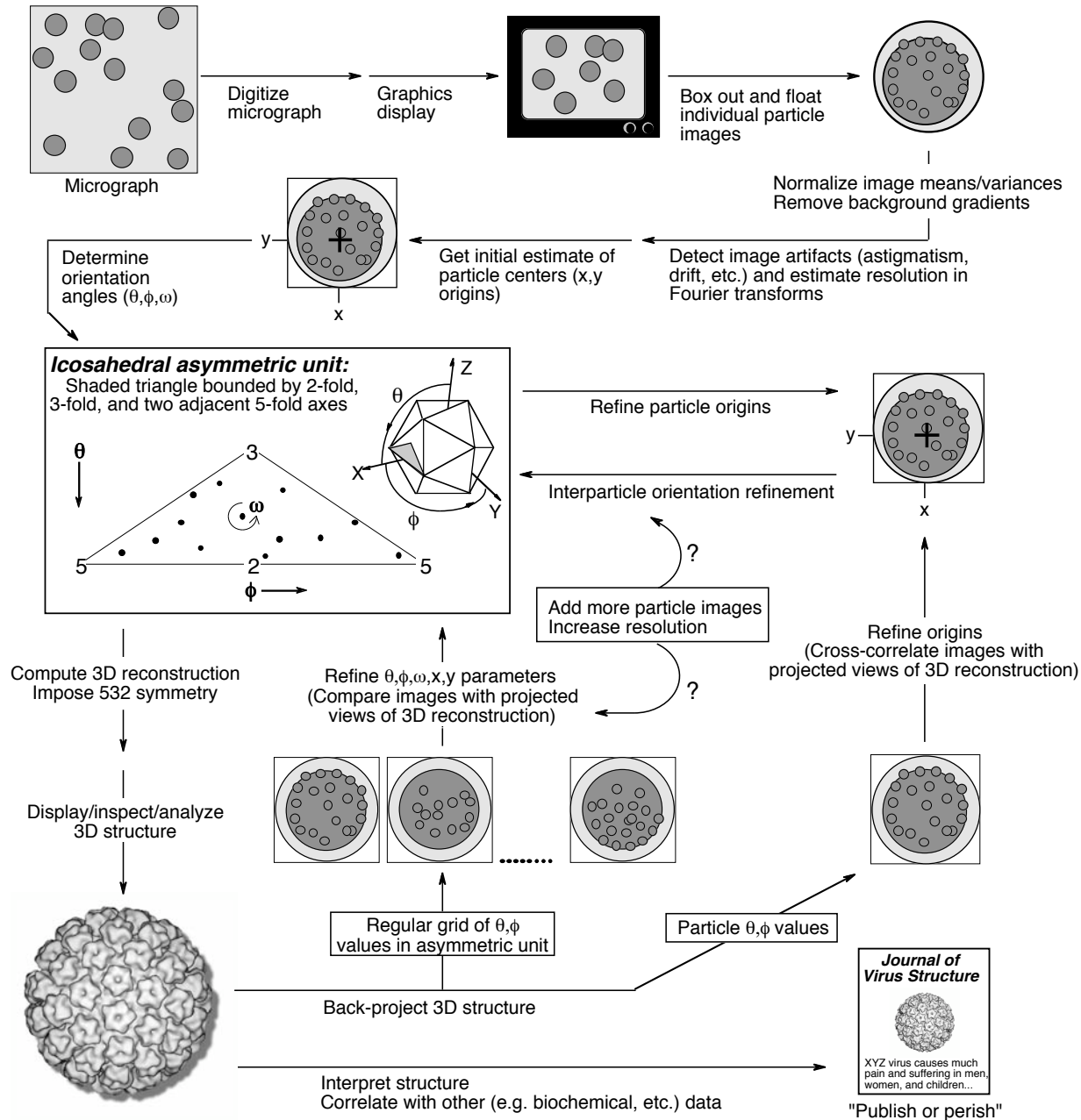
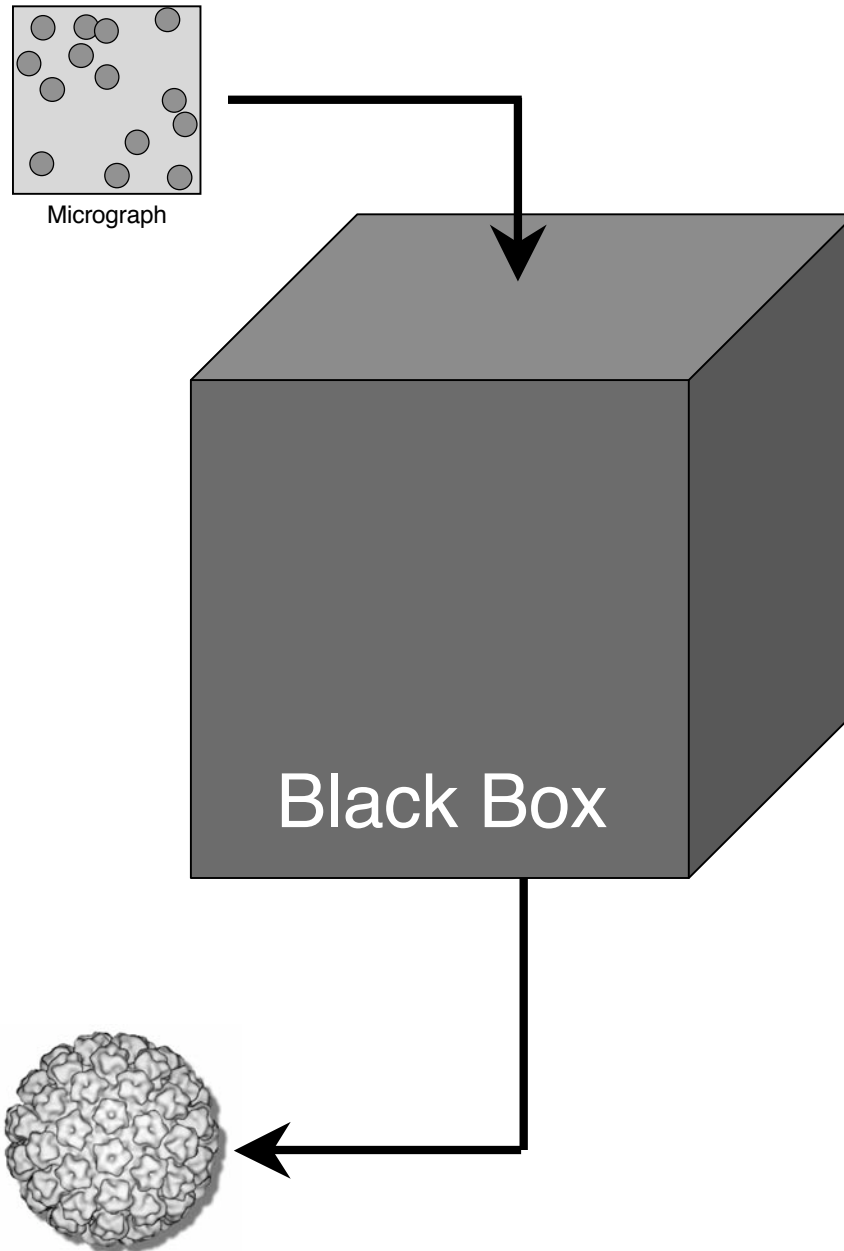


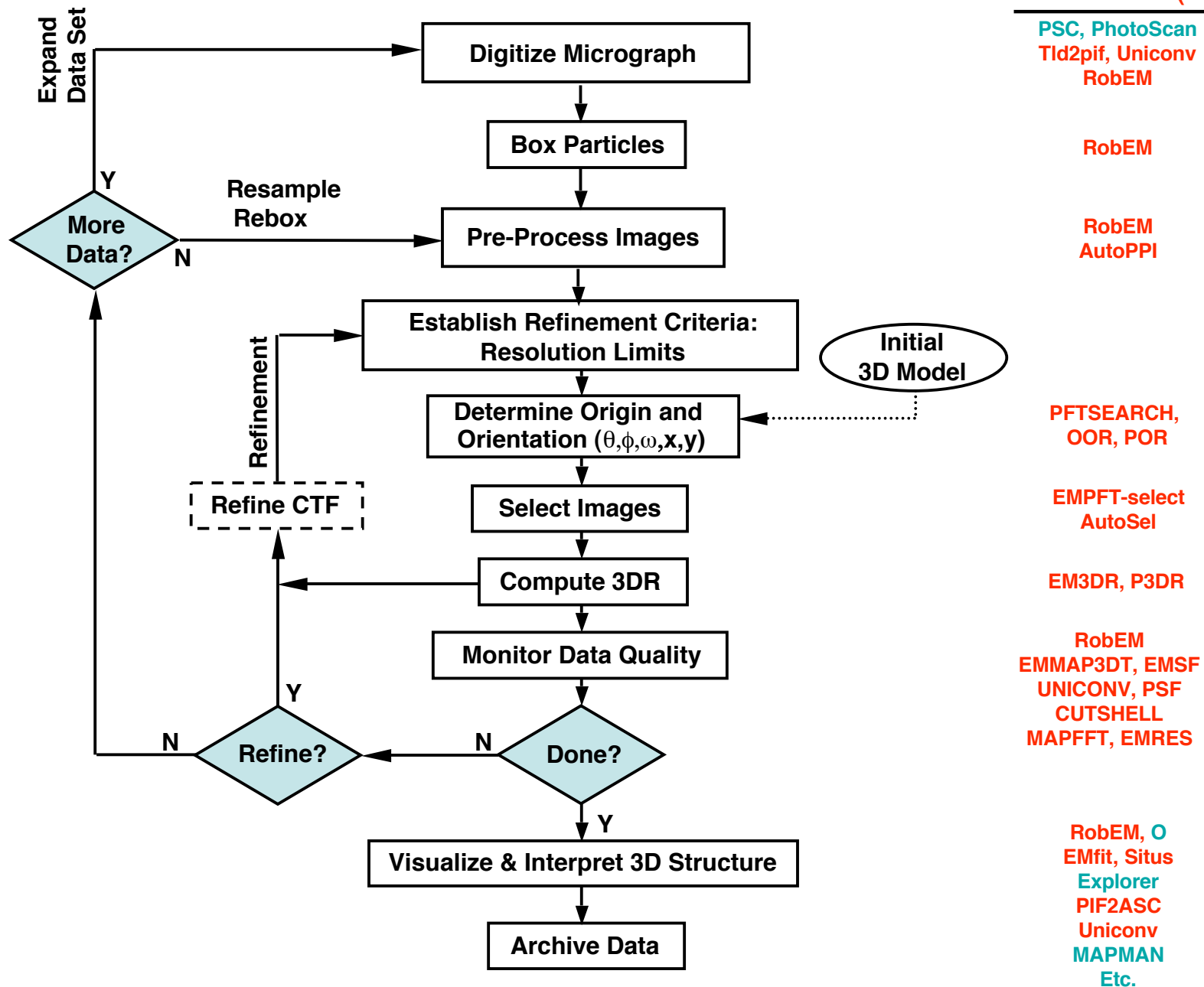
Icosahedral Particle Image Reconstruction Scheme



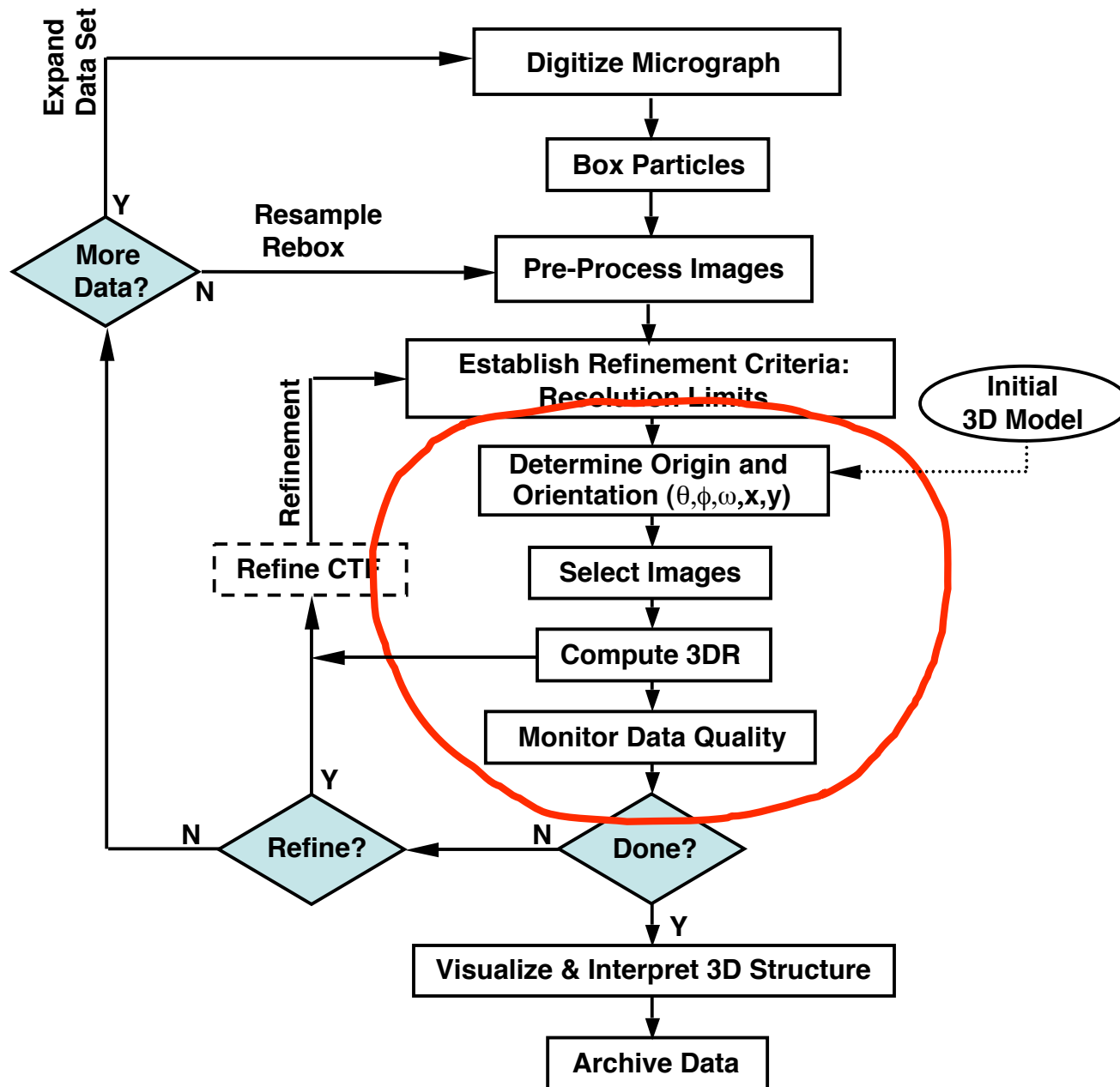
Icosahedral Particle Image Reconstruction Scheme



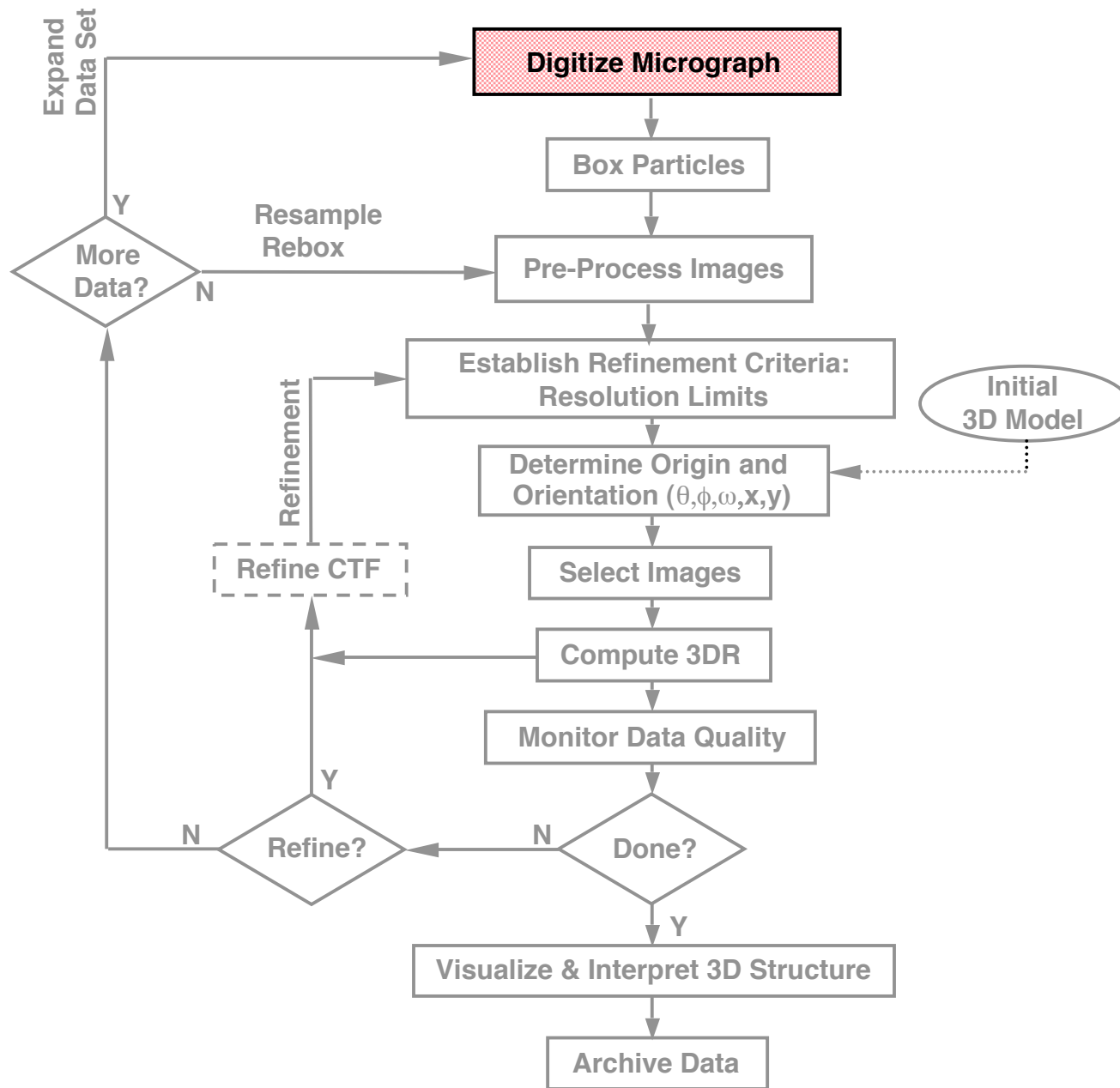
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



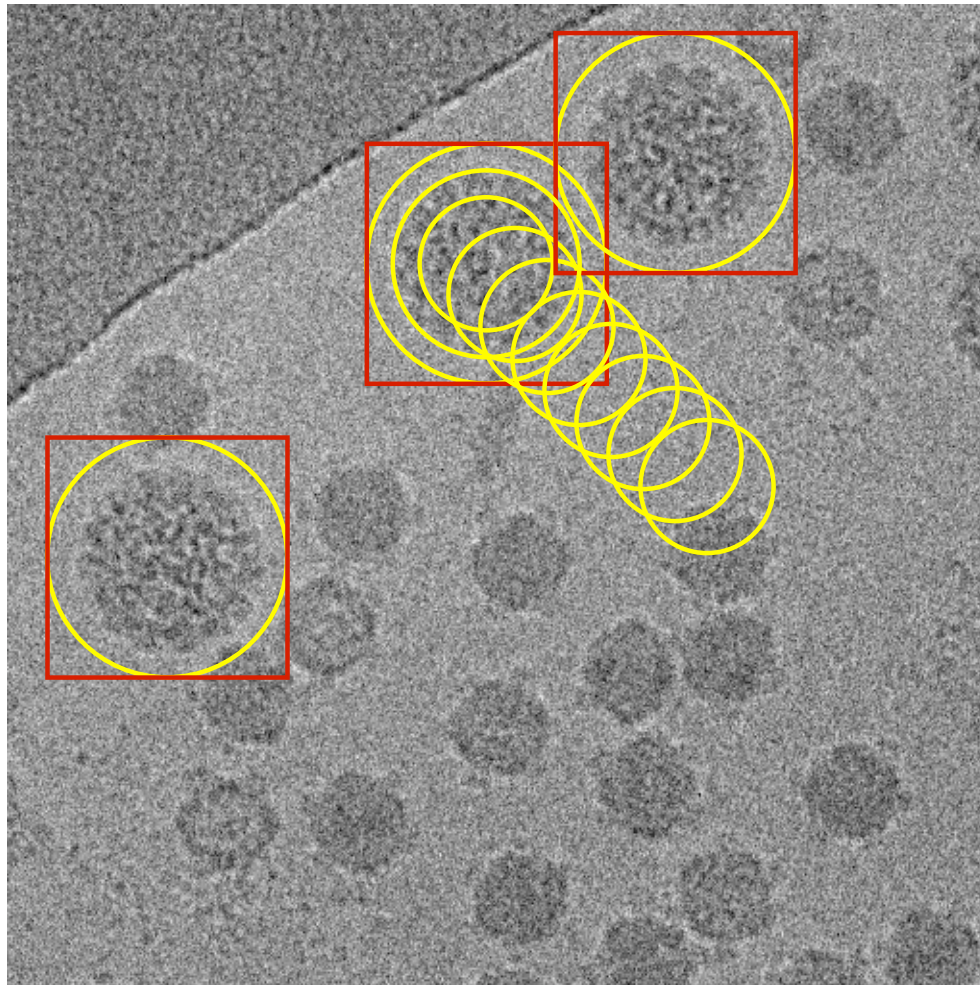
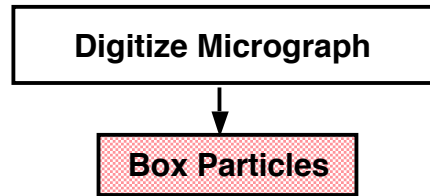
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

Icosahedral Virus 3D Reconstruction Scheme

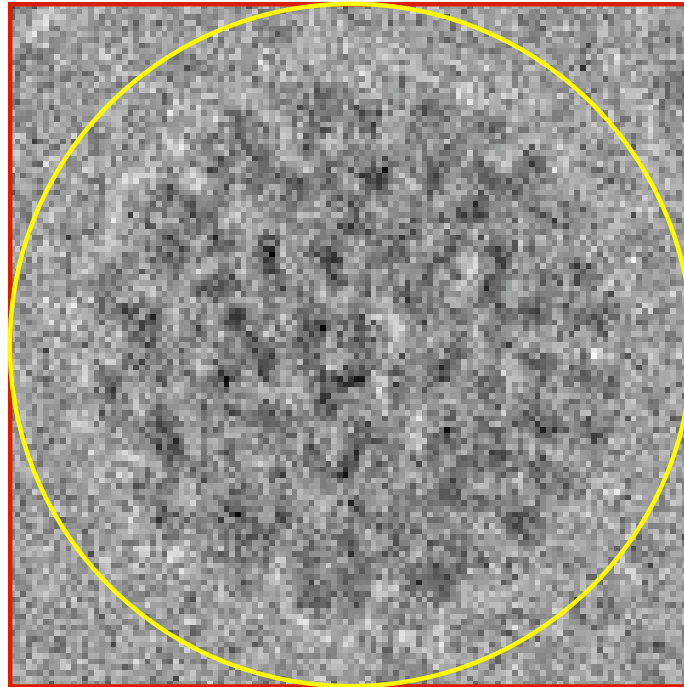


Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph



Box Particles



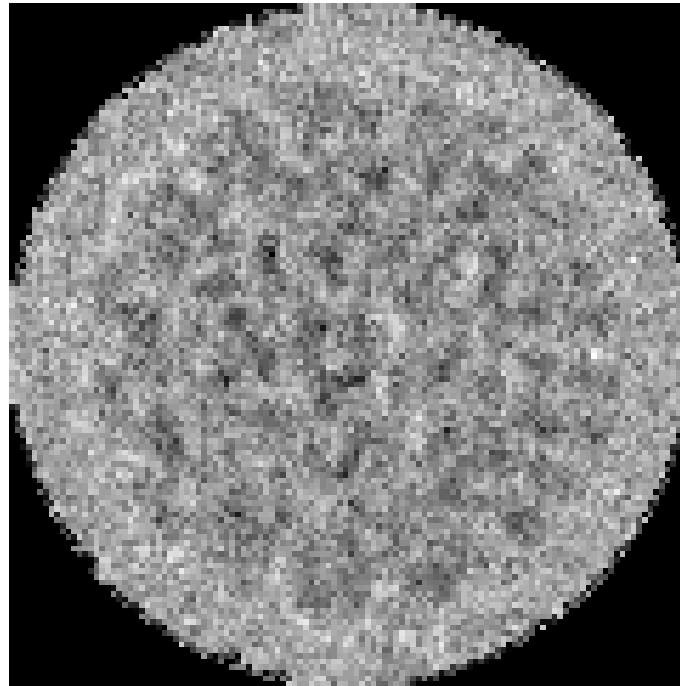
Extracted

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph



Box Particles



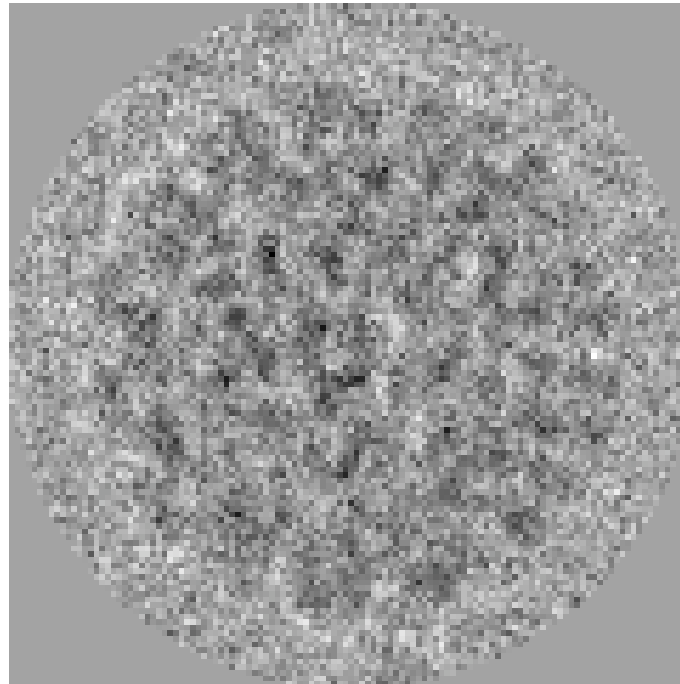
Masked

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph



Box Particles



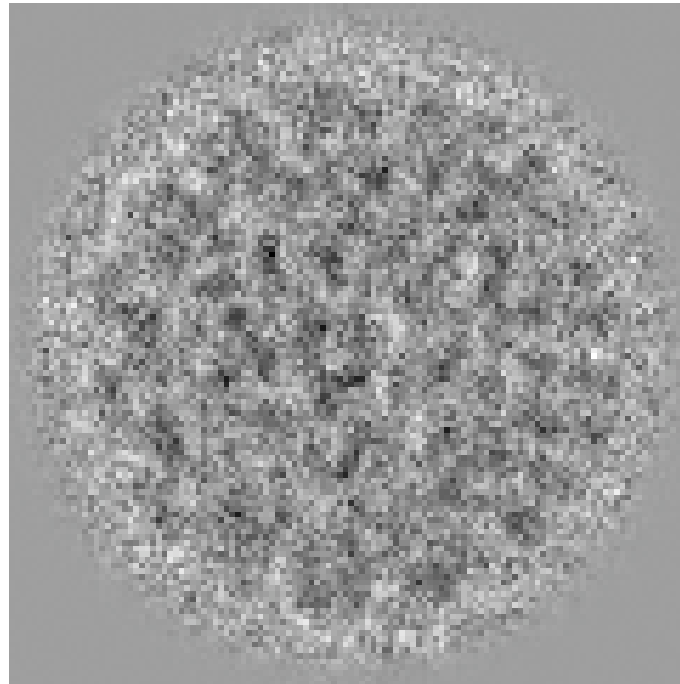
Floated

Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

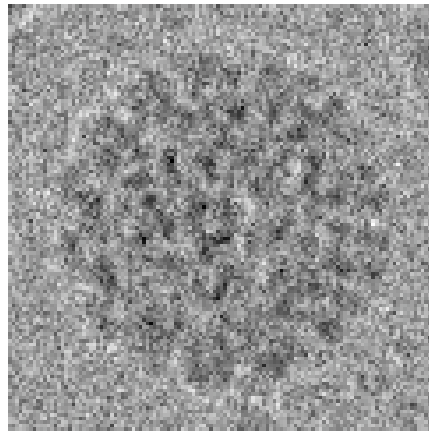
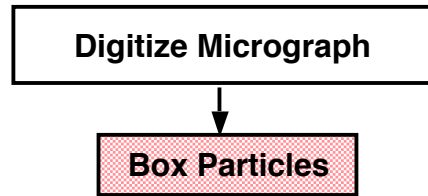


Box Particles

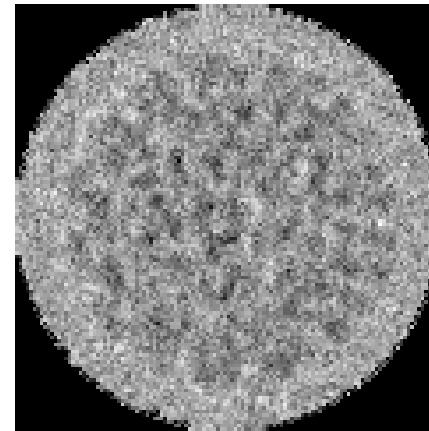


Apodized

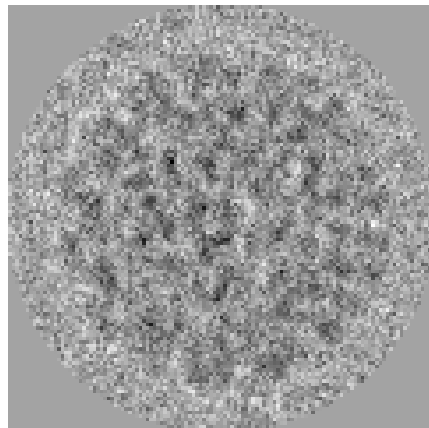
Icosahedral Virus 3D Reconstruction Scheme



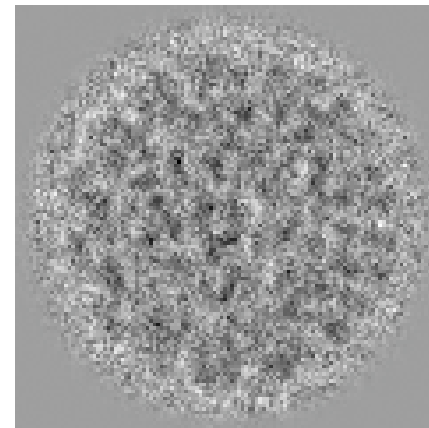
Extracted



Masked

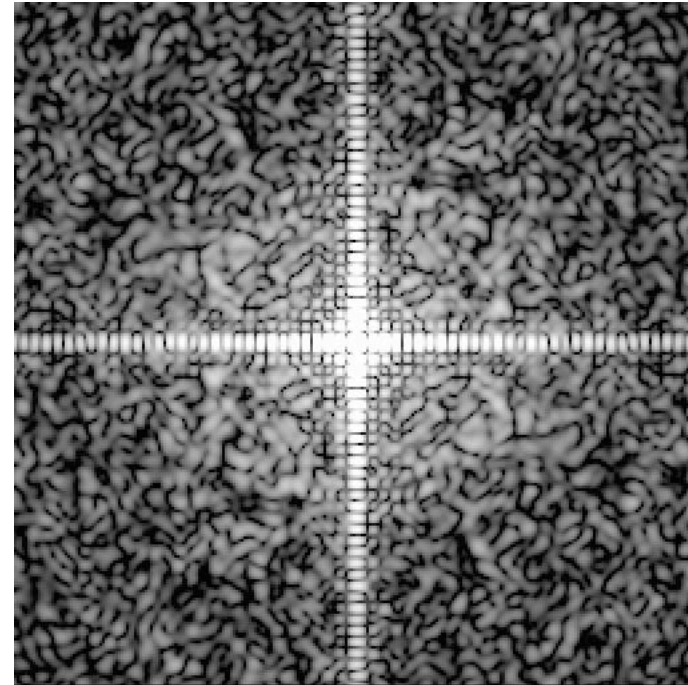
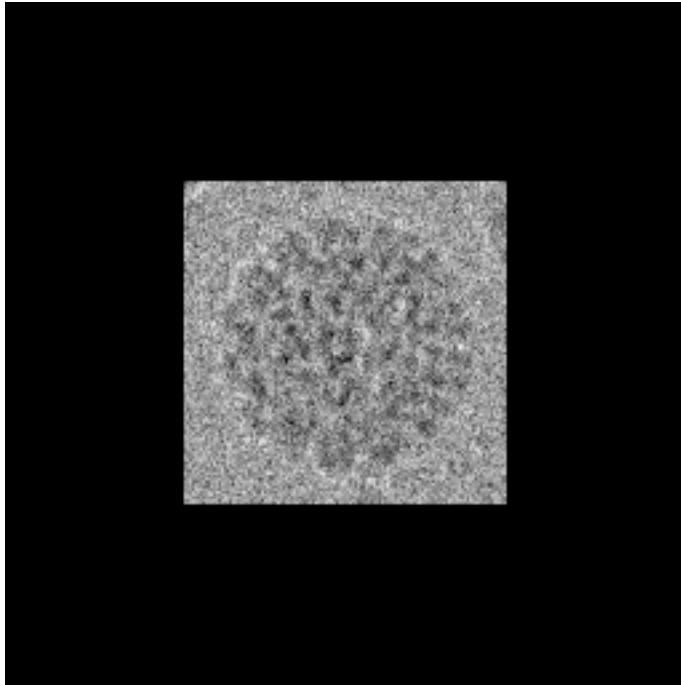
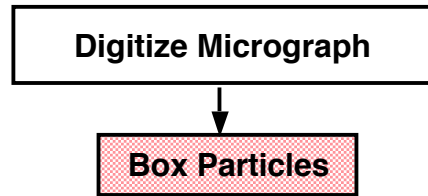


Floated



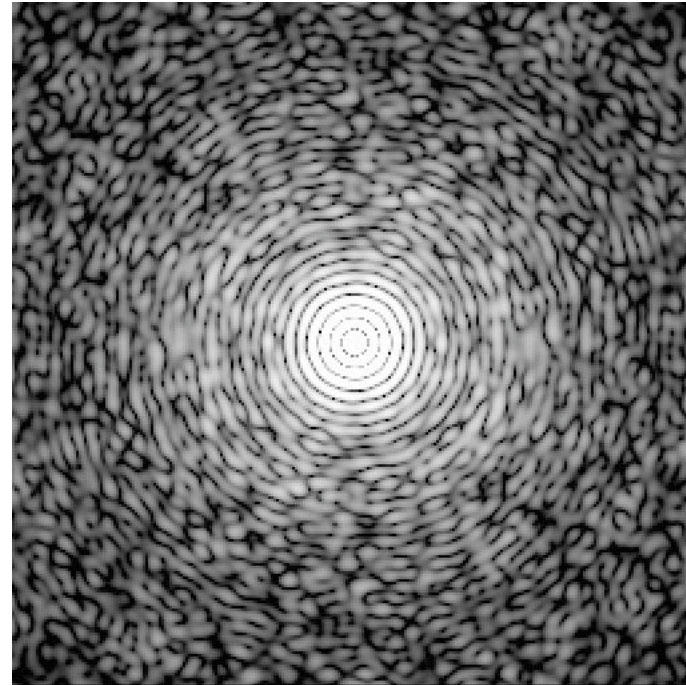
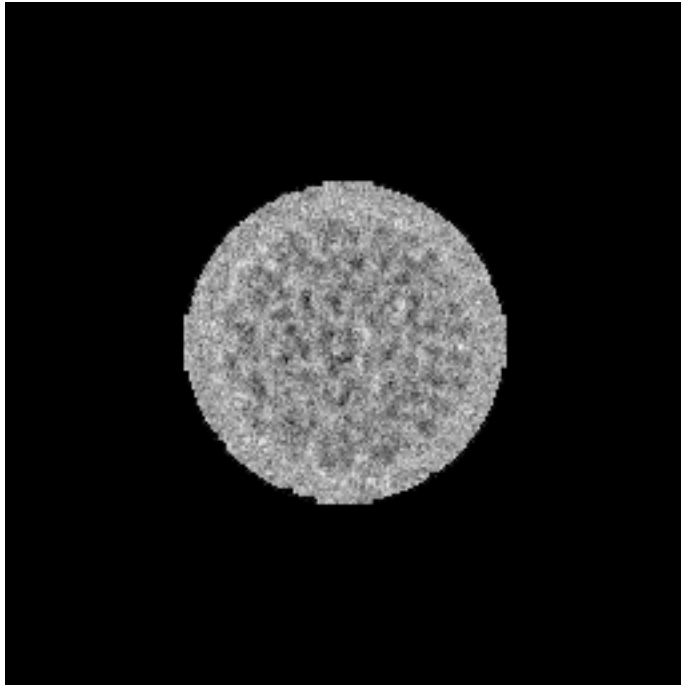
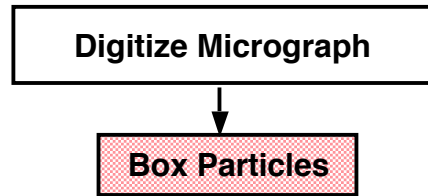
Apodized

Icosahedral Virus 3D Reconstruction Scheme



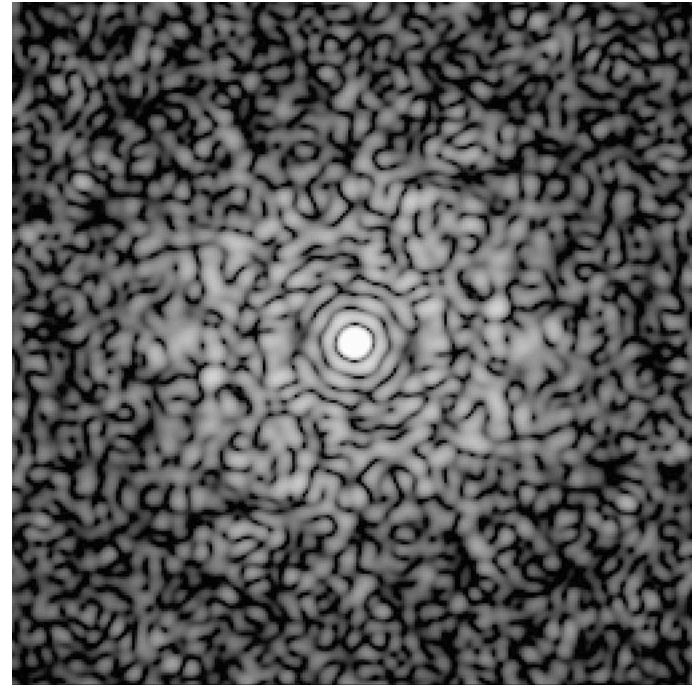
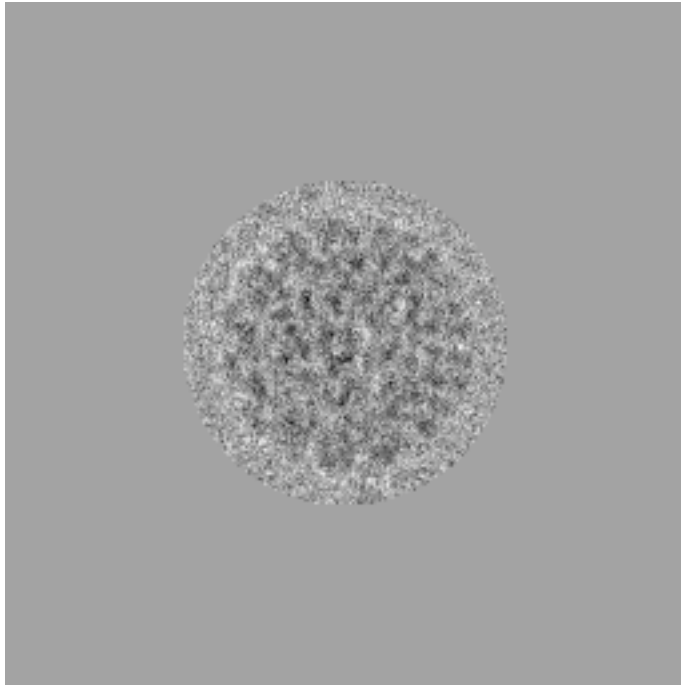
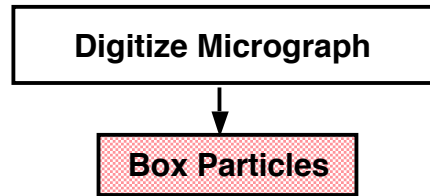
Square mask; unfloated

Icosahedral Virus 3D Reconstruction Scheme



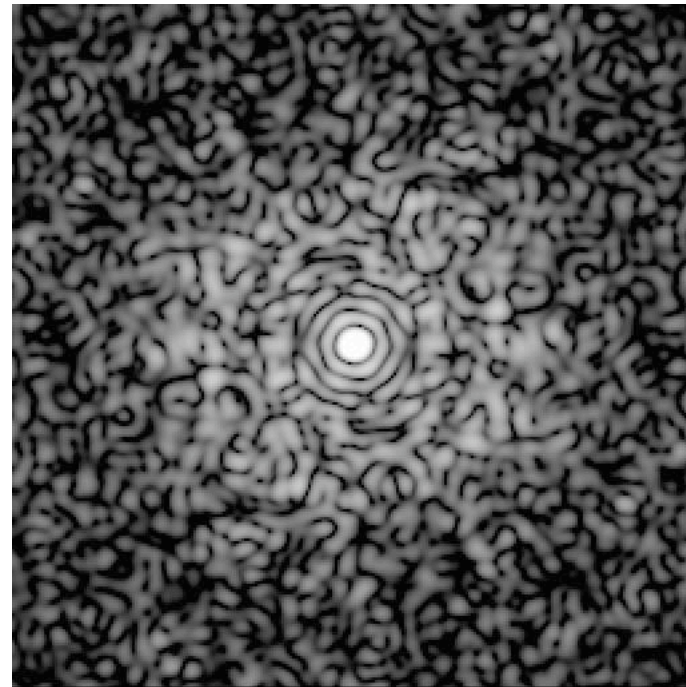
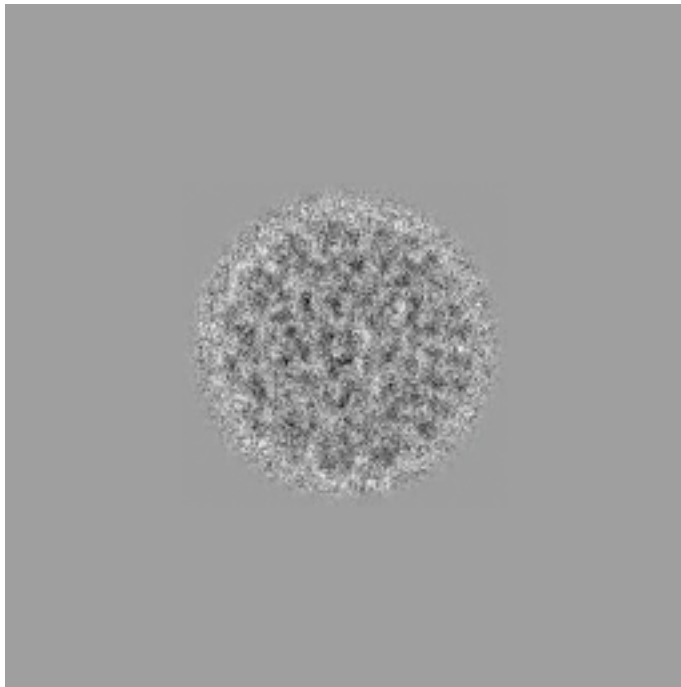
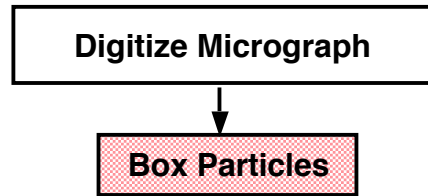
Circular mask; unfloated

Icosahedral Virus 3D Reconstruction Scheme



Circular mask; floated

Icosahedral Virus 3D Reconstruction Scheme



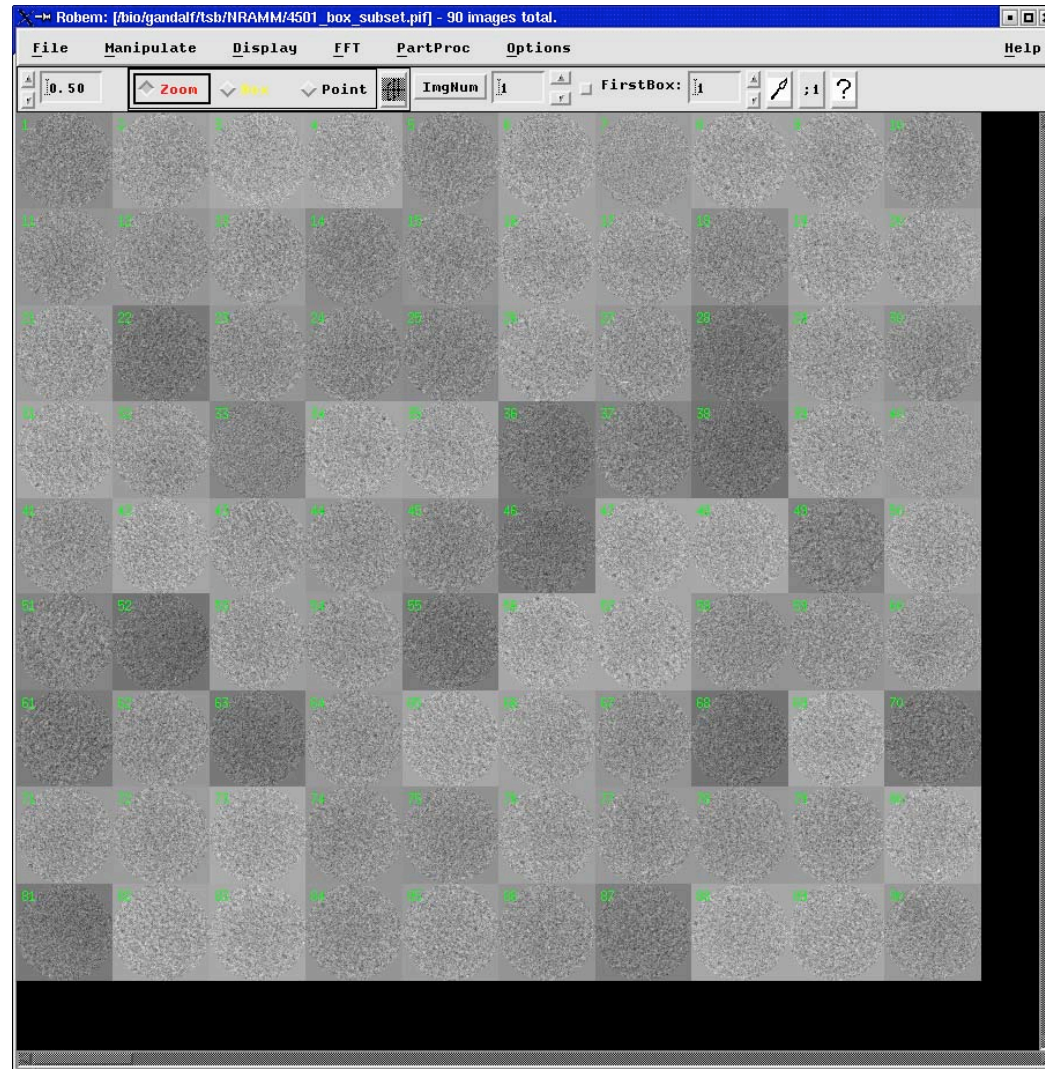
Circular mask; floated & apodized

Icosahedral Virus 3D Reconstruction Scheme

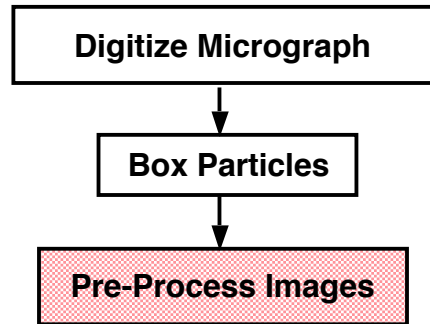
Digitize Micrograph



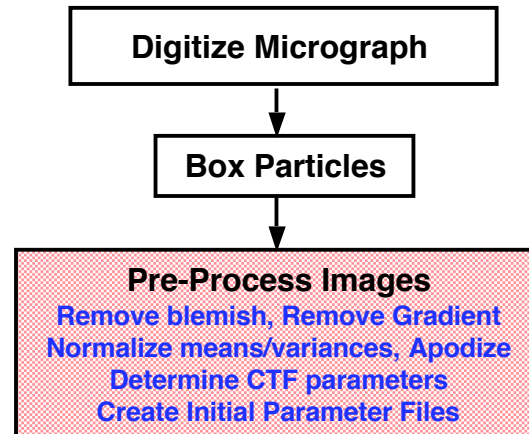
Box Particles



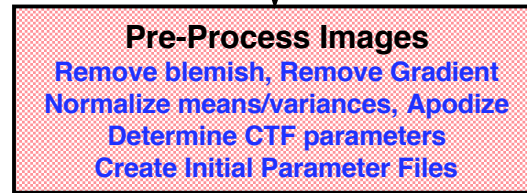
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



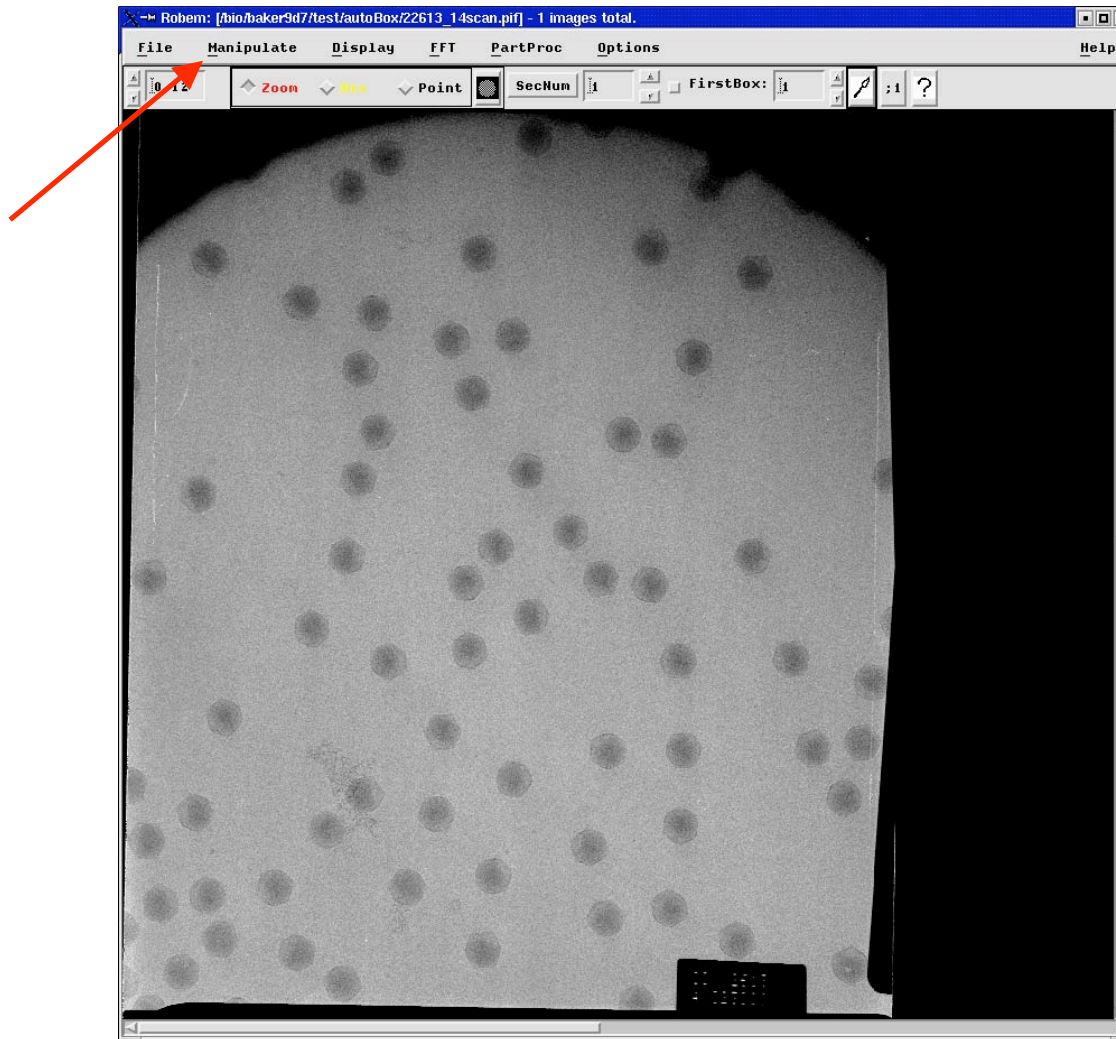
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



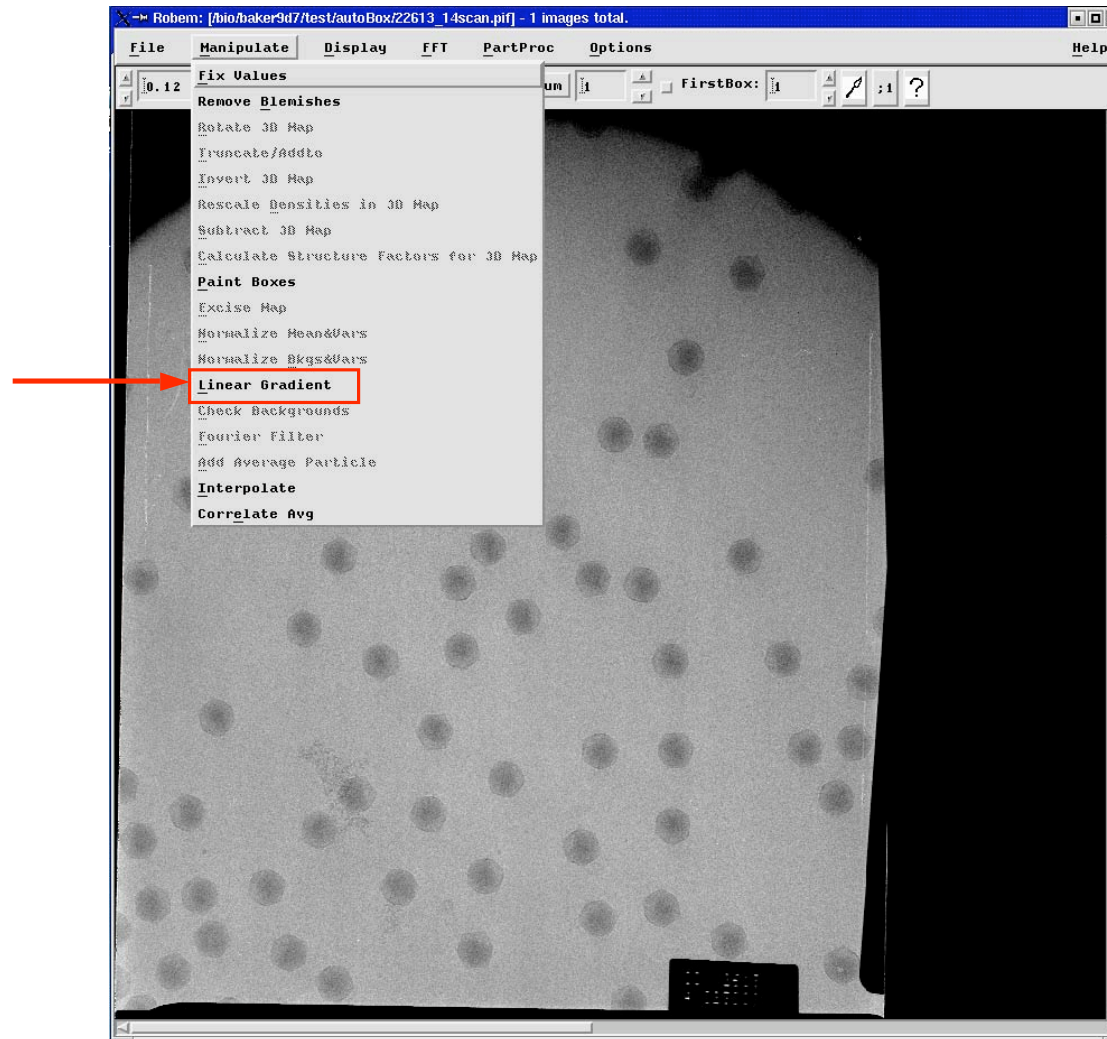
Pre-Process images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files



Icosahedral Virus 3D Reconstruction Scheme



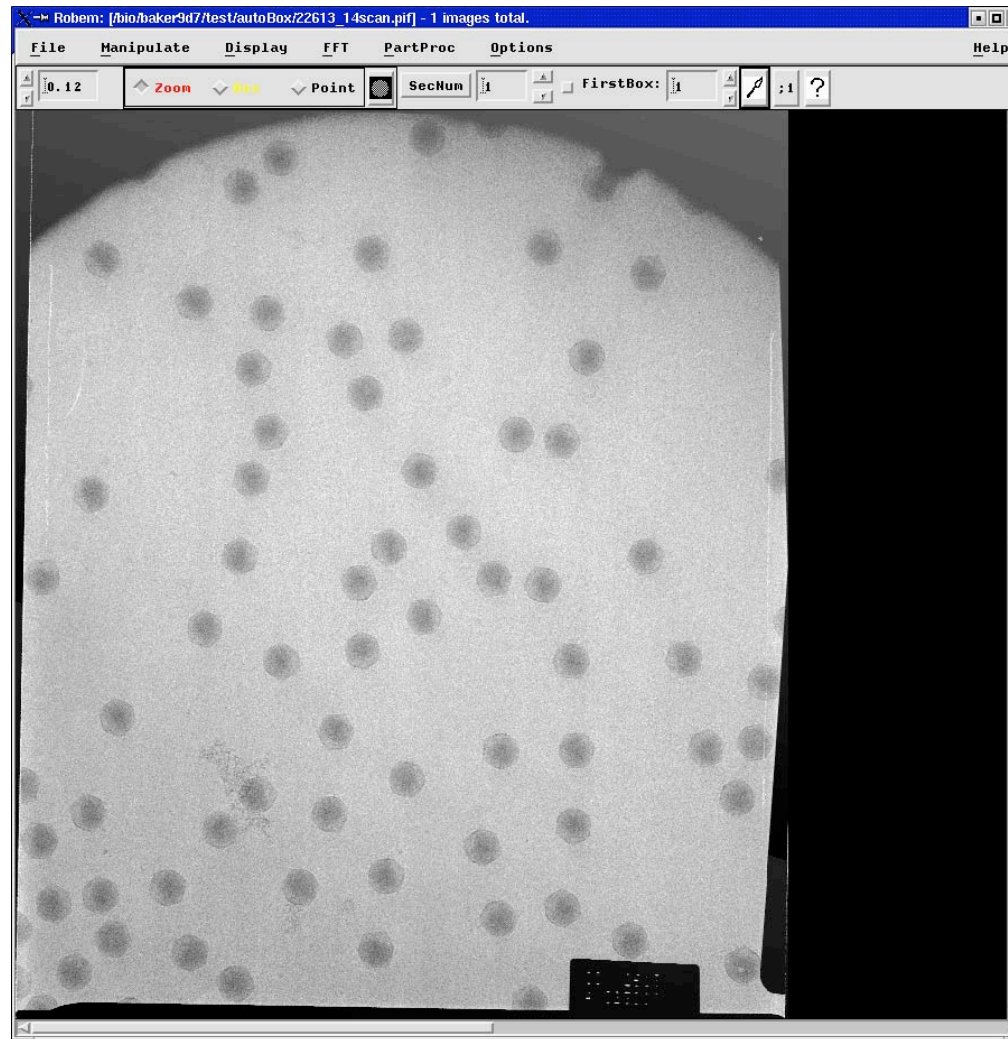
Pre-Process images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files



Icosahedral Virus 3D Reconstruction Scheme



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

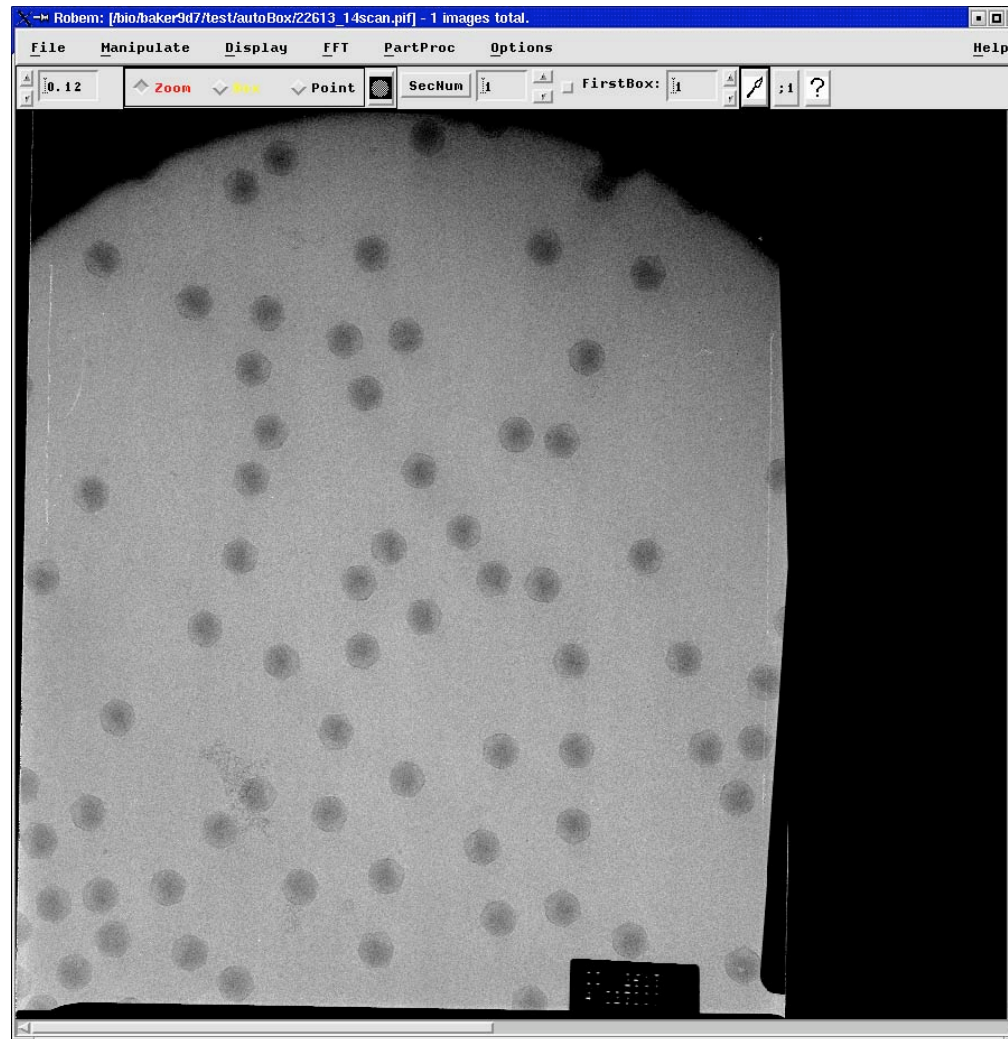


Gradient
removed

Icosahedral Virus 3D Reconstruction Scheme



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

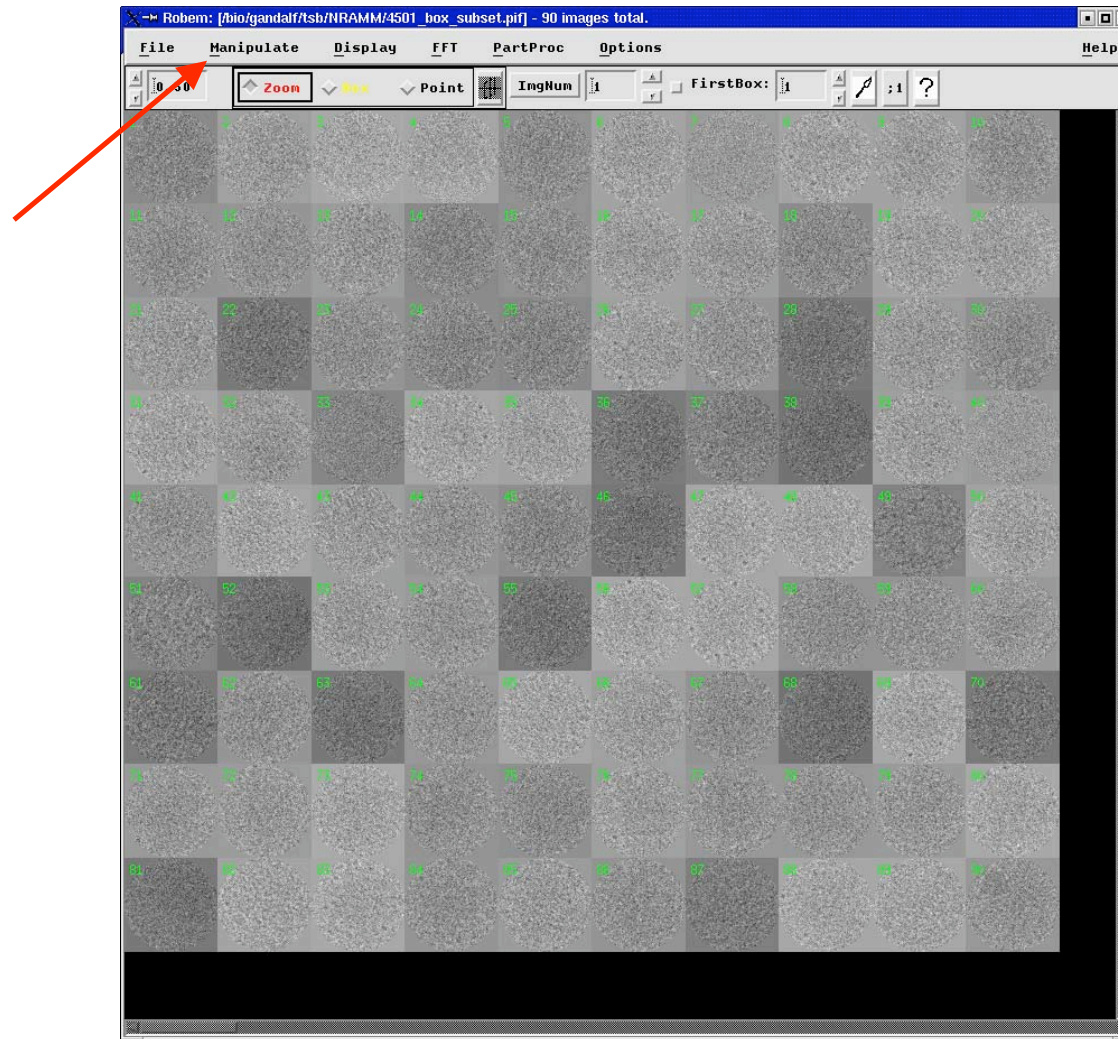


Gradient
not removed

Icosahedral Virus 3D Reconstruction Scheme



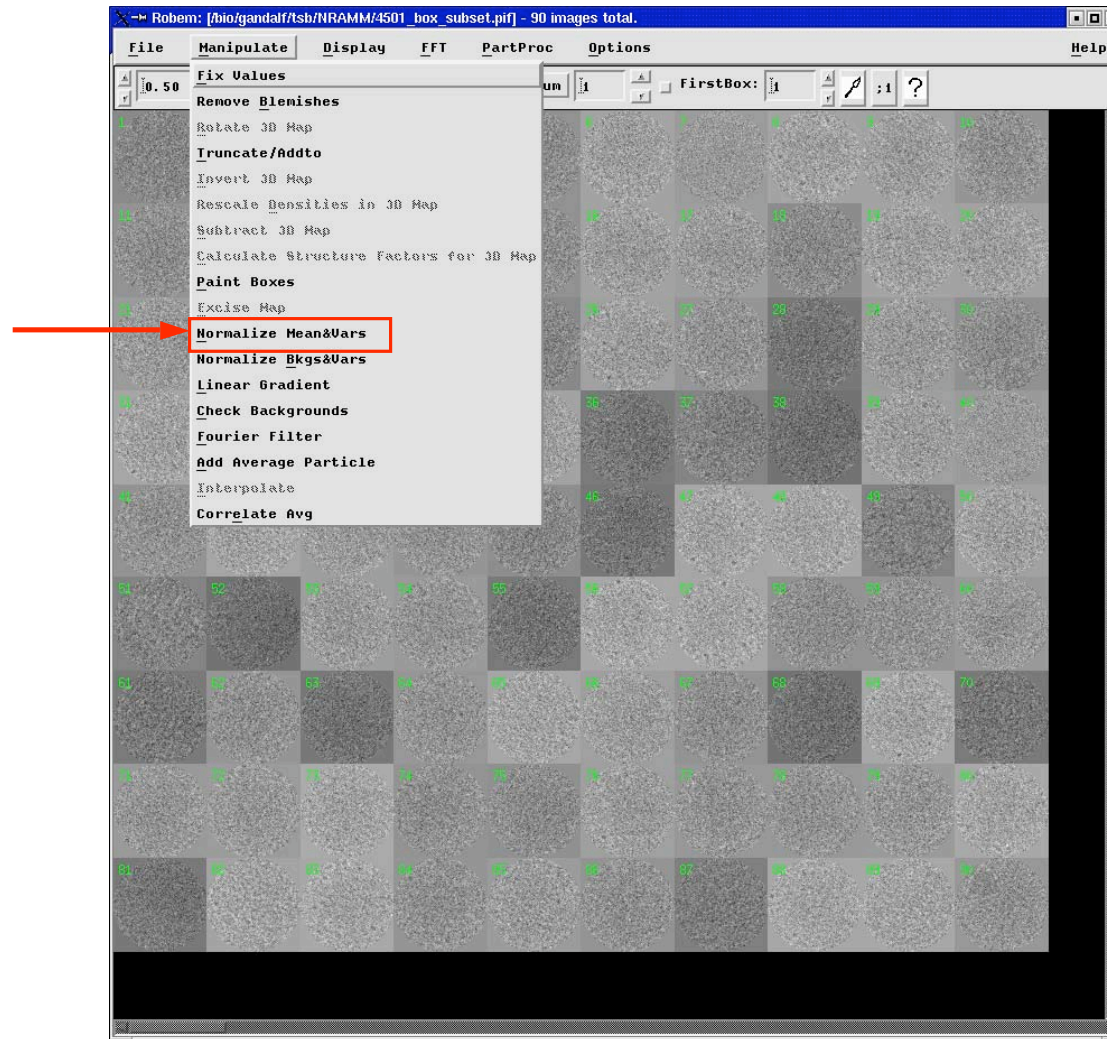
Pre-Process images
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files



Icosahedral Virus 3D Reconstruction Scheme



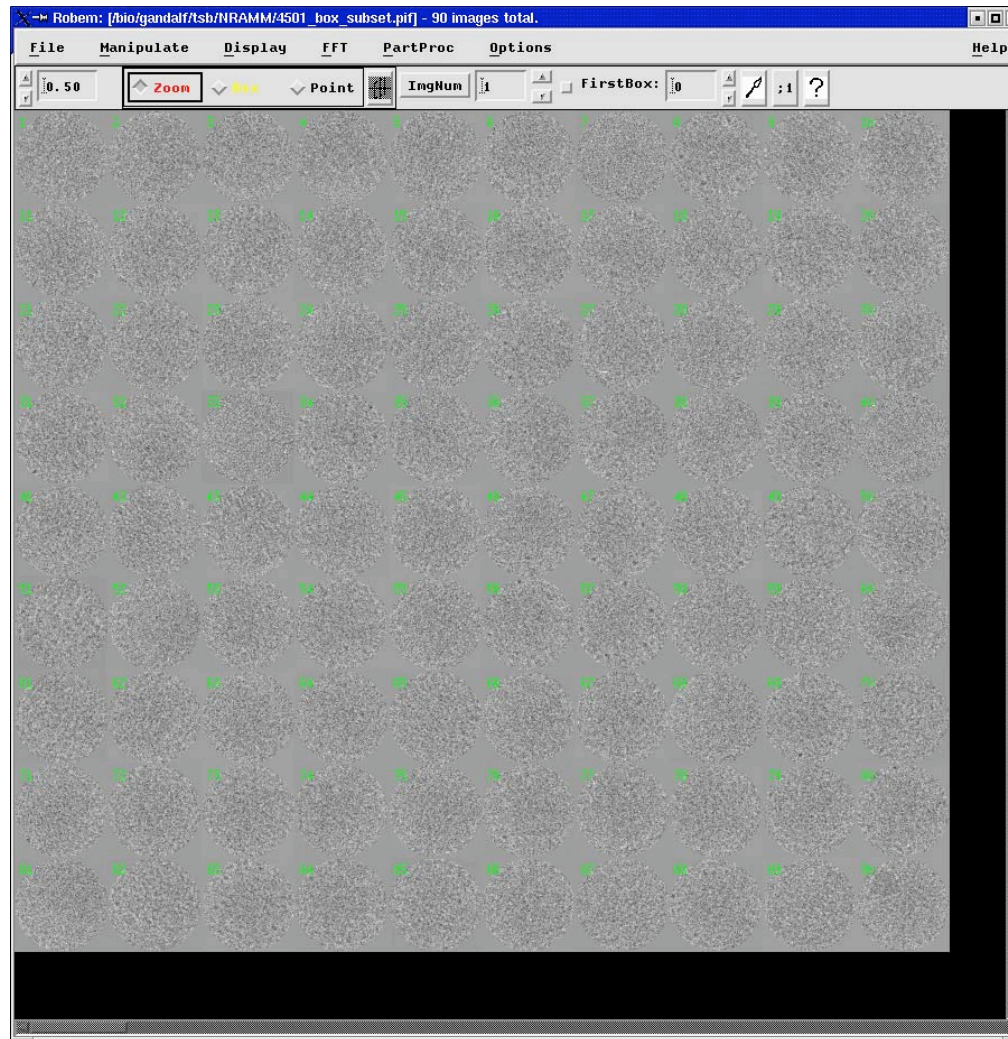
Pre-Process Images
Remove blenish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files



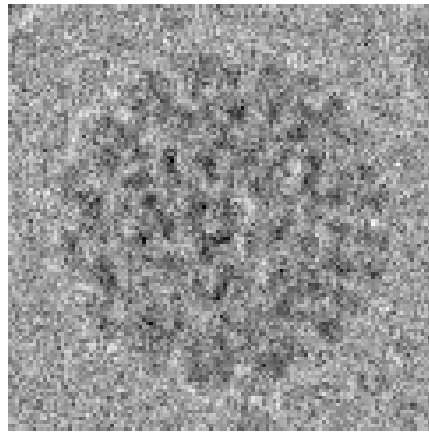
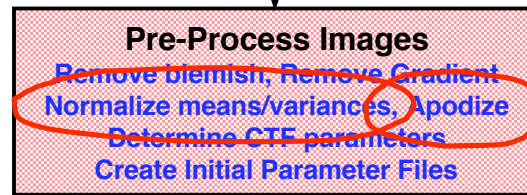
Icosahedral Virus 3D Reconstruction Scheme



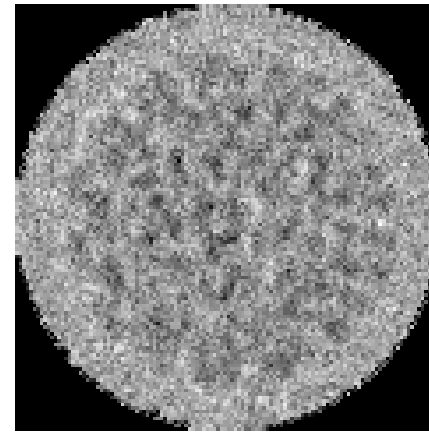
Pre-Process Images
~~Remove blurriness, Remove Gradient~~
Normalize means/variances, Apodize
~~Determine CTF parameters~~
Create Initial Parameter Files



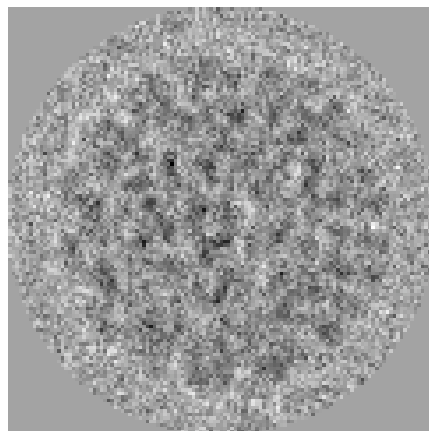
Icosahedral Virus 3D Reconstruction Scheme



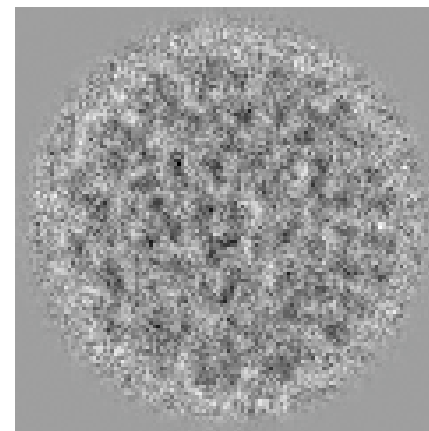
Extracted



Masked



Floated

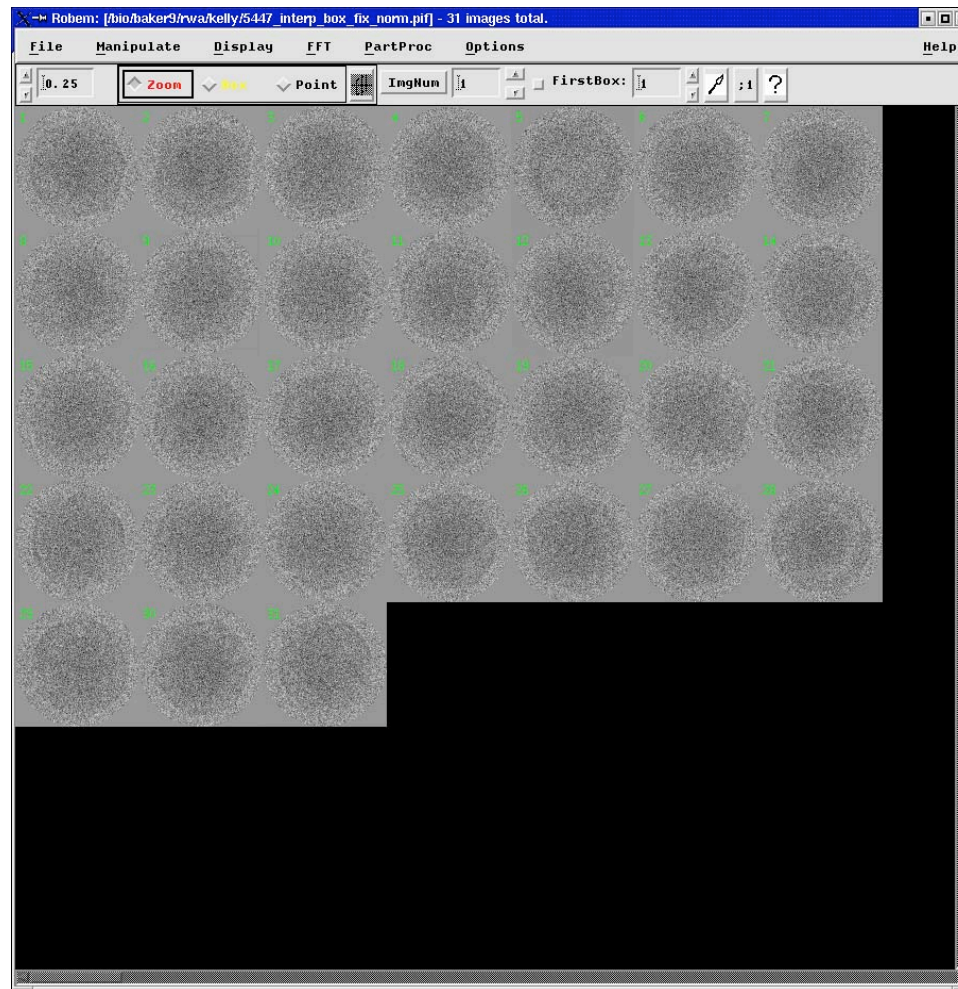


Apodized

Icosahedral Virus 3D Reconstruction Scheme



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



Icosahedral Virus 3D Reconstruction Scheme



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

FFT - CTF Estimation

Linear
Log
Non-linear

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

Intensity
Recalc FFT

Lock Scrolls

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

Back Transform
Pointer Detail
Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom
Disp 1D CA
Contour 1D CircAvg

Pick CTF Pts
Flicker
Determine Image CTF

Ang Major
Focus Major
Focus Minor

CTF Node Num:
1

FFT Average
Average FFTs
Incoherent Hvg

ScStp (um) 1.4
Mag 33019
PxSiz (nm) 0.424
Wiener 0.2
AmpCont 0.07
AngMaj 0.0
Volts (kV) 300
tFac 0
Wave (nm) 0.001
Mode 1
Cs (nm) 2.0
ctf 1/ctf
FocMaj (um) 0.0
PixelSize in nanometers
FocMin (um) 0.0

Icosahedral Virus 3D Reconstruction Scheme



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

FFT - CTF Estimation

Linear
 Log
 Non-linear

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

Intensity
Recalc FFT

Lock Scrolls

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

Back Transform
Pointer Detail

Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom

Disp 1D CA
Contour 1D CircAvg

Pick CTF Pts
Flicker Determine Image CTF

Ang Major
Focus Major
Focus Minor

CTF Node Num: 1

FFT Average
Aver Ing#
Average FFTs
Incoherent hvv

ScStp (um) 1.4 Mag 33019
PxSiz (nm) 0.424 Wiener 0.2
AmpCont 0.07 AngMaj 0.0
Volts (kV) 300 tFac 0
Wave (nm) 0.001 Mode 1
Cs (nm) 2.0 ctf 1/ctf
FocMaj (um) 0.0 PixelSize i
FocMin (um) 0.0 nanometers

Icosahedral Virus 3D Reconstruction Scheme



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

The screenshot shows the 'FFT - CTF Estimation' software interface. The main window displays a power spectrum with concentric rings and a red circle highlighting the central region. The interface includes various control panels for image processing, CTF estimation, and parameter adjustment.

Linear/Log/Non-linear (dropdowns)
1024 x 1024 (dropdown)
512 x 512 (dropdown)
256 x 256 (dropdown)
128 x 128 (dropdown)
Intensity (dropdown)
Recalc FFT (button)
Lock Scrolls (checkbox)
Min (slider) 14.71
Max (slider) 17.42
TFac (slider) 0
Range Min: 14.71 **Max:** 25.16

Back Transform (checkbox)
Pointer Detail (button)
Generate Default Param File (button)
Defocus Refinement (button)

Overlay Intensity: (slider)
Zoom (checkbox)
Disp 1D CA (checkbox)
Contour 1D CircAvg (checkbox)

Pick CTF Pts (checkbox) **Flicker** (button) **Determine Image CTF** (button)
Ang Major (slider) 359.4
Focus Major (slider) 2.16
Focus Minor (slider) 3.31

CTF Node Num: (input) 1
FFT Average (checkbox)
Aver (checkbox) **Ing#** (input)
Average FFTs (checkbox) **Incoherent** (checkbox) **Hvg** (checkbox)

ScStp (um) 1.4 **Mag** 33019
PxSiz (nm) 0.424 **Wiener** 0.2
AmpCont 0.07 **AngMaj** 359.4
Volts (kV) 300 **tFac** 0
Wave (nm) 0.001 **Mode** 1
Cs (mm) 2.0 **ctf** 1/ctf
FocMaj (um) 2.16 **PixelSize i**
FocMin (um) 3.31 **nanometers**

Icosahedral Virus 3D Reconstruction Scheme



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

FFT - CTF Estimation

Linear
Log
Non-linear

Min
Max
TFac
Range

Back Transf
Pointer Detail

Overlay Intensity

Zoom
Disp 1D CA
Contour 1D Ci

Pick CTF Pts
Flicker
Determine Image CTF

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

CTF Node Num: 1

FFT Average
Average FFTs
Incoherent hv9

Automatic Defocus Refinement

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

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

Highest Resolution (Angs): 6.480000

Estimated Defocus (um):
Chi squared:

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

Zoom FFT image
Change Overlay Intensity to see CTF curves.

Estimate the Defocus *1
Update FFT screen

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

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

Icosahedral Virus 3D Reconstruction Scheme



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

FFT - CTF Estimation

Linear
Log
Non-Linear

Min
Max
TFac
Range

Back Transf
Pointer Detail

Overlay Intensity

Zoom

Disp 1D CA

Contour 1D Ci

Pick CTF Pts
Flicker
Determine Image CTF

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

CTF Node Num: 1

FFT Average
Aver Ing#
Average FFTs
Incoherent hv9

Automatic Defocus Refinement

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

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

Highest Resolution (Angs): 8.480000

Estimated Defocus (um): 2.204652
Chi squared: 0.111960

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

Zoom FFT image
Change Overlay Intensity to see CTF curves.

Estimate the Defocus *1
Update FFT screen

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

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

Icosahedral Virus 3D Reconstruction Scheme



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

FFT - CTF Estimation

Linear
 Log
 Non-linear

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

Intensity
Recalc FFT

Lock Scrolls

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

Back Transform
Pointer Detail

Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom
 Disp 1D CA
 Contour 1D CircAvg

Pick CTF Pts
Flicker Determine Image CTF

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

CTF Node Num: 1

FFT Average
Aver Inq#
Average FFTs
 Incoherent hvg

ScStp (um): 1.4 Mag: 33019
PxSiz (nm): 0.424 Wiener: 0.2
AmpCont: 0.07 AngMaj: 359.4
Volts (kV): 300 tFac: 0
Wave (nm): 0.001 Mode: 1
Cs (mm): 2.0 ctf: 1/ctf
FocMaj (um): 2.20 PixelSize in nanometers
FocMin (um): 2.20

Icosahedral Virus 3D Reconstruction Scheme



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

FFT - CTF Estimation

Linear
 Log
 Non-linear

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

Intensity
Recalc FFT

Lock Scrolls

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

Back Transform
Pointer Detail

Generate Default Param File

Defocus Refinement

Overlay Intensity:

Zoom

Disp 1D CA Contour 1D CircAvg

Pick CTF Pts **Flicker** Determine Image CTF

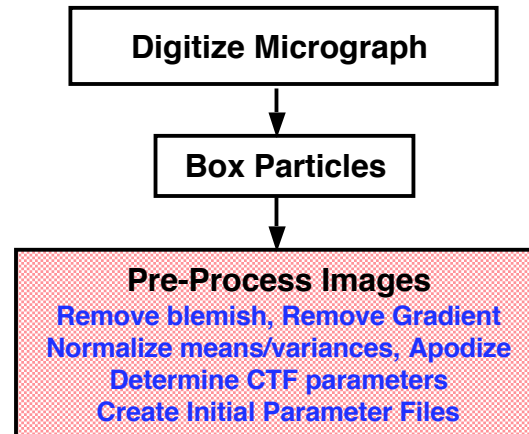
Ang Major: 359.4
Focus Major: 2.204
Focus Minor: 2.204

CTF Node Num: 1

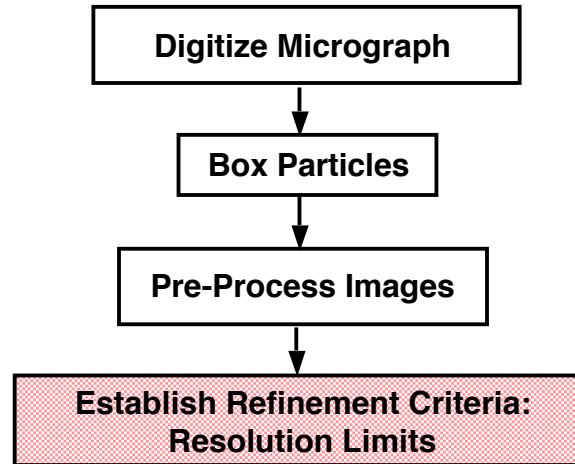
FFT Average
Aver Ing#
Average FFTs
Incoherent hv9

ScStp (um): 1.4 Mag: 33019
PxSiz (nm): 0.424 Wiener: 0.2
AmpCont: 0.07 AngMaj: 359.4
Volts (kV): 300 tFac: 0
Wave (nm): 0.001 Mode: 1
Cs (nm): 2.0 ctf: 1/ctf
FocMaj (um): 2.20 PixelSize in nanometers
FocMin (um): 2.20

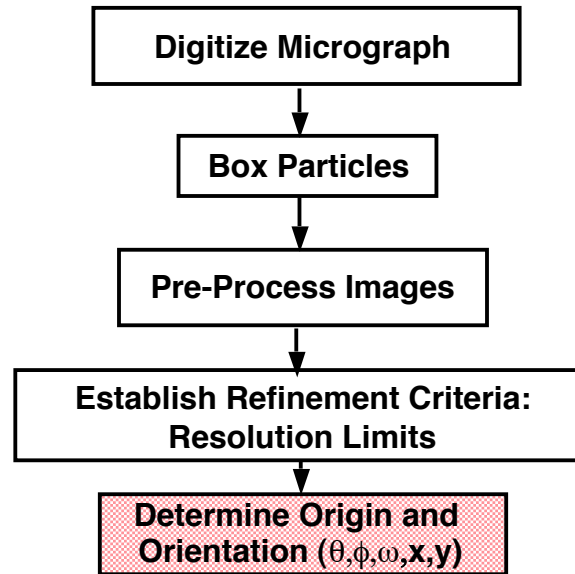
Icosahedral Virus 3D Reconstruction Scheme



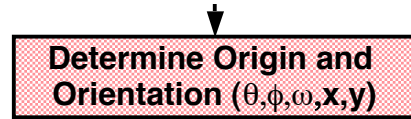
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

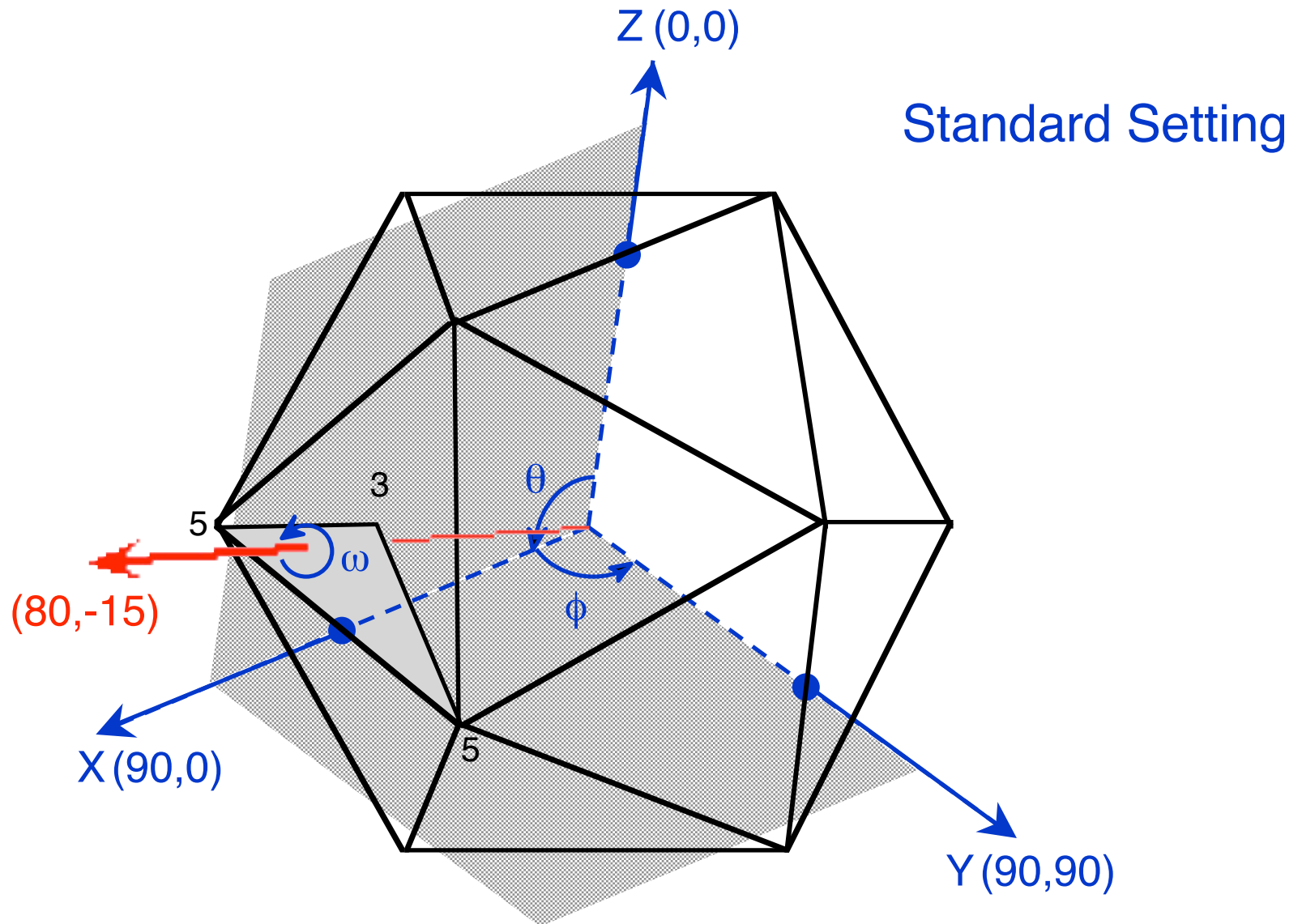


Goal: determine phase origin and view orientation for each boxed particle

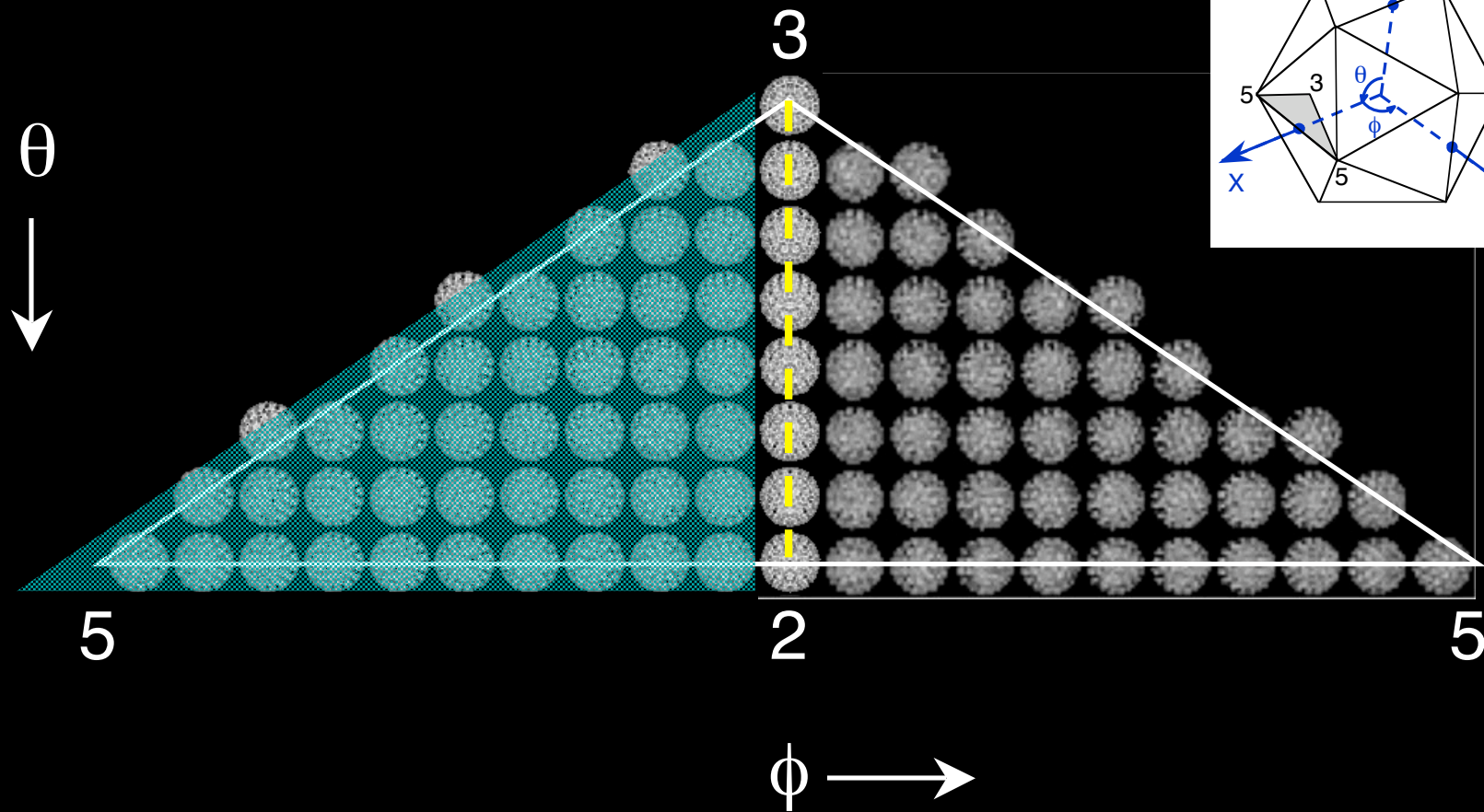
MOST IMPORTANT STEP?

Garbage in -----> garbage out

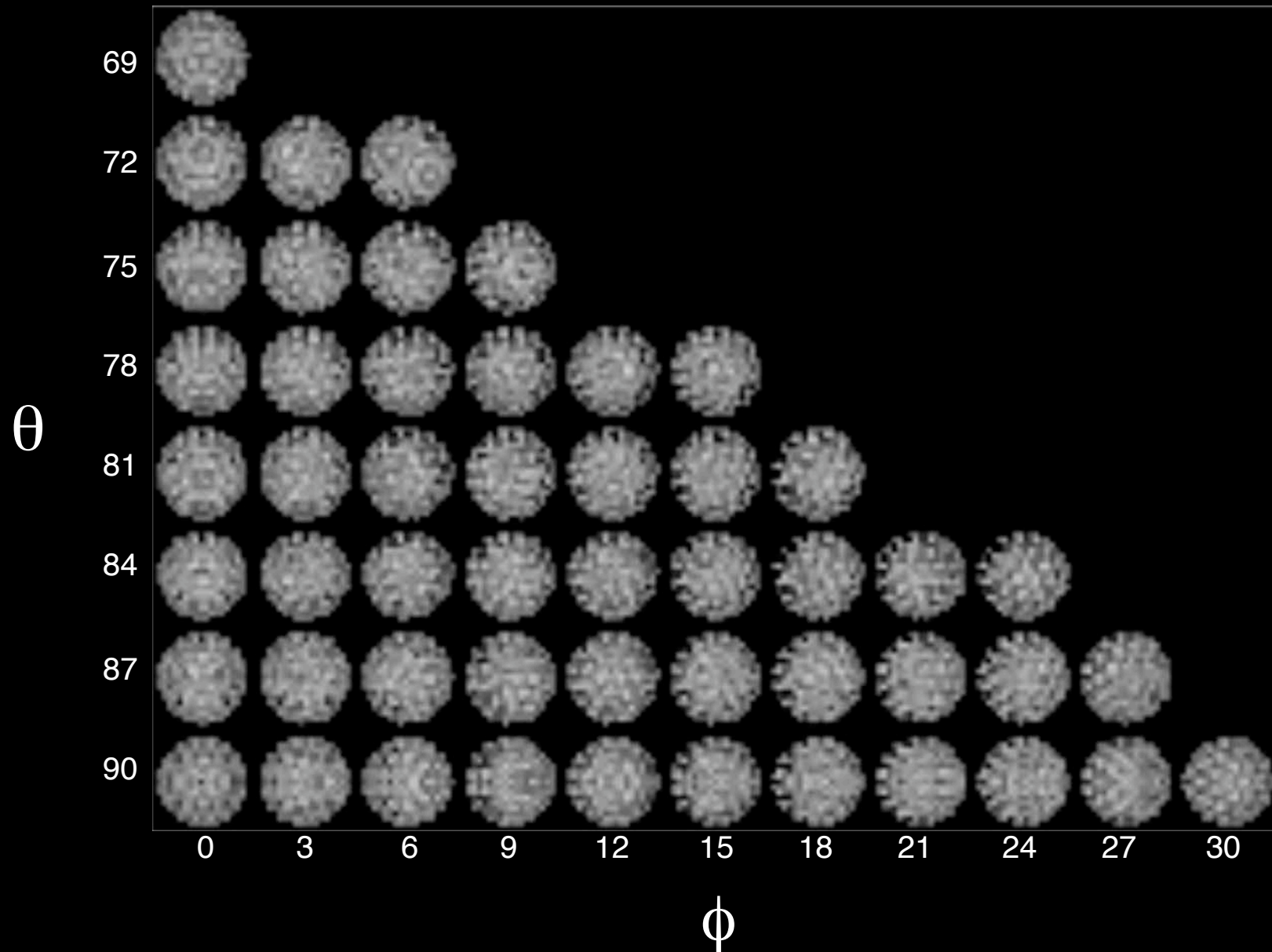
Specifying Direction of View: (θ, ϕ, ω) Orientation



BPV Projections: Icosahedral ASU

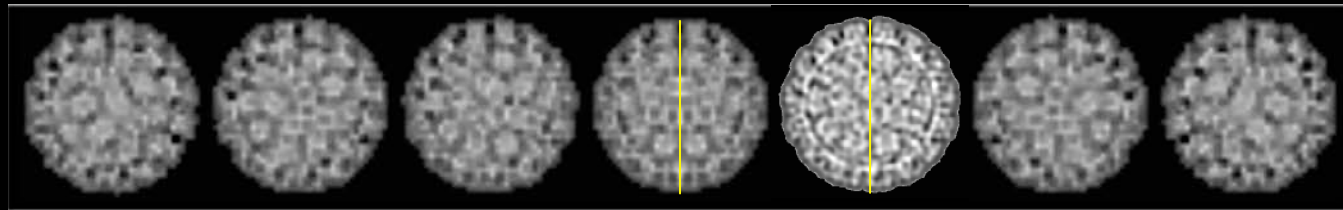


BPV Projections: 1/2 Icosahedral ASU



$\pm\phi$ Images Related by Mirror Symmetry

$\theta=75^\circ$



-9

-6

-3

0

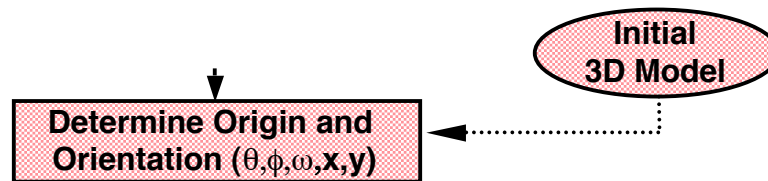
3

6

9

ϕ

Icosahedral Virus 3D Reconstruction Scheme



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

Two methods:

1. Common lines

New or unknown structure

2. Model-based (template) matching

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

Icosahedral Virus 3D Reconstruction Scheme



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

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

Common Lines

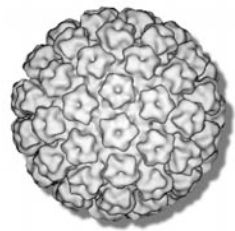
The ‘gospel’ continued:

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

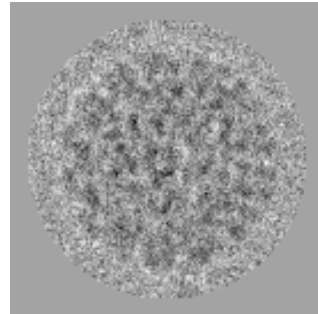
“A similar pair of lines will be generated by each possible choice of pairs of symmetry operations”

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

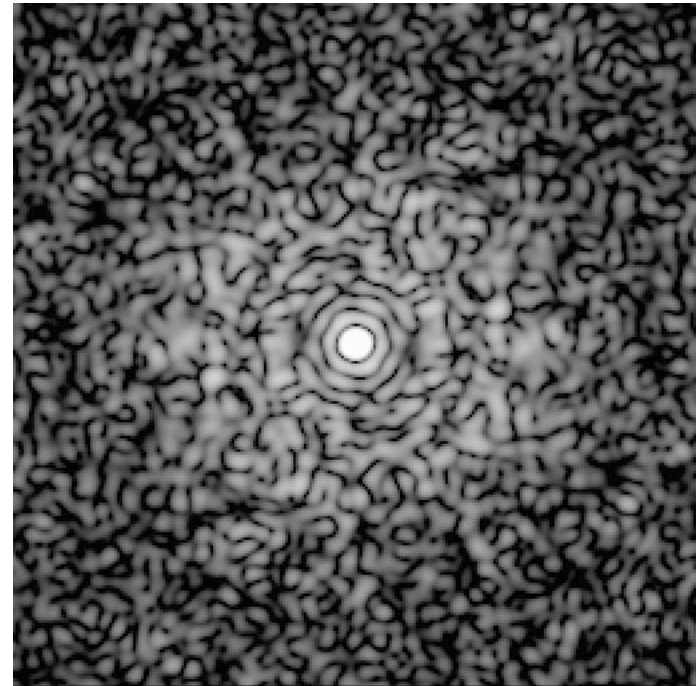
Orientation Determination by Common Lines



3D Object



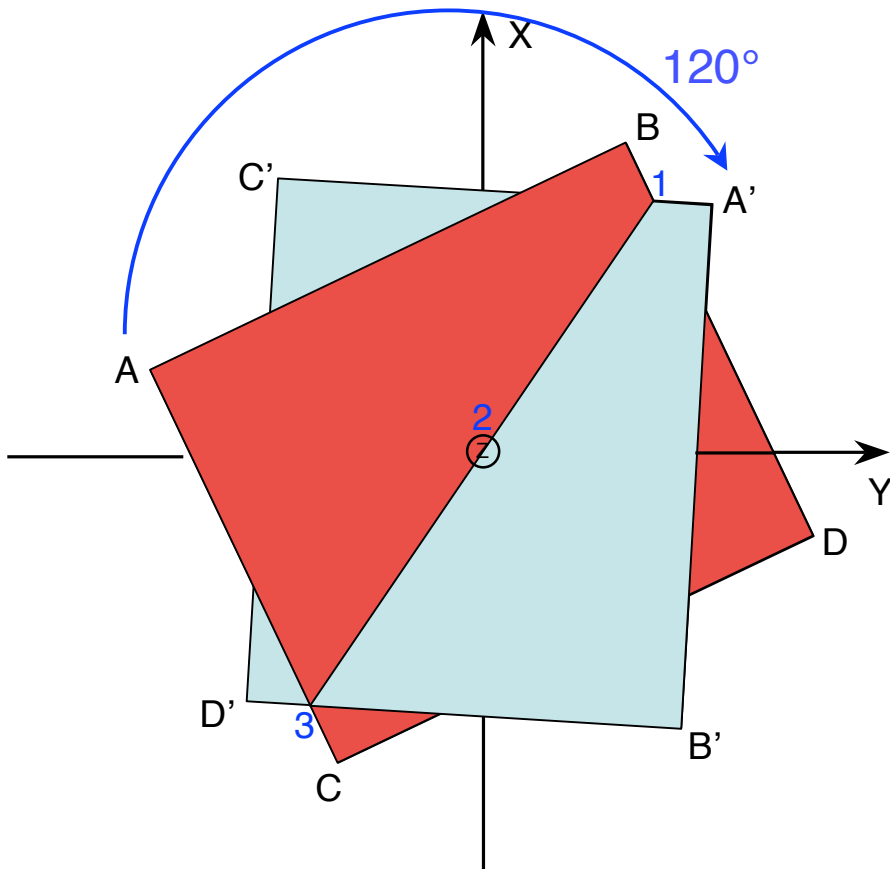
2D Projection
(θ, ϕ, ω)



2D Fourier Transform

Orientation Determination by Common Lines

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



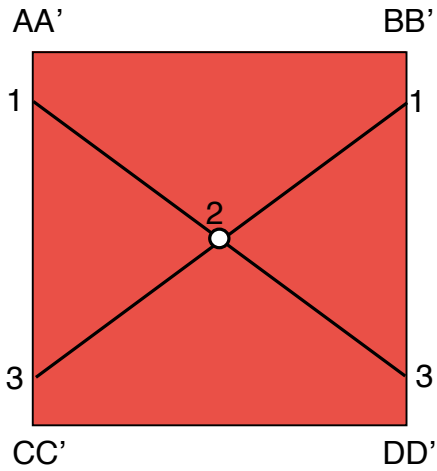
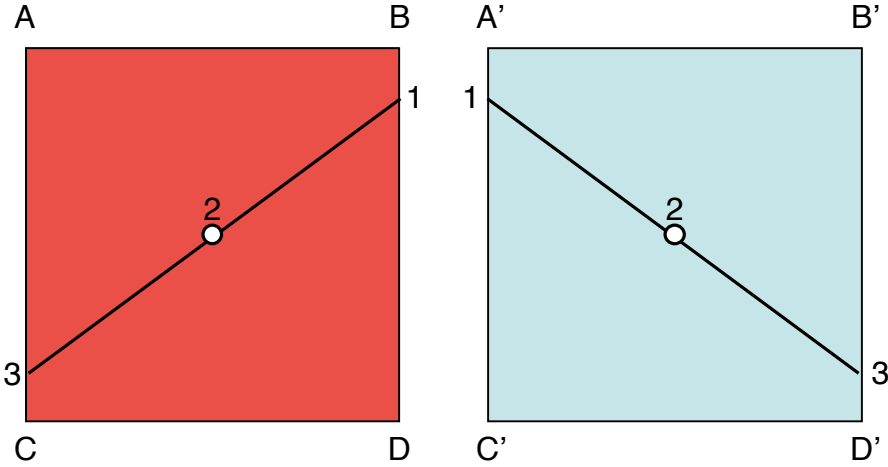
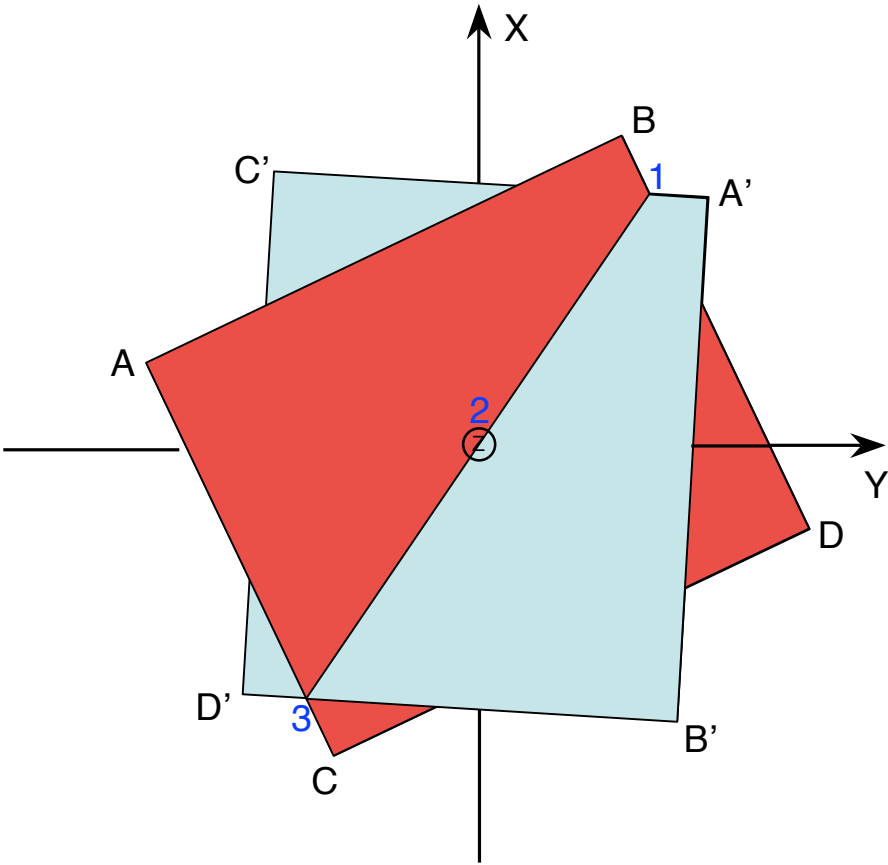
ABCD = 2D transform of image from particle **not** viewed along an axis of symmetry

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

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

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

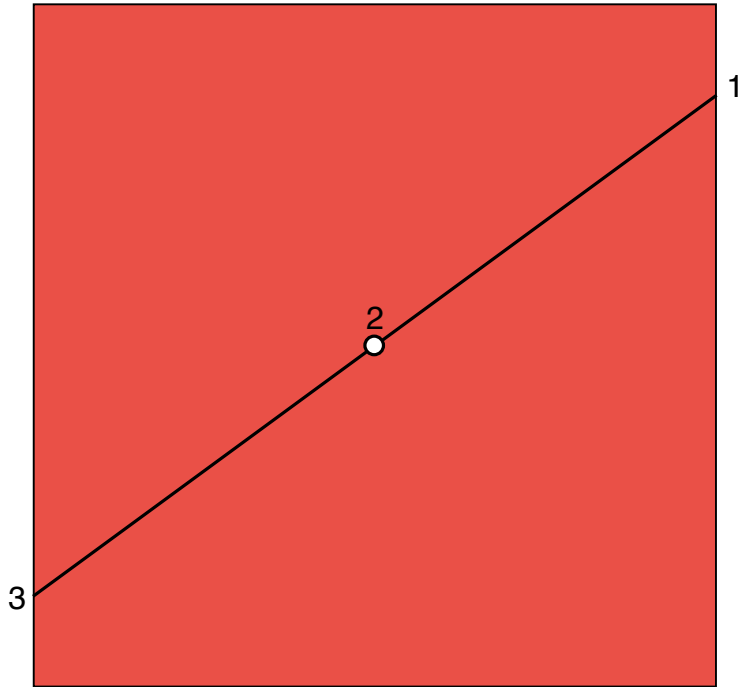
Orientation Determination by Common Lines



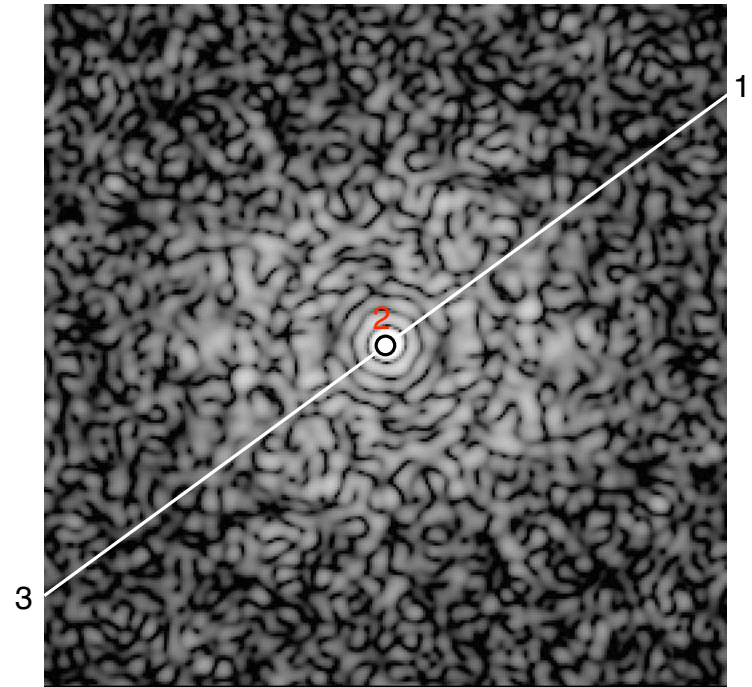
Adapted from Moody (1990) Fig. 7.68, p.245

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

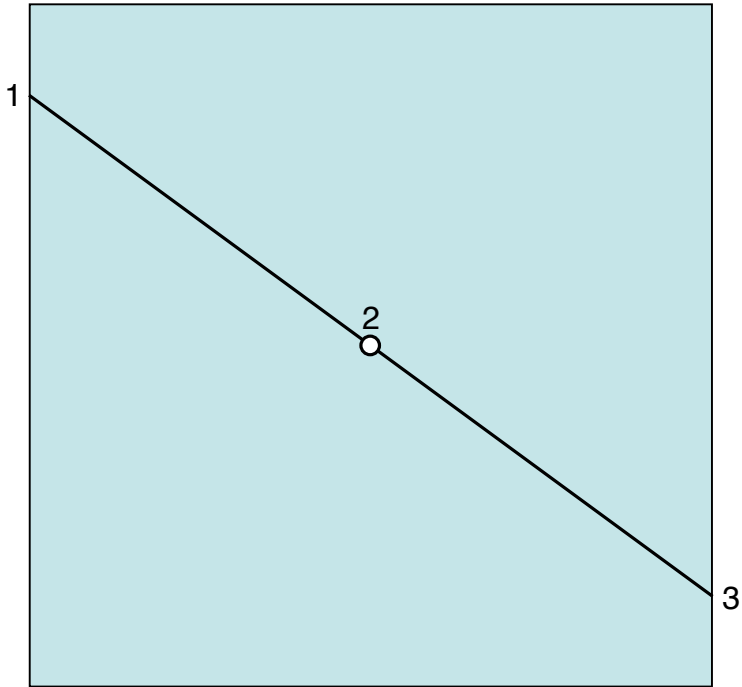
Orientation Determination by Common Lines



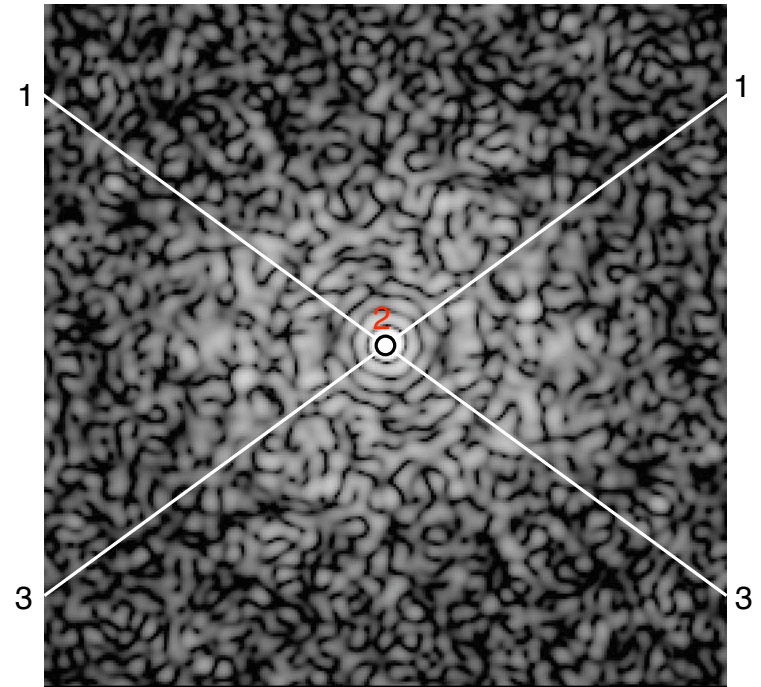
Original Transform Plane



Orientation Determination by Common Lines



Symmetry-Related
Transform Plane



Orientation Determination by Common Lines

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

What about an object with 532 symmetry?

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

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

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

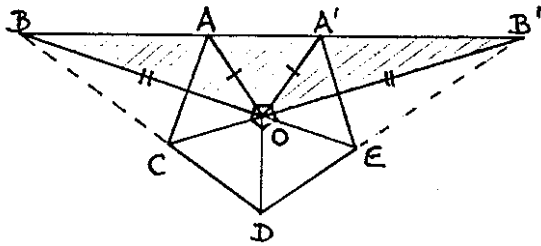
$$\text{2-folds: } \frac{30}{2} \times 1 = \underline{15} \text{ real lines}$$

37 common lines

III.D.5 3D Fourier Reconstruction Methods

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

Orientation Determination by Common Lines

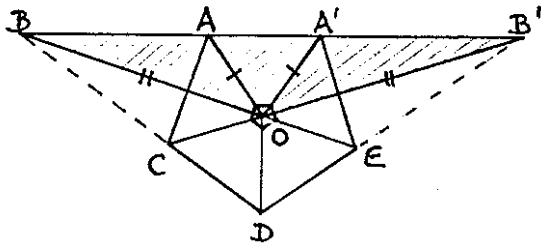


Each **5-fold** generates **2 pairs** of common lines (OA, OA') and (OB, OB')

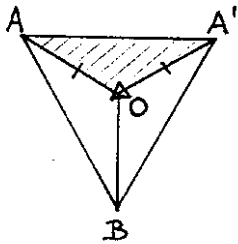
III.D.5 3D Fourier Reconstruction Methods

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')

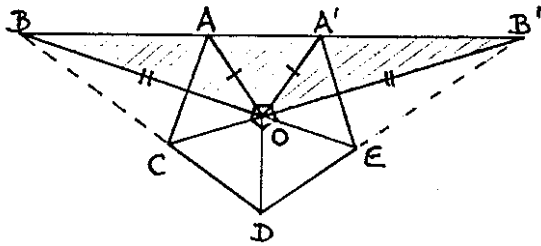


Each **3-fold** generates **1 pair** of common lines (OA,OA')

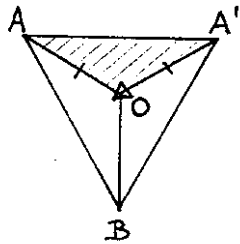
III.D.5 3D Fourier Reconstruction Methods

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

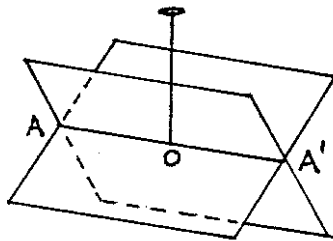
Orientation Determination by Common Lines



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

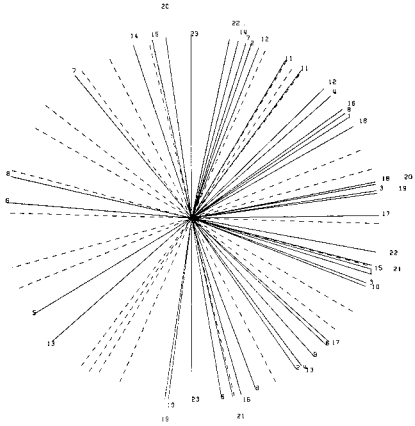


Each **3-fold** generates **1 pair** of common lines (OA,OA')

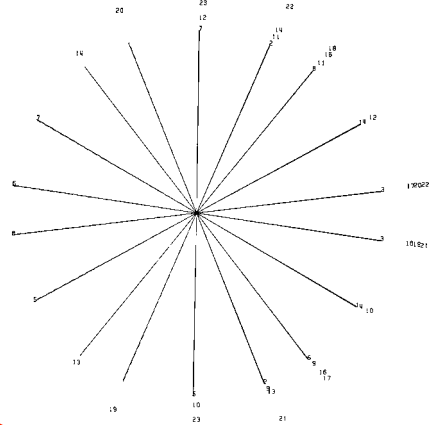


Each **2-fold** generates **1 real line**
 $F(OA) = F(OA') = F^*(OA)$
2-fold Friedel

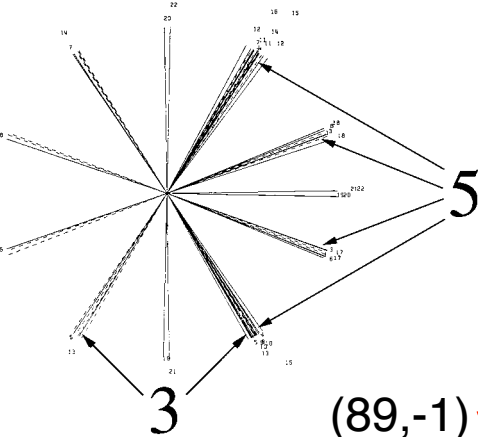
Orientation Determination by Common Lines



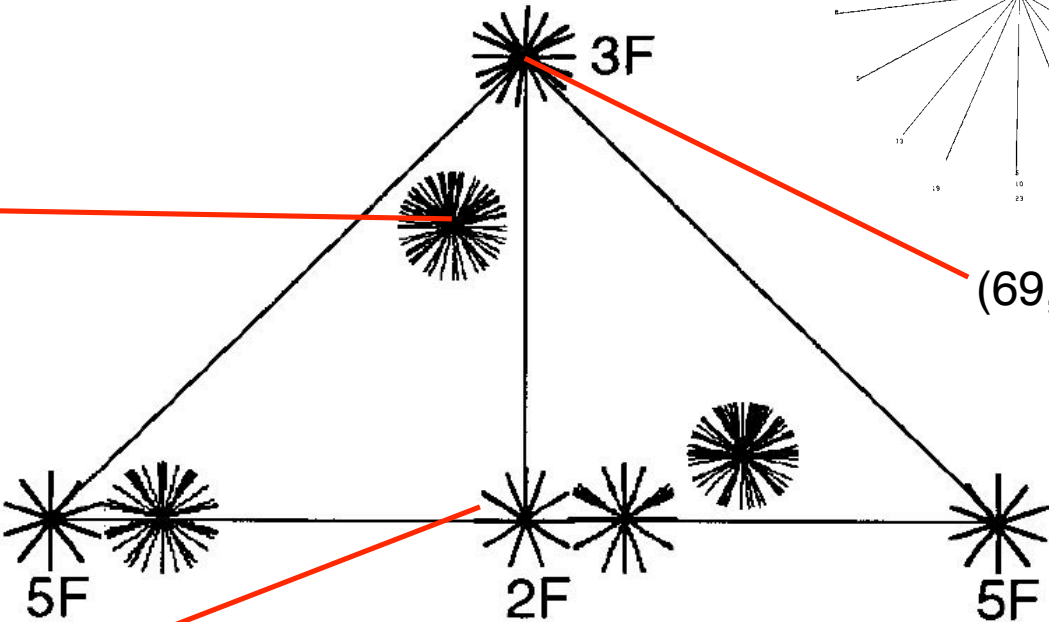
(80,11)



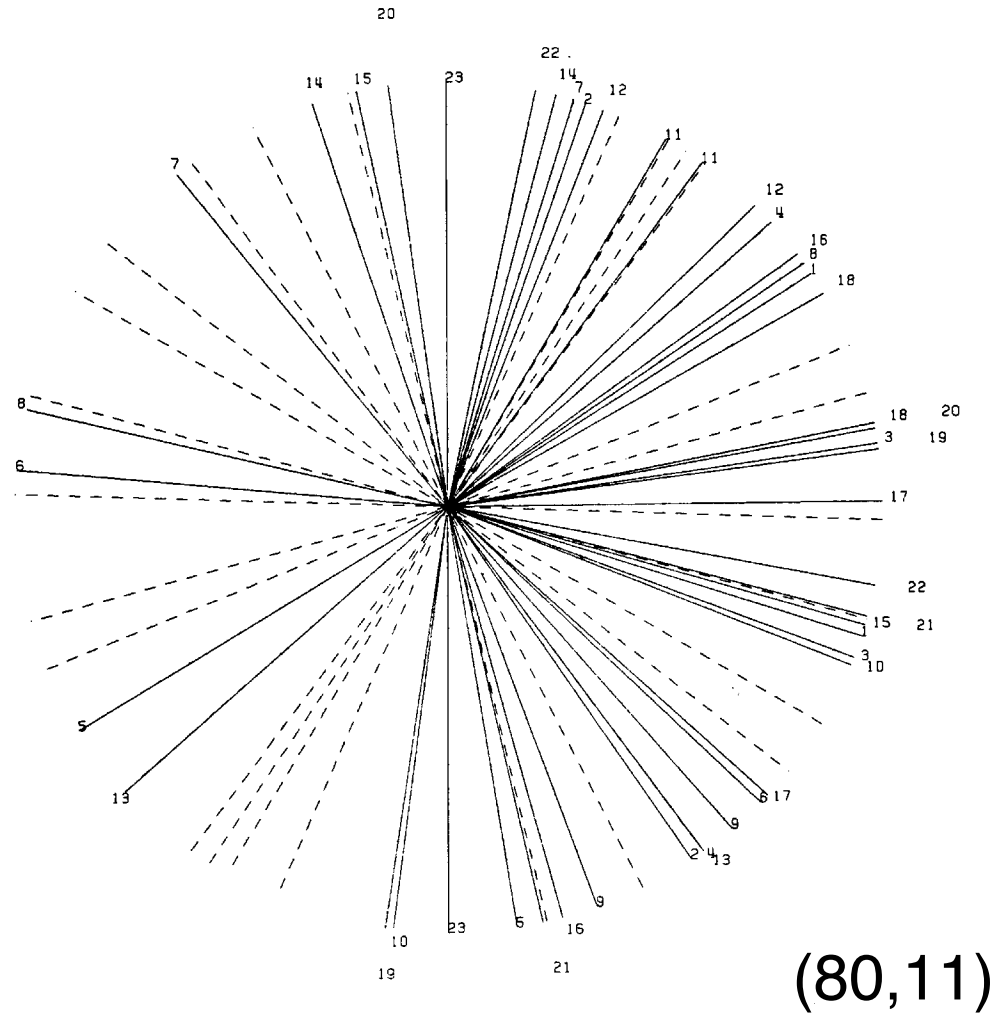
(69,0)



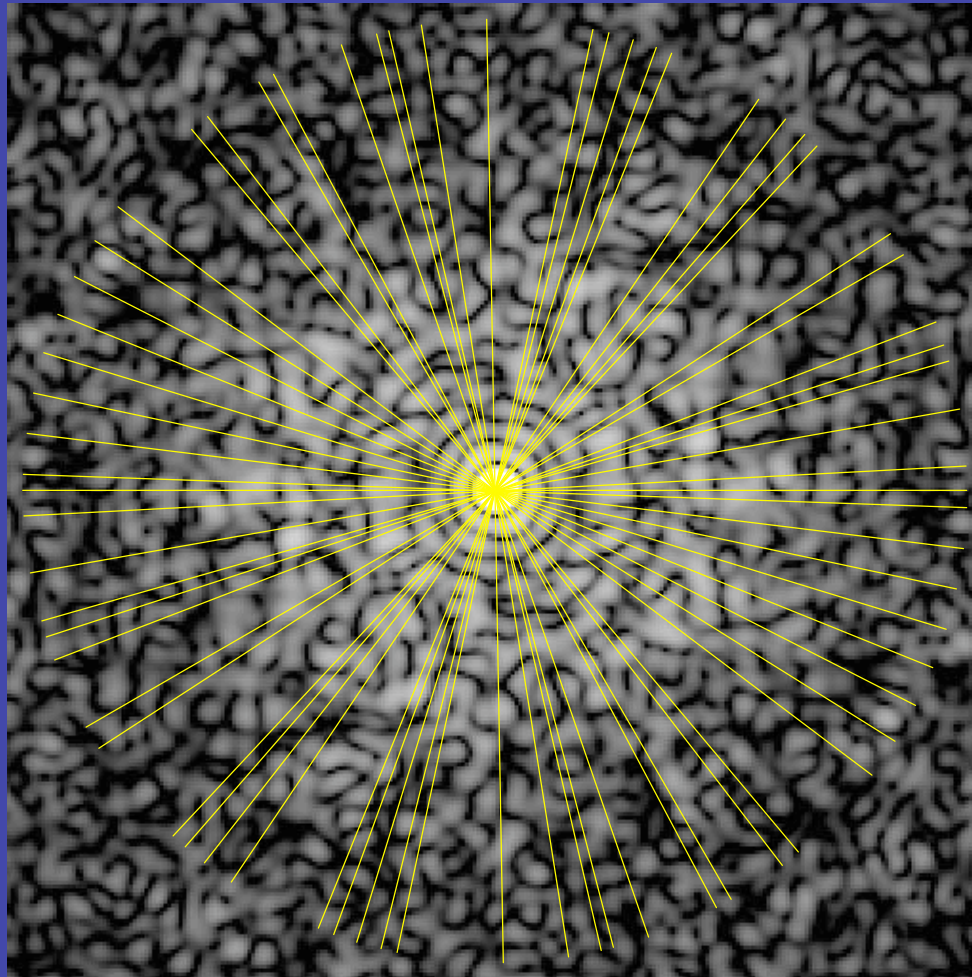
(89,-1)



Orientation Determination by Common Lines



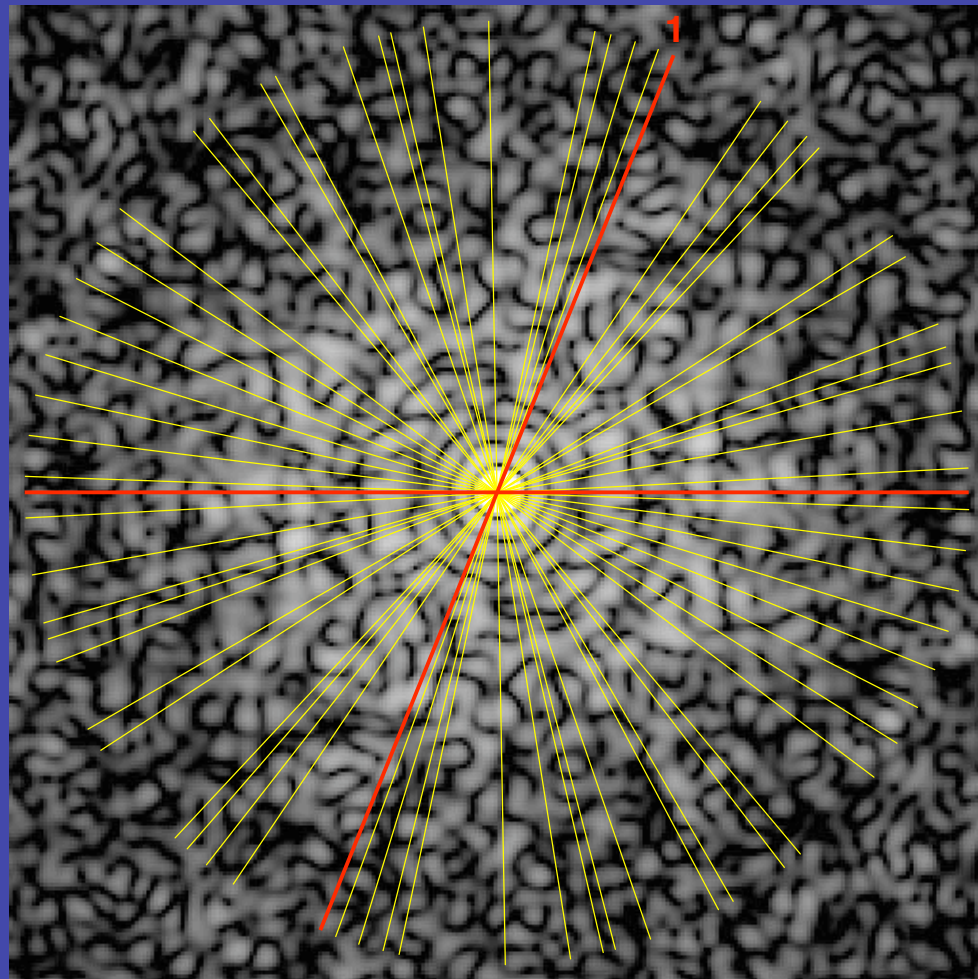
Orientation Determination by Common Lines



(80,11)

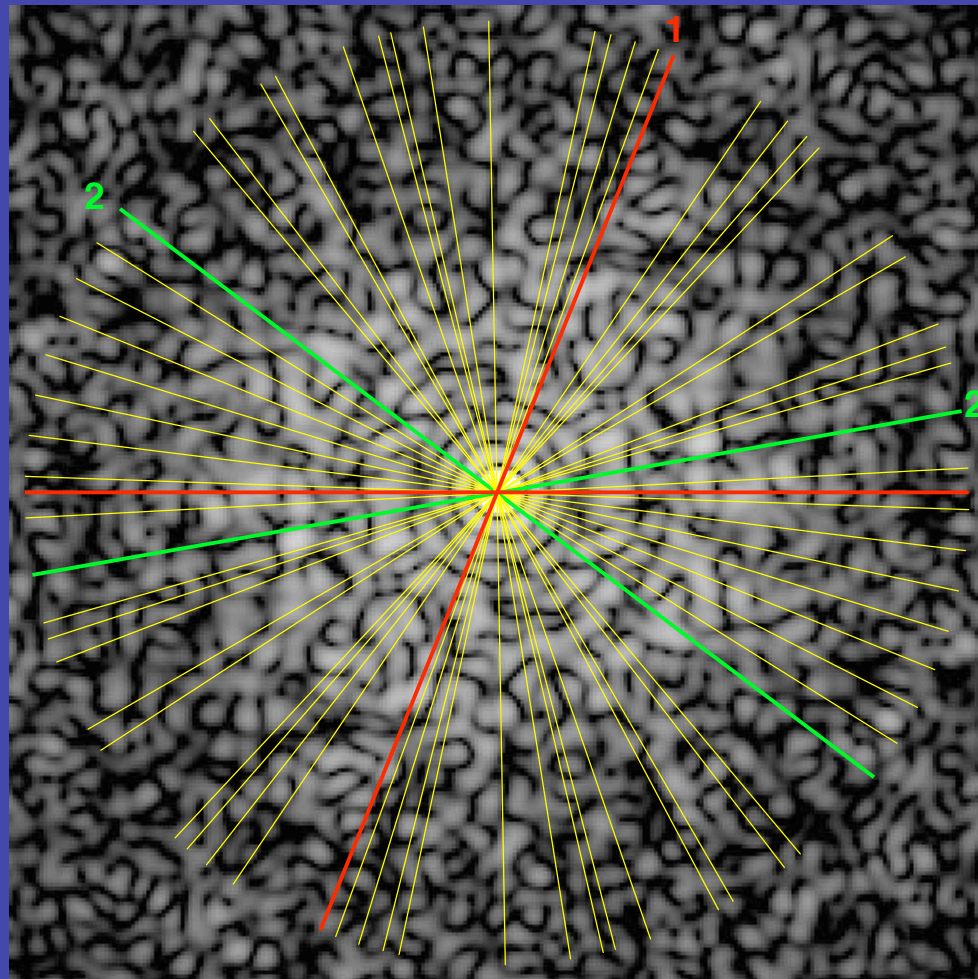
What is (θ, ϕ, ω) for this particle?

Orientation Determination by Common Lines

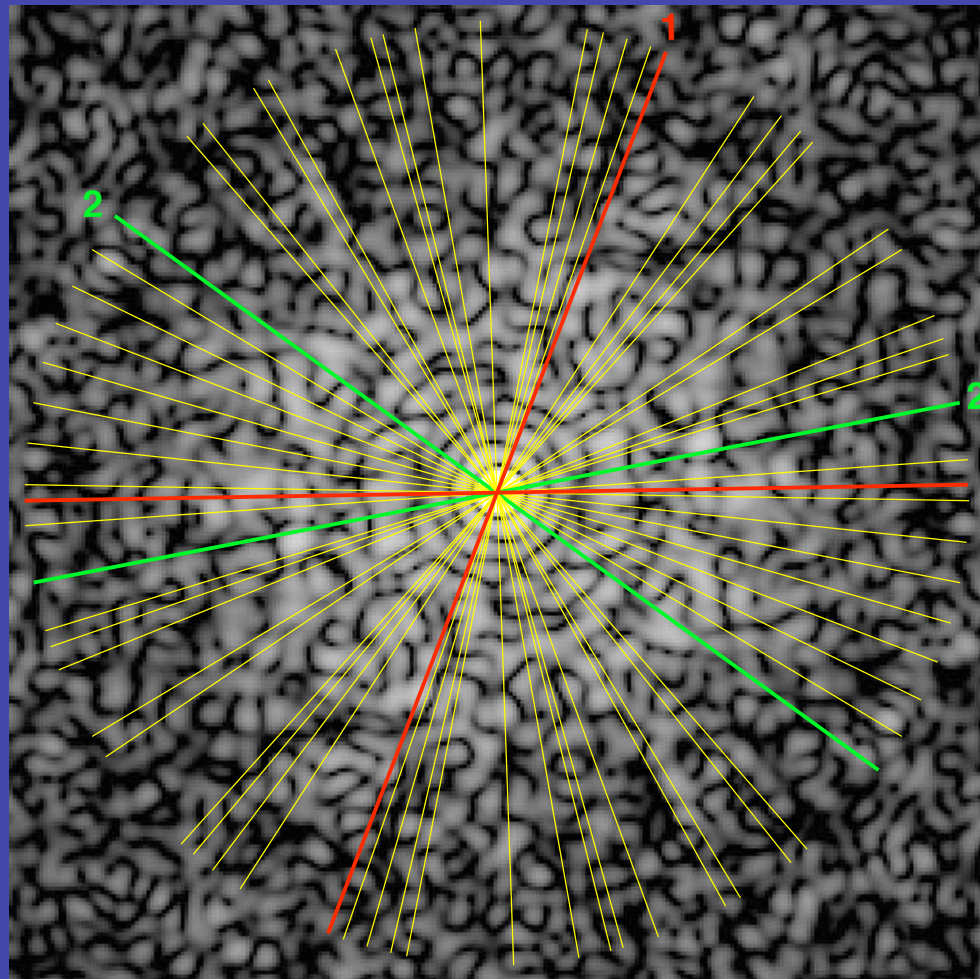


(80,11,0)

Orientation Determination by Common Lines

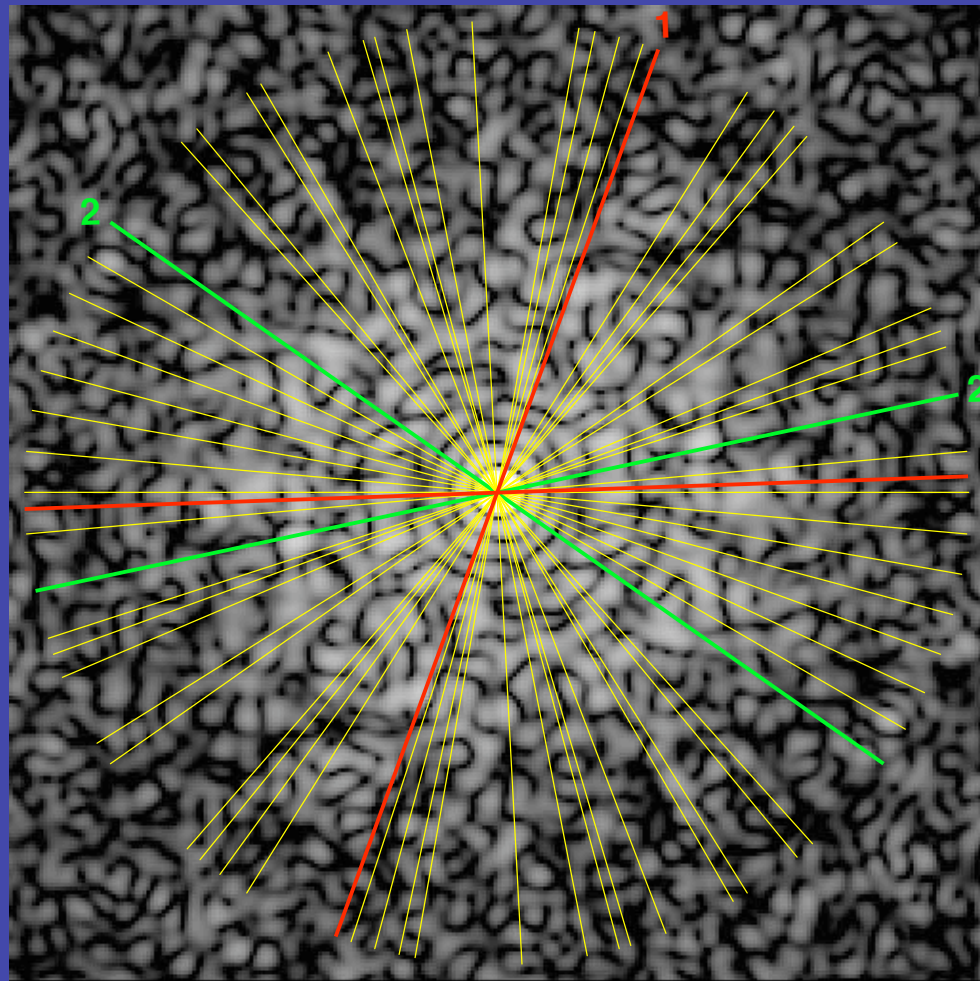


Orientation Determination by Common Lines

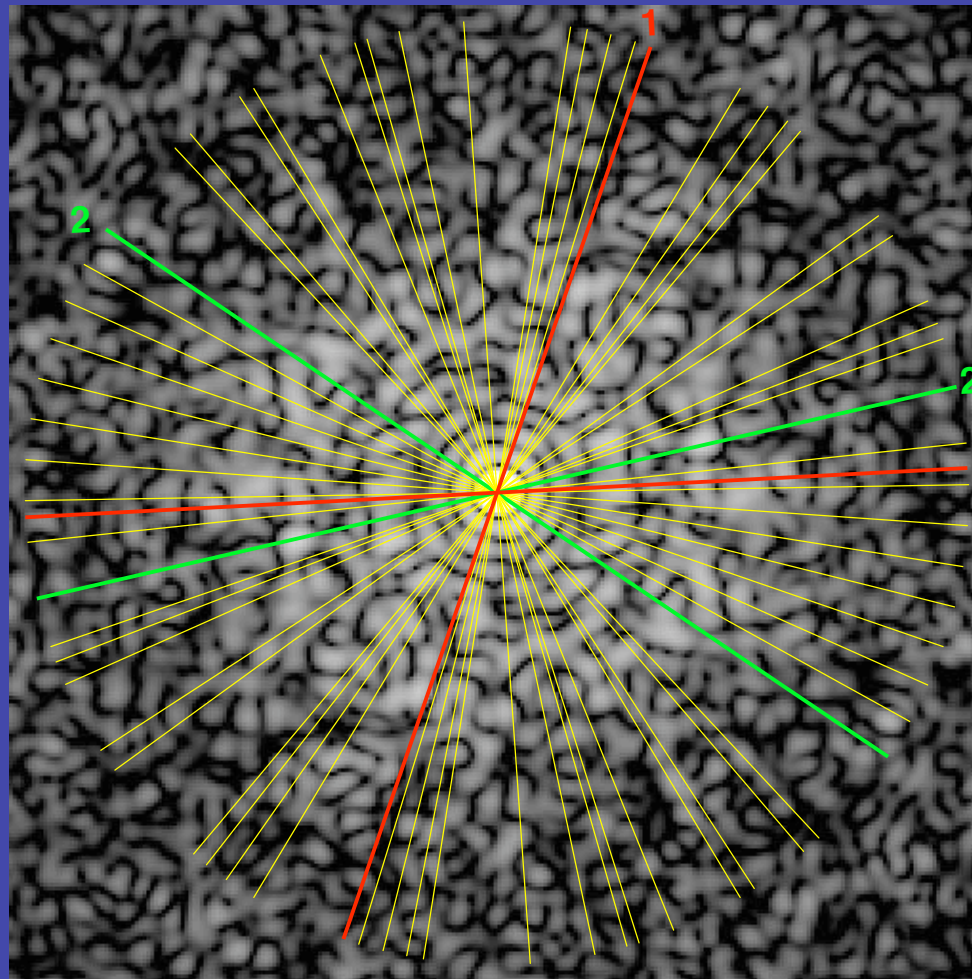


ω
↓
(80,11,1)

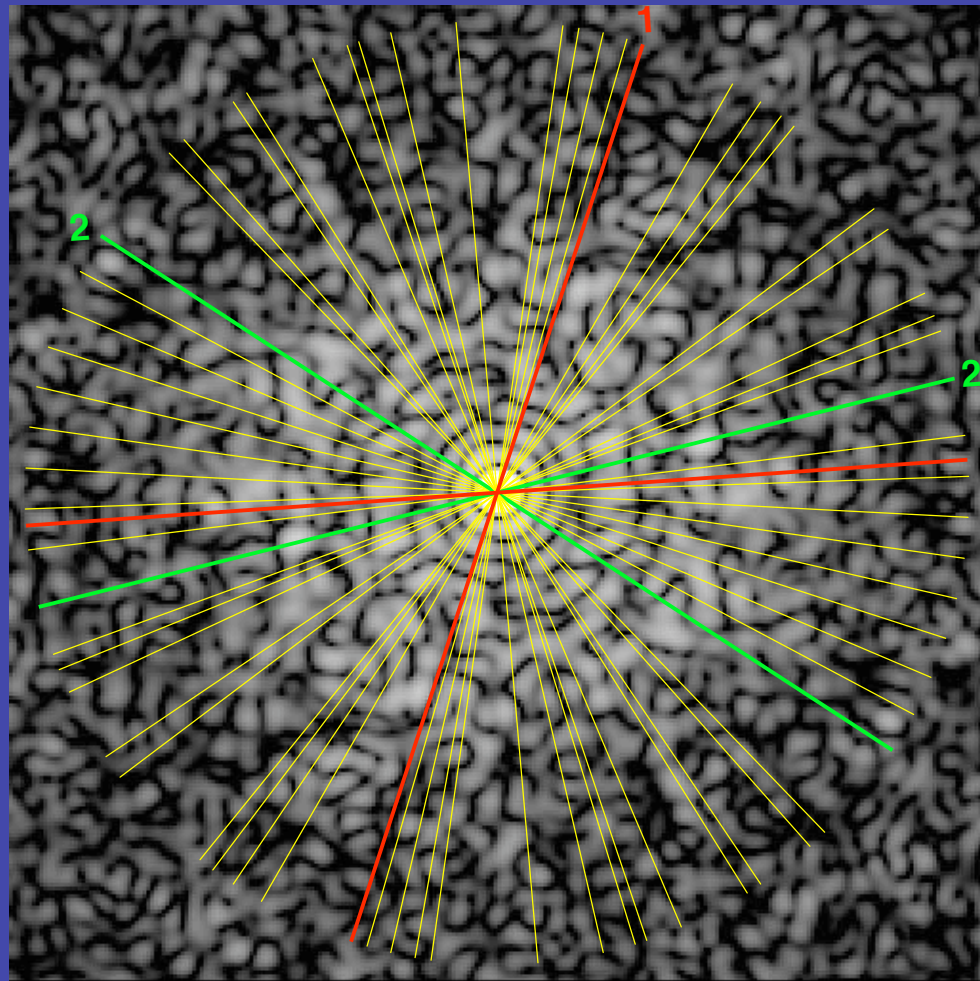
Orientation Determination by Common Lines



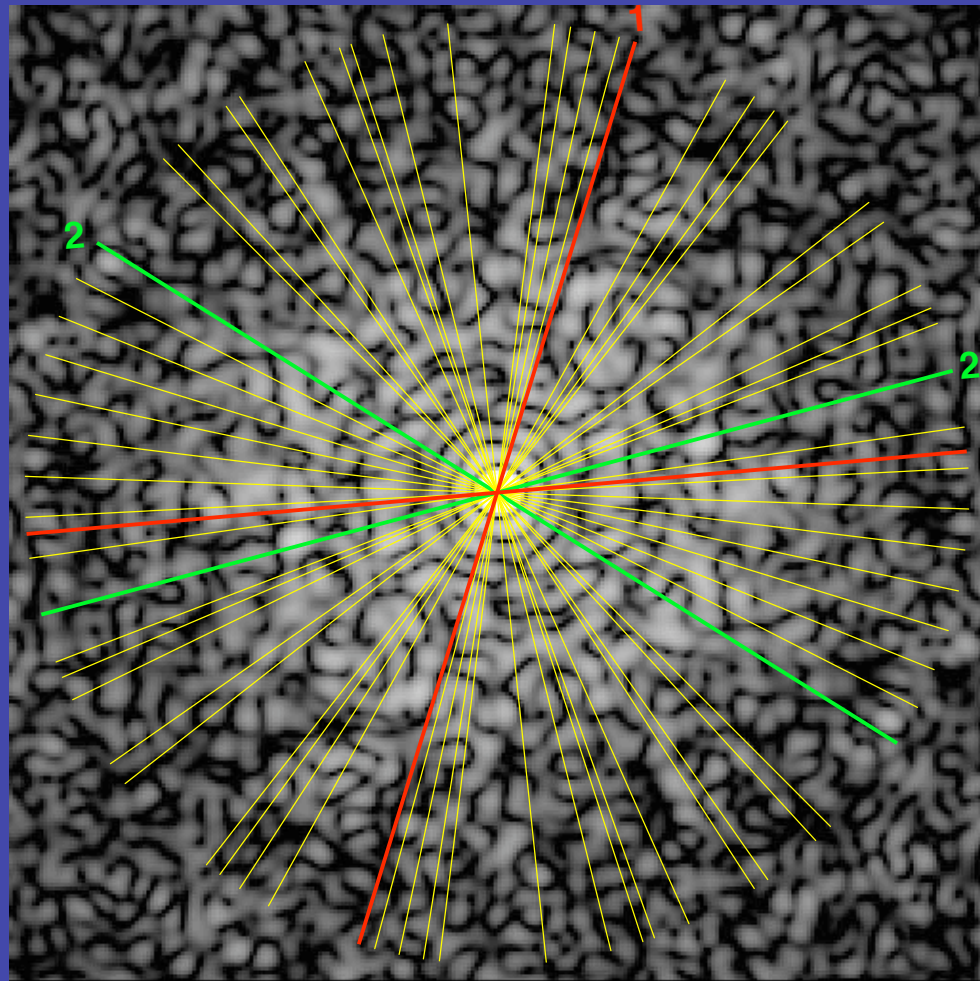
Orientation Determination by Common Lines



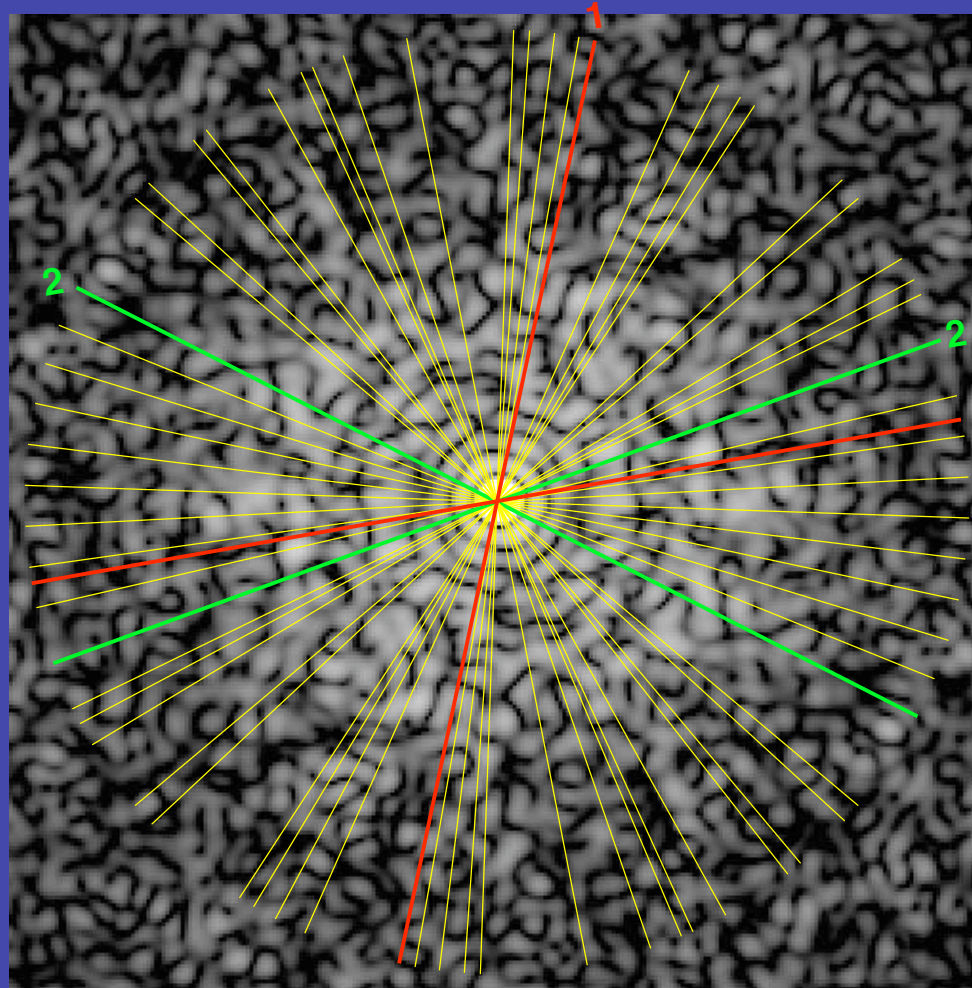
Orientation Determination by Common Lines



Orientation Determination by Common Lines

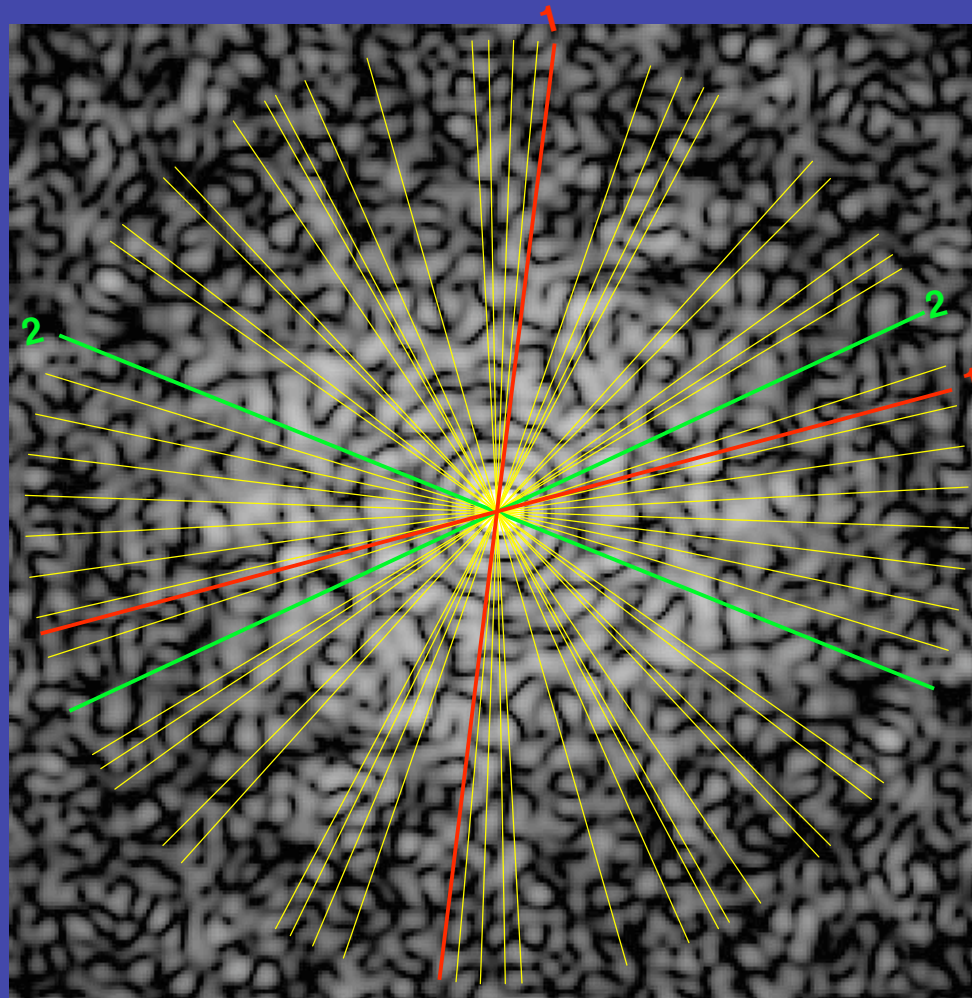


Orientation Determination by Common Lines



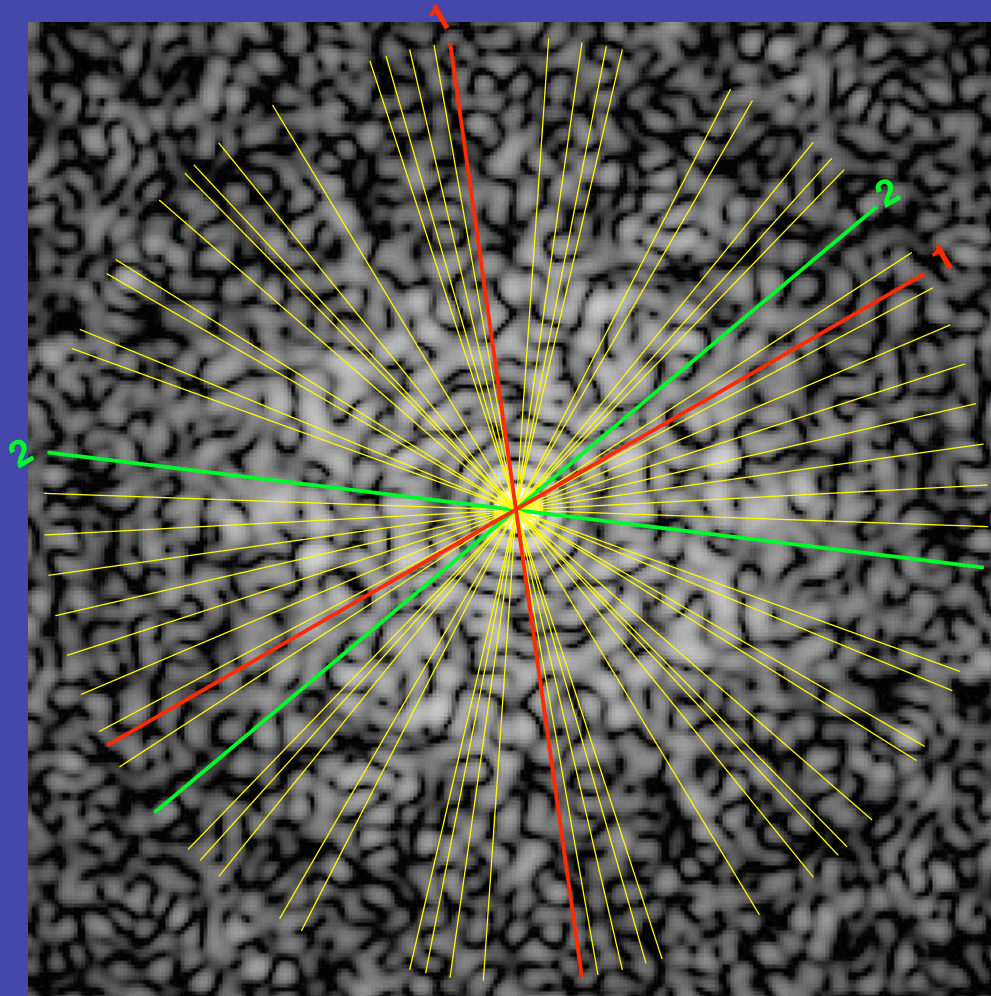
ω
↓
(80, 11, 10)

Orientation Determination by Common Lines



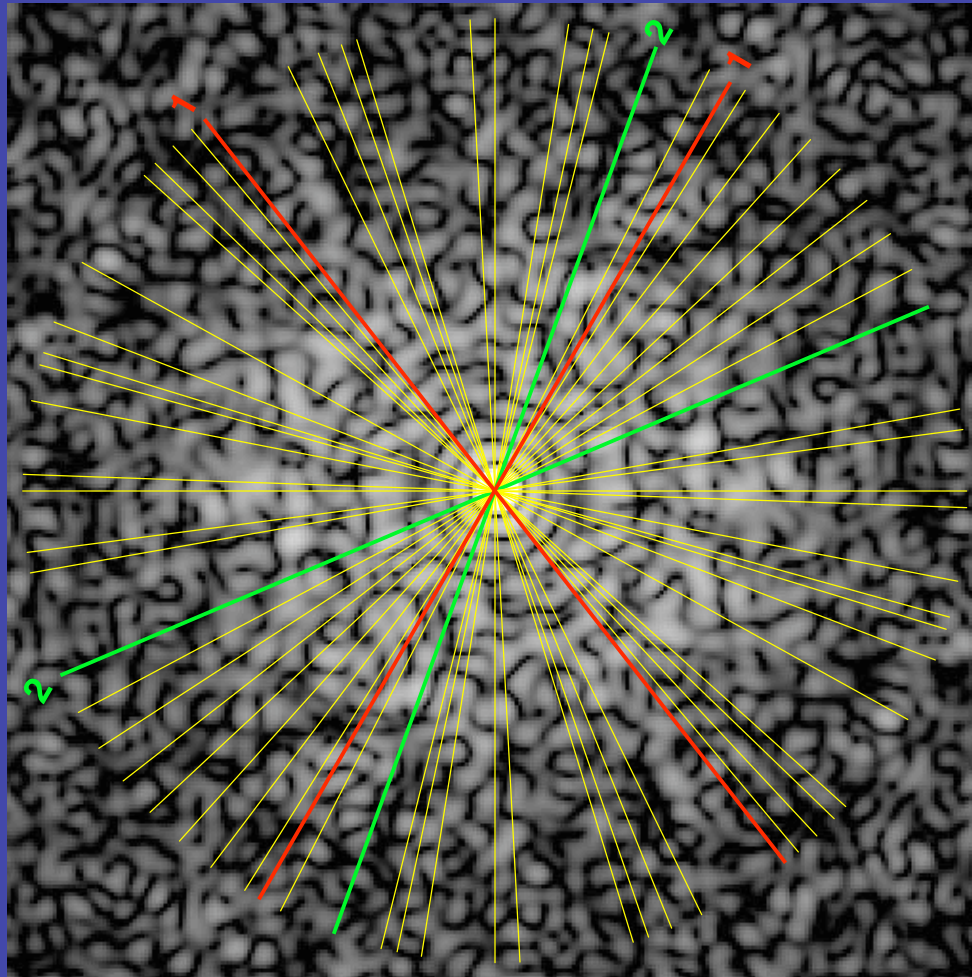
ω
↓
(80,11,15)

Orientation Determination by Common Lines



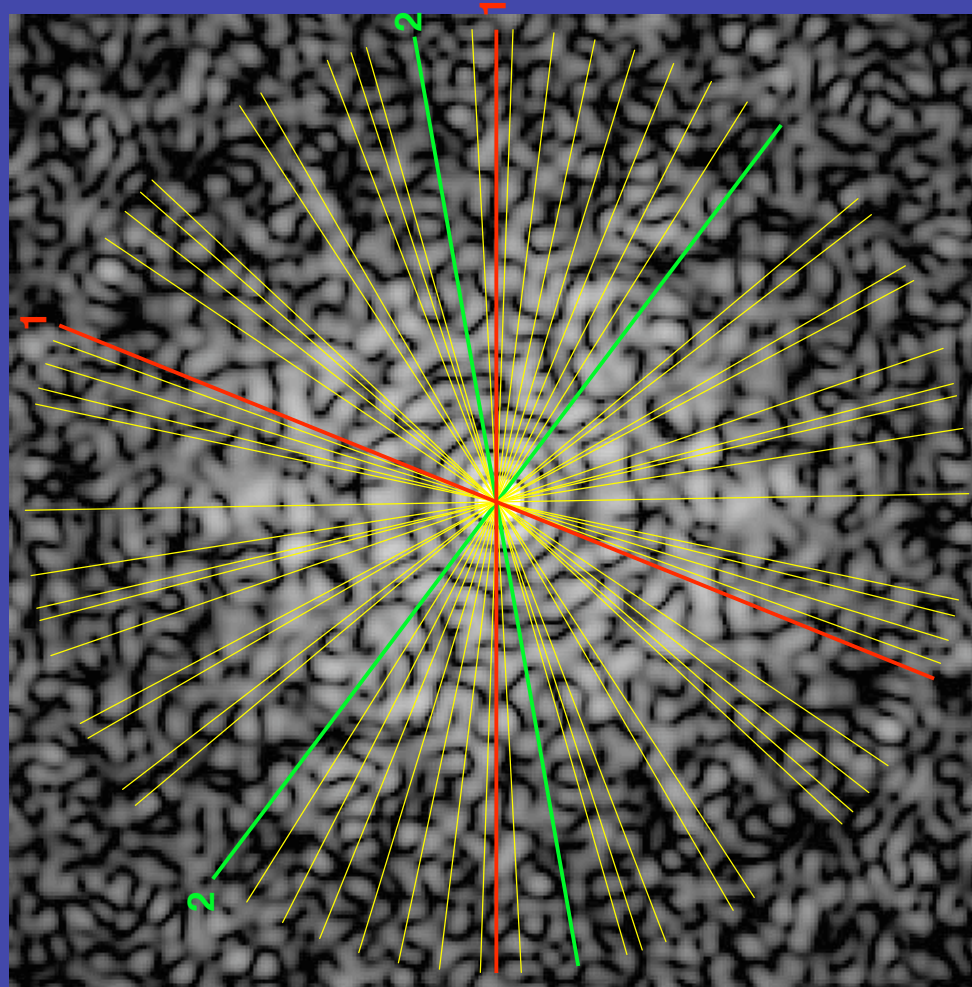
ω
↓
(80, 11, 30)

Orientation Determination by Common Lines



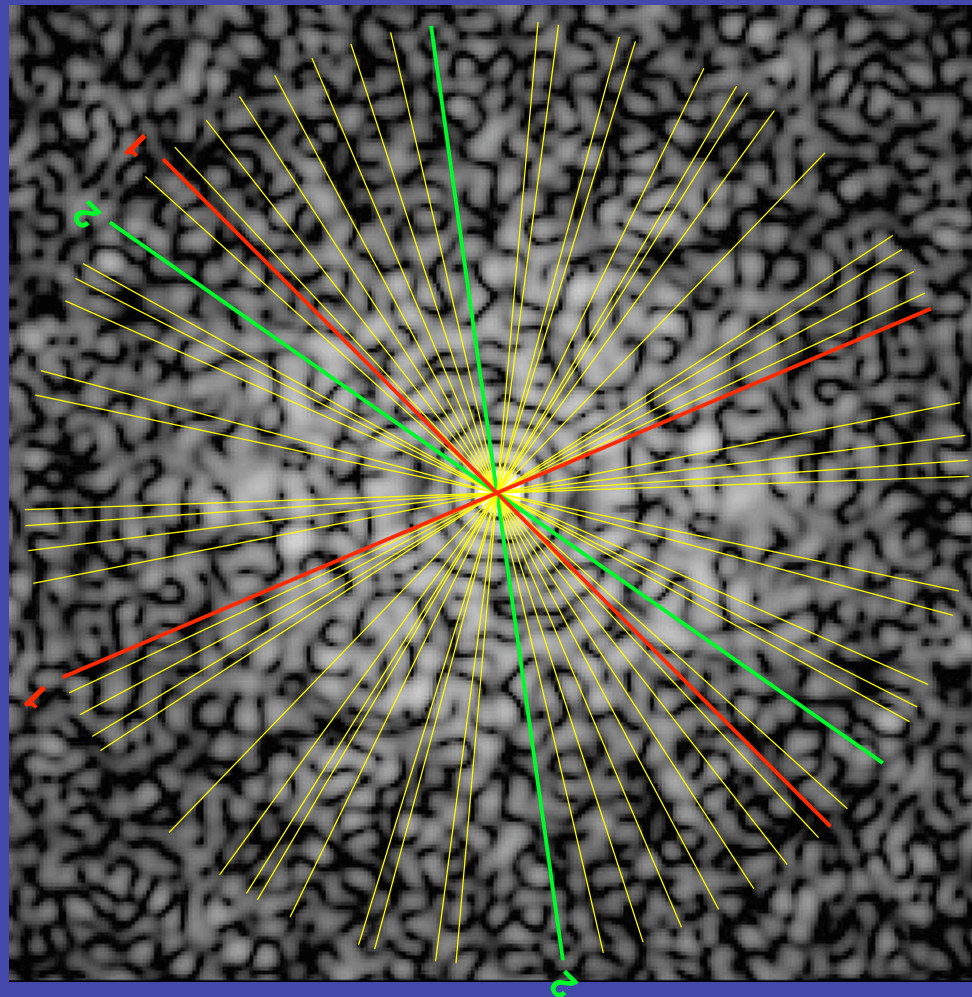
ω
↓
 $(80, 11, 60)$

Orientation Determination by Common Lines



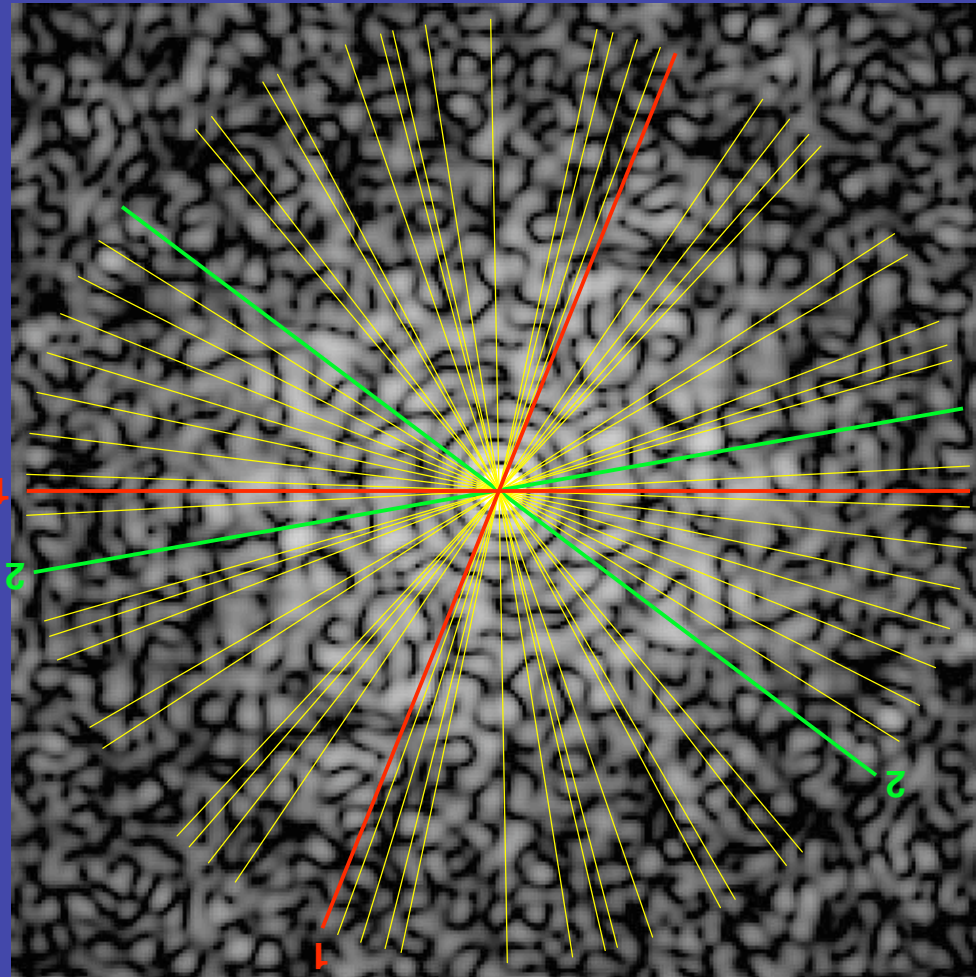
ω
↓
(80, 11, 90)

Orientation Determination by Common Lines



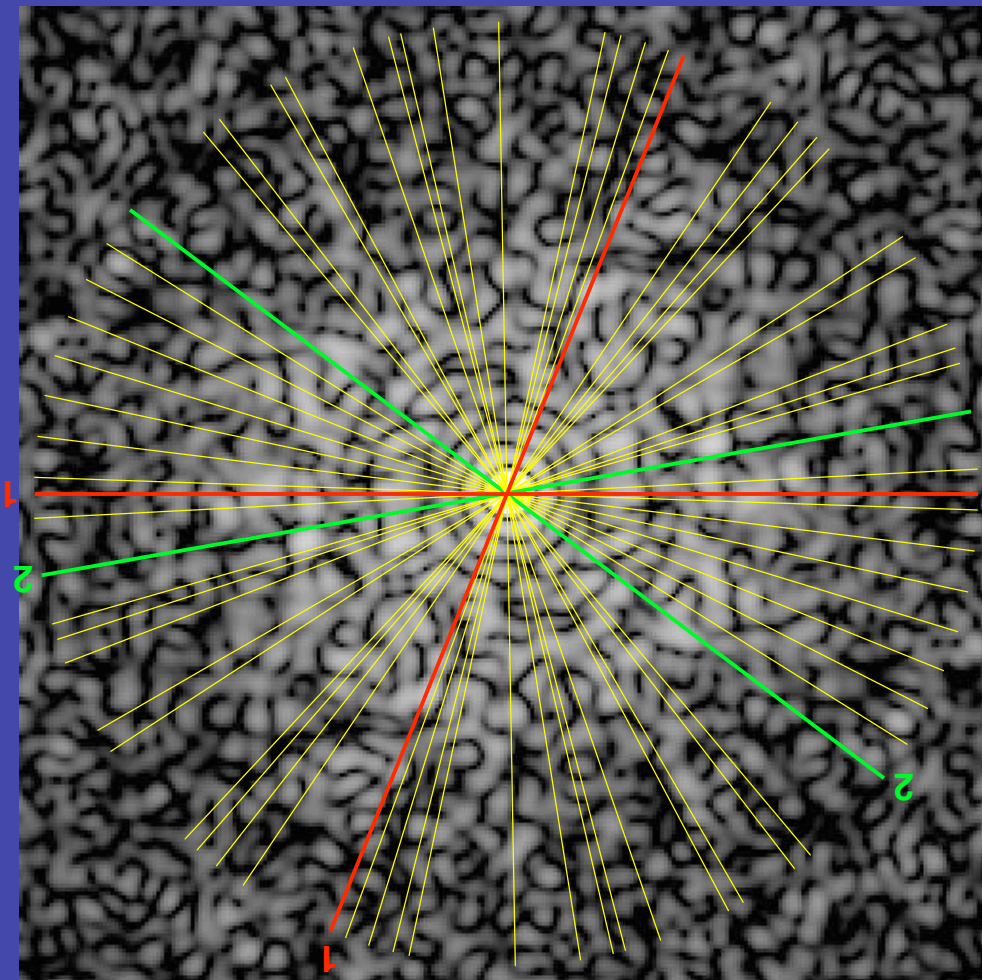
ω
↓
(80,11,135)

Orientation Determination by Common Lines



ω
↓
(80, 11, 180)

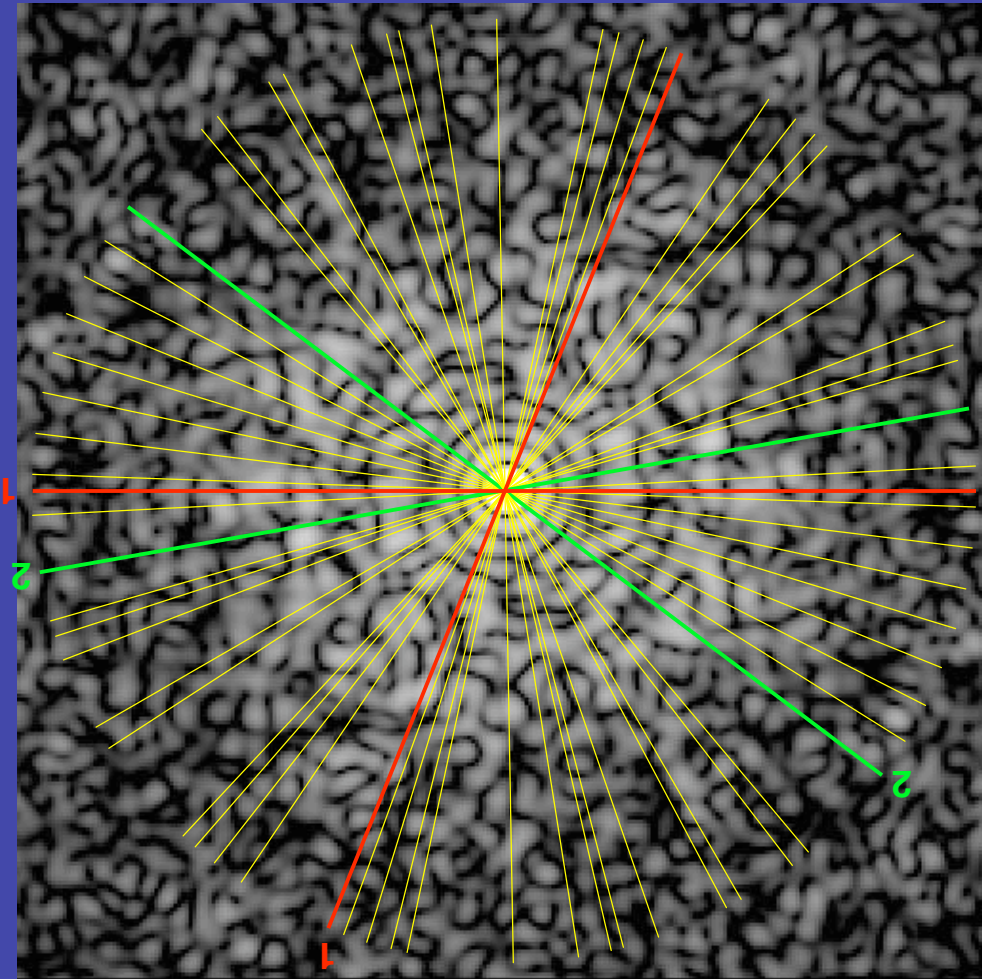
Orientation Determination by Common Lines



(80,11, ω)

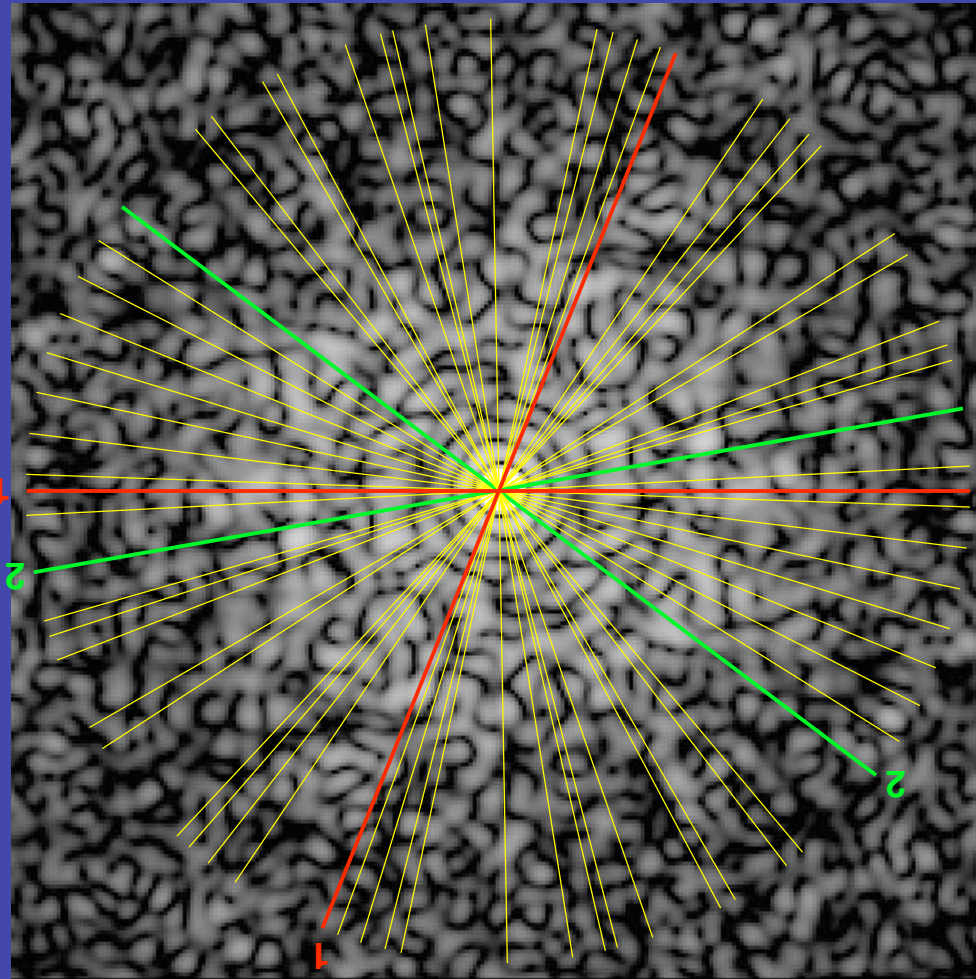
Metric: Identify ω that gives lowest phase residual

Orientation Determination by Common Lines



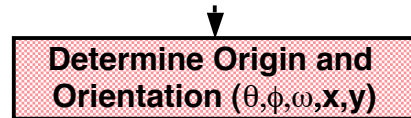
Repeat process for all possible (θ, ϕ, ω) combinations

Orientation Determination by Common Lines



> 250,000 combinations for 1° angular search intervals

Icosahedral Virus 3D Reconstruction Scheme



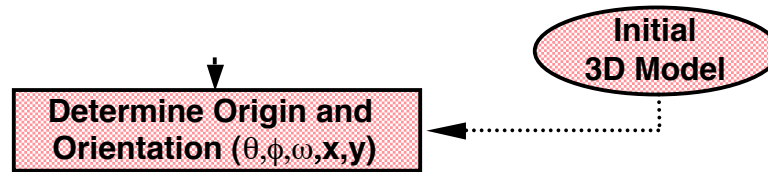
Common Lines

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



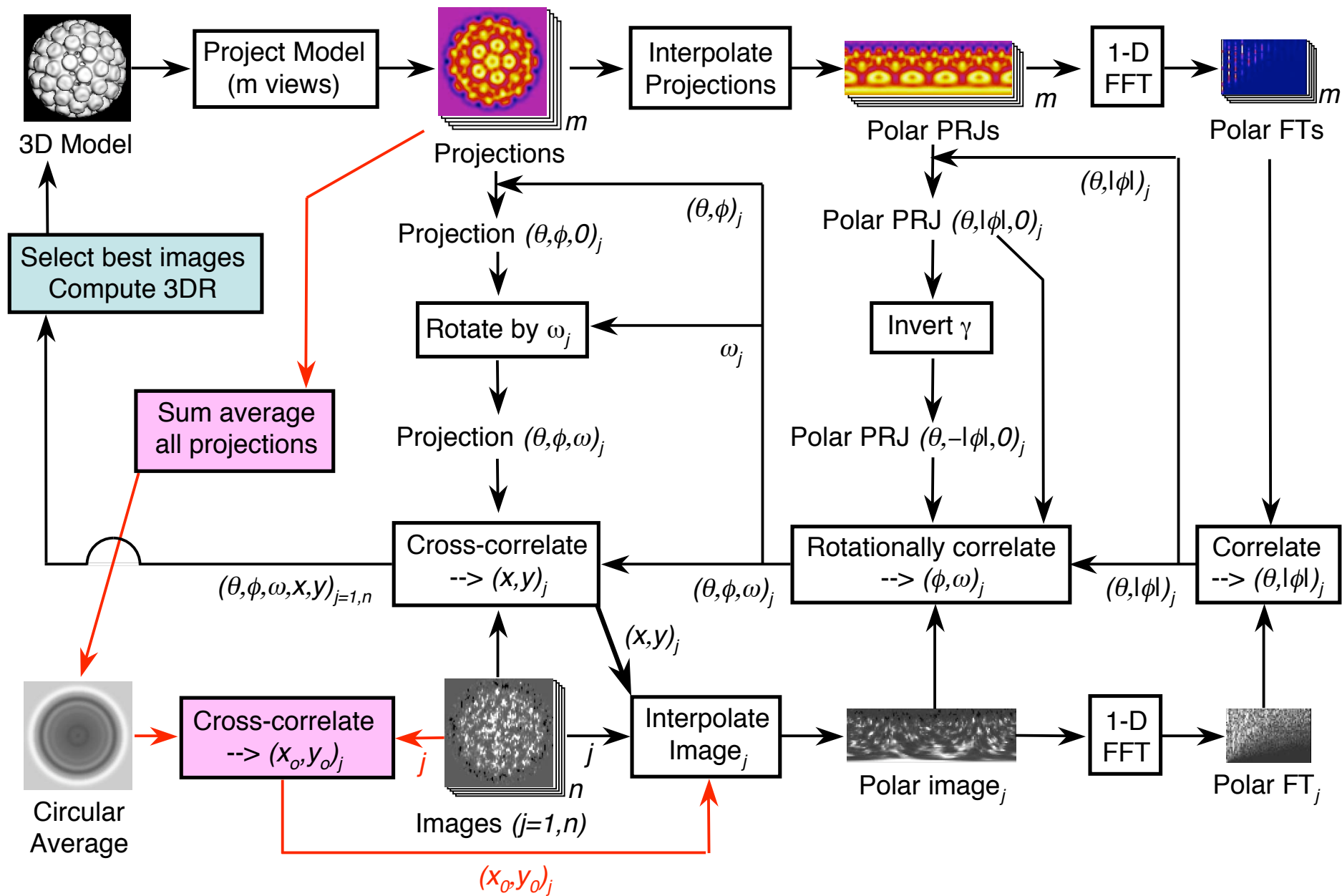
Recall: two methods to determine $(\theta, \phi, \omega, x, y)$:

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

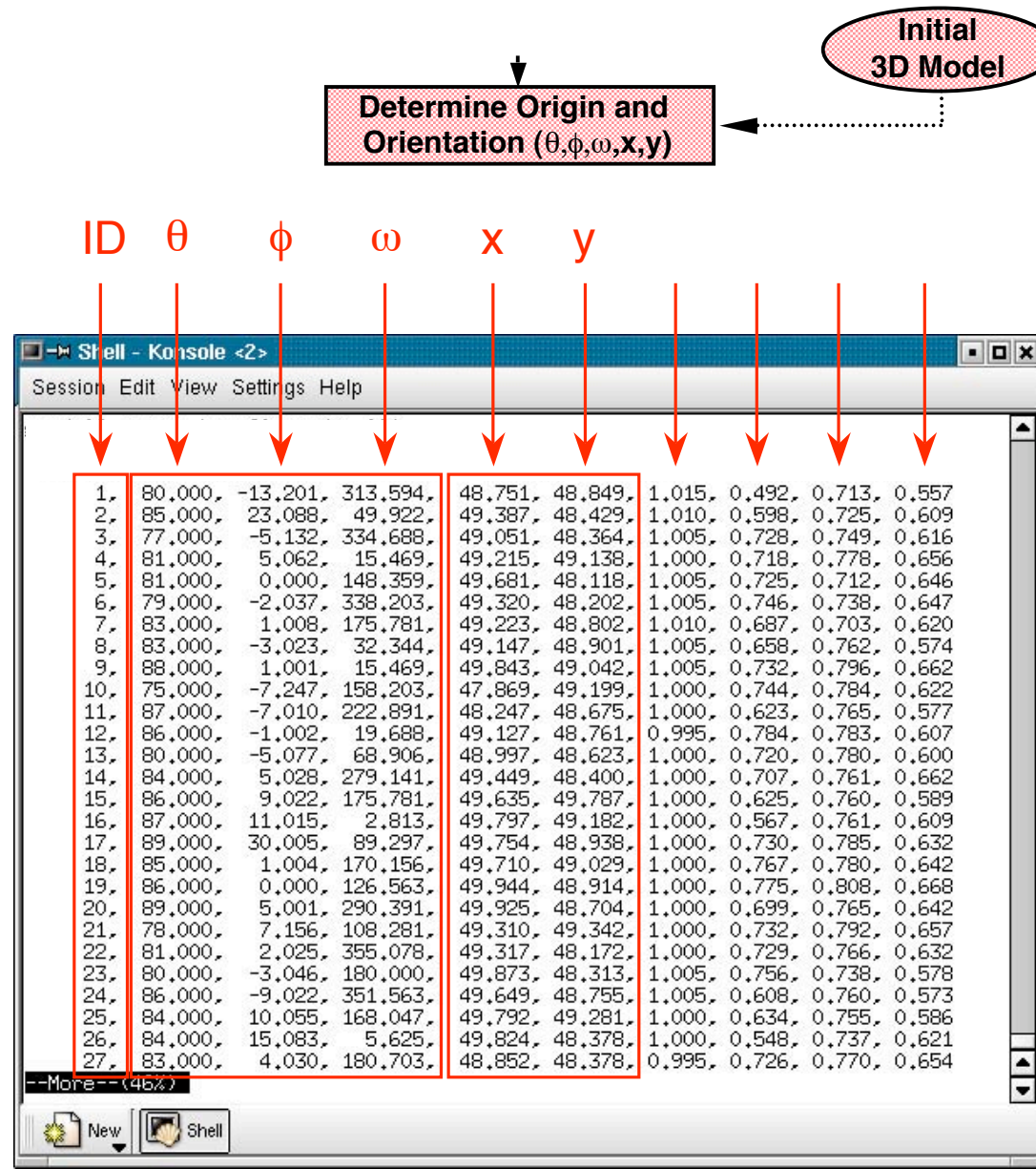
Bulk of structures now solved this way

Details discussed later if you like!

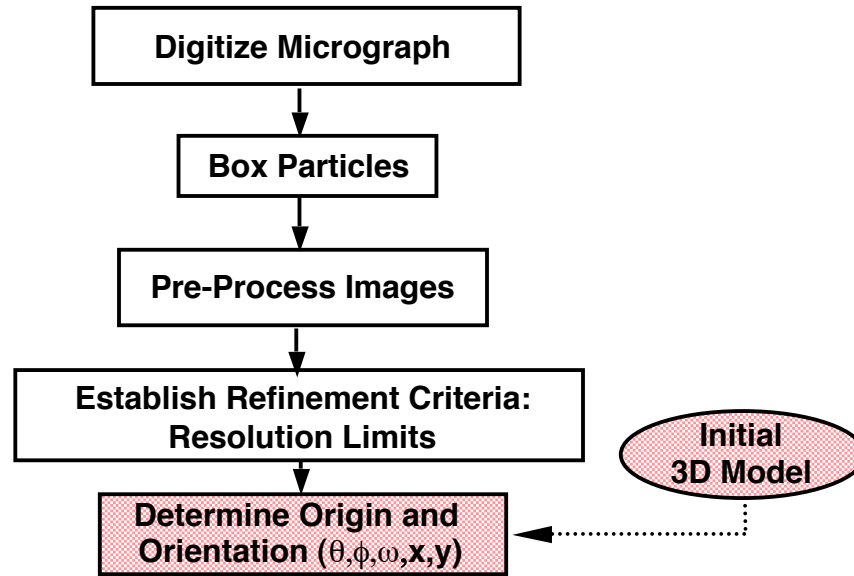
PFT Program Flow Chart



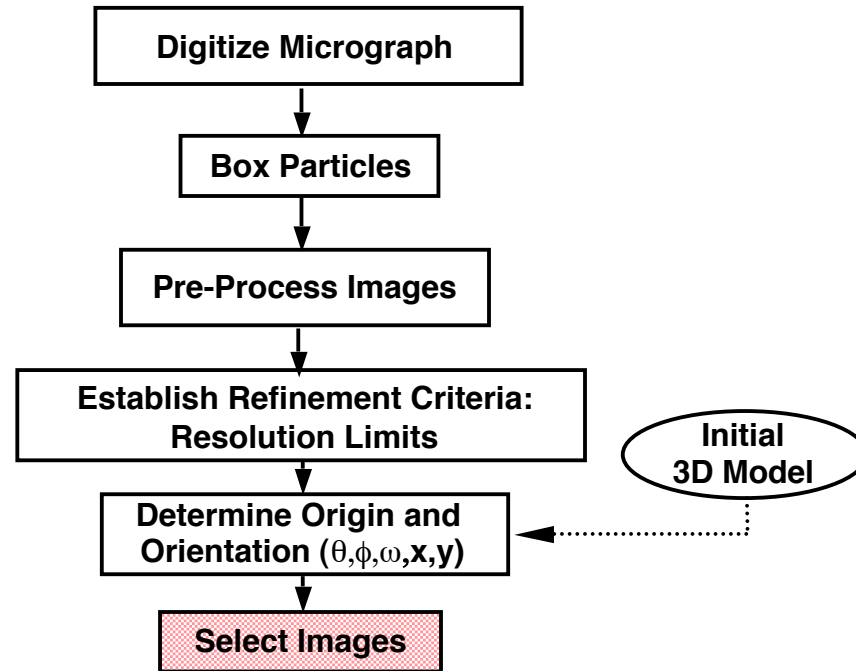
Icosahedral Virus 3D Reconstruction Scheme



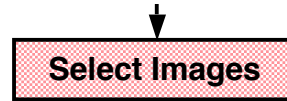
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



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

Icosahedral Virus 3D Reconstruction Scheme

Select Images

ID	θ	ϕ	ω	x	y				
1,	80,000,	-13,201,	313,594,	48,751,	48,849,	1,015,	0,492,	0,713,	0,557
2,	85,000,	23,088,	49,922,	49,387,	48,429,	1,010,	0,598,	0,725,	0,609
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

--More-- (46%)

Icosahedral Virus 3D Reconstruction Scheme

Select Images

PRJ CC

PFT CC

CMP CC

1,	80,000,	-13,201,	313,594,	48,751,	48,849,	1,015,	0,492,	0,713,	0,557
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

0.784

0.567 ?

0.775

0.548 ?

Icosahedral Virus 3D Reconstruction Scheme

Select Images

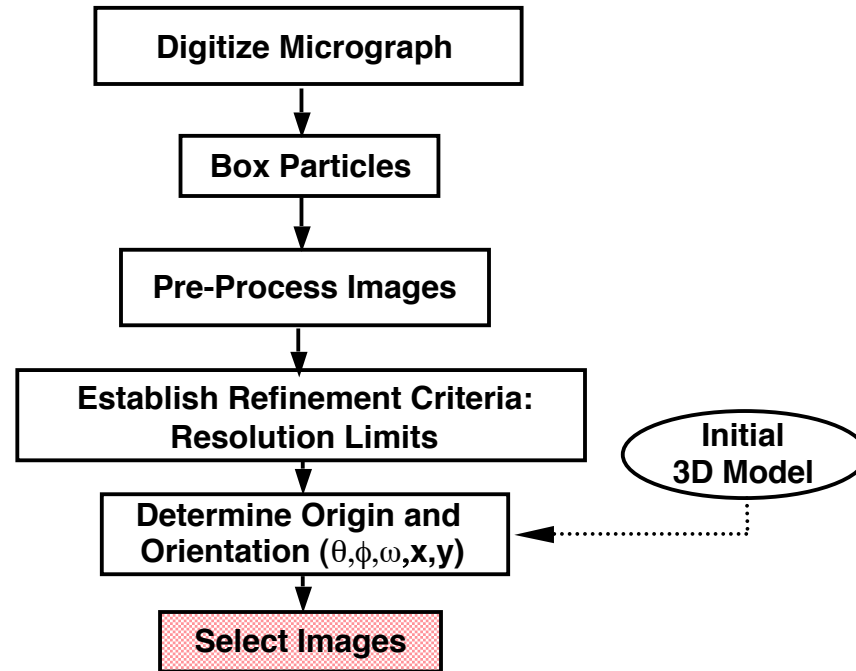
```
Shell - Konsole <2>
Session Edit View Settings Help
gandalf # more hsv_float.dat_001
1856_grad_float.pif
13.8800, 1, 100000.0, 0.0700, 1.5000, 1.5000, 0.0000, 2.00
1, 80.000, -13.201, 313.594, 48.751, 48.849, 1.015, 0.492, 0.713, 0.557
2, 85.000, 23.088, 49.922, 49.387, 48.429, 1.010, 0.598, 0.725, 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
```

```
Shell - Konsole <2>
Session Edit View Settings Help
42, 82.000, -7.069, 175.781, 49.389, 48.980, 1.000, 0.678, 0.762, 0.593
43, 84.000, 16.088, 50.625, 49.775, 48.918, 1.000, 0.543, 0.735, 0.562
44, 80.000, 7.108, 239.063, 48.996, 49.075, 0.995, 0.690, 0.761, 0.623
45, 74.000, 1.040, 142.734, 49.824, 48.960, 0.990, 0.685, 0.718, 0.637
46, 81.000, 13.162, 242.578, 49.019, 49.032, 1.000, 0.533, 0.758, 0.558
47, 73.000, -1.046, 173.672, 48.797, 47.683, 1.000, 0.687, 0.744, 0.569
48, 71.000, -3.173, 296.016, 50.072, 48.370, 1.000, 0.717, 0.788, 0.614
49, 82.000, -6.059, 199.688, 49.642, 49.156, 1.000, 0.719, 0.764, 0.633
50, 73.000, 2.091, 170.859, 49.110, 48.884, 1.000, 0.681, 0.741, 0.589
51, 84.000, 20.110, 215.156, 49.076, 48.673, 1.000, 0.576, 0.764, 0.608
52, 79.000, 14.262, 21.094, 49.088, 49.107, 1.005, 0.536, 0.745, 0.560
53, 89.000, -5.001, 187.031, 48.970, 49.204, 1.005, 0.602, 0.744, 0.551
54, 79.000, 6.112, 227.109, 49.223, 48.719, 1.000, 0.721, 0.755, 0.636
55, 88.000, 21.013, 162.422, 48.765, 49.285, 0.995, 0.557, 0.749, 0.580
56, 84.000, -3.017, 251.016, 49.831, 50.002, 0.995, 0.745, 0.776, 0.616
57, 80.000, -2.031, 213.047, 47.831, 48.686, 0.995, 0.722, 0.744, 0.590
58, 72.000, -3.154, 295.313, 48.862, 47.839, 0.995, 0.680, 0.741, 0.546
59, 83.000, -1.008, 102.656, 48.957, 48.904, 1.005, 0.713, 0.767, 0.575
60, 89.000, -6.001, 73.828, 48.802, 48.736, 1.010, 0.680, 0.693, 0.544

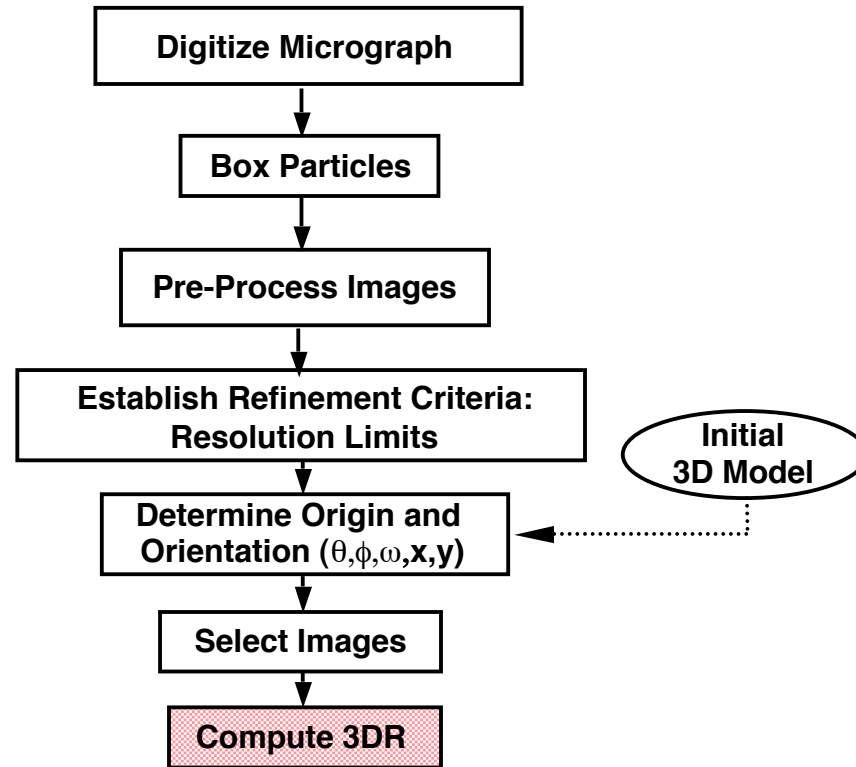
-----
Compute average MAG factor and coefficients, N = 120
-----
Average Std. Dev.
MAG Factor 1.000 0.006
PFT Coefficient 0.679 0.075
PRJ Coefficient 0.756 0.023
CMP Coefficient 0.609 0.038
PFT finished.
*****
gandalf #
```

PFT Coefficient 0.679 ± 0.075

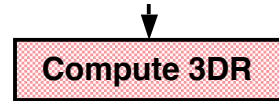
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

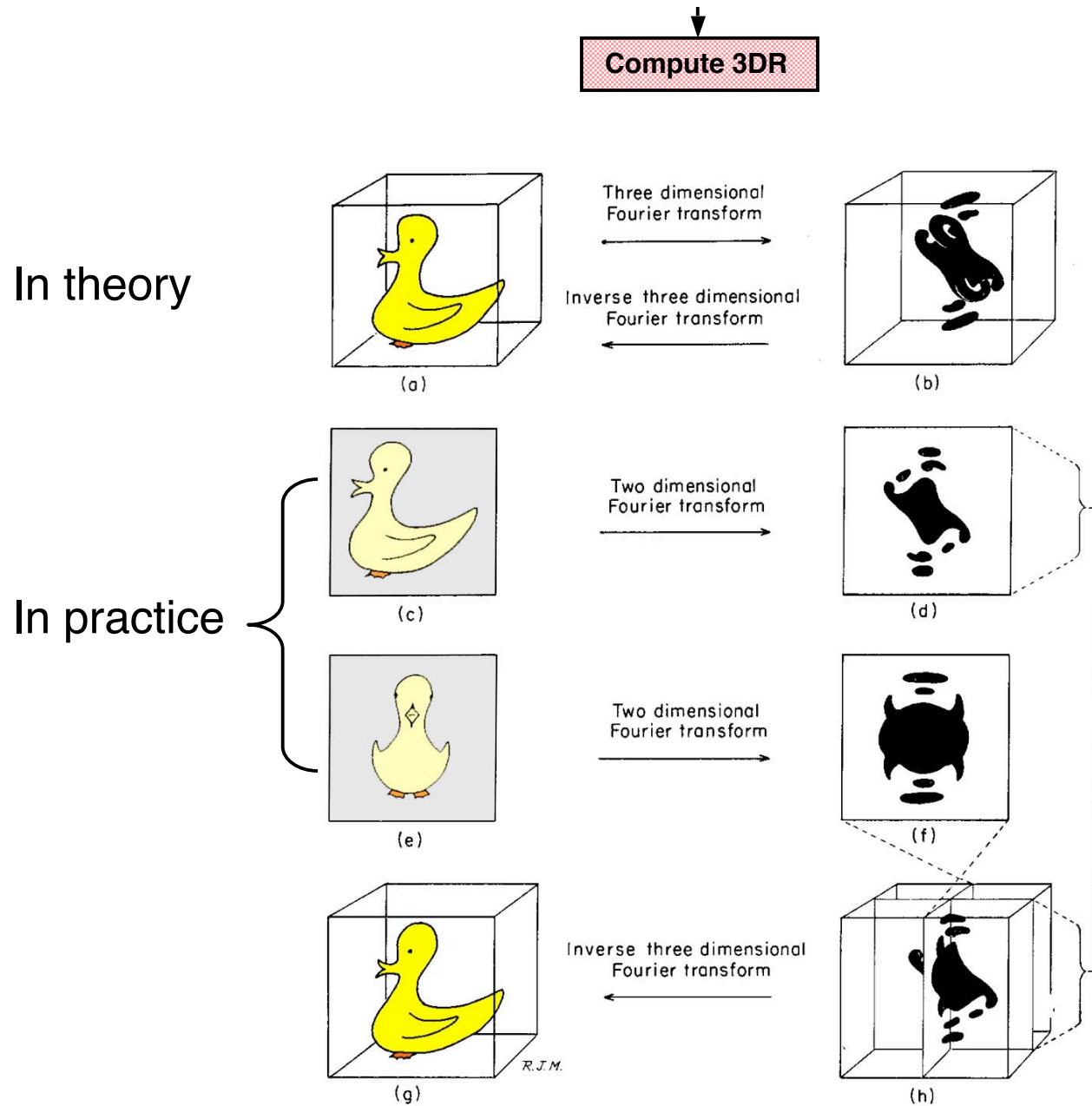


Icosahedral Virus 3D Reconstruction Scheme

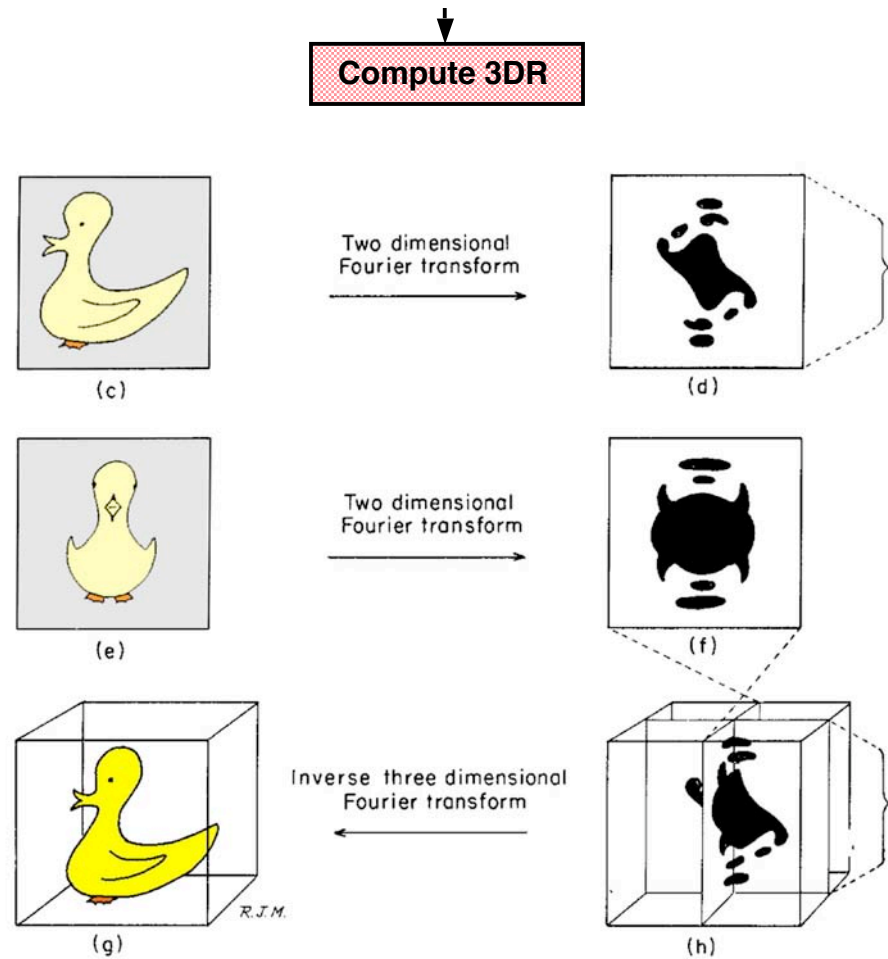


Goal: combine “good” particle images to compute a 3D density map

Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



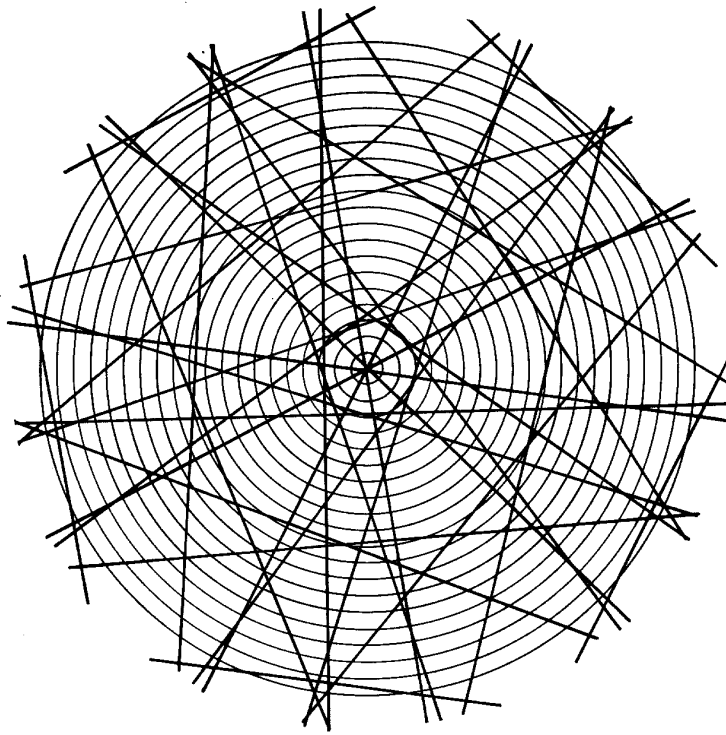
Overall scheme: $\rho \leftarrow g \leftarrow G \leftarrow F$

Icosahedral Virus 3D Reconstruction Scheme

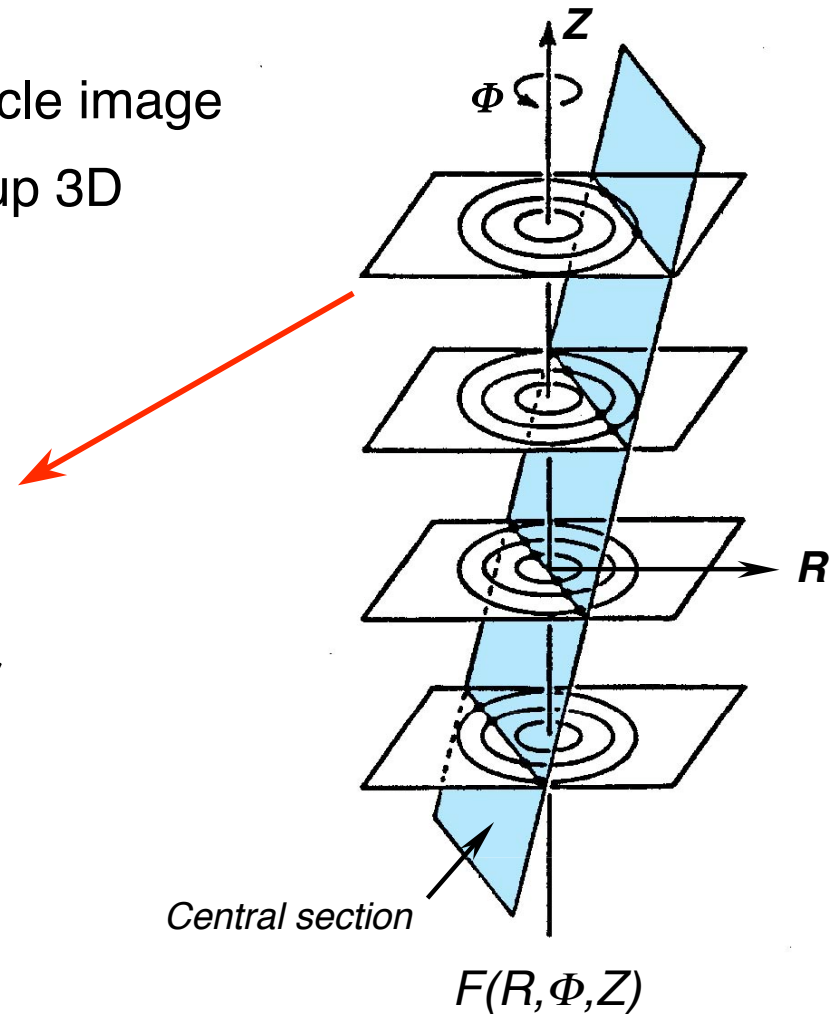
↓
Compute 3DR

Steps:

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

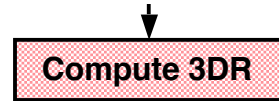


Crowther, DeRosier and Klug, 1970, p.329



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

Icosahedral Virus 3D Reconstruction Scheme

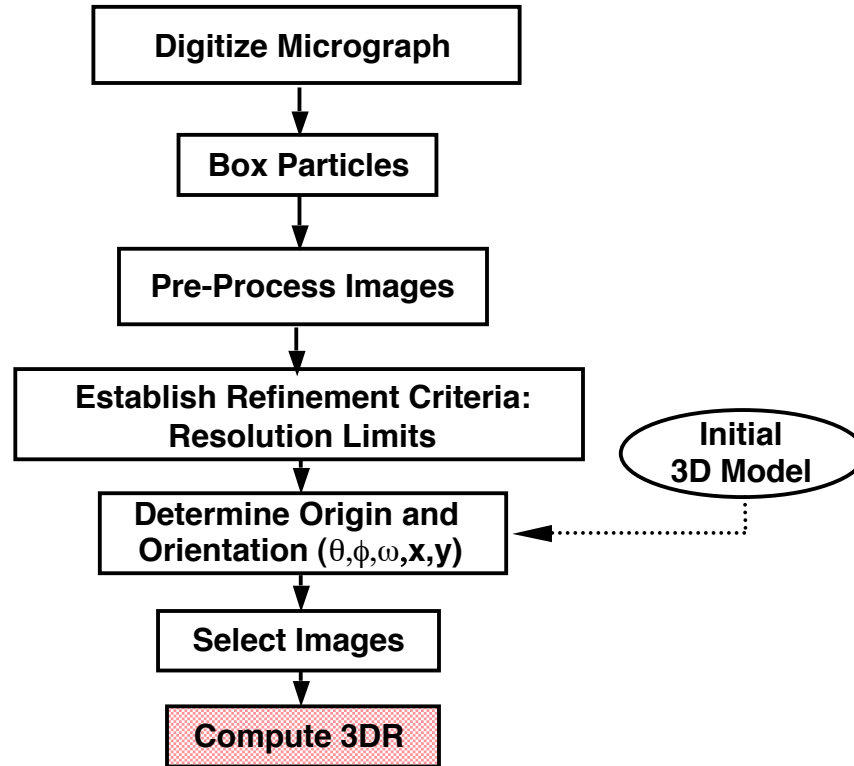


$$\rho \leftarrow \mathbf{g} \leftarrow \mathbf{G} \leftarrow \mathbf{F}$$

Steps:

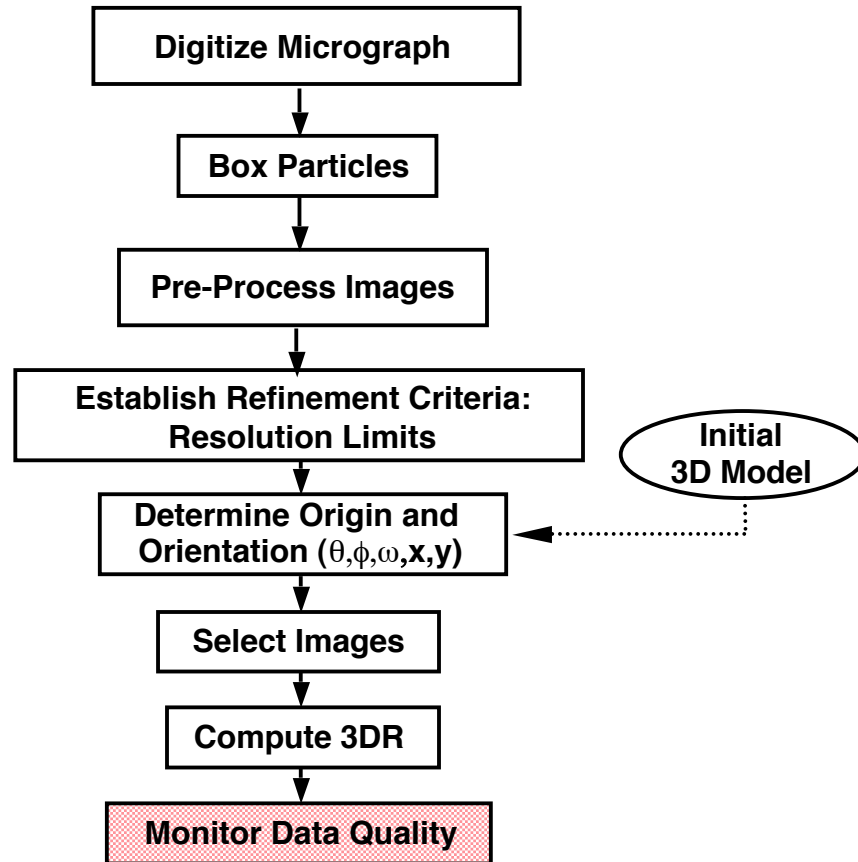
1. Compute 2D FFT of each particle image
2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform
3. Compute G_n 's on each annulus $G = (B^\dagger B)^{-1} B^\dagger F$
4. Compute g_n 's from G_n 's (Fourier-Bessel transform)
5. Compute polar density map ($\rho(r, \phi, z)$) from g_n 's
6. Convert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

Icosahedral Virus 3D Reconstruction Scheme



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

Icosahedral Virus 3D Reconstruction Scheme



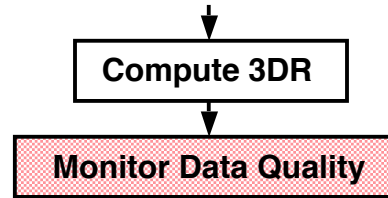
Icosahedral Virus 3D Reconstruction Scheme



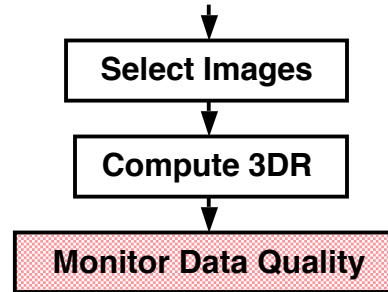
Monitor Data Quality

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

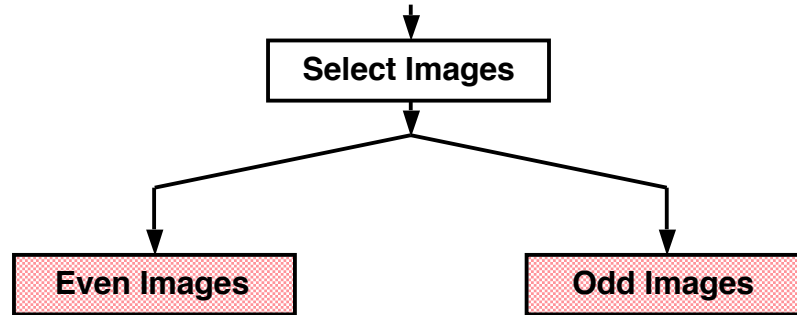
Icosahedral Virus 3D Reconstruction Scheme



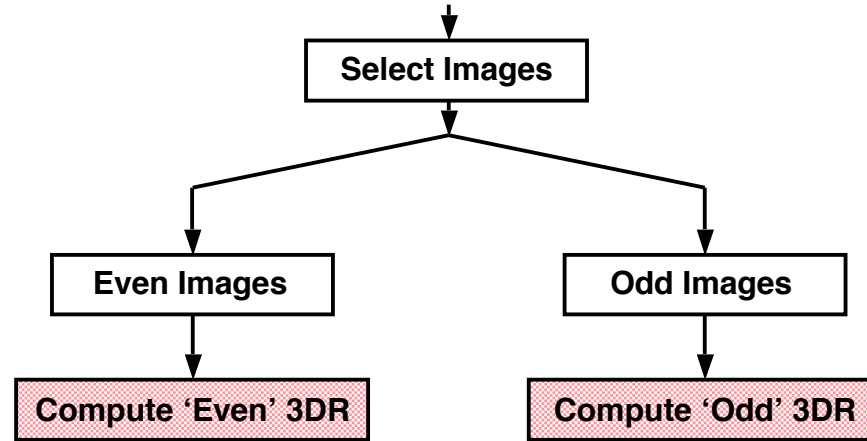
Icosahedral Virus 3D Reconstruction Scheme



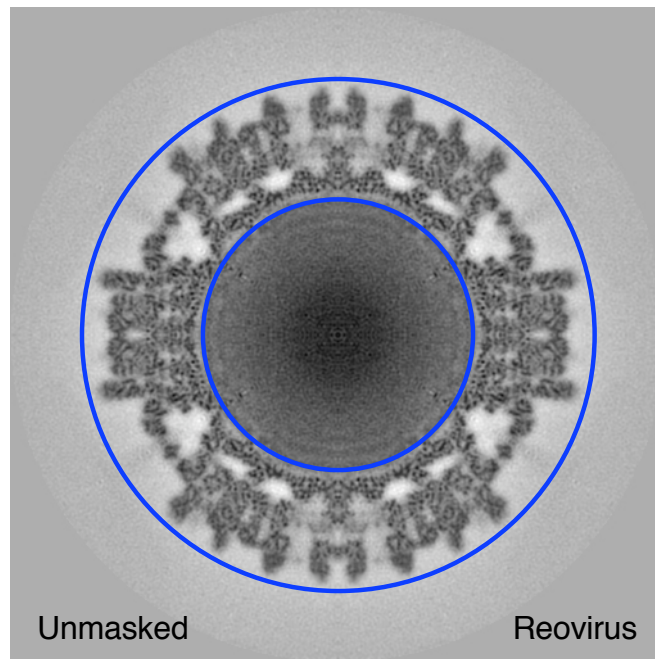
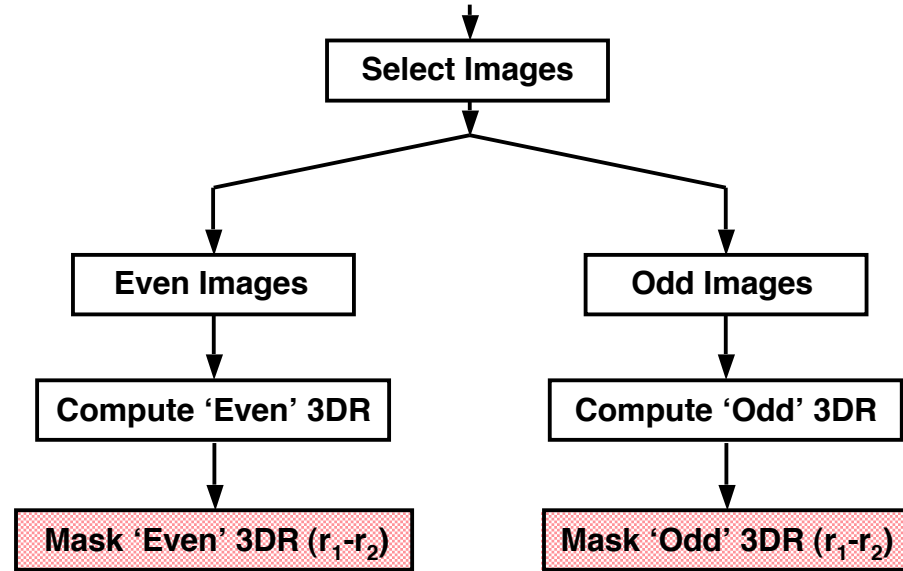
Icosahedral Virus 3D Reconstruction Scheme



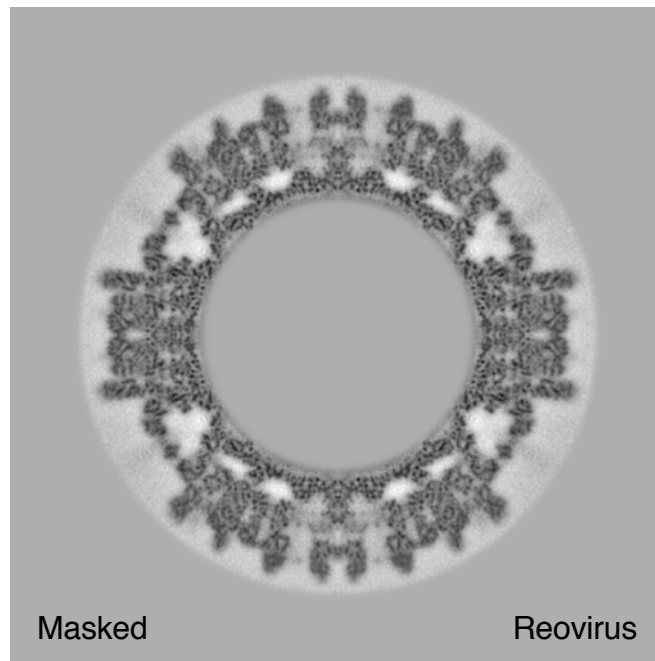
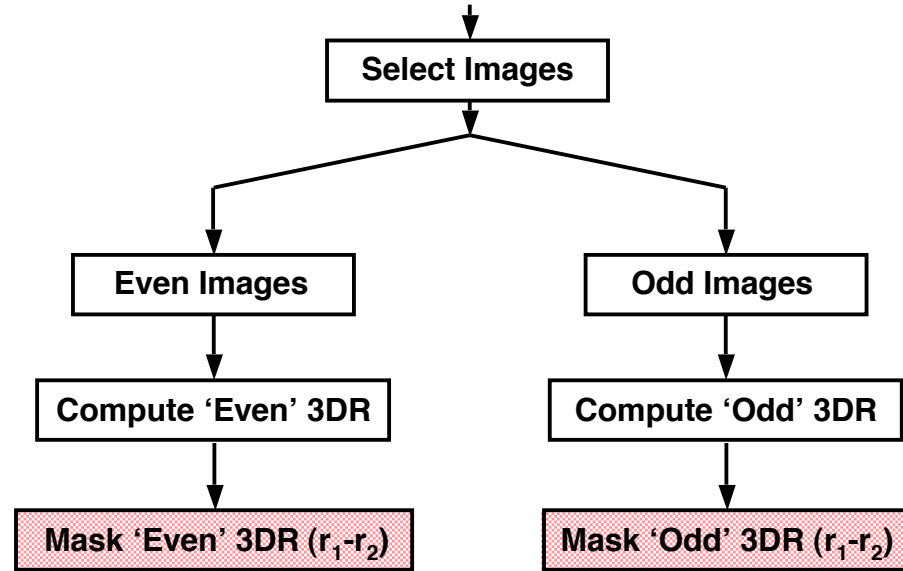
Icosahedral Virus 3D Reconstruction Scheme



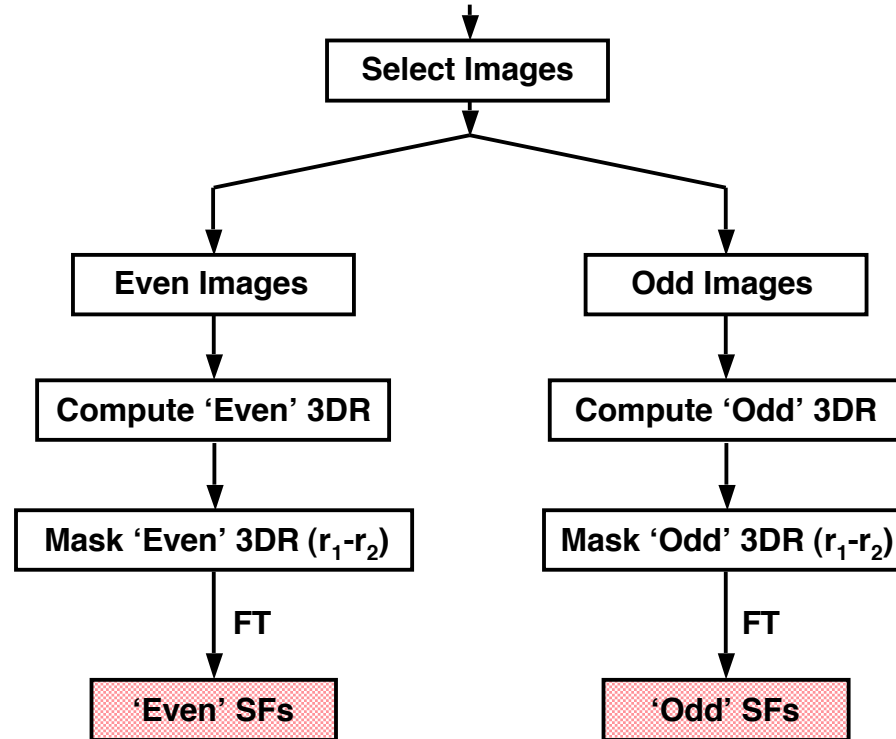
Icosahedral Virus 3D Reconstruction Scheme



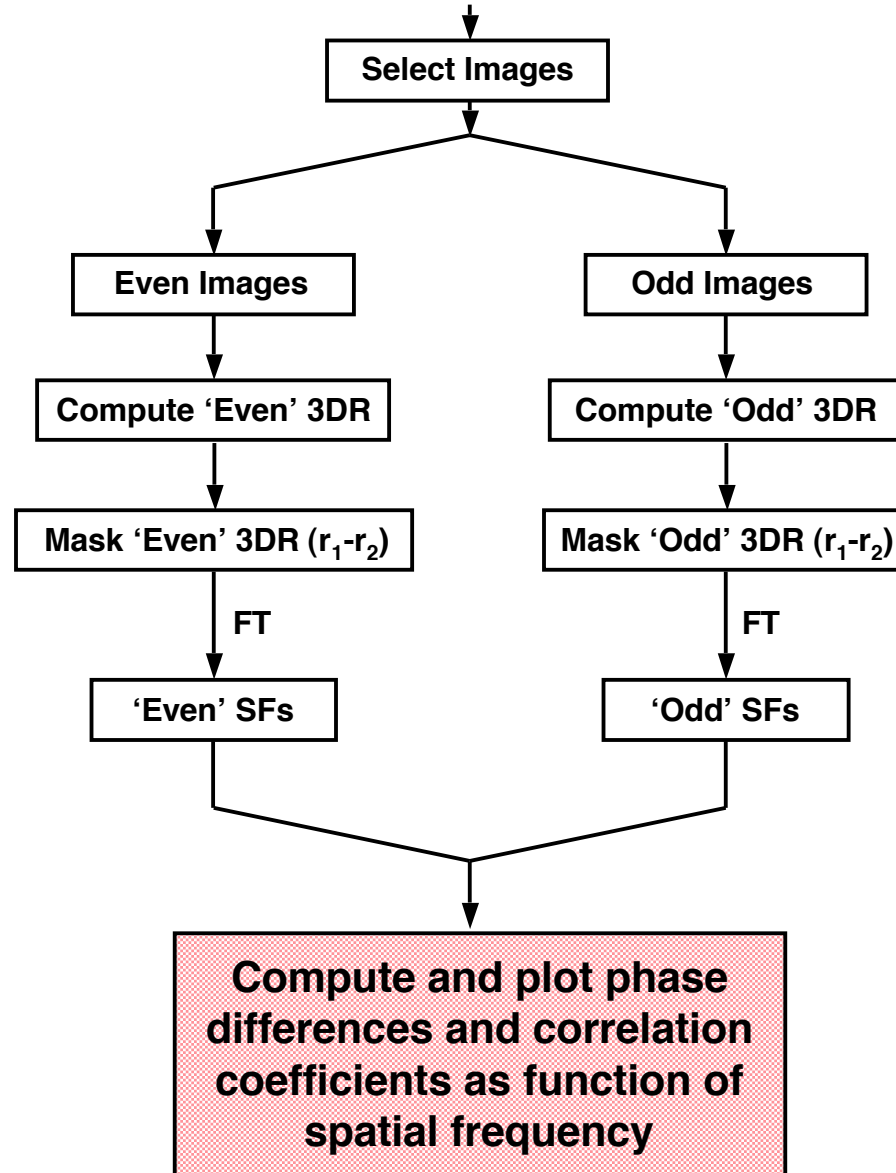
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



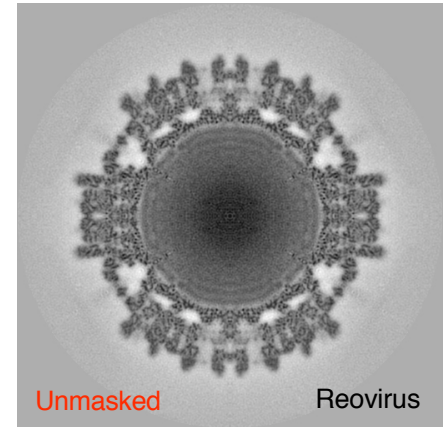
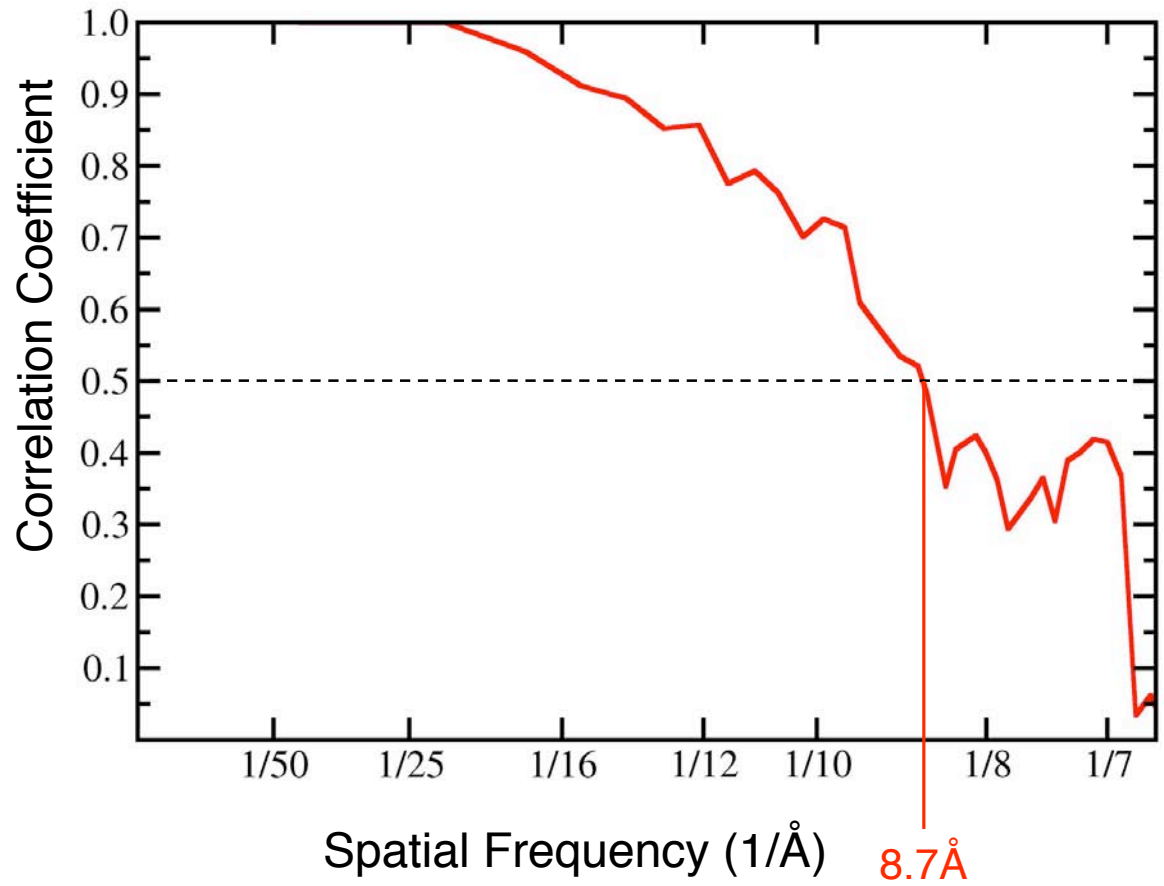
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



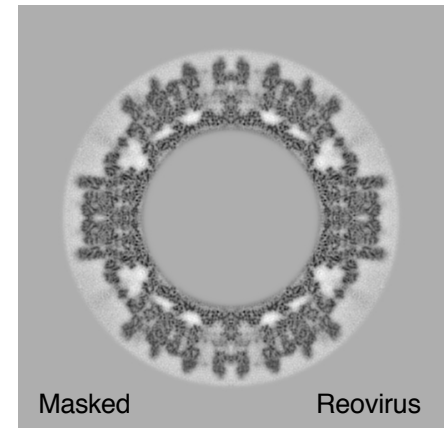
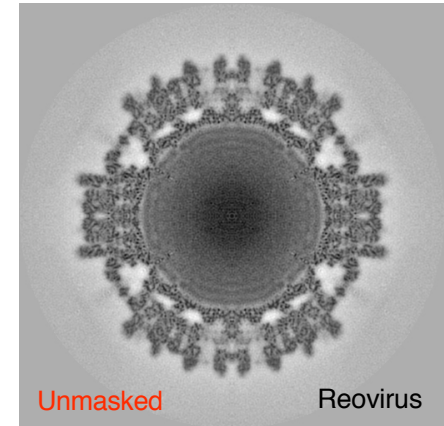
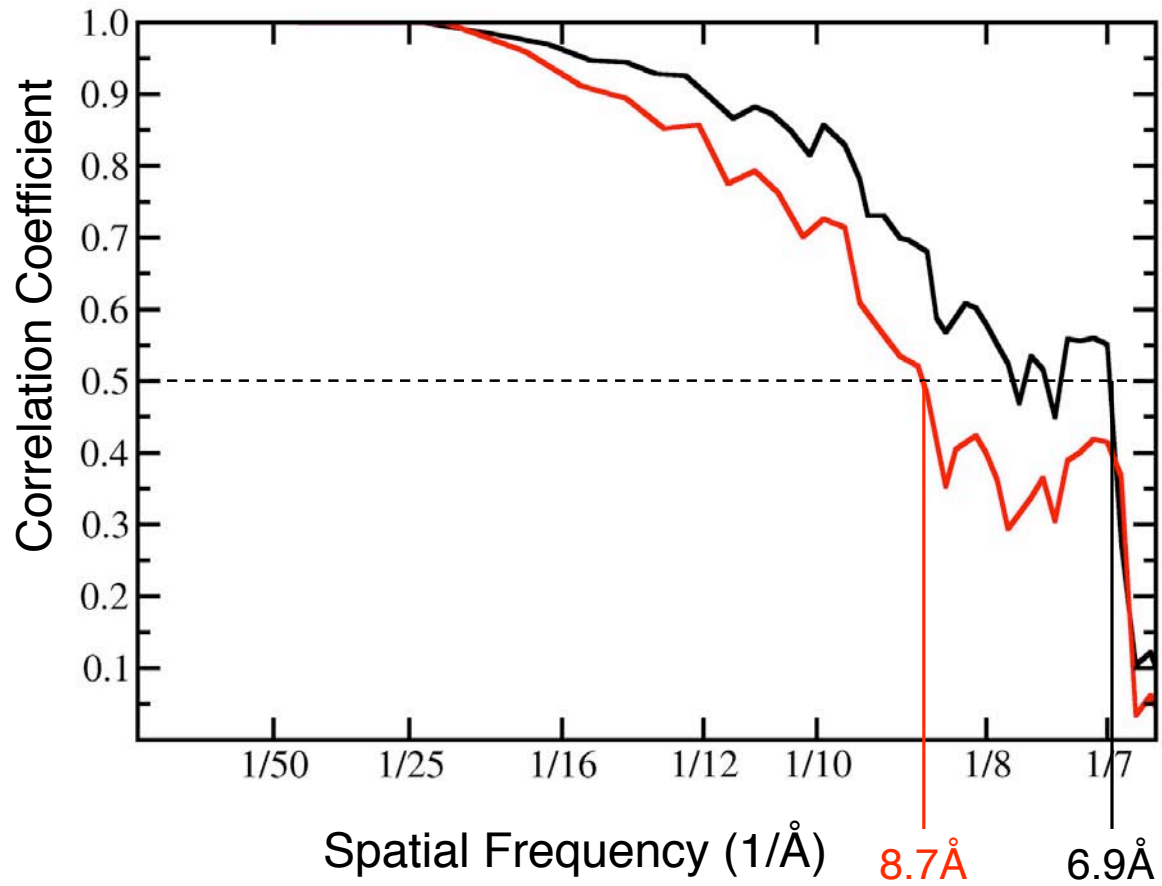
Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme



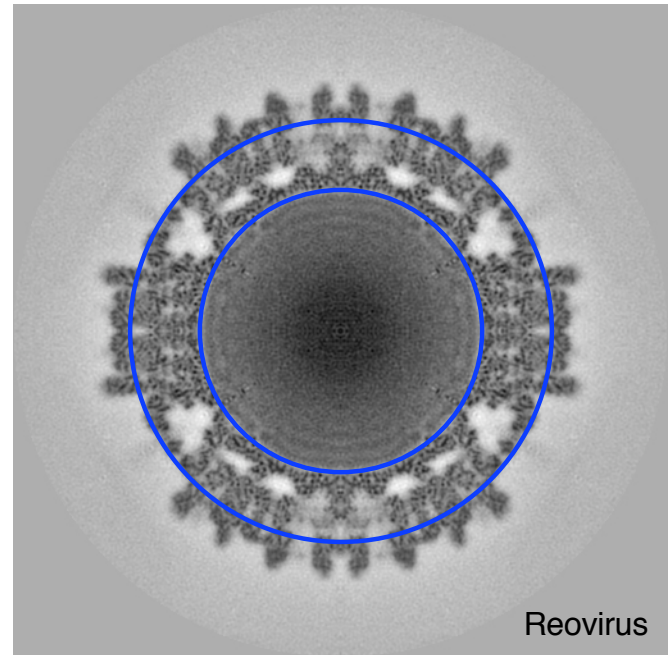
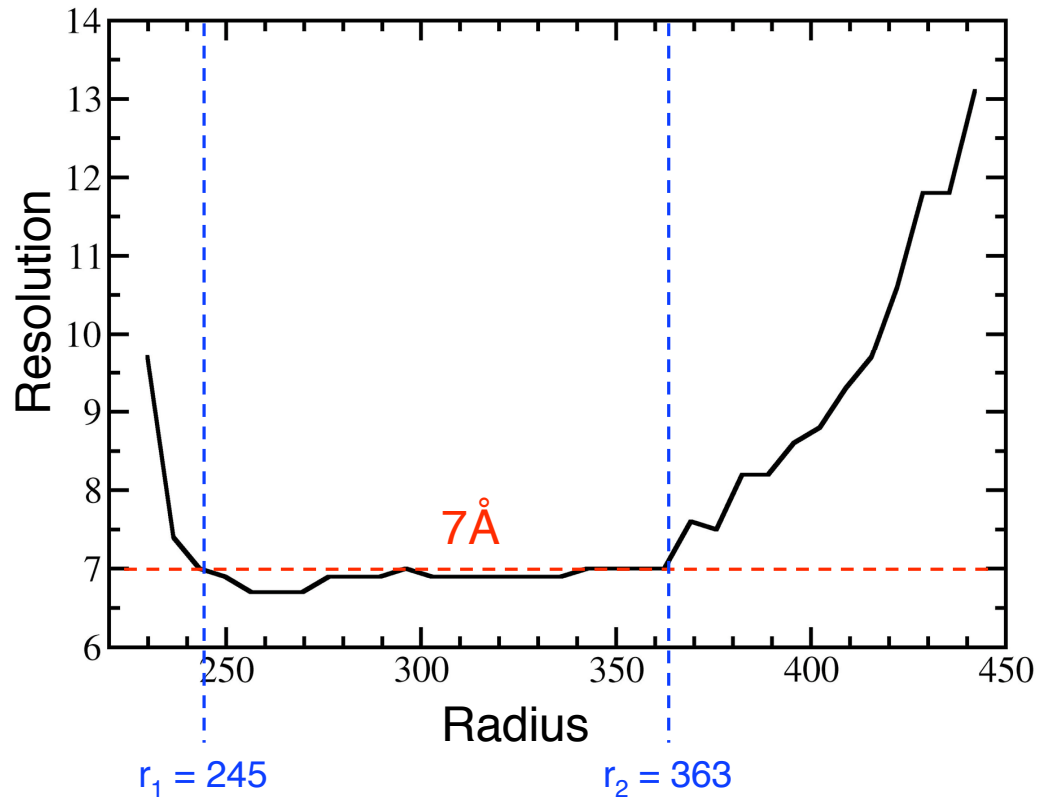
Monitor Data Quality

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

Icosahedral Virus 3D Reconstruction Scheme



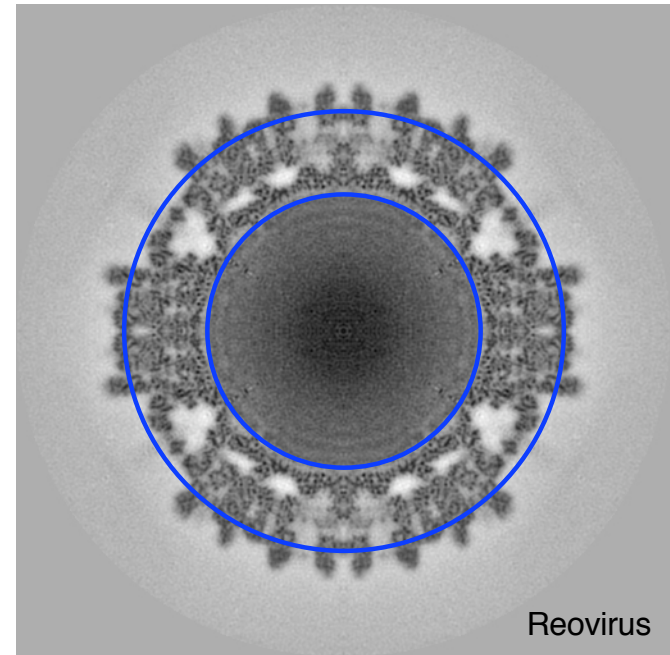
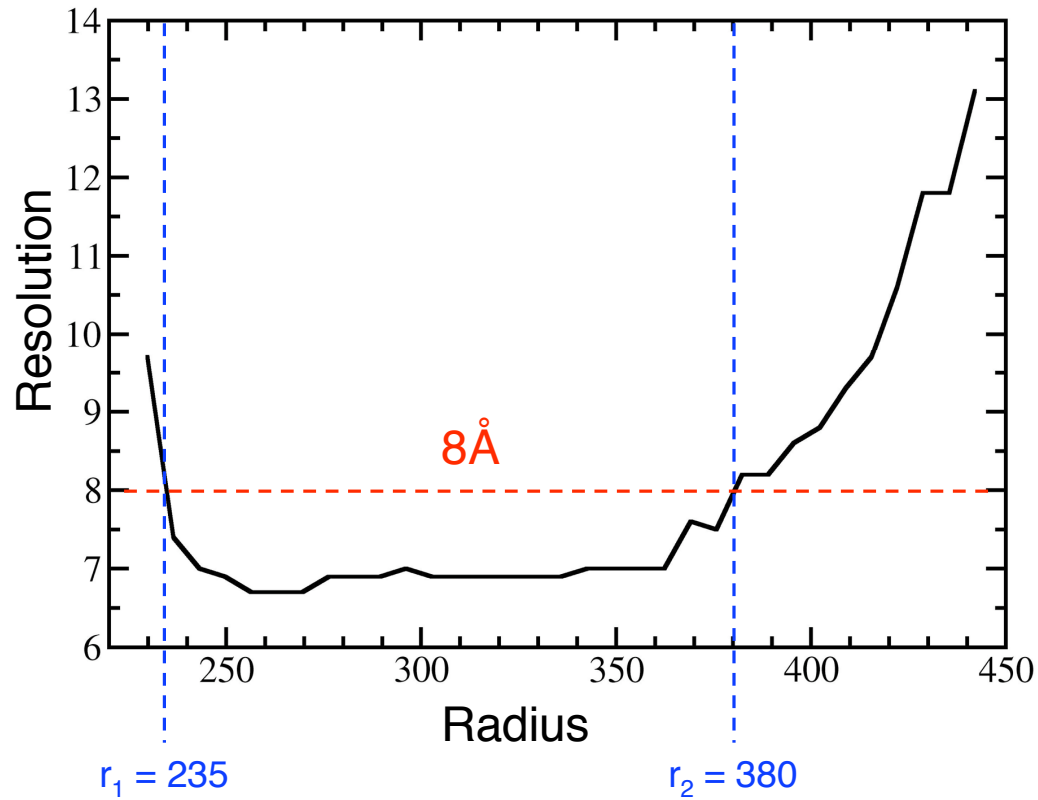
Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme



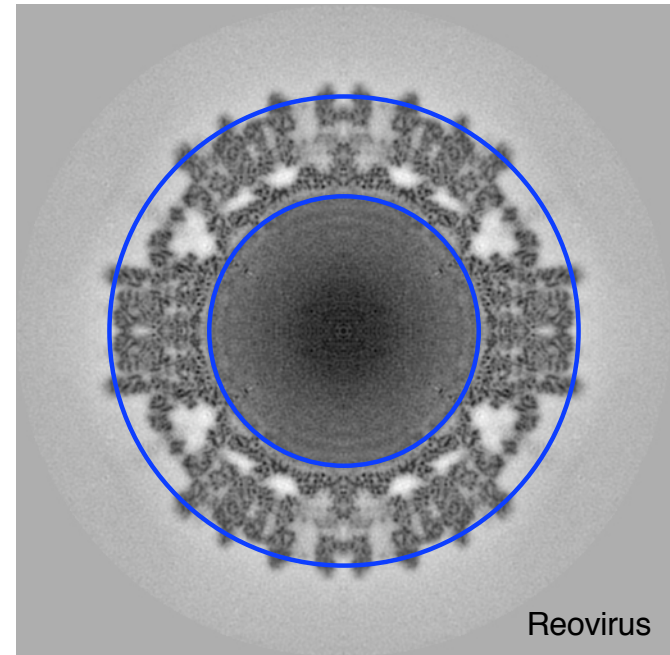
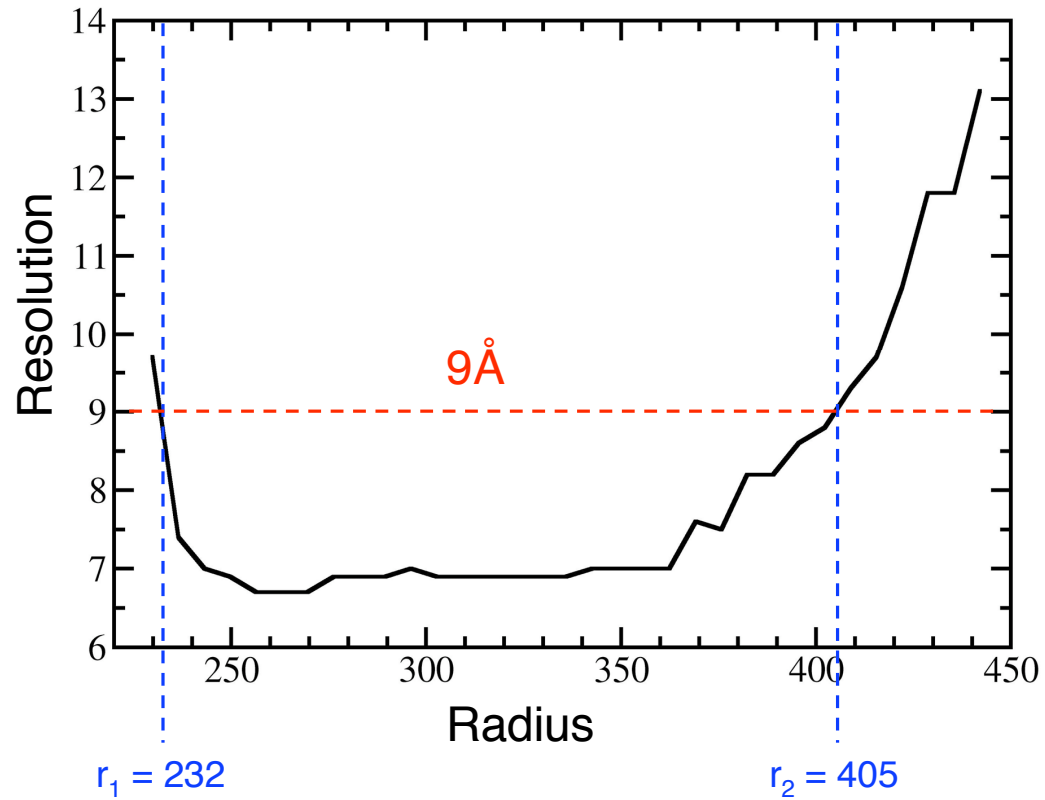
Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme



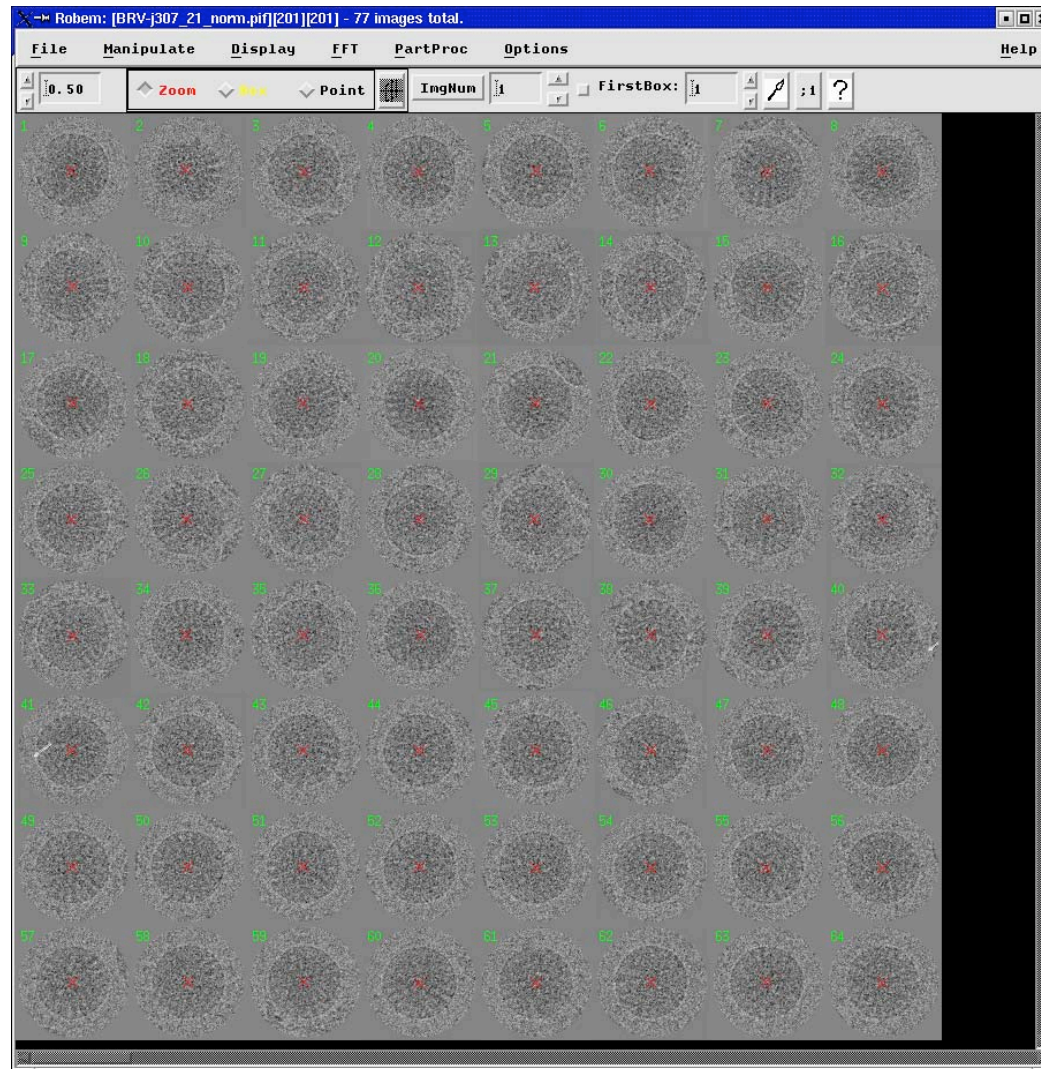
Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme



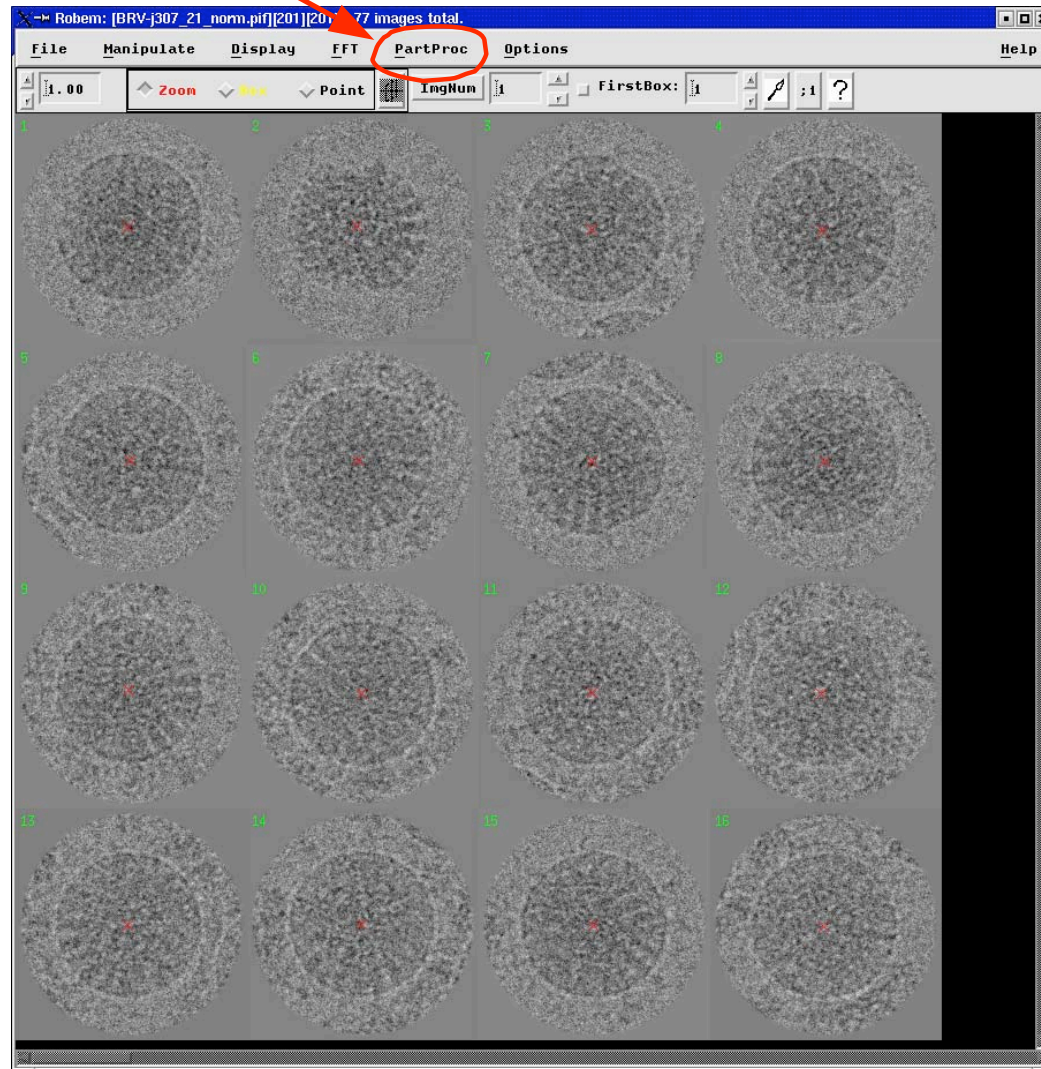
Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality



Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality

The screenshot displays the PartProc software interface. The main window is titled "PartProc" and contains several panels:

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

The right side of the interface features a large triangular plot with a black background and a blue dashed triangle. Red 'x' marks represent particle positions. A red arrow points to a specific data point in the bottom right corner of the triangle.

Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

The screenshot displays the PartProc software interface. On the left, a grid of micrographs is shown. The central panel contains various controls and parameters:

- Parameter File: `/bio/gandalf/tsb/NRAHM/B`
- Map File: `/bio/gandalf/tsb/NRAHM/B`
- Scan File:
- Display centers
- Pick new particle center
- MAP Projections
- Projection Contour
- Parameter table:

PixSize	6.0000	Units	1
Voltage	100000.0		
Amp Factor	0.0700		
Δf major	1.6910		
Δf minor	1.6910		
Ang major	0.0000		
Cs Coef	2.00		
- Buttons: Compute Circular Avg, Add delta omega, Manipulate Particle Params, Add delta X,Y to centers, Rebox ALL Particles, Rebox THIS Particle, Write New Parm File, EXIT

The right-side plot shows a triangular distribution of red 'x' marks representing particle parameters. A blue circle is visible in the upper right of the plot. A red arrow points to a small square on the x-axis at the value 31.

```
Particle[ 44] Theta [ 90.000] Phi [ 31.000] Omega [ 55.141]
X [ 98.694] Y [ 99.617] Mag [ 1.000]
pft_cc [ 0.536] proj_cc [ 0.559] cmp_cc [ 0.567]
delta_k [ -1.306] delta_l [ -0.383] distance [ 1.361]
state [ ON] rank [ 44]
```

Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

The screenshot displays a software interface for 3D reconstruction. The main window, titled "X-robem: [BRV-j307_21_norm.pif][201][201] - 77 images total.", contains two image thumbnails on the left and a large black area on the right. The "Partproc" sub-window is open, showing various parameters and a triangular grid of particles.

Partproc Parameters:

- Parameter File: `/bio/gandalf/tsb/NRAHM/B`
- Map File: `/bio/gandalf/tsb/NRAHM/B`
- Scan File:
- Display centers:
- Pick new particle center:
- MAP Projections: Projection Contour:

Particle Parameters Table:

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

Grid Parameters:

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

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

The triangular grid of particles is shown in the bottom right of the Partproc window. The vertices are labeled with coordinates: top vertex is `90,0,90,9`, bottom-left is `-31`, and bottom-right is `31`. A red arrow points to a small square icon at the bottom-right vertex of the grid.

Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

The screenshot displays a software interface for 3D reconstruction. The main window, titled "X Robem: [BRV-j307_21_norm.pif][201][201] - 77 images total.", contains two image thumbnails: a grayscale micrograph of a virus particle and a corresponding 2D reconstruction. The "Partproc" window is open, showing various parameters and a 2D plot.

Partproc Parameters:

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

Particle Parameters (Particle[26]):

Particle[26]	Theta [76.644]	Phi [11.927]	Omega [92.277]
X [97.863]	Y [99.927]	Mag [1.000]	
pft_cc [0.542]	prj_cc [0.605]	cmp_cc [0.578]	
deltaX [-2.137]	deltaY [-0.073]	distance [2.138]	
state [0N]	rank [26]		

2D Plot: A 2D plot showing a triangular arrangement of red 'x' marks representing particle centers. A blue dashed triangle outlines the arrangement. A red arrow points to a specific particle center. The plot includes two icosahedral virus models (one blue, one red) and a coordinate system with axes ranging from -31 to 31.

Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

The screenshot displays a software interface for 3D reconstruction. The main window, titled "X Robem: [BRV-j307_21_norm.pif][201][201] - 77 images total.", contains two image thumbnails: a grayscale micrograph of a virus particle on the left and a corresponding reconstruction on the right. The "Partproc" window is open, showing various parameters and a plot of particle distribution.

Partproc Parameters:

- Parameter File: `/bio/gandalf/tsb/NRAHM/B`
- Map File: `/bio/gandalf/tsb/NRAHM/B`
- Scan File:
- Display centers:
- Pick new particle center:
- MAP Projections: Projection Contour:

Particle Parameters Table:

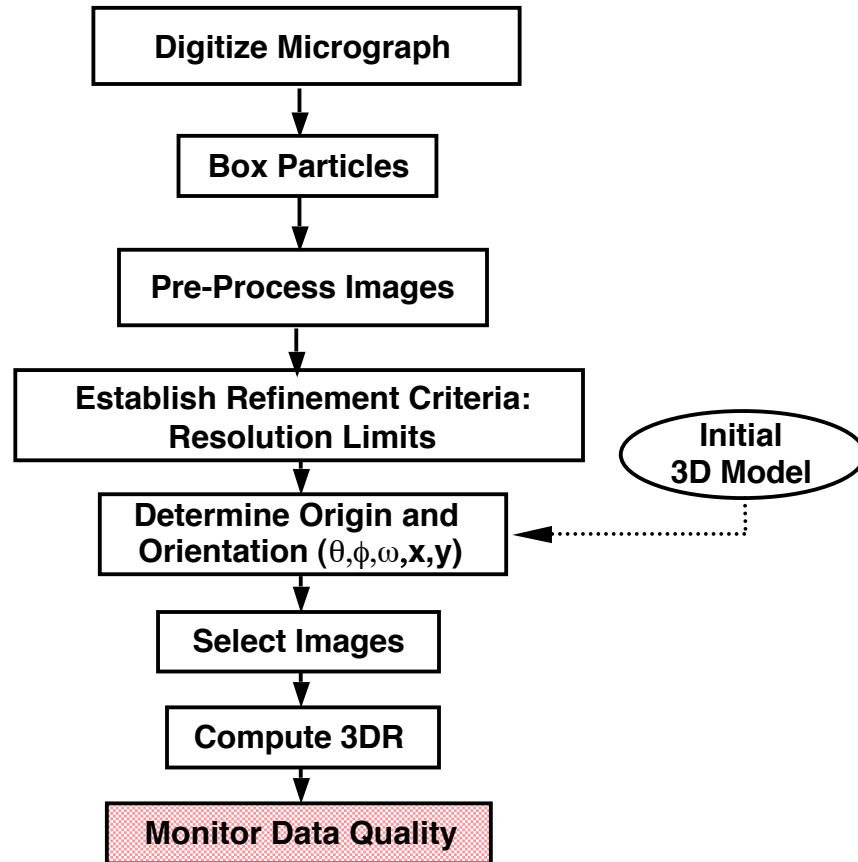
Particle	X	Y	Theta	Phi	Omega	Mag	Distance
20	99.732	99.992	70.000	-1.064	181.813	1.000	0.488
pft_cc[0.560	0.623		0.577			
deltaK[-0.268	-0.408					
state[ON						

Plot Parameters:

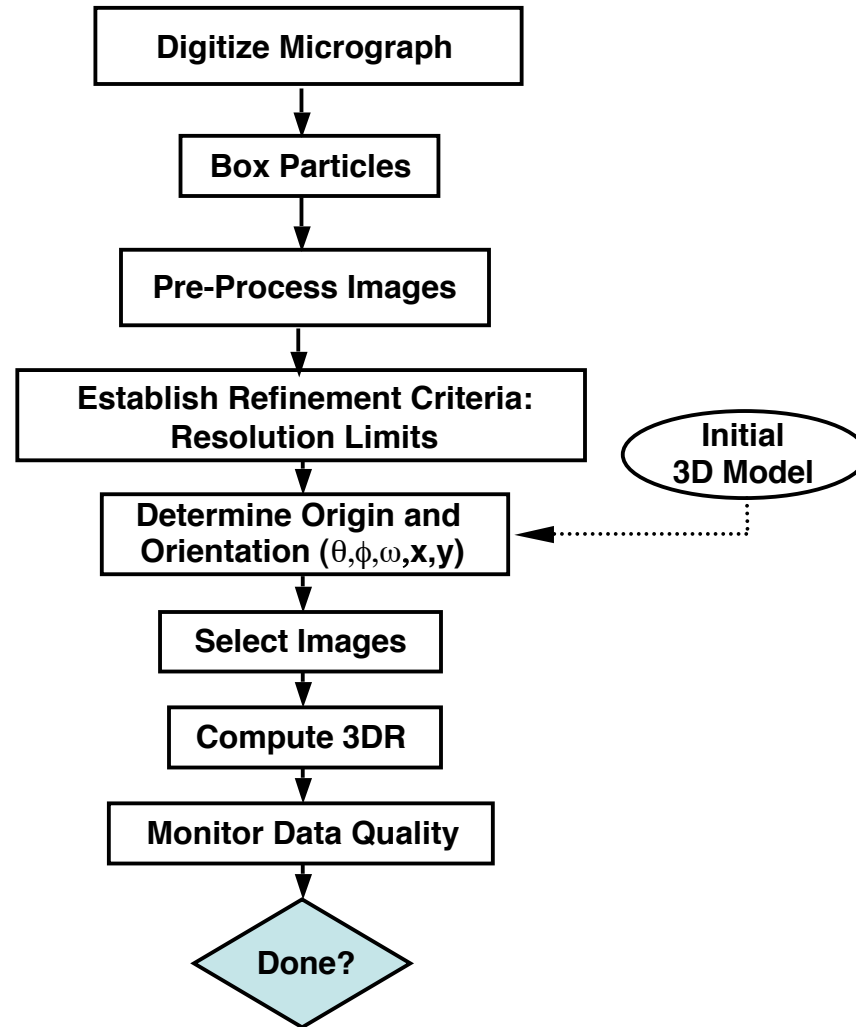
- PixSize: `6.0000` Units: `1`
- Voltage: `100000.0`
- Amp Factor: `0.0700`
- Δf major: `1.6910`
- Δf minor: `1.6910`
- Ang major: `0.0000`
- Cs Coef: `2.00`

Plot Description: The plot shows a distribution of particles (red 'x' marks) within a triangular region. A blue dashed line outlines the triangle. A red arrow points to a specific particle at the top vertex. Two icosahedral virus models are shown in the top right corner: a blue one and a red one. The plot axes are labeled with values: 69, 90, -31, 0, 31.

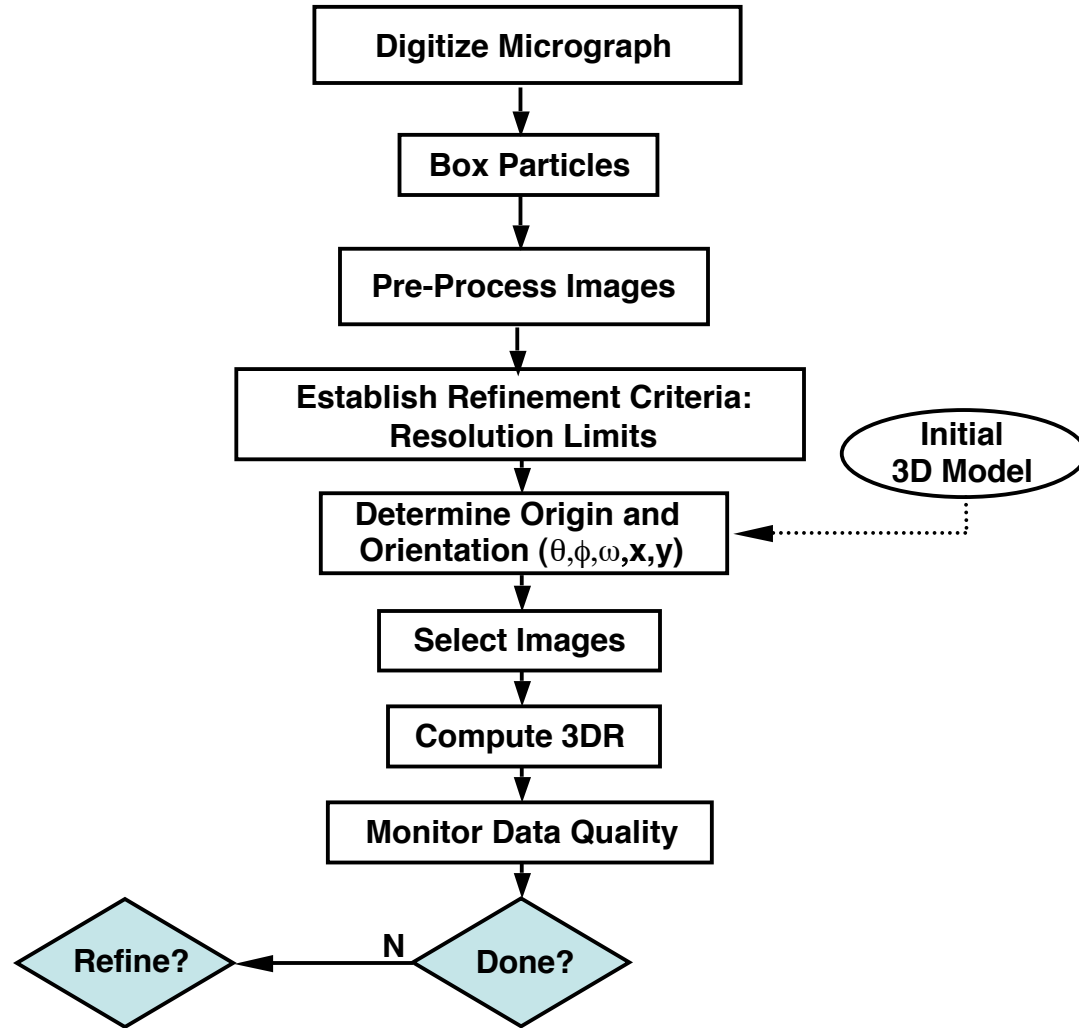
Icosahedral Virus 3D Reconstruction Scheme



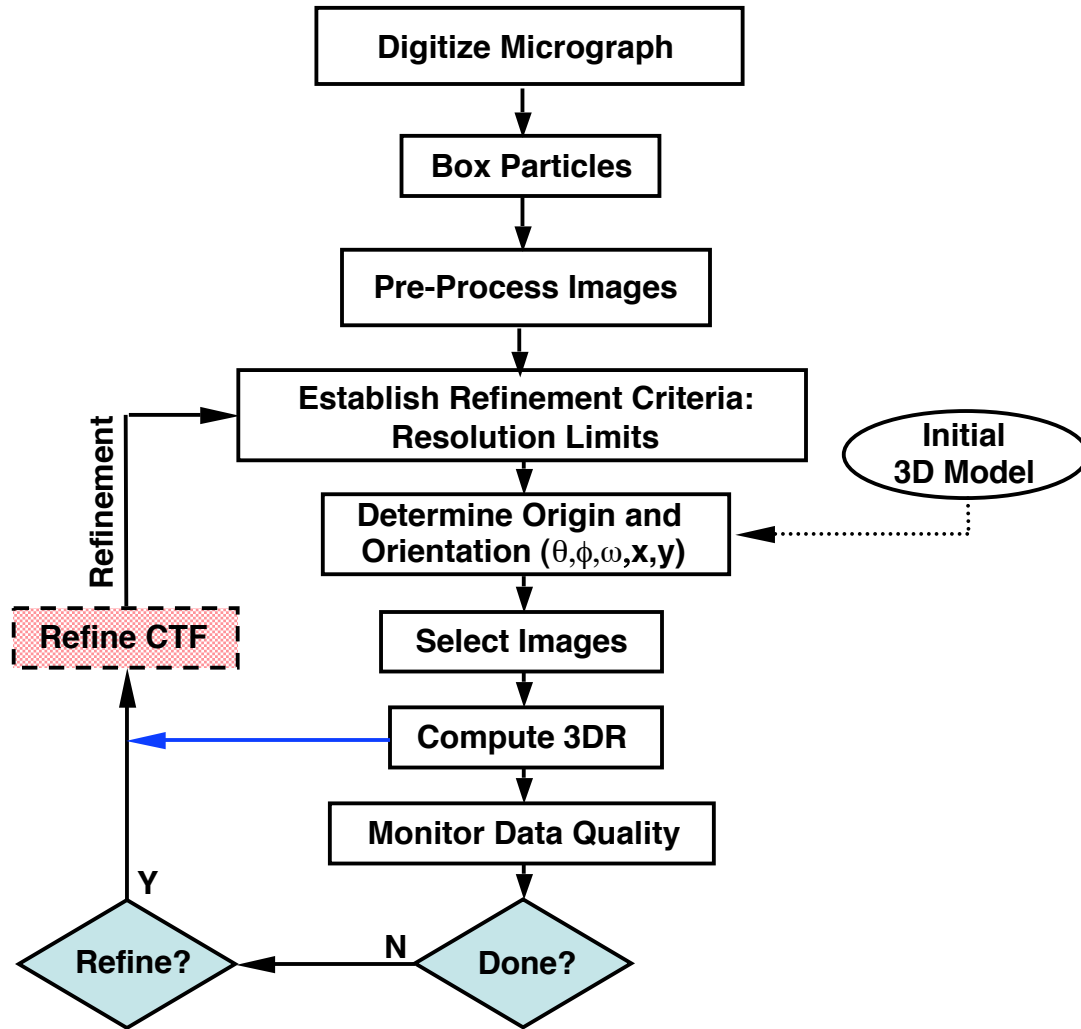
Icosahedral Virus 3D Reconstruction Scheme



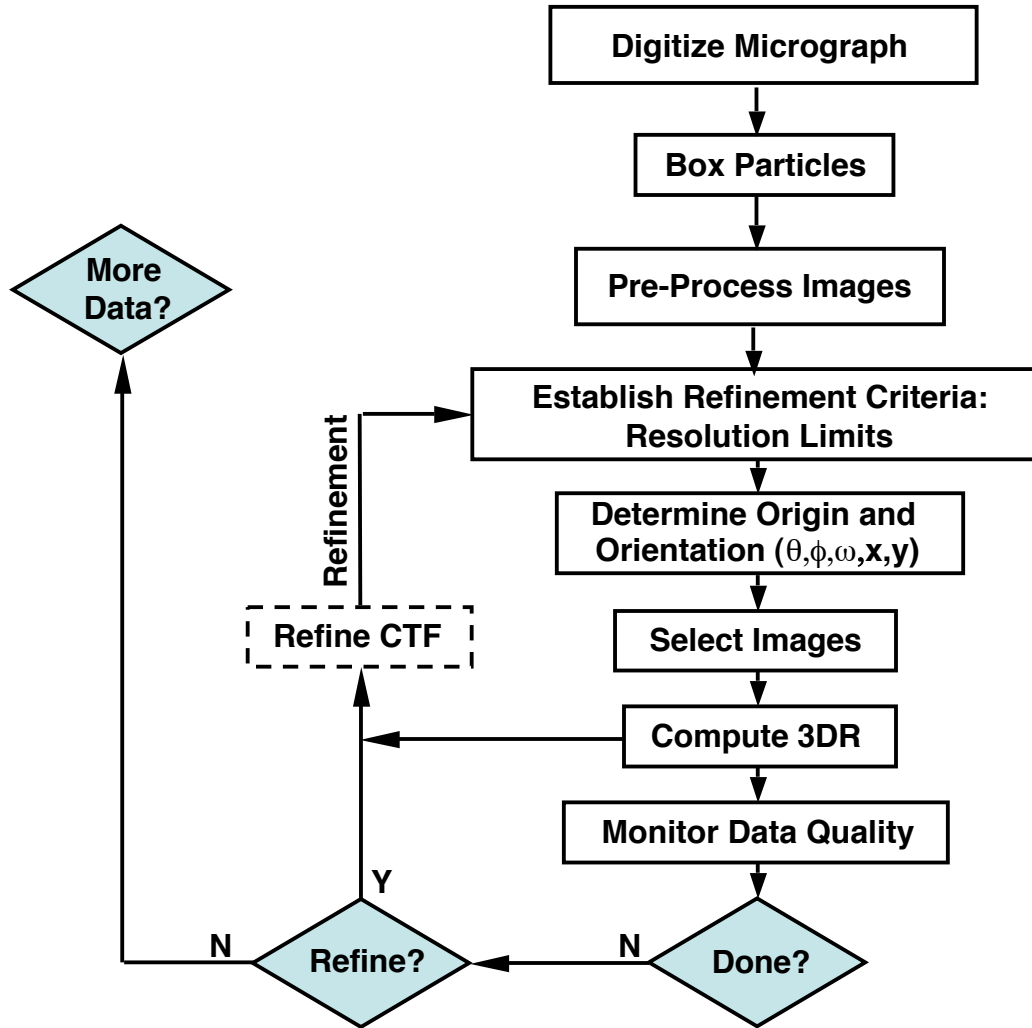
Icosahedral Virus 3D Reconstruction Scheme



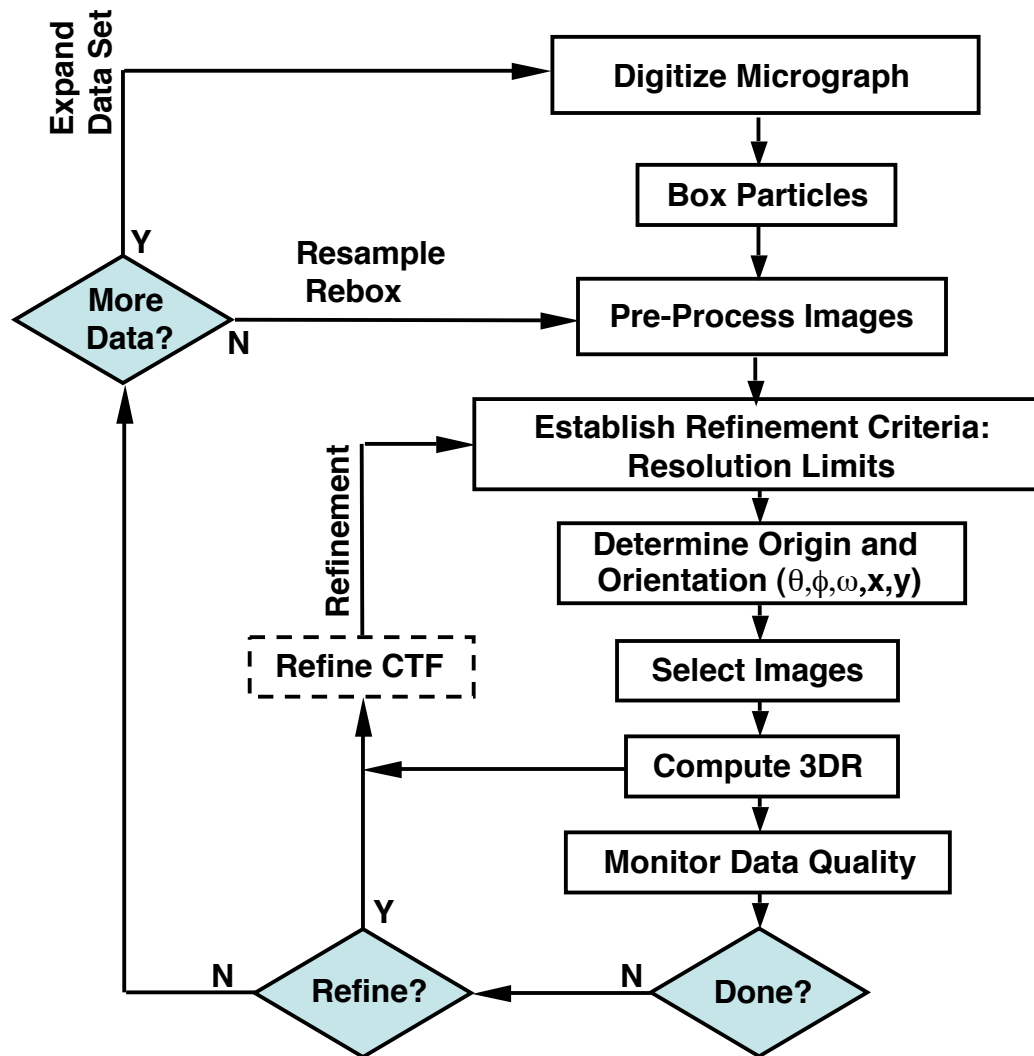
Icosahedral Virus 3D Reconstruction Scheme



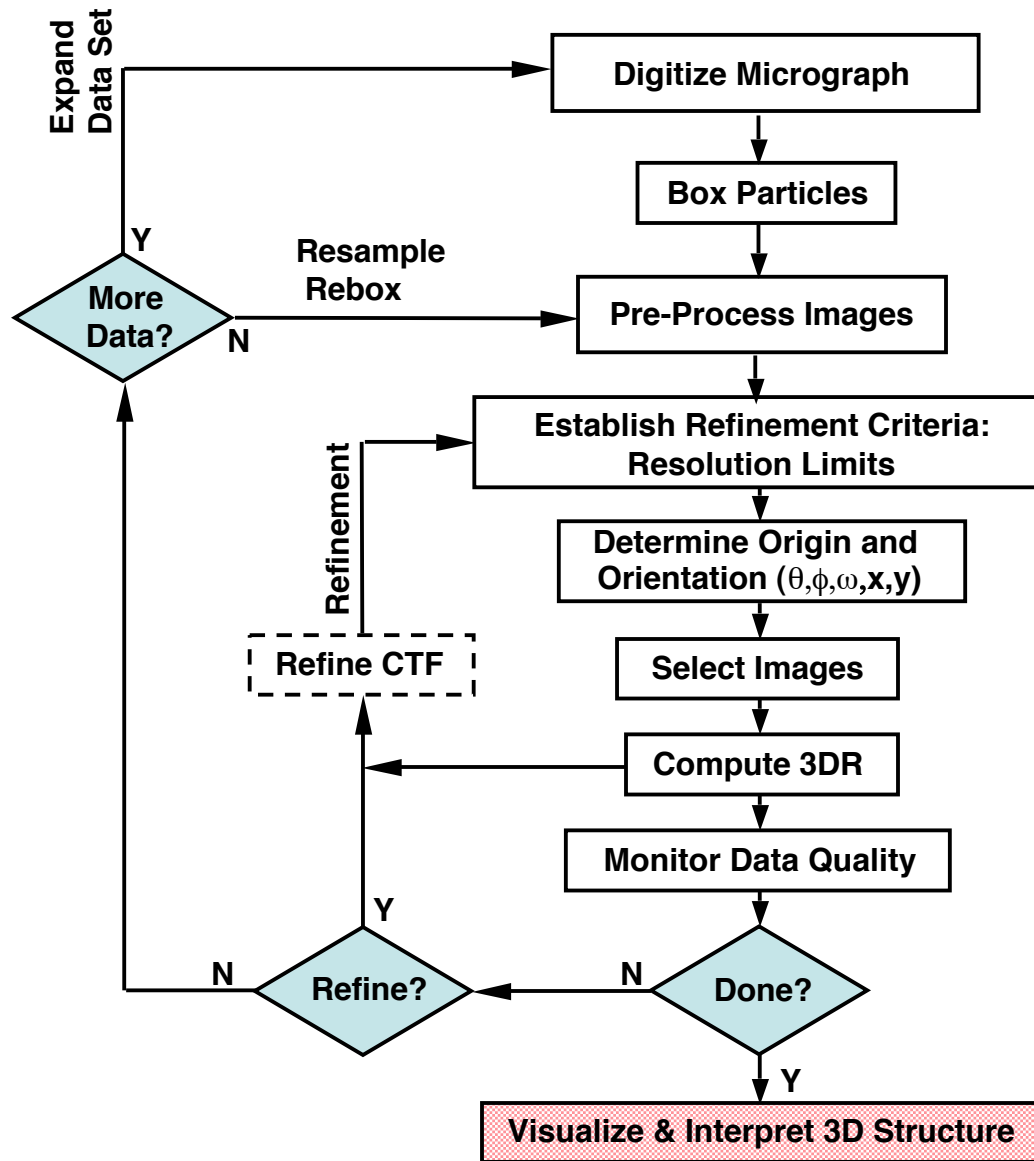
Icosahedral Virus 3D Reconstruction Scheme



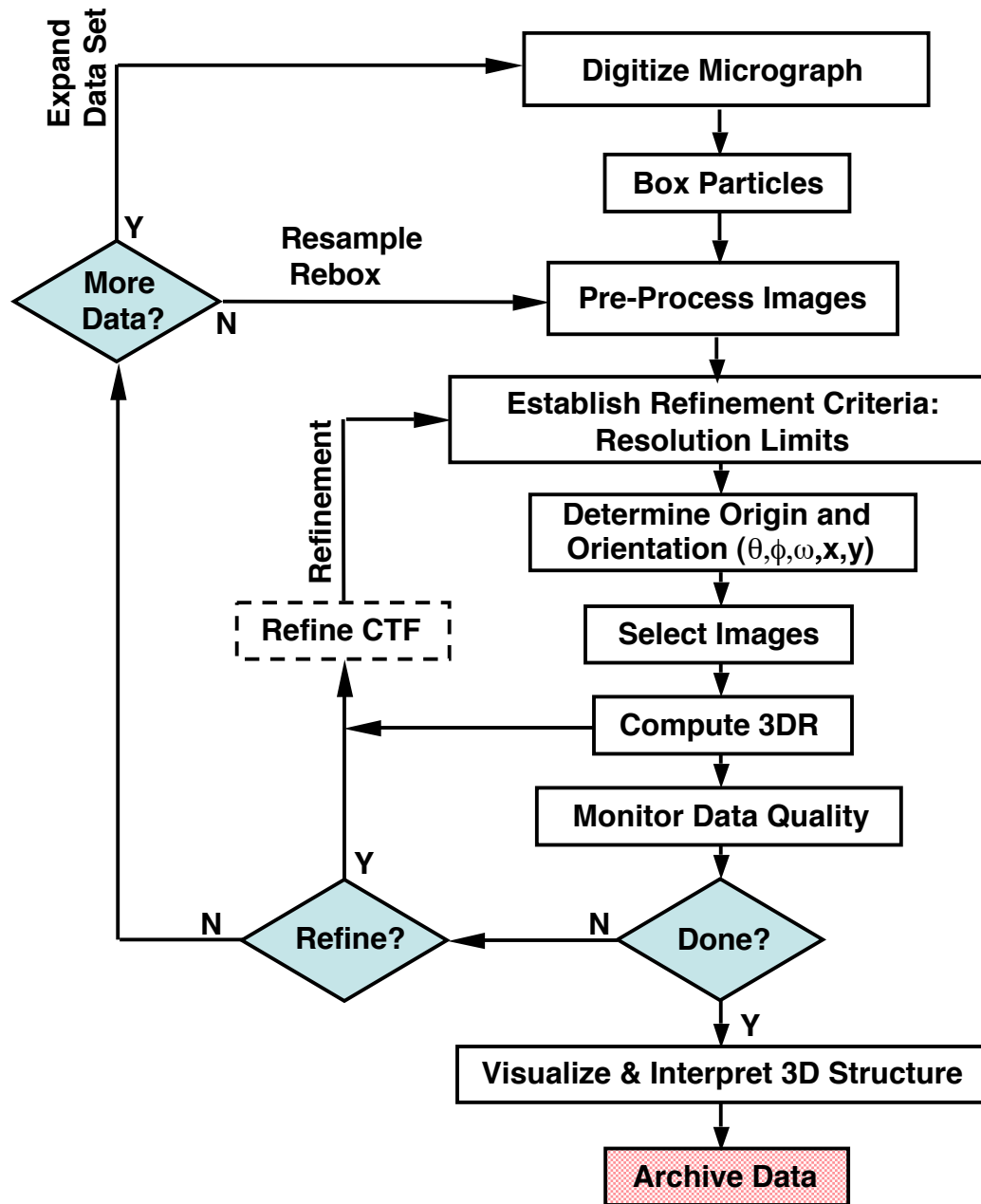
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



3D Reconstruction of Icosahedral Particles

Outline

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

3D Reconstruction of Icosahedral Particles

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

