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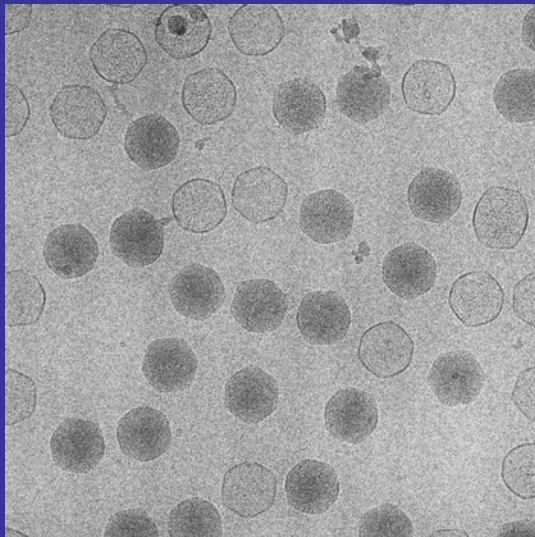
Berkeley, CA 94720-3206

Tel: (510) 642-2905

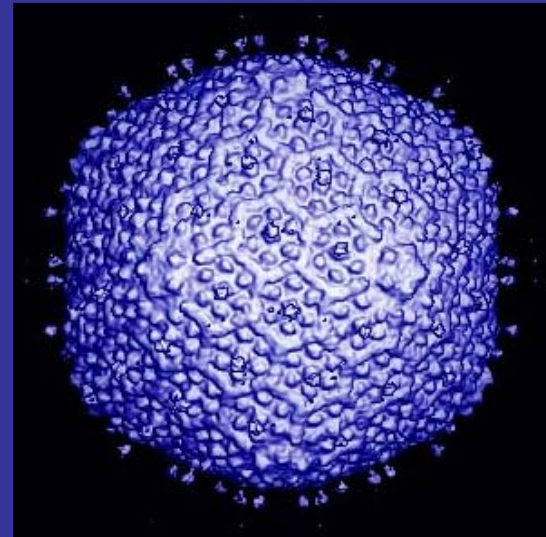
Fax: (510) 486-6488

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# 3D Reconstruction of Icosahedral Particles



2D



3D

# 3D Reconstruction of Icosahedral Particles

## Outline

### - Background

- References; examples; etc.

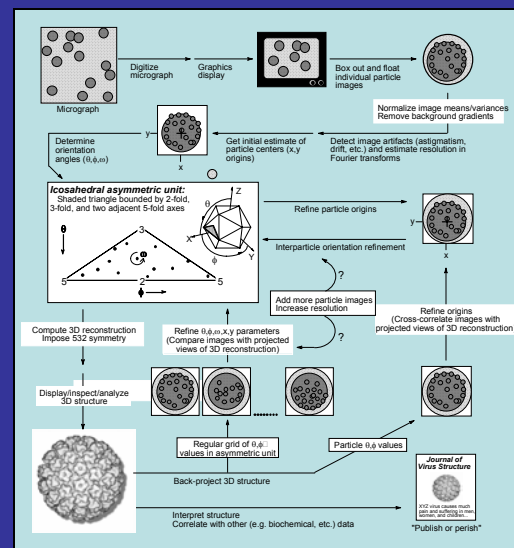
### - Symmetry

- Icosahedral (532) point group symmetry
- Triangulation symmetry

### - “Typical” procedure (flow chart)

- Digitization and boxing
- Image preprocessing / CTF estimation
- Initial particle orientation/origin search
- Orientation/origin refinement
- 3D reconstruction with CTF corrections
- Validation (resolution assessment)

### - Current and future strategies



# 3D Reconstruction of Icosahedral Particles

## REFERENCES

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

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

- Human wart virus
- Tomato bushy stunt

# 3D Reconstruction of Icosahedral Particles

## REFERENCES

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

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

General principles of 3DR method

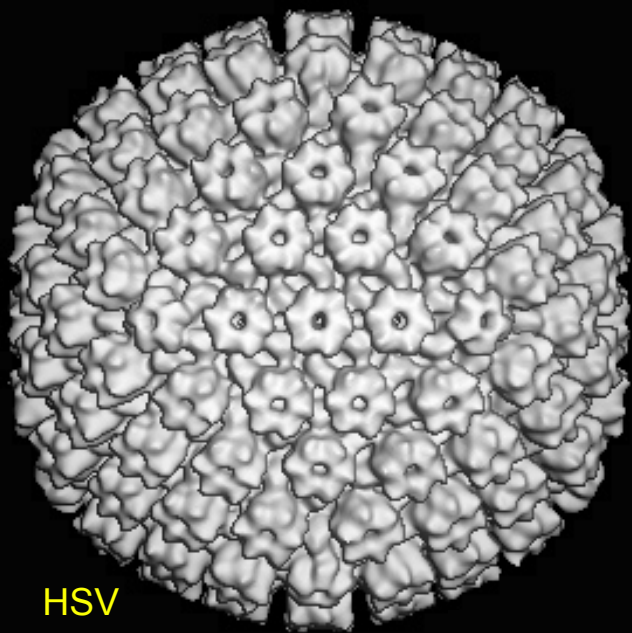
- Fourier-Bessel mathematics
- Common lines

# 3D Reconstruction of Icosahedral Particles

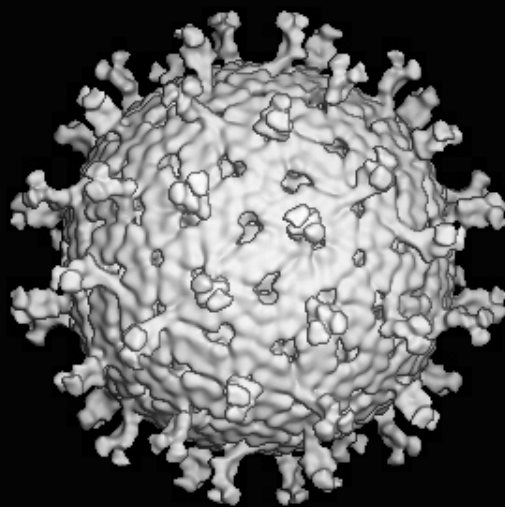
## REFERENCES

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

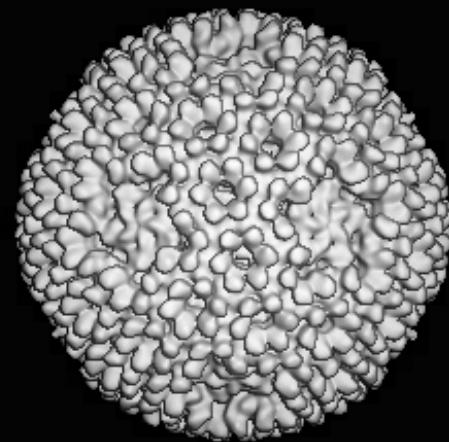
Baker, T. S., N. H. Olson, and S. D. Fuller (1999) Adding the third dimension to virus life cycles: Three-Dimensional reconstruction of icosahedral viruses from cryo-electron micrographs. *Microbiol. Molec. Biol. Reviews* 63:862-922



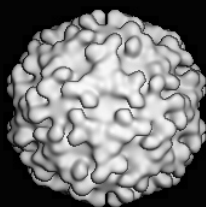
HSV



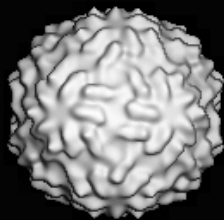
Rotavirus



Reovirus



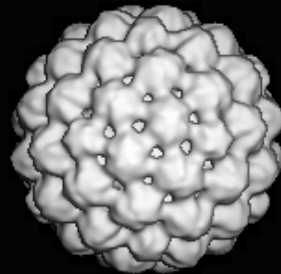
N<sub>CoV</sub>



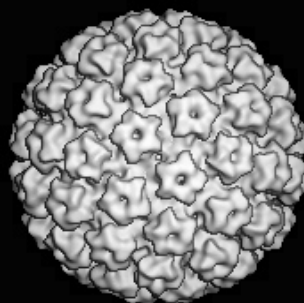
LA-1



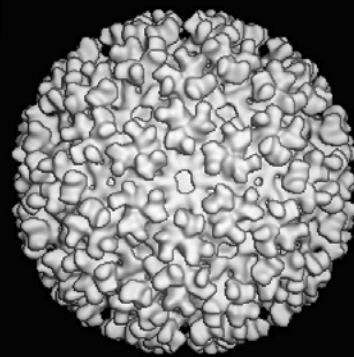
Polyoma



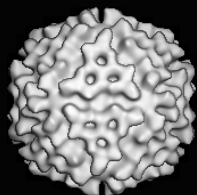
CaMV



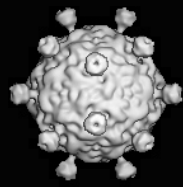
HPV



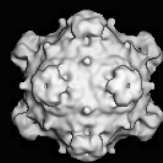
Ross River



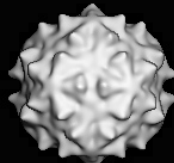
N $\beta$ V



SpV-4



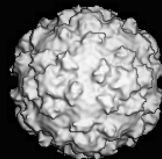
$\phi$ X174



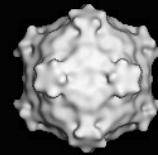
FHV



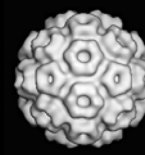
HRV-14



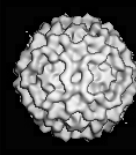
Polio



CPMV



CCMV



B19

500 Å

# 3D Reconstruction of Icosahedral Particles

## Outline

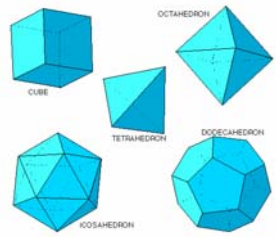
- Background
  - References; examples; etc.
- **Symmetry**
  - Icosahedral (532) point group symmetry
  - Triangulation symmetry
- “Typical” procedure (flow chart)
  - Digitization and boxing
  - Image preprocessing / CTF estimation
  - Initial particle orientation/origin search
  - Orientation/origin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

## Symmetry

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



# Regular Polyhedra (Platonic Solids)

There are just five platonic solids:

From **equilateral triangles** you can make:

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

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

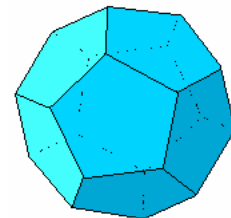
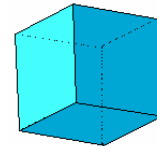
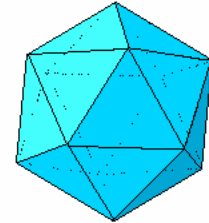
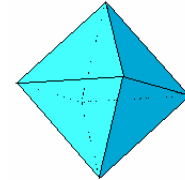
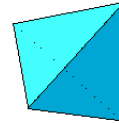
with 5 faces at each vertex, an **icosahedron**

From **squares** you can make:

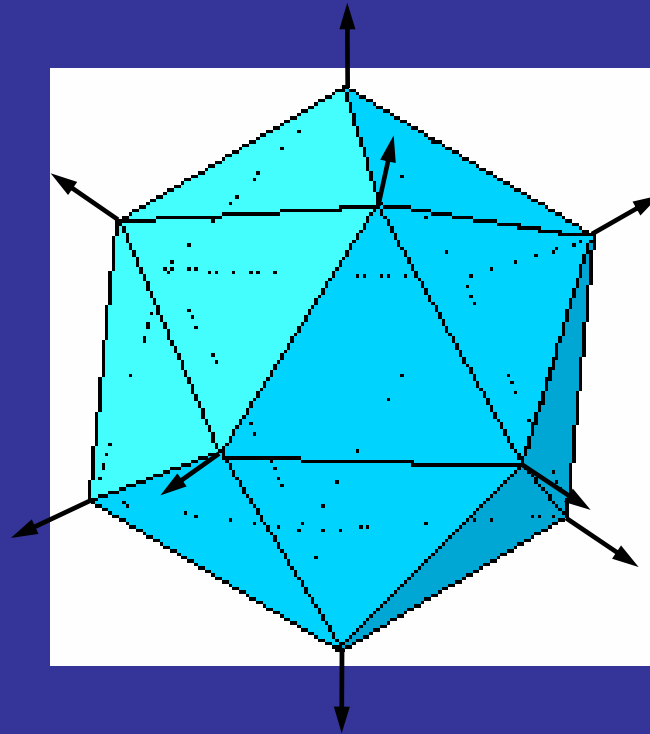
with 3 faces at each vertex, a **cube**

From **pentagons** you can make:

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

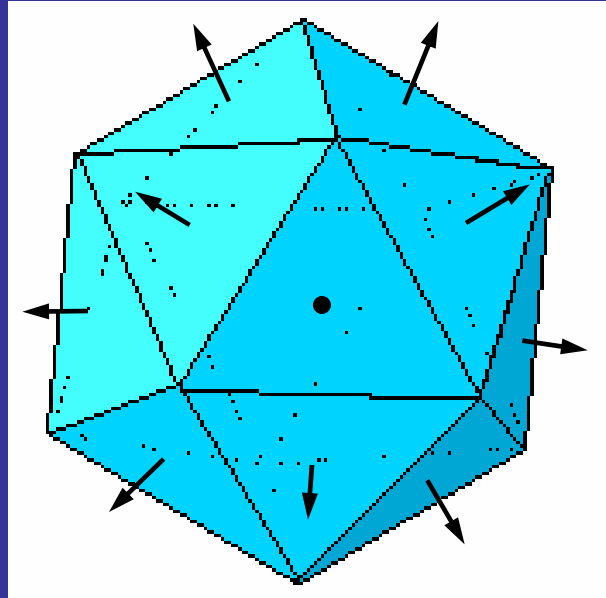


# Icosahedral ( $532$ ) Point Group Symmetry



12 vertices (5-fold)

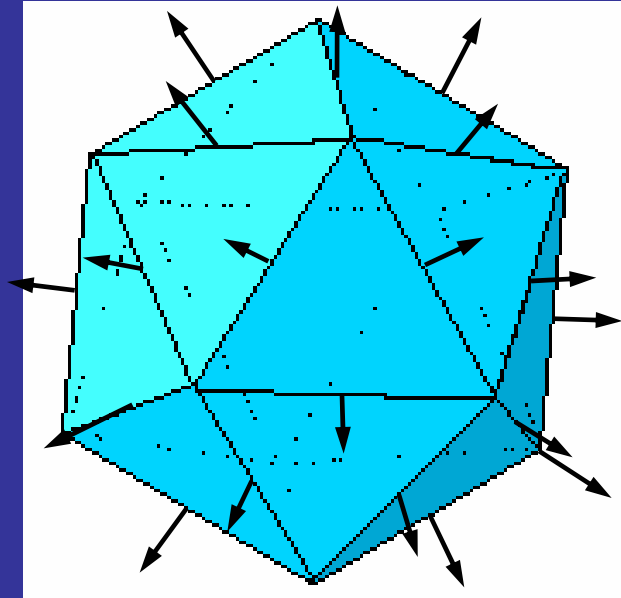
# Icosahedral (532) Point Group Symmetry



12 vertices (5-fold)

20 faces (3-fold)

# Icosahedral (532) Point Group Symmetry

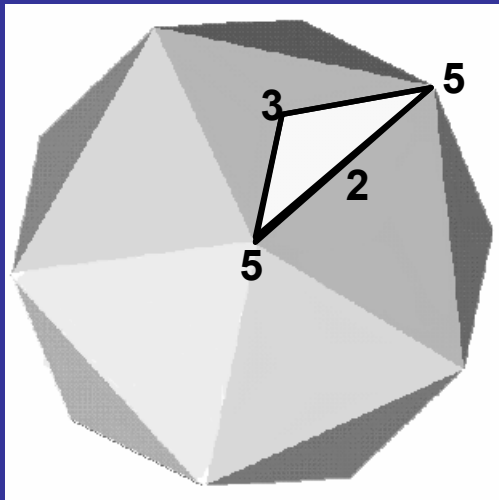


12 vertices (5-fold)

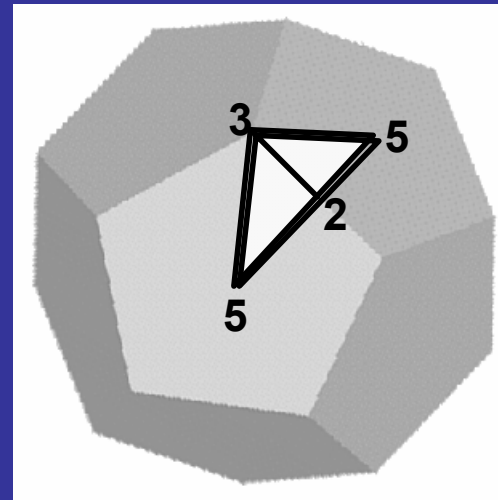
20 faces (3-fold)

30 edges (2-fold)

# Icosahedron



# Dodecahedron



Different shapes, but both have  $532$  symmetry

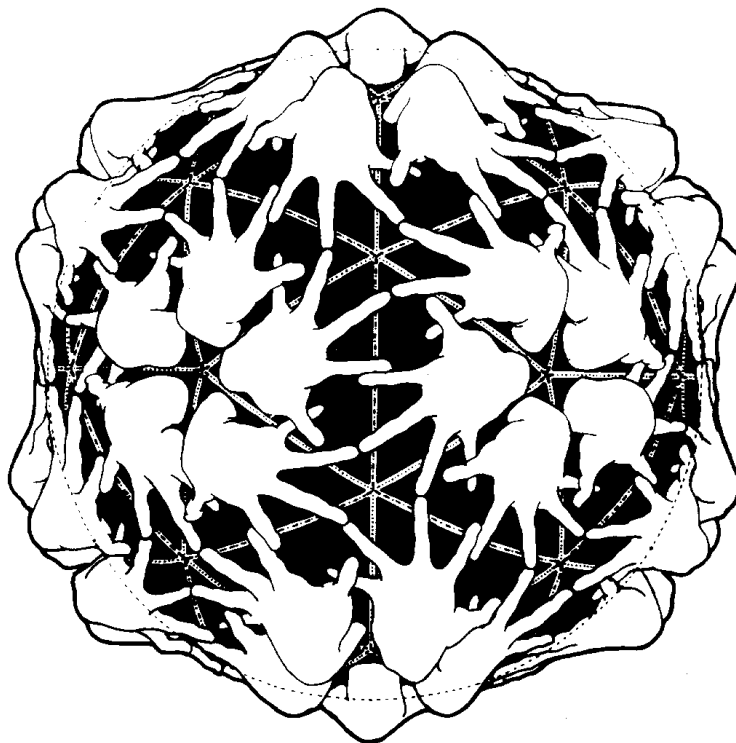
12 vertices, 20 faces, 30 edges  
(6 5-folds, 10 3-folds, 15 2-folds)

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

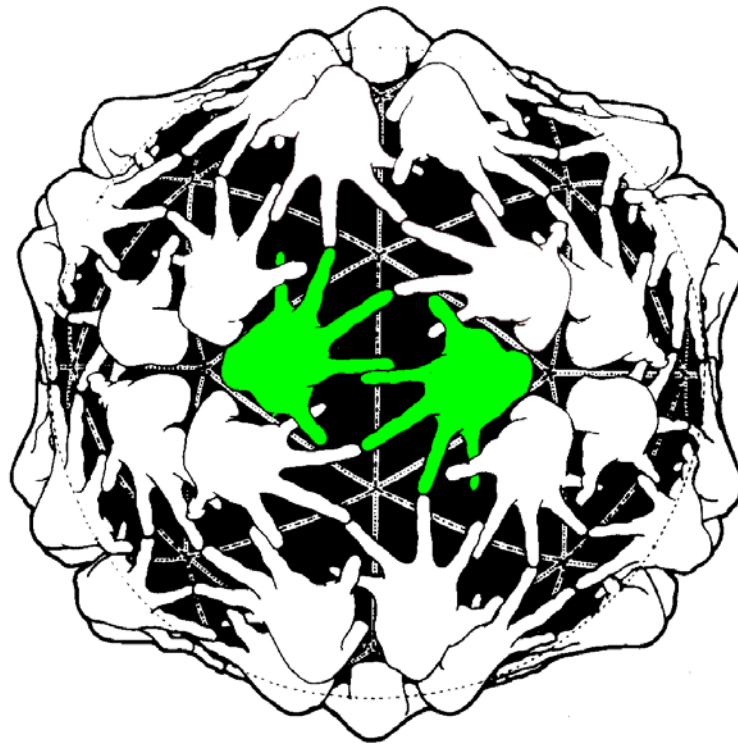
Asymmetric unit is  $1/60^{\text{th}}$  of whole object

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

# Icosahedral ( $532$ ) Point Group Symmetry



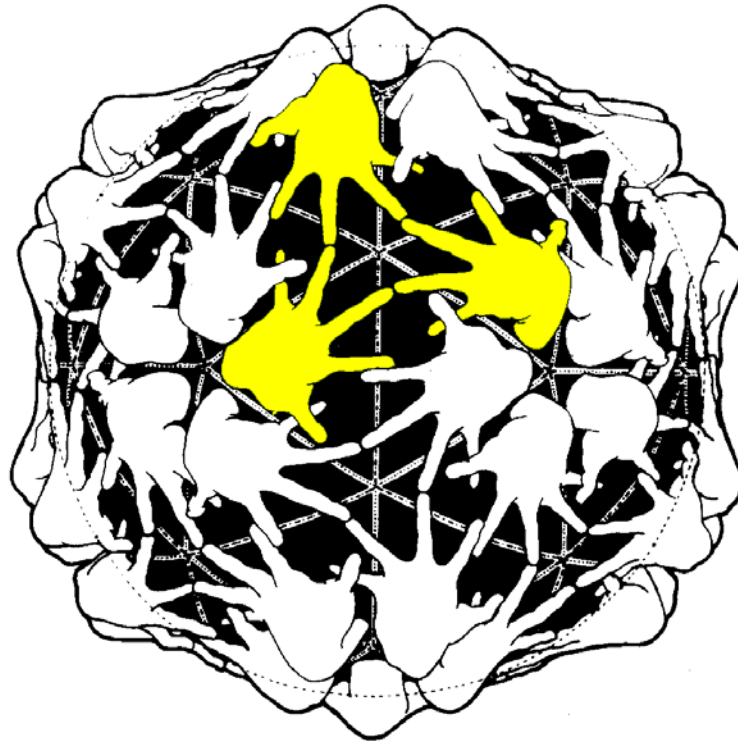
# Icosahedral (532) Point Group Symmetry



30 dimers

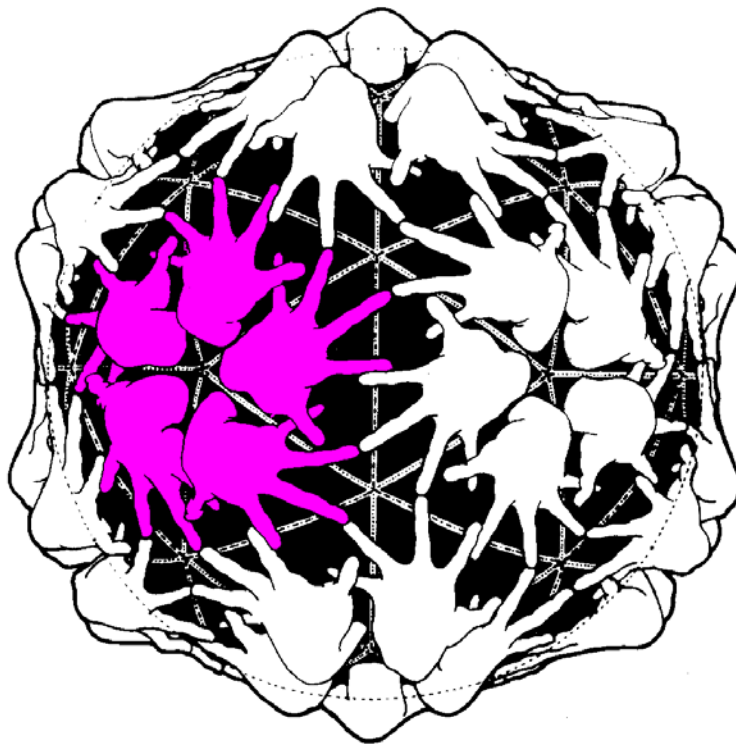


# Icosahedral (532) Point Group Symmetry



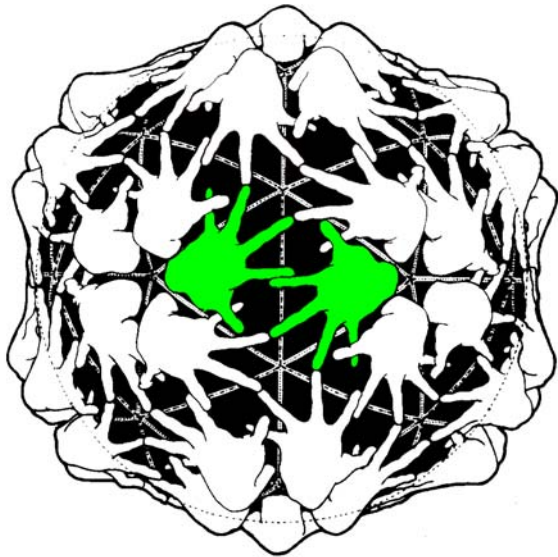
20 trimers

# Icosahedral (532) Point Group Symmetry

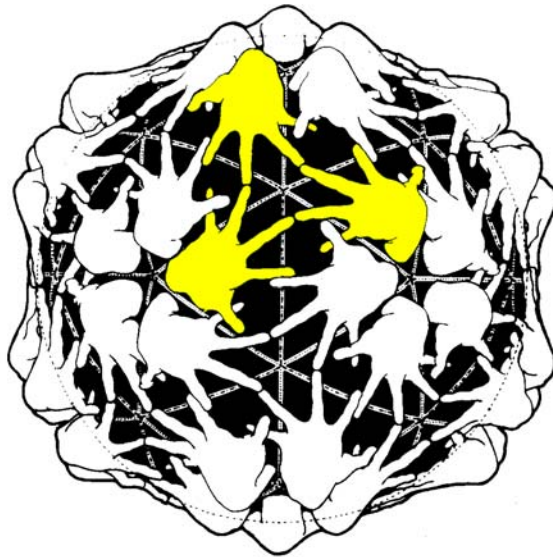


12 pentamers

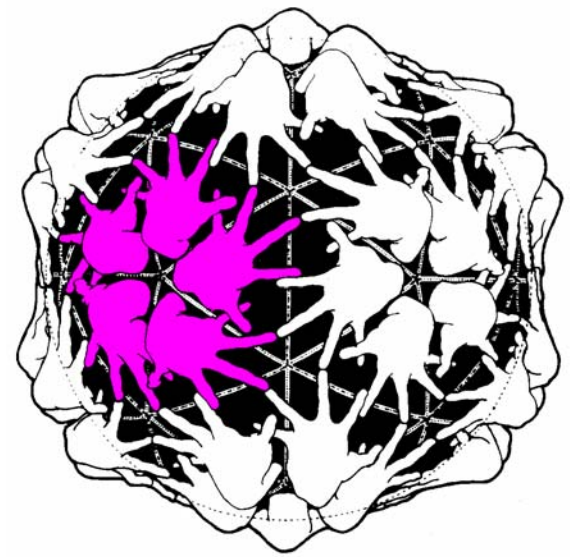
# Icosahedral (532) Point Group Symmetry



30 dimers



20 trimers



12 pentamers

# 3D Reconstruction of Icosahedral Particles

## Symmetry

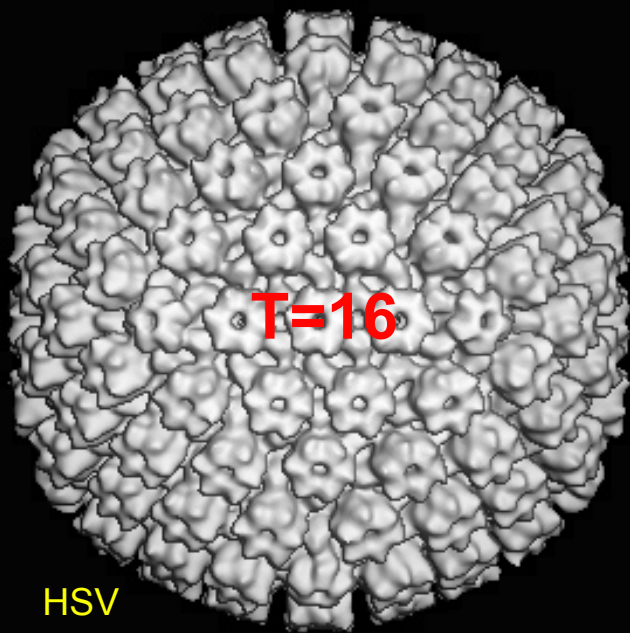
1. Icosahedral (532) point group symmetry

➡ 2. Triangulation symmetry

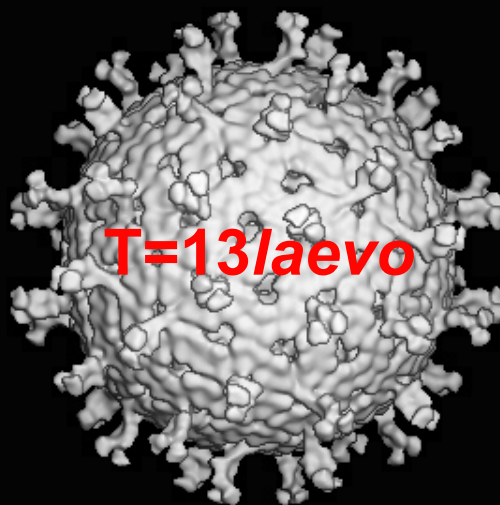
Purely mathematical concept (concerns lattices)

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

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



HSV



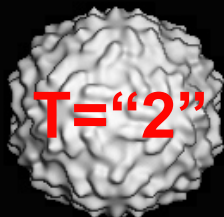
Rotavirus



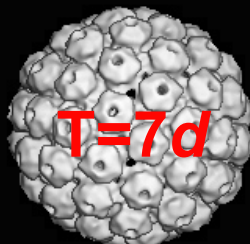
Reovirus



NoV



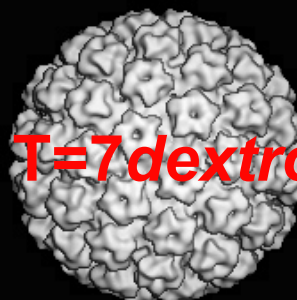
LA-1



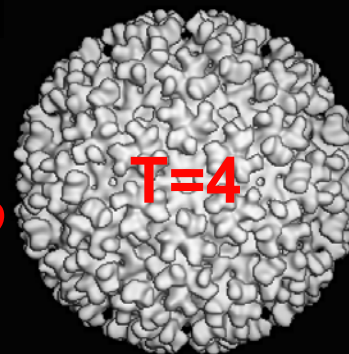
Polyoma



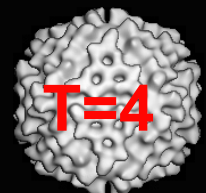
CaMV



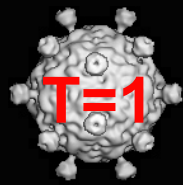
HPV



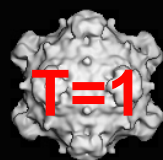
Ross River



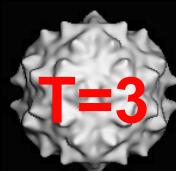
NβV



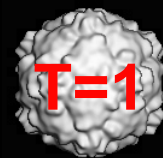
SpV-4



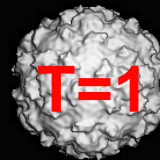
φX174



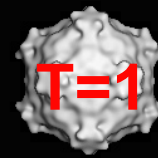
FHV



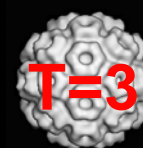
HRV-14



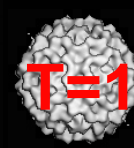
Polio



CPMV

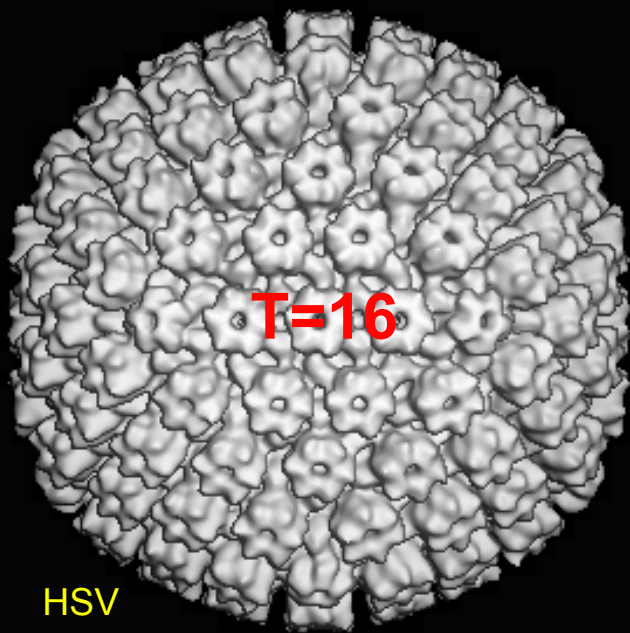


CCMV

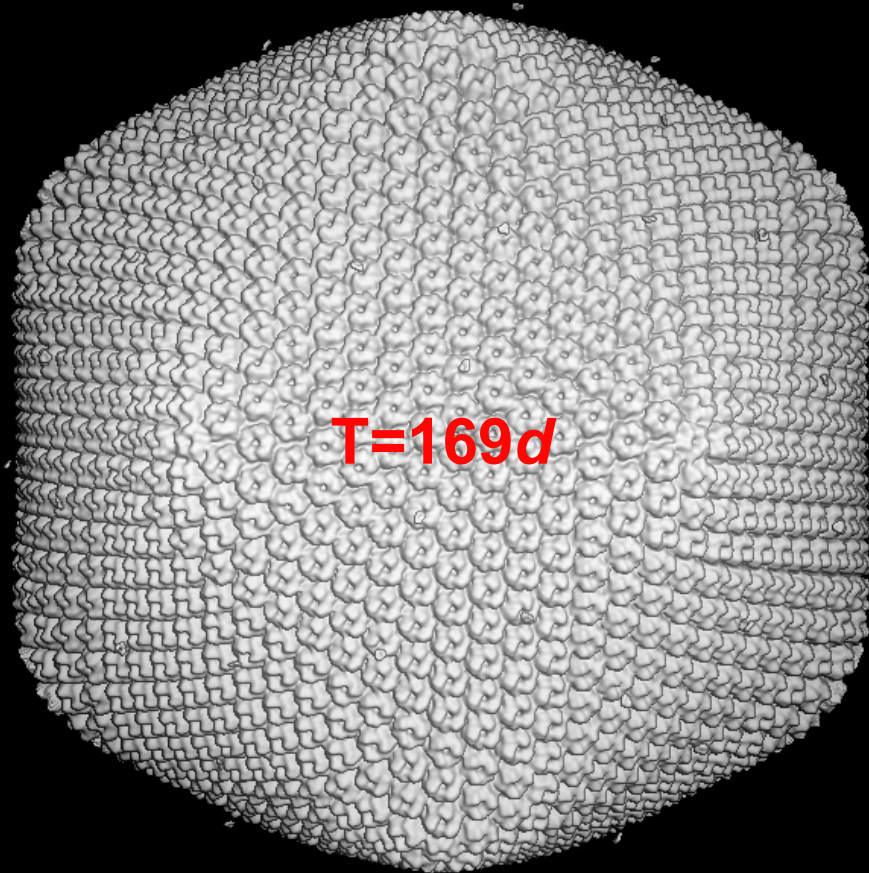


B19

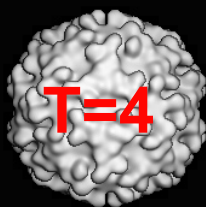
500 Å



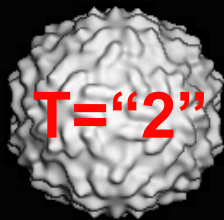
HSV



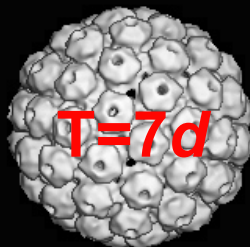
PBCV-1



NoV



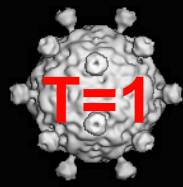
LA-1



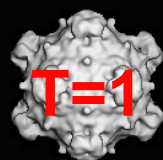
Polyoma



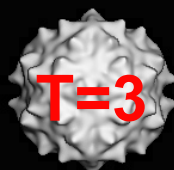
NβV



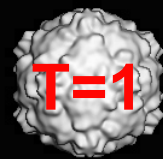
SpV-4



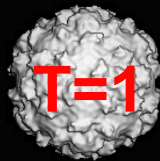
φX174



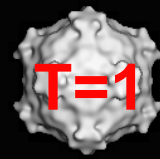
FHV



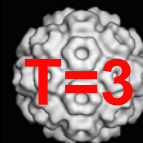
HRV-14



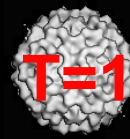
Polio



CPMV



CCMV



B19

500 Å

# 3D Reconstruction of Icosahedral Particles

Triangulation Number

## Key Concept:

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

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

# 3D Reconstruction of Icosahedral Particles

## Two Basic Assumptions:

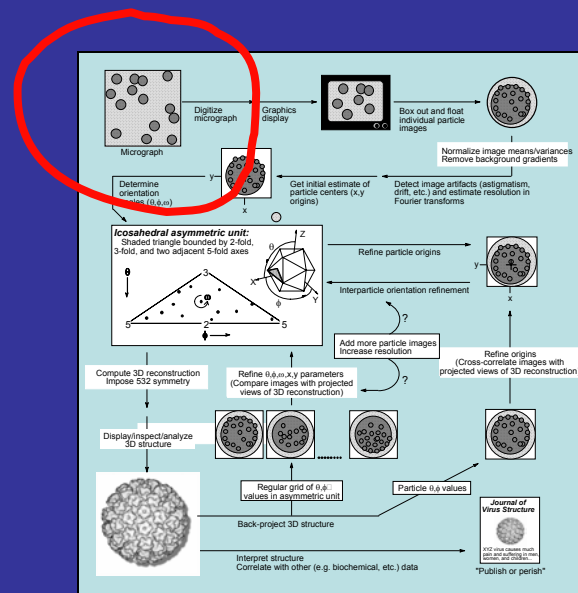
- Specimen consists of stable particles with 'identical' structures (else averaging is invalid)
- Programs test for and *assume* presence of icosahedral (532) symmetry



# 3D Reconstruction of Icosahedral Particles

## Outline

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  - References; examples; etc.
- Symmetry
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  - Triangulation symmetry
- “Typical” procedure (flow chart)
  - Digitization and boxing
  - Image preprocessing / CTF estimation
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  - Orientation/origin refinement
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  - Validation (resolution assessment)
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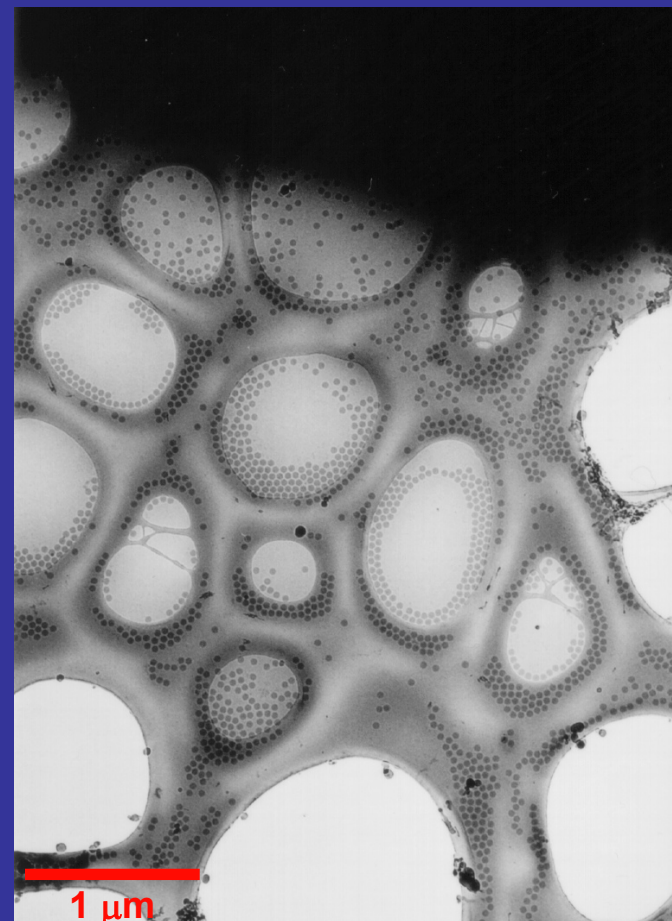
# 3D Reconstruction of Icosahedral Particles

## Protocol

### Electron Cryo-Microscopy

Sample : ~2-3  $\mu\text{l}$  at 1-5 mg/ml

Specimen support: holey carbon film (1-2  $\mu\text{m}$ )



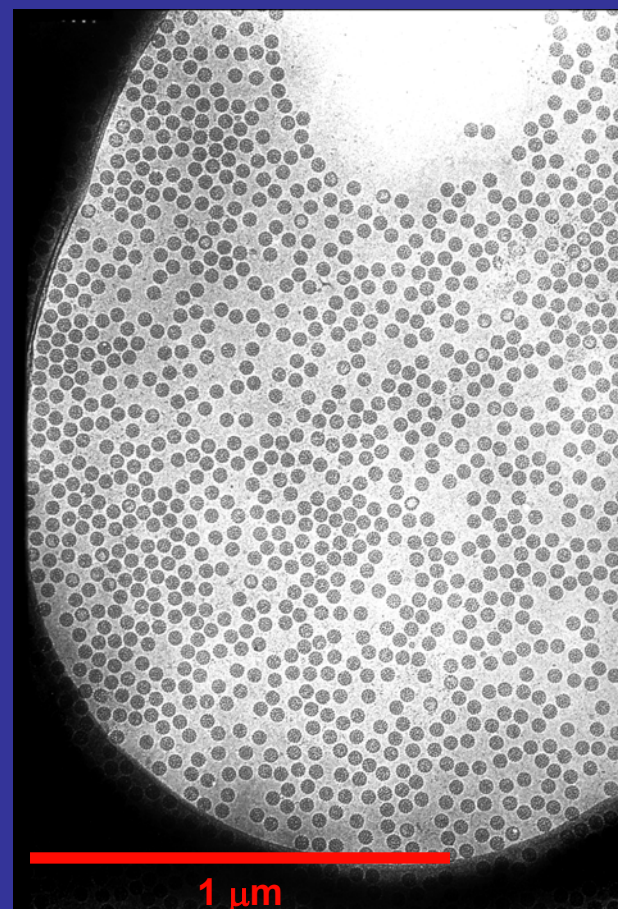
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Specimen support: holey carbon film (1-2  $\mu\text{m}$ )



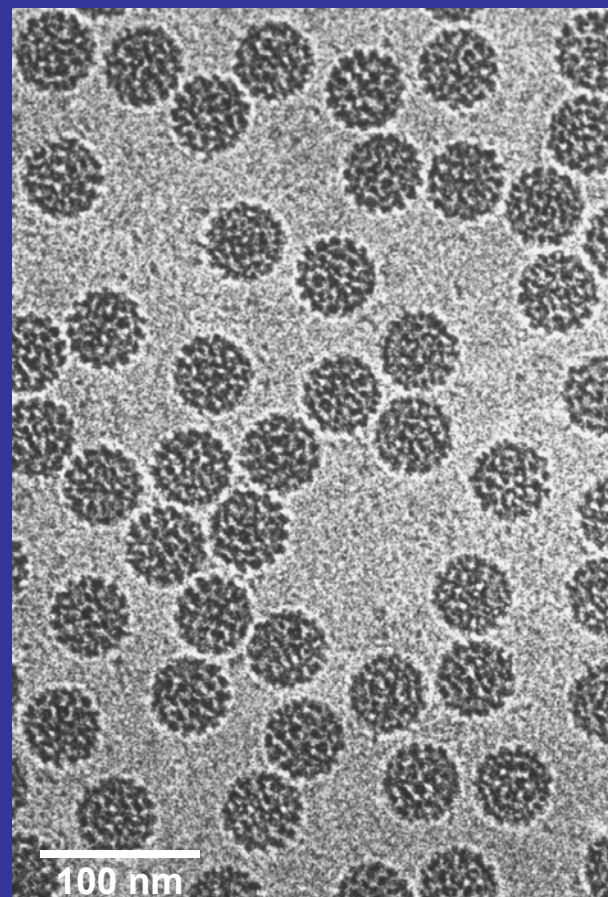
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Specimen support: holey carbon film (1-2  $\mu\text{m}$ )



# 3D Reconstruction of Icosahedral Particles

## Protocol

### Electron Cryo-Microscopy

Sample : ~2-3  $\mu\text{l}$  at 1-5 mg/ml

Specimen support: holey carbon film (1-2  $\mu\text{m}$ )

Microscope: 200-300 keV with FEG

Defocus range: 1-3  $\mu\text{m}$  underfocus

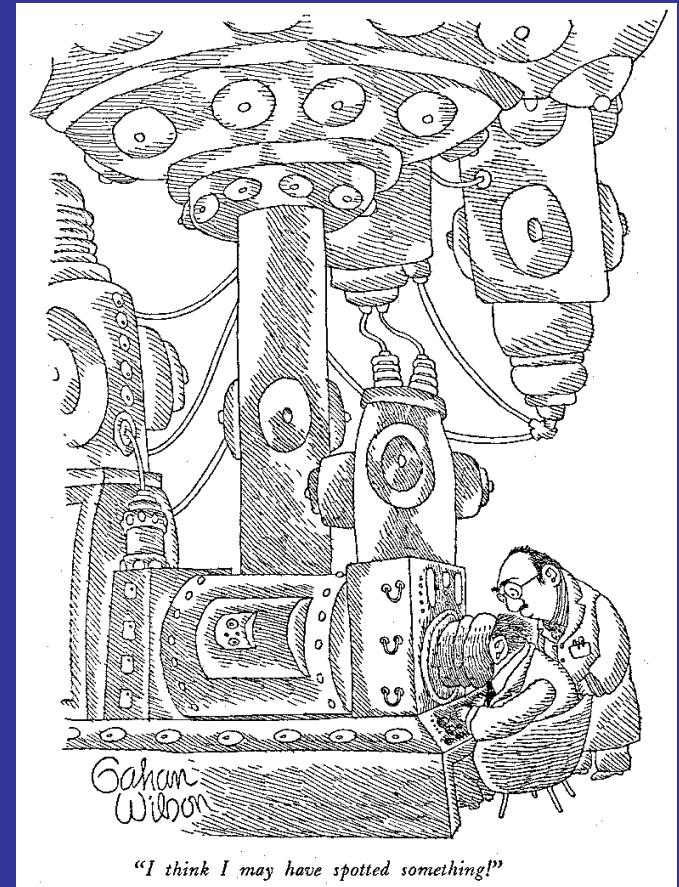
Dose: 10-20  $\text{e}/\text{\AA}^2$

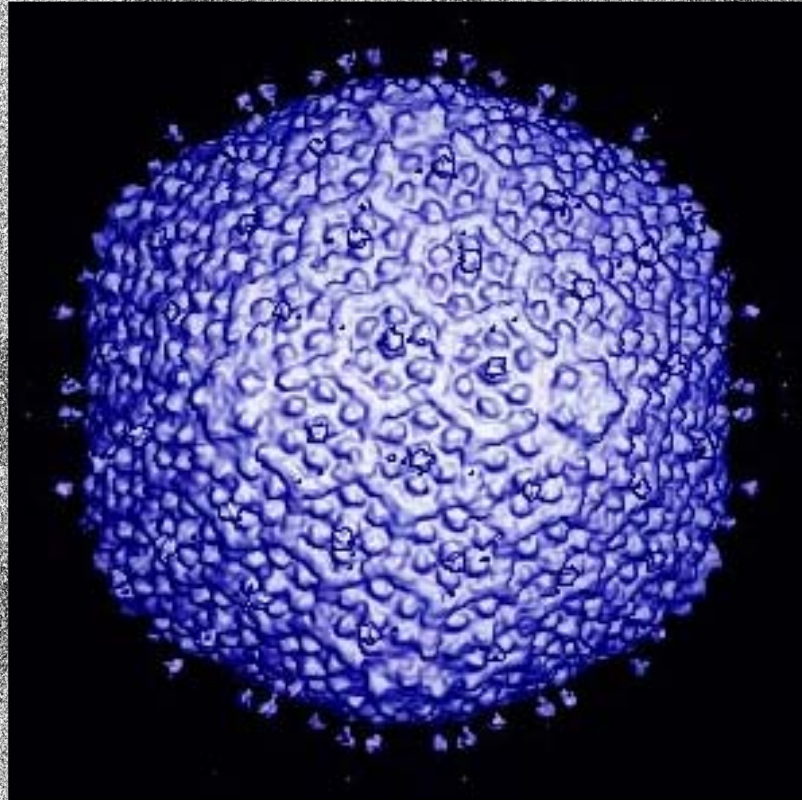
Film: SO-163 (12 min, full strength)

Micrographs: 25-100

Particles:  $10^3$ - $10^4$

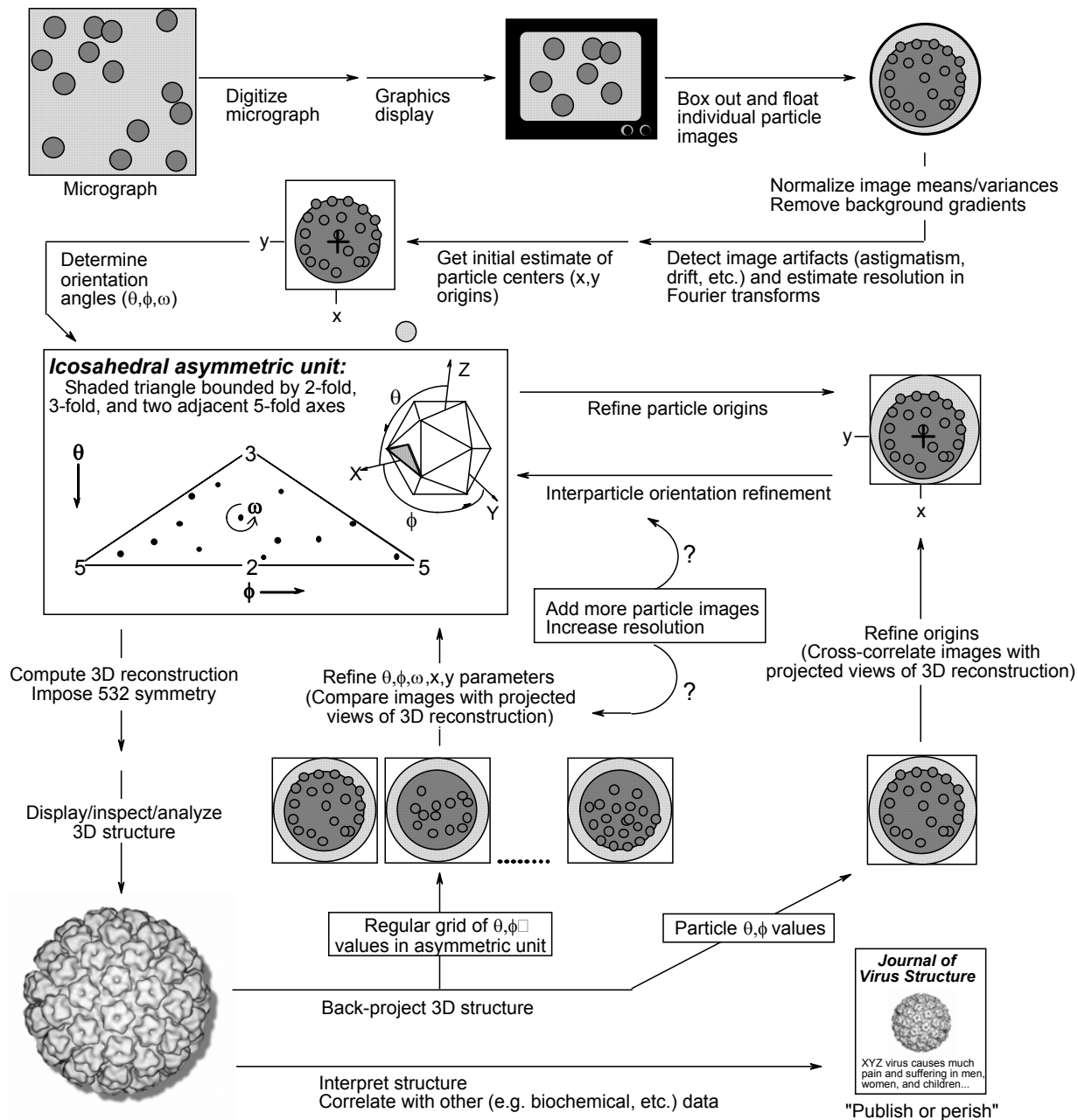
Target resolution: 12 - 6  $\text{\AA}$



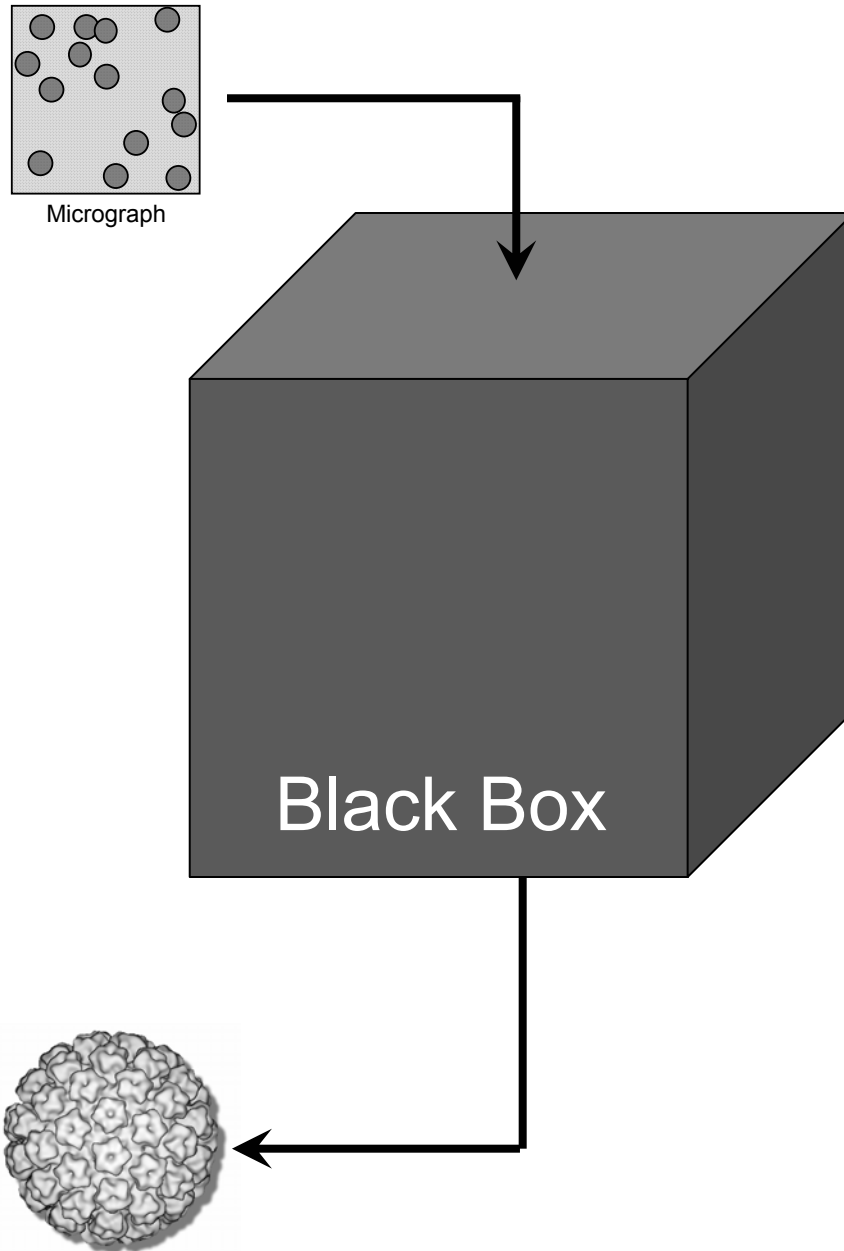


100 nm

# Icosahedral Particle Image Reconstruction Scheme



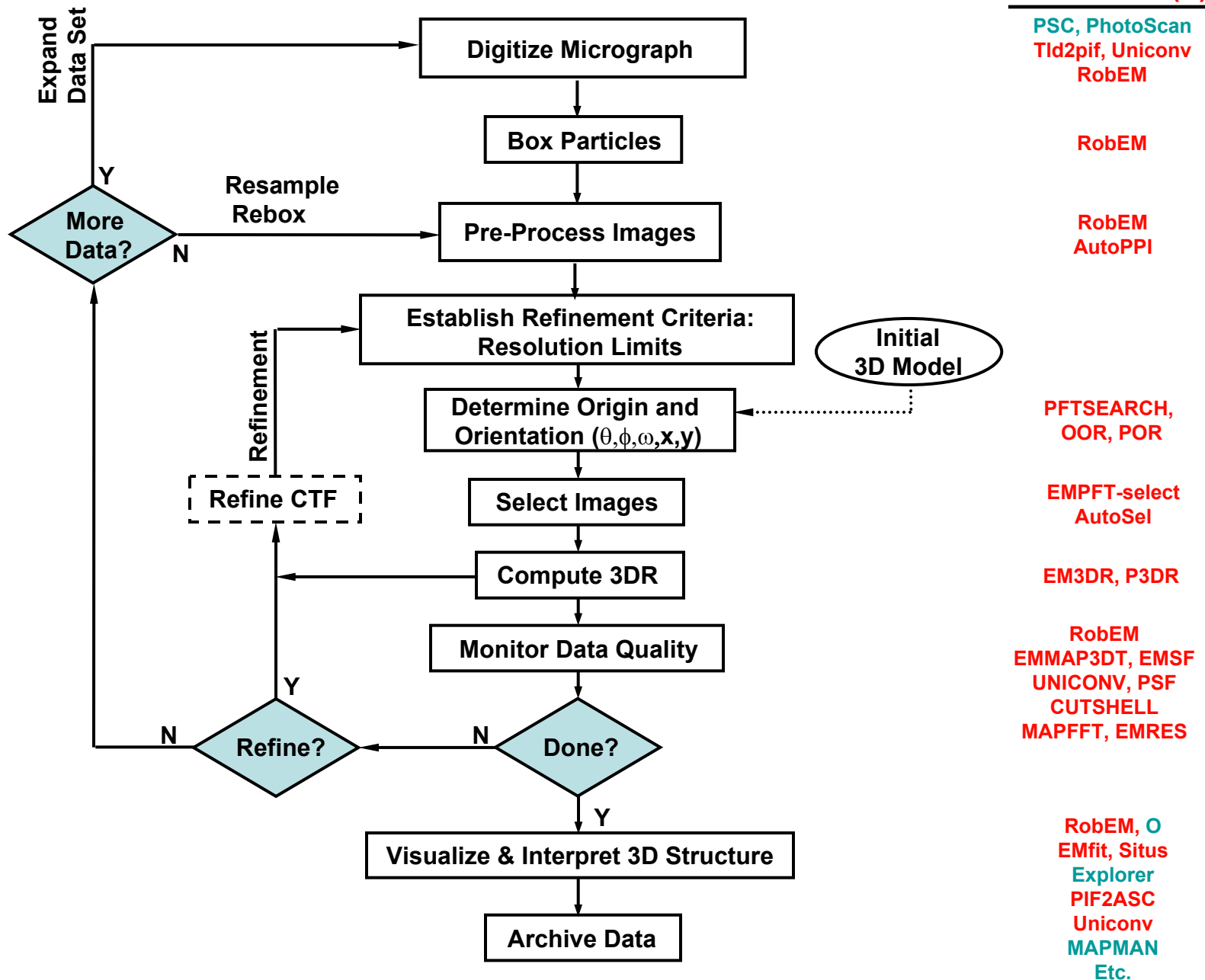
# Icosahedral Particle Image Reconstruction Scheme



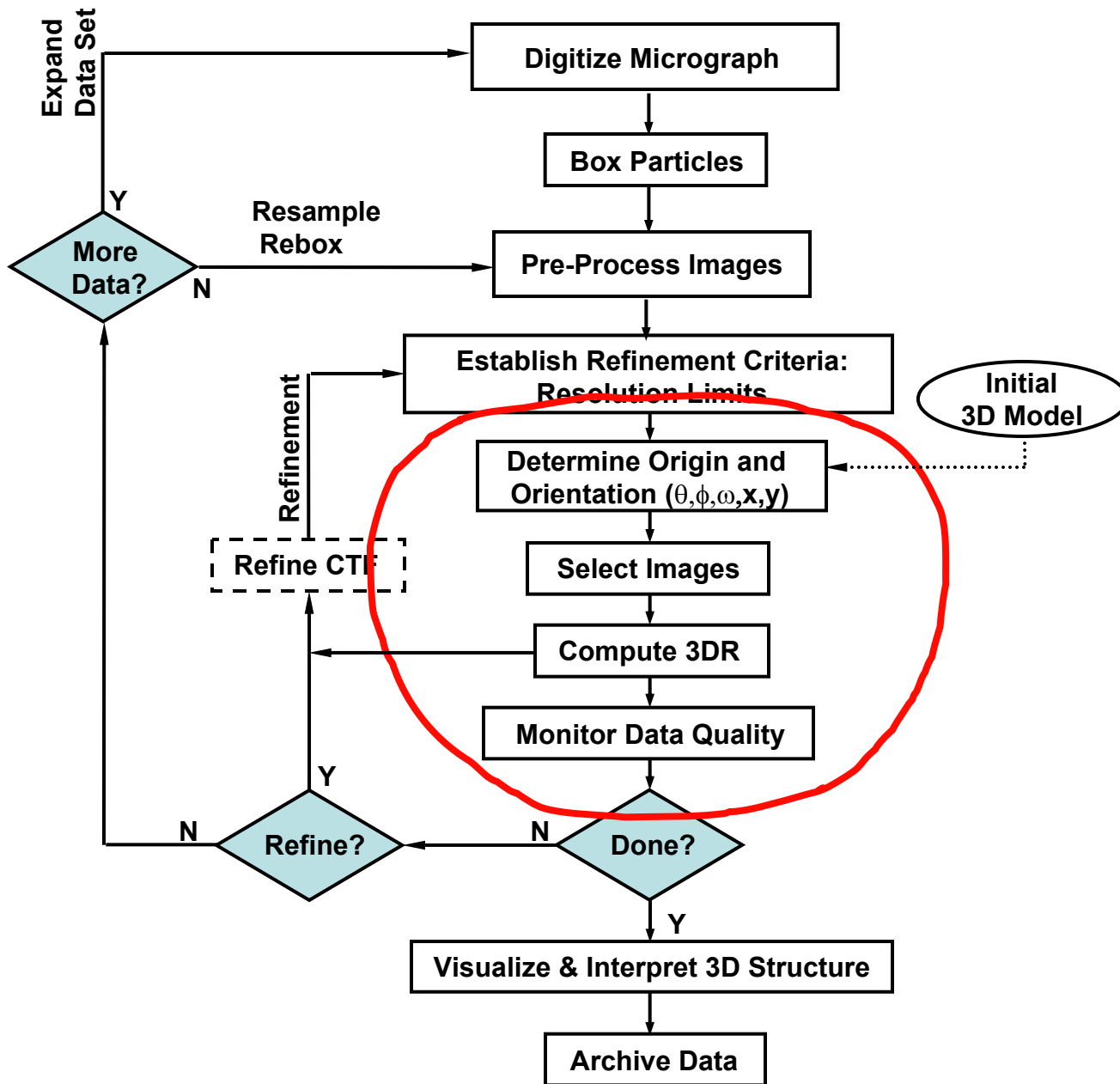


# Icosahedral Virus 3D Reconstruction Scheme

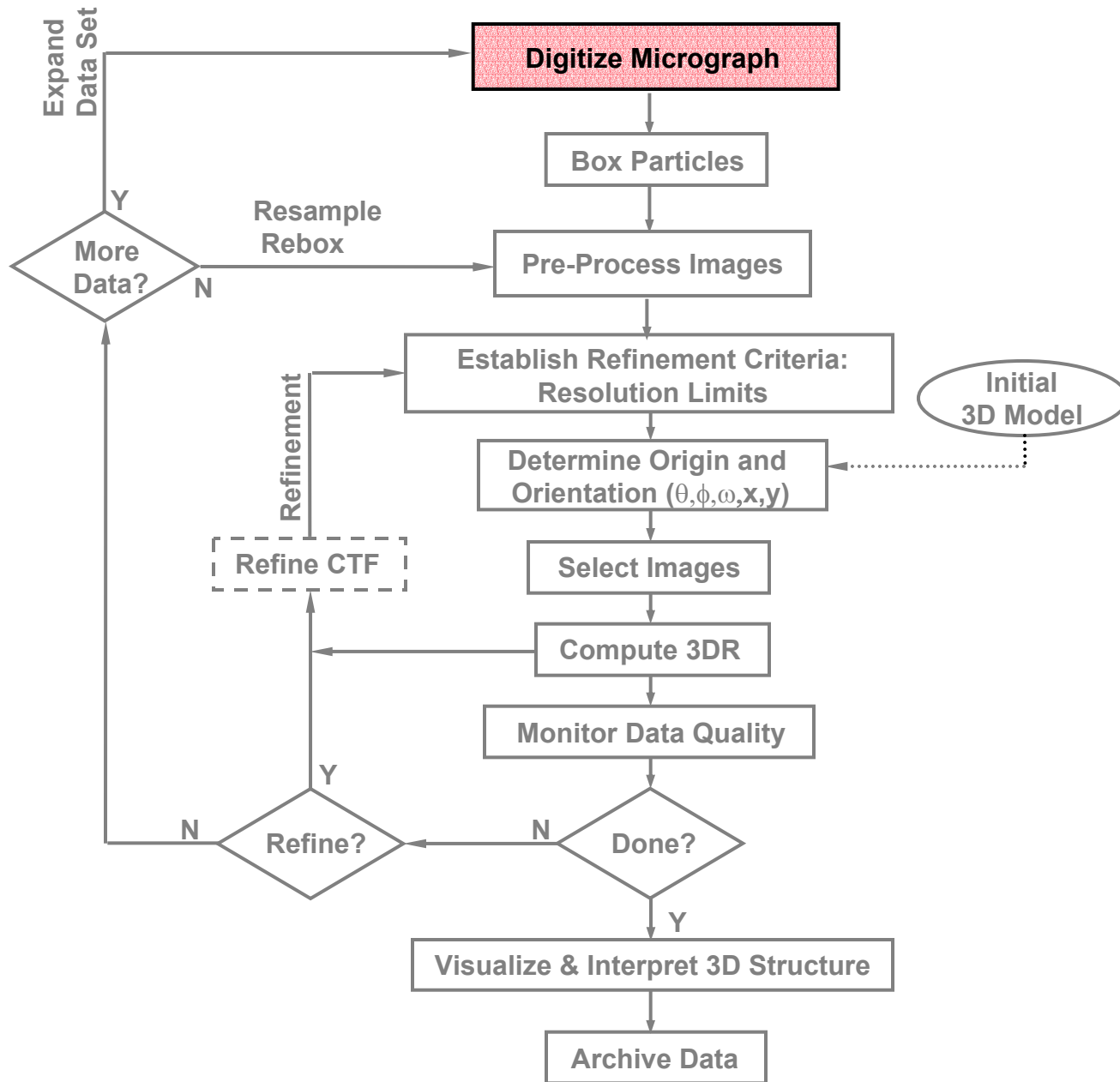
## PROGRAM(S)



# Icosahedral Virus 3D Reconstruction Scheme



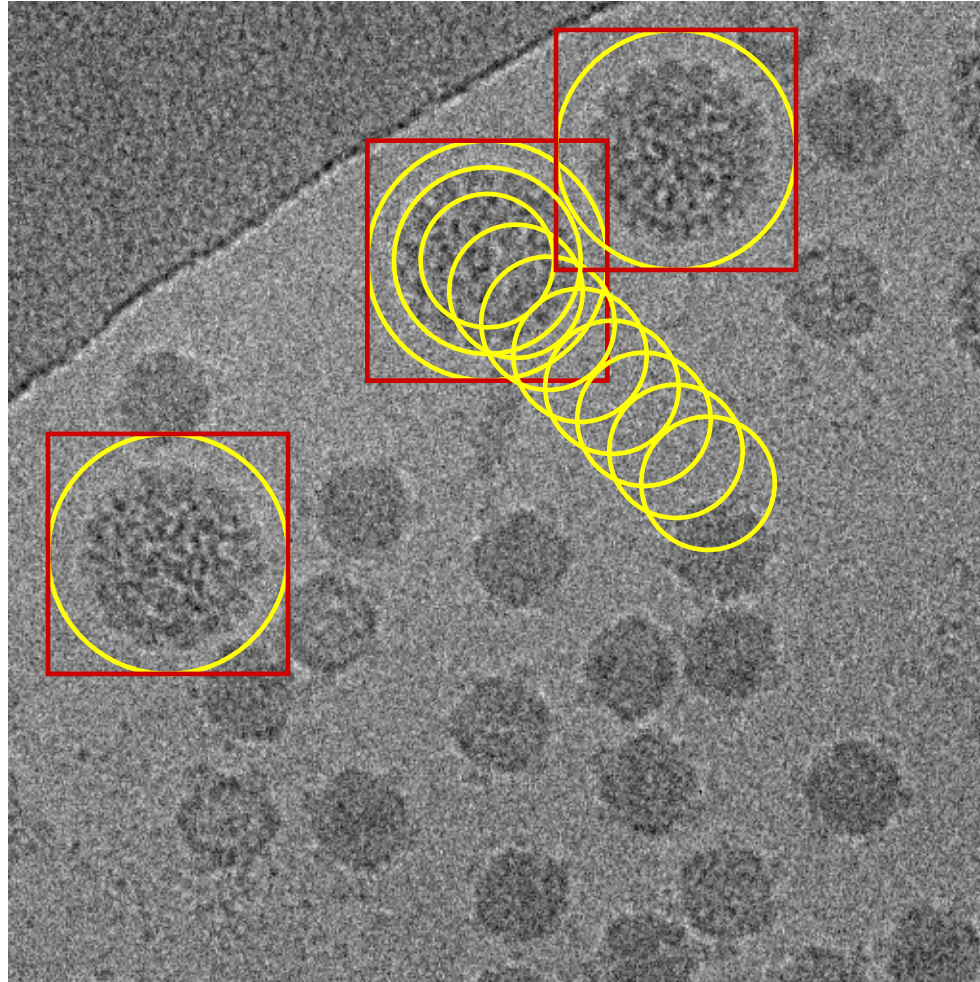
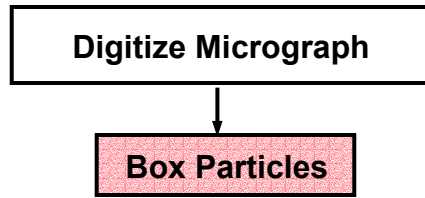
# Icosahedral Virus 3D Reconstruction Scheme



# Icosahedral Virus 3D Reconstruction Scheme

**Digitize Micrograph**

# Icosahedral Virus 3D Reconstruction Scheme

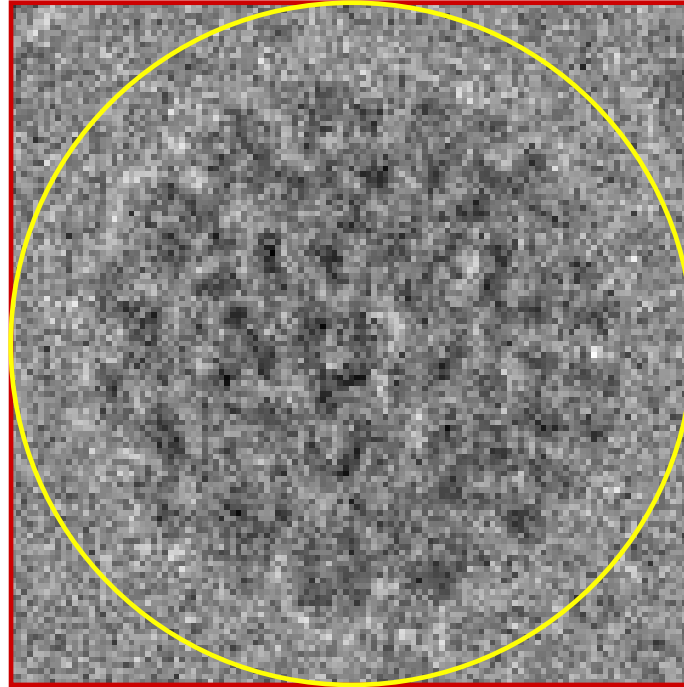


# Icosahedral Virus 3D Reconstruction Scheme

Digitize Micrograph

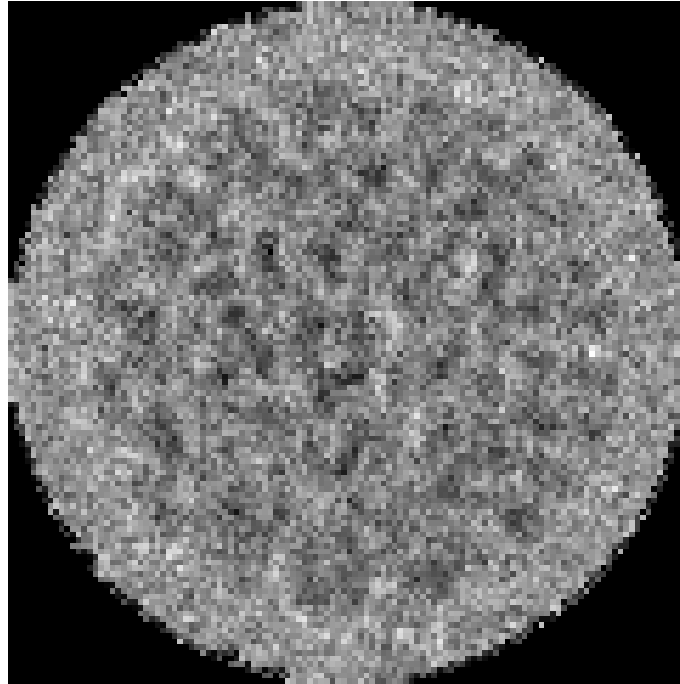
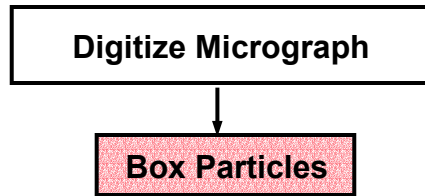


Box Particles



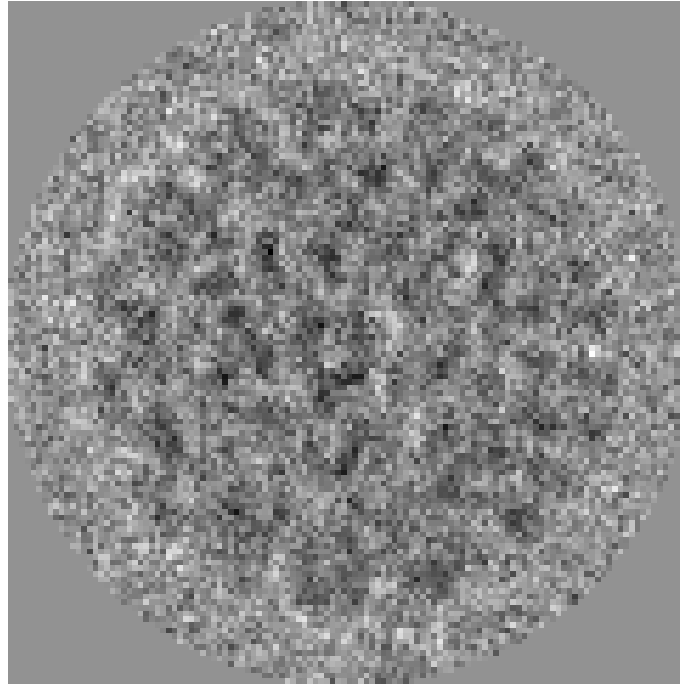
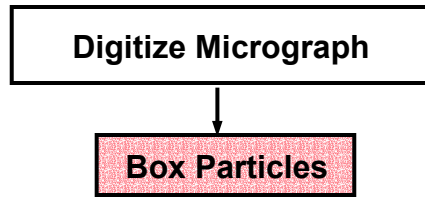
Extracted

# Icosahedral Virus 3D Reconstruction Scheme



Masked

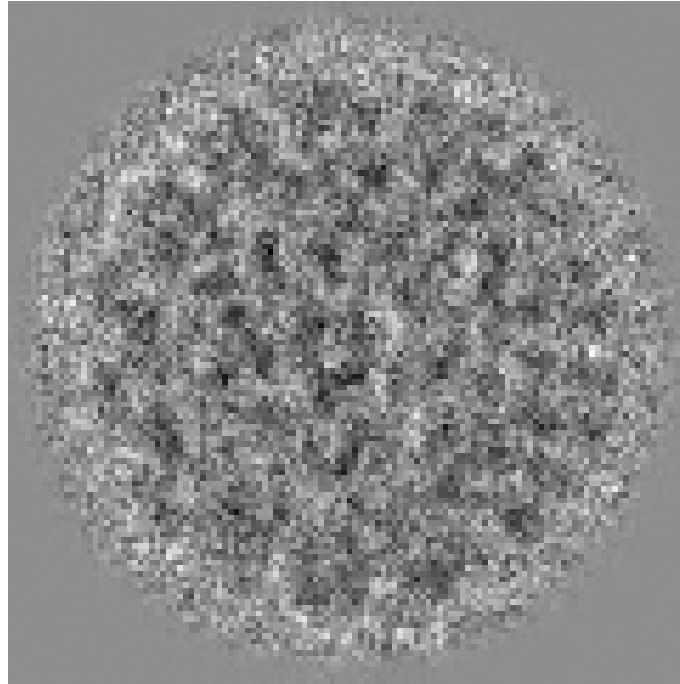
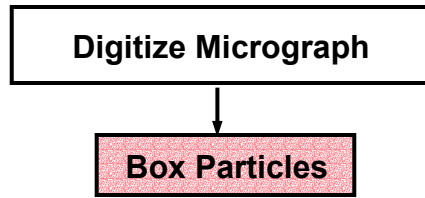
# Icosahedral Virus 3D Reconstruction Scheme



Floated

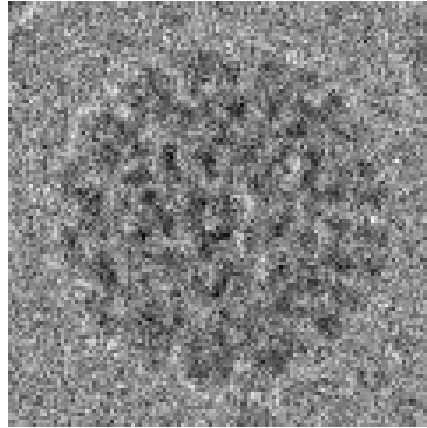
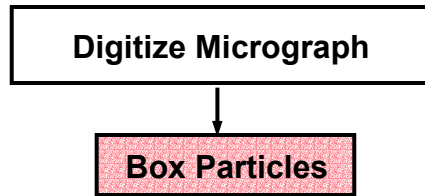


# Icosahedral Virus 3D Reconstruction Scheme

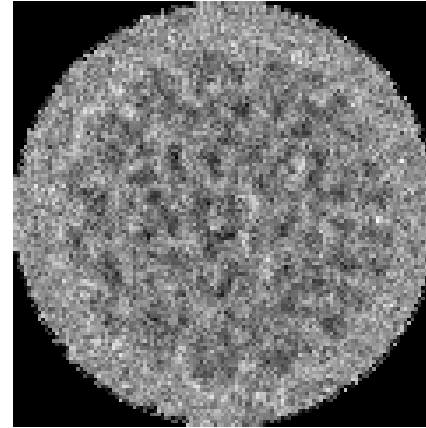


Apodized

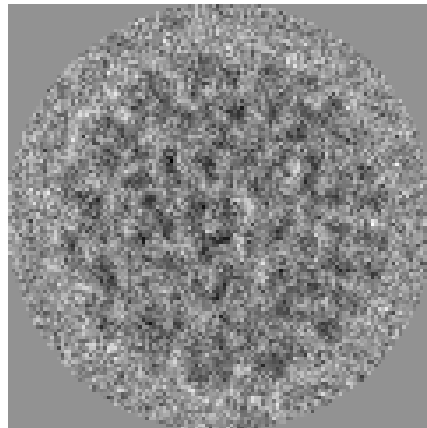
# Icosahedral Virus 3D Reconstruction Scheme



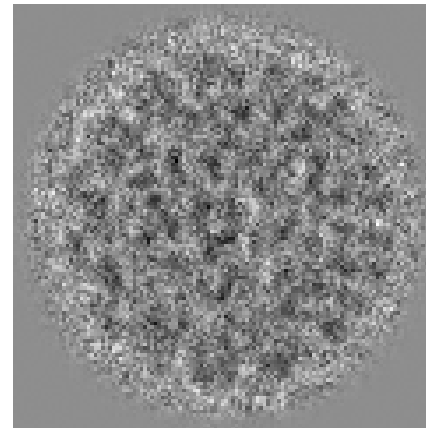
Extracted



Masked

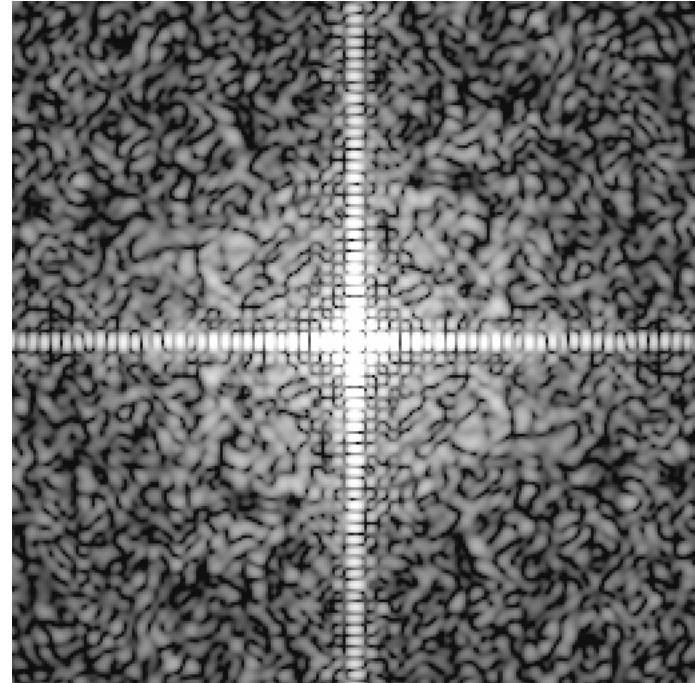
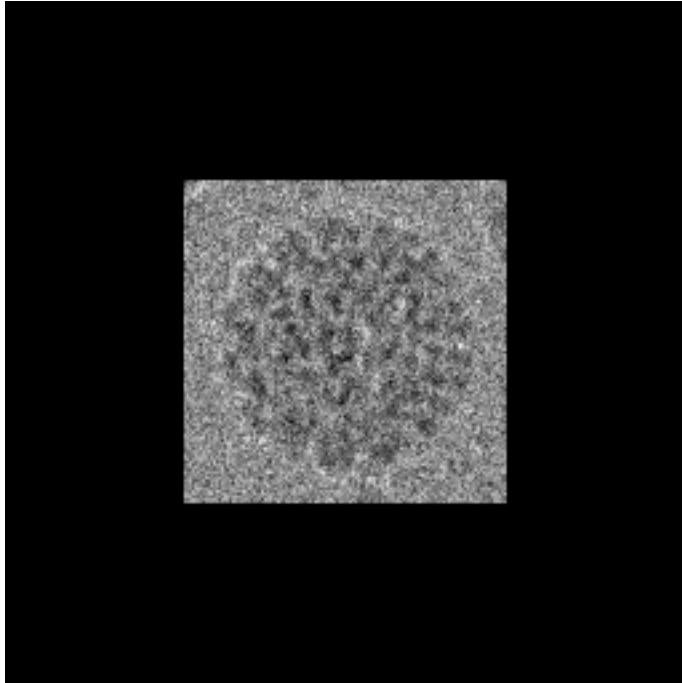
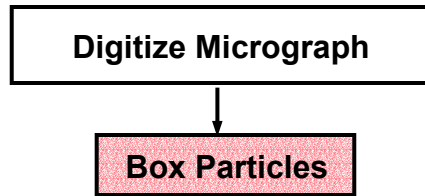


Floated



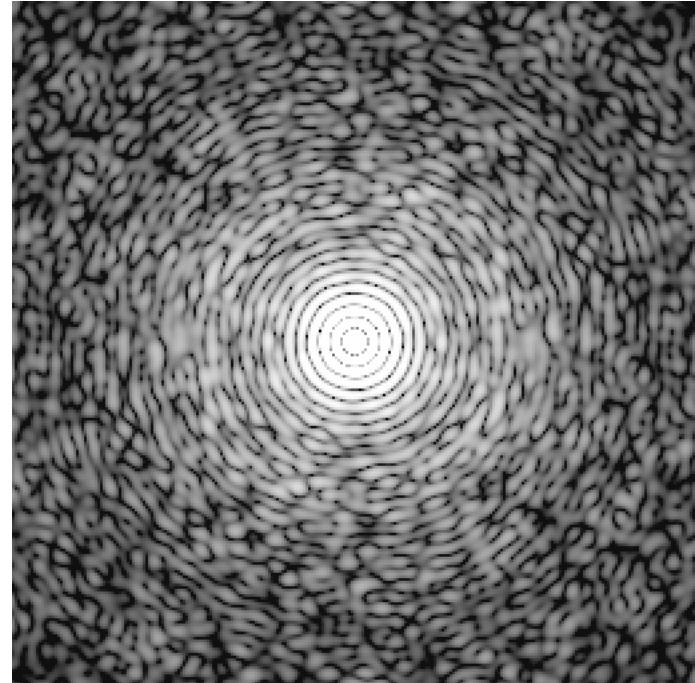
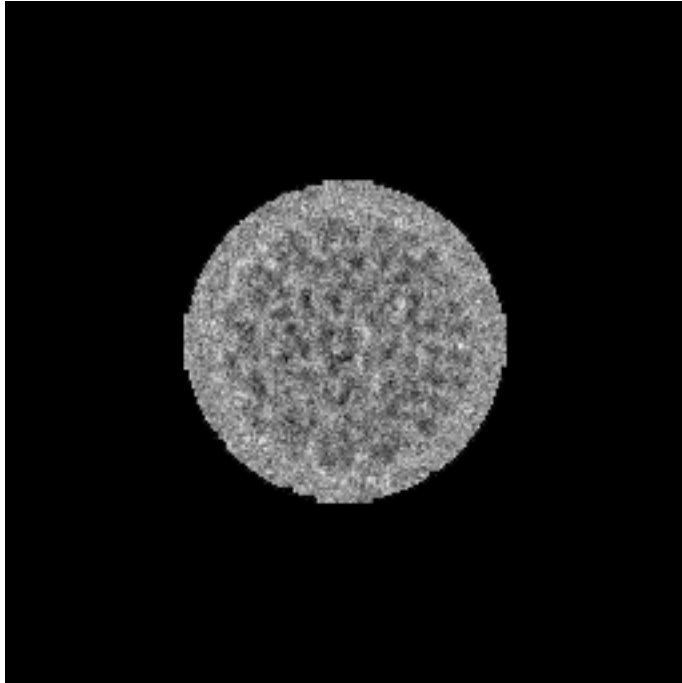
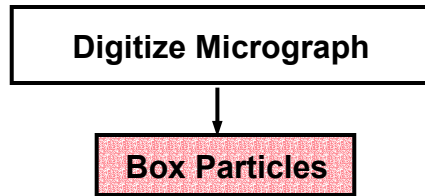
Apodized

# Icosahedral Virus 3D Reconstruction Scheme



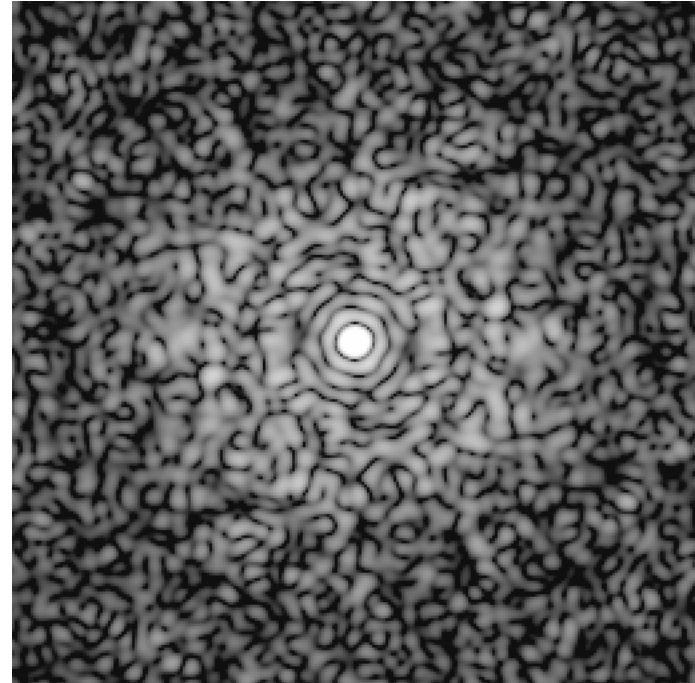
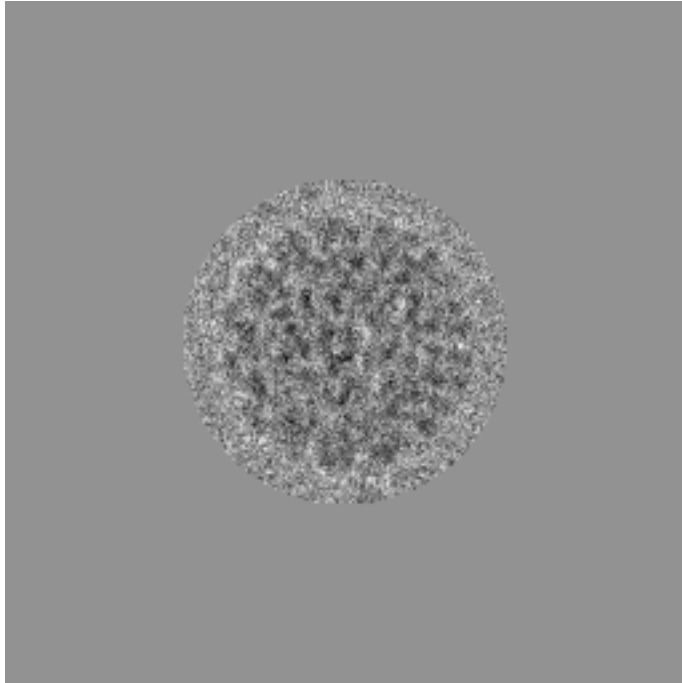
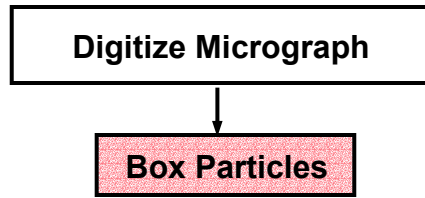
Square mask; unfloated

# Icosahedral Virus 3D Reconstruction Scheme



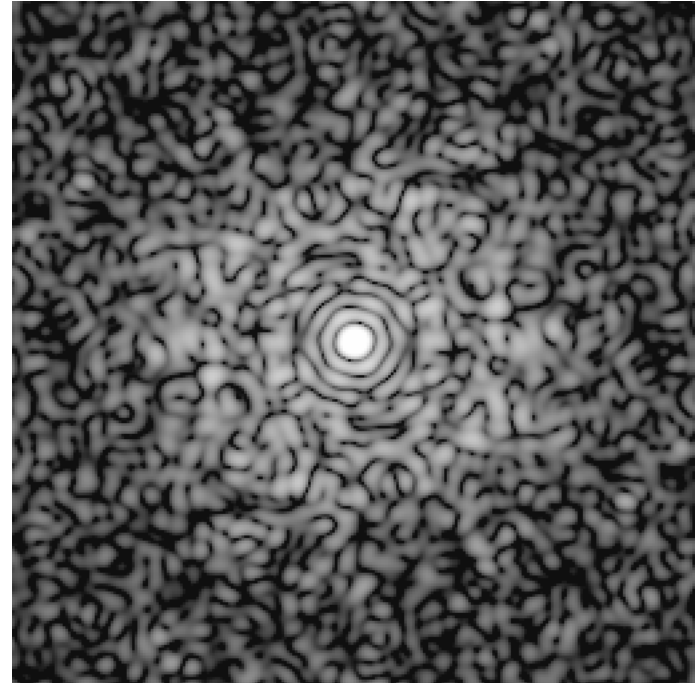
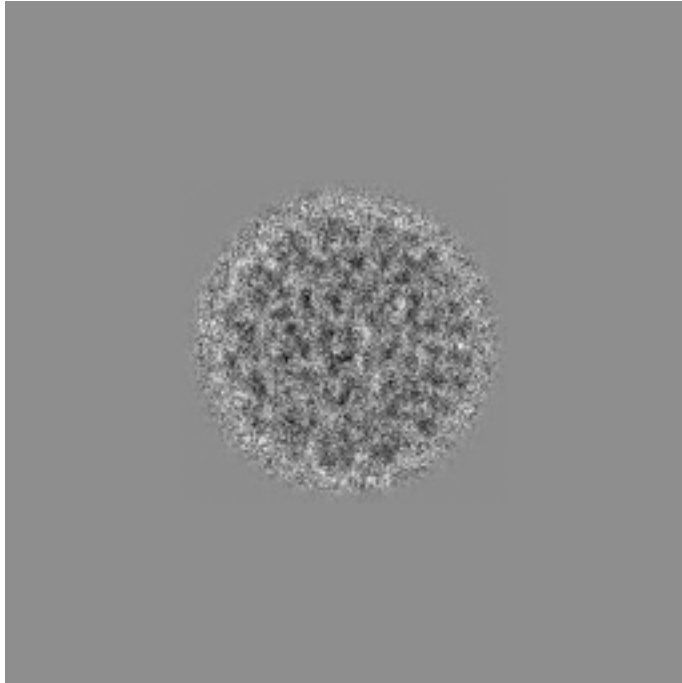
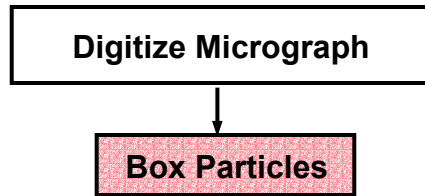
Circular mask; unfloated

# Icosahedral Virus 3D Reconstruction Scheme



Circular mask; floated

# Icosahedral Virus 3D Reconstruction Scheme



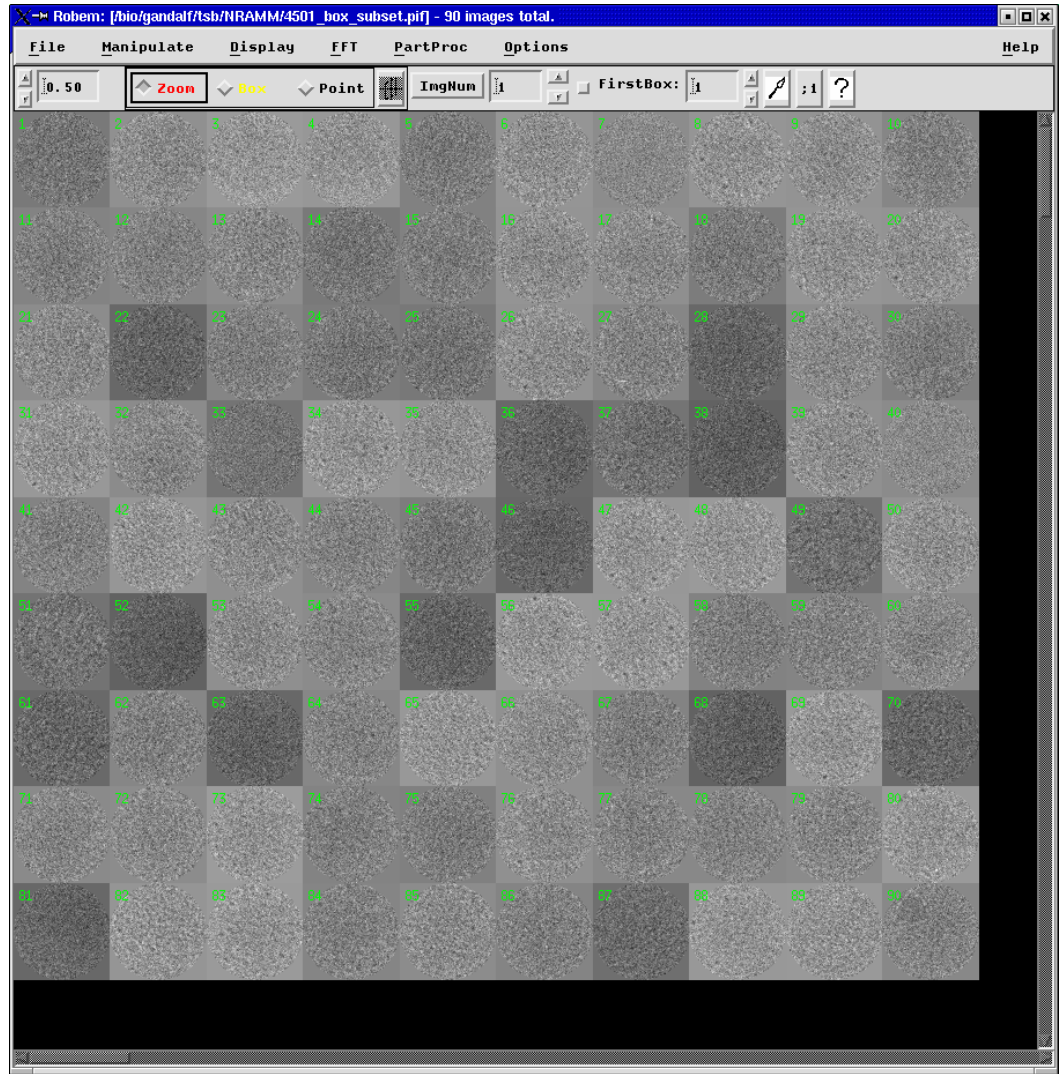
Circular mask; floated & apodized

# Icosahedral Virus 3D Reconstruction Scheme

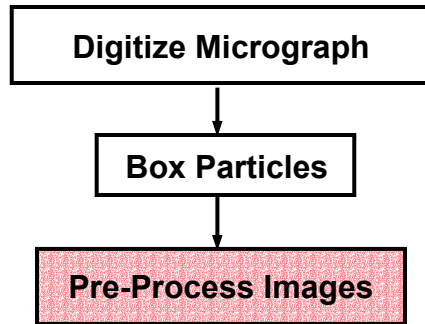
Digitize Micrograph



Box Particles

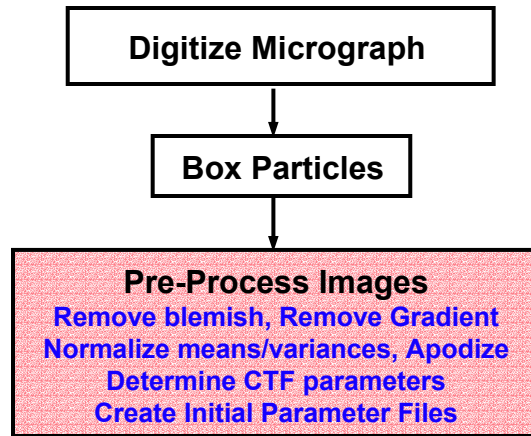


# Icosahedral Virus 3D Reconstruction Scheme

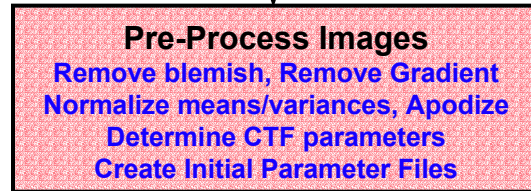




# Icosahedral Virus 3D Reconstruction Scheme



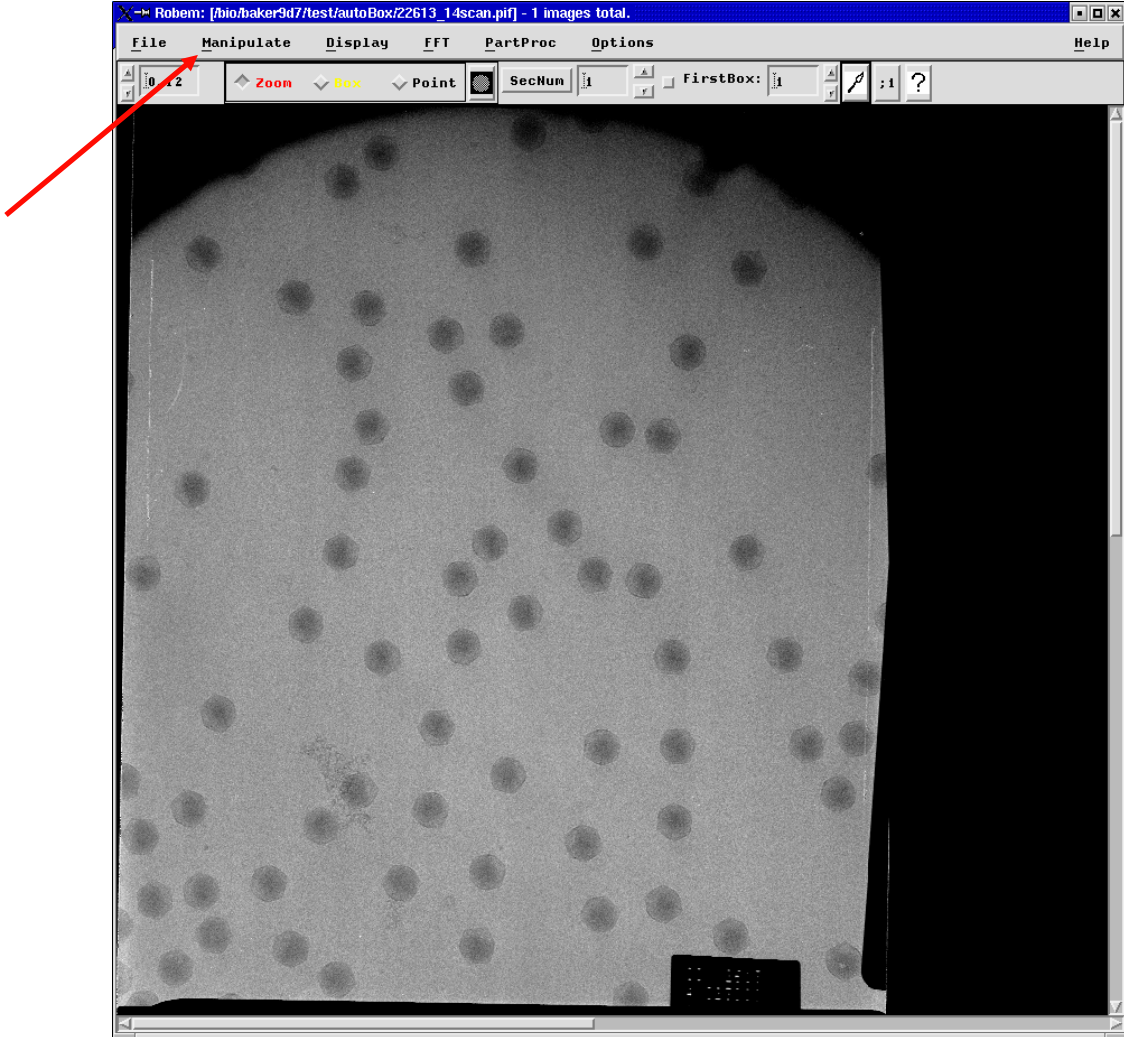
# Icosahedral Virus 3D Reconstruction Scheme



# Icosahedral Virus 3D Reconstruction Scheme



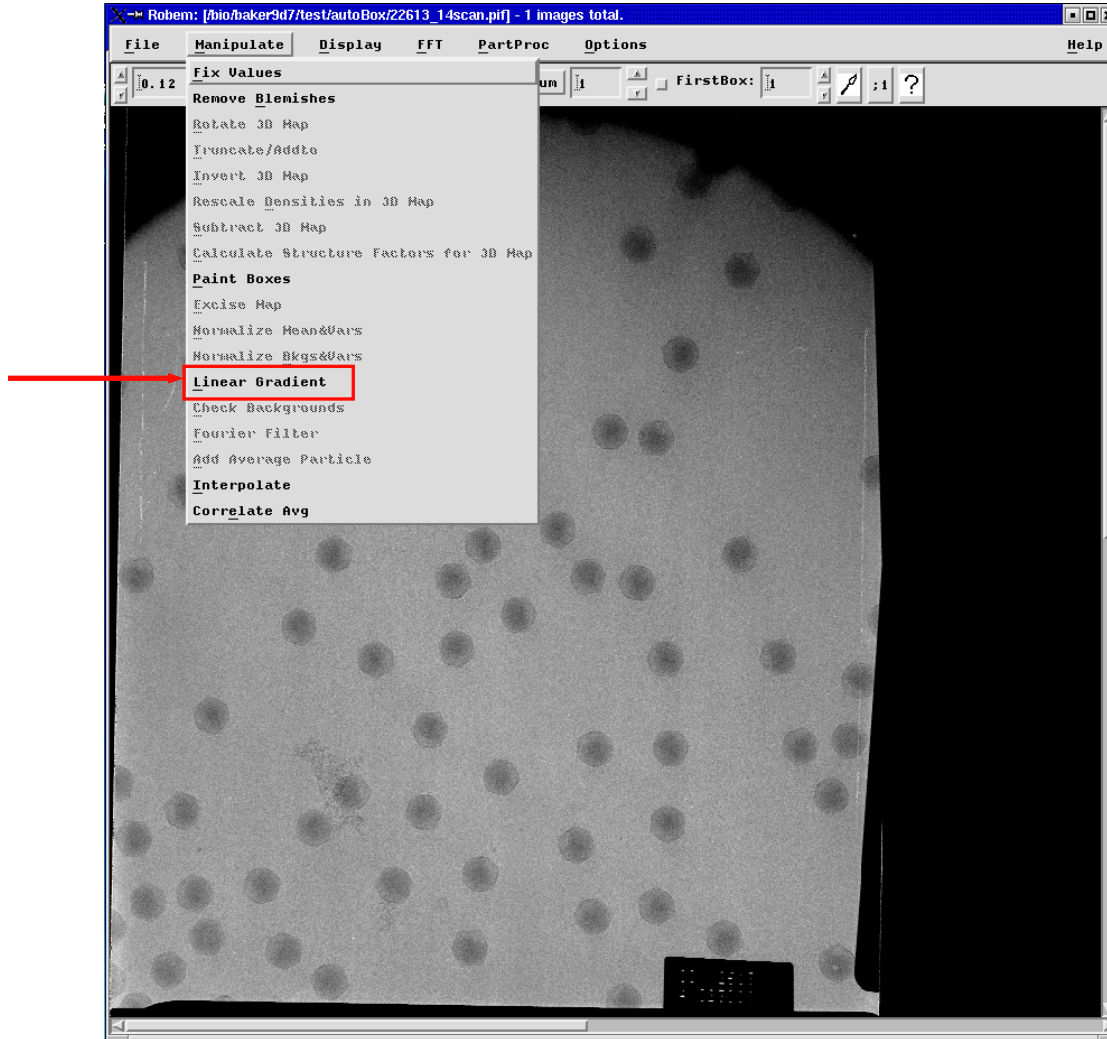
**Pre-Process images**  
Remove blemish, Remove Gradient  
Normalize means/variances, Apodize  
Determine CTF parameters  
Create Initial Parameter Files



# Icosahedral Virus 3D Reconstruction Scheme



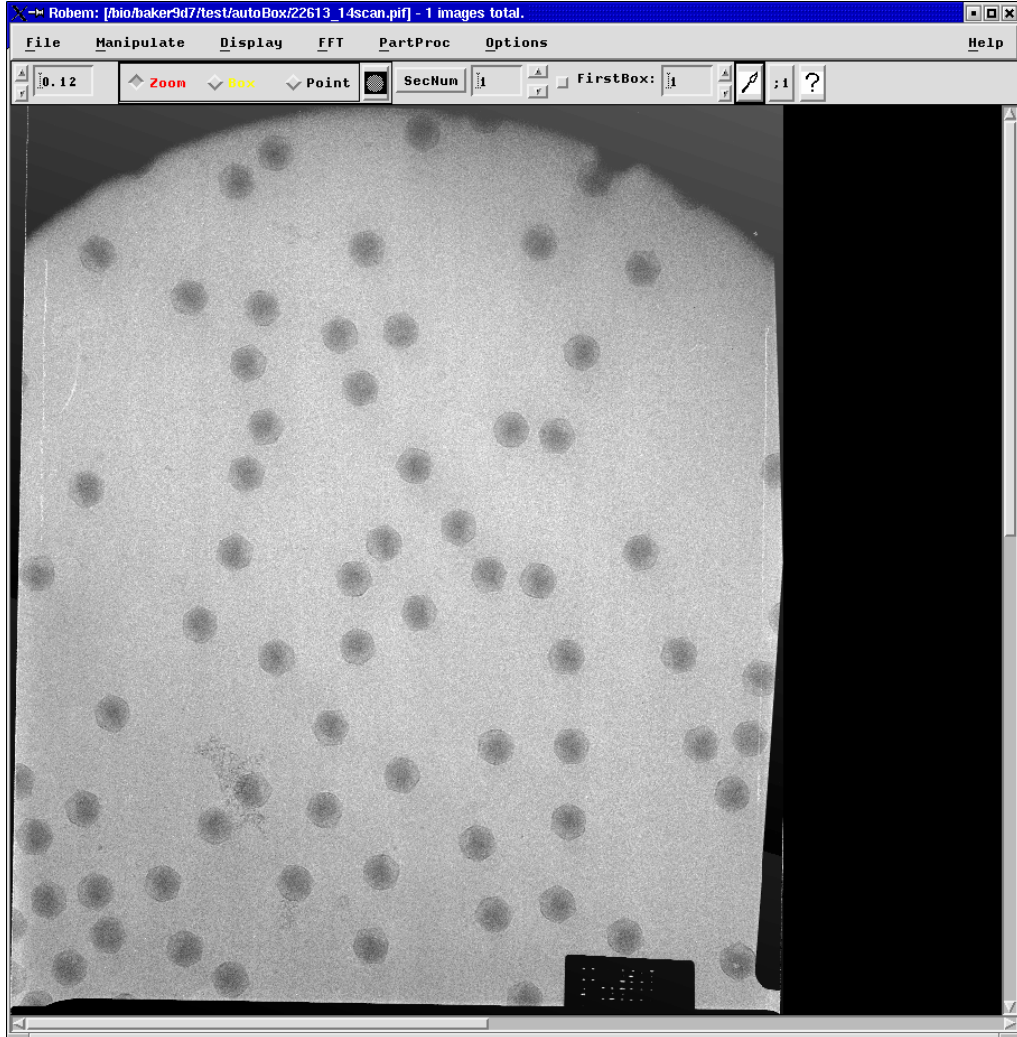
**Pre-Process images**  
Remove blemish, Remove Gradient  
Normalize means/variances, Apodize  
Determine CTF parameters  
Create Initial Parameter Files



# Icosahedral Virus 3D Reconstruction Scheme

↓

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

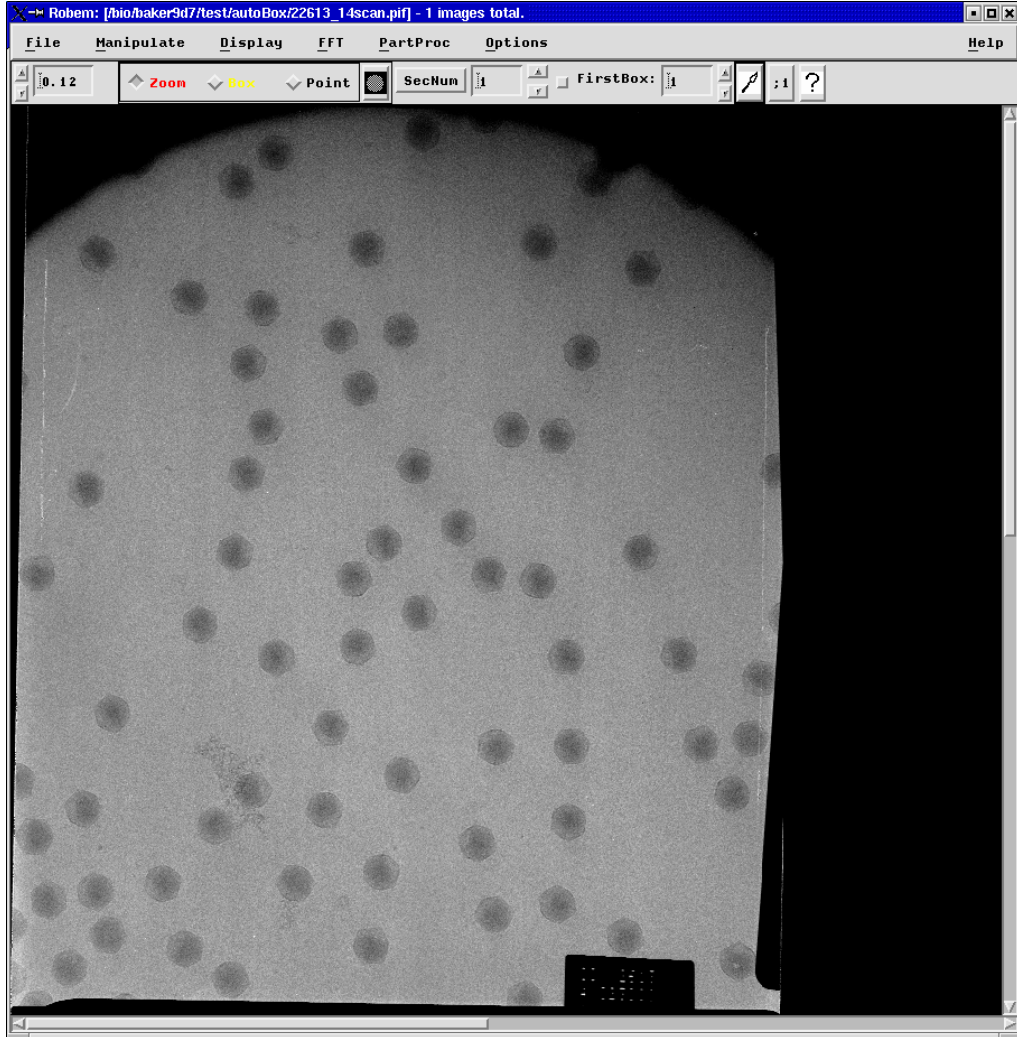


Gradient removed

# Icosahedral Virus 3D Reconstruction Scheme

↓

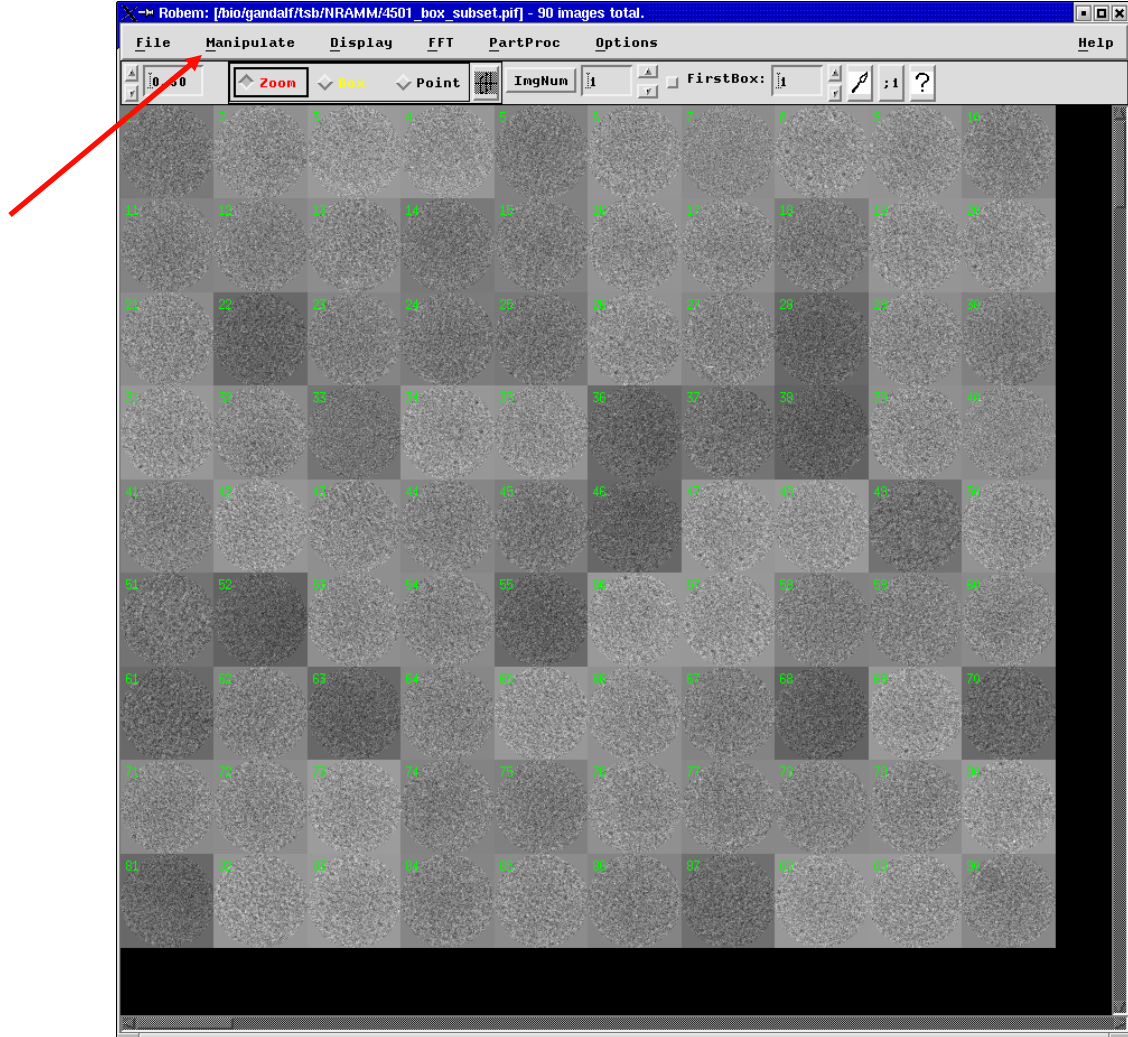
**Pre-Process images**  
Remove blemish, Remove Gradient  
Normalize means/variances, Apodize  
Determine CTF parameters  
Create Initial Parameter Files



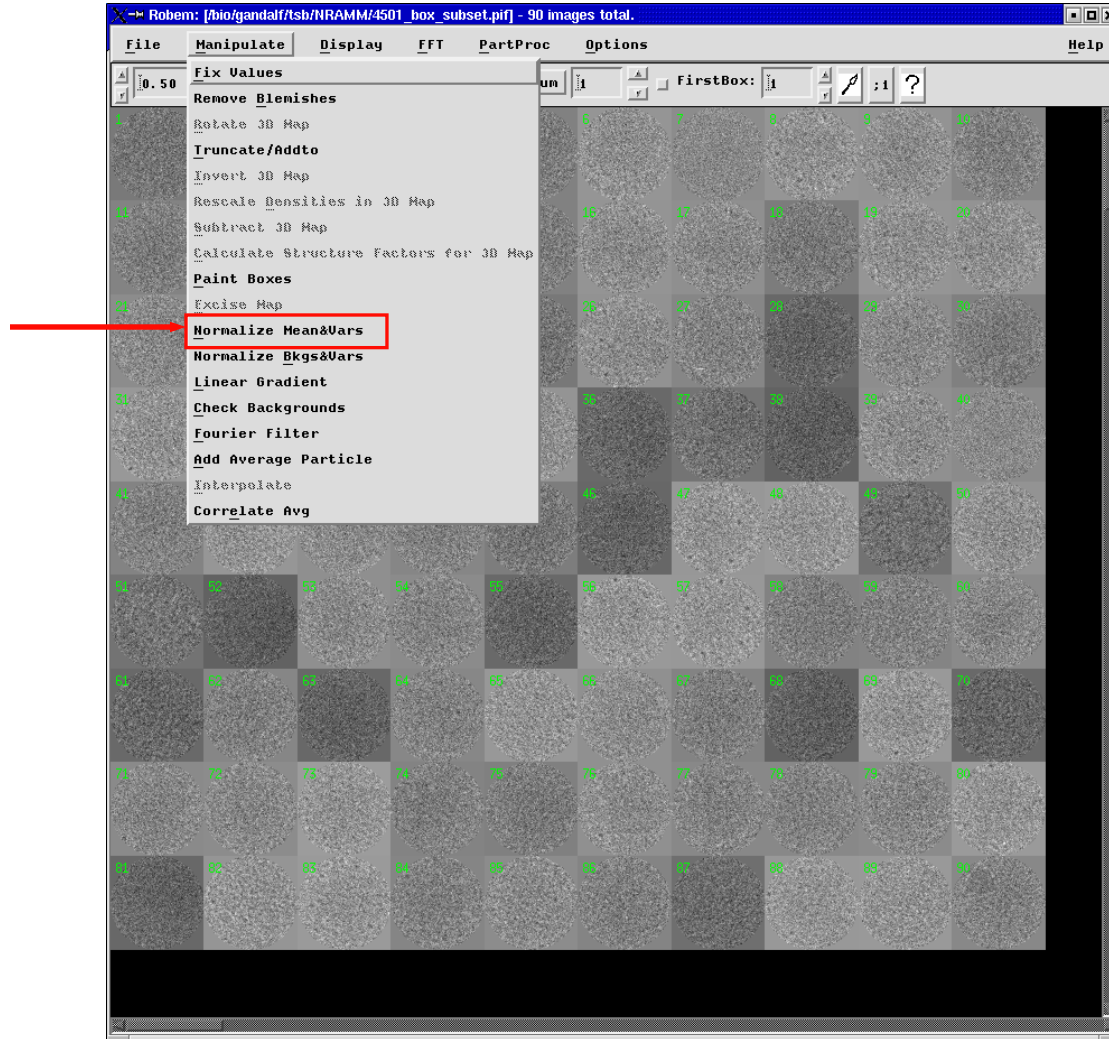
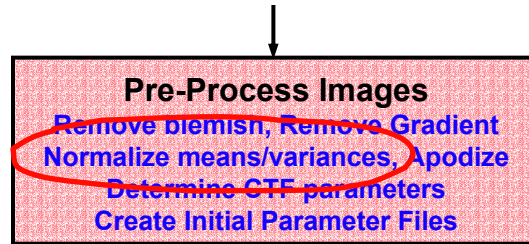
Gradient  
not removed

# Icosahedral Virus 3D Reconstruction Scheme

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



# Icosahedral Virus 3D Reconstruction Scheme

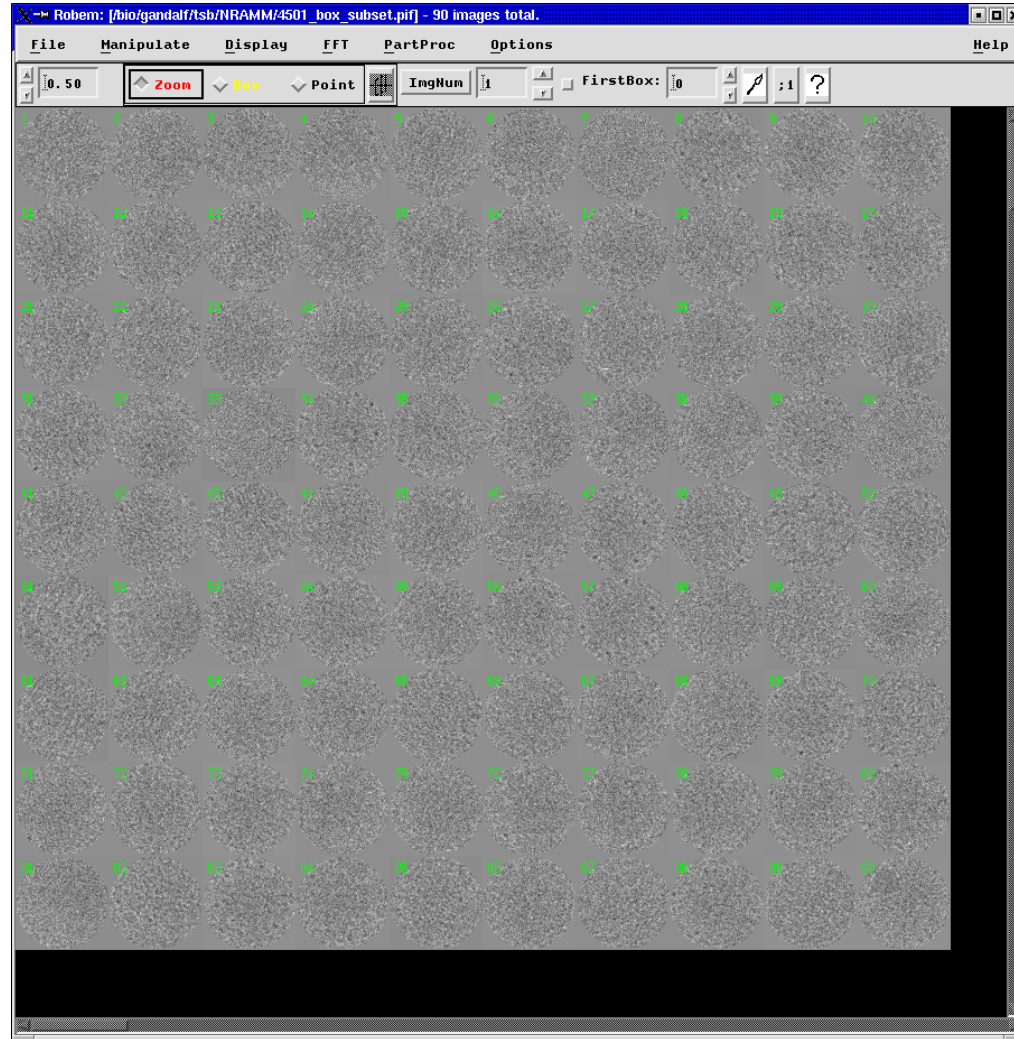




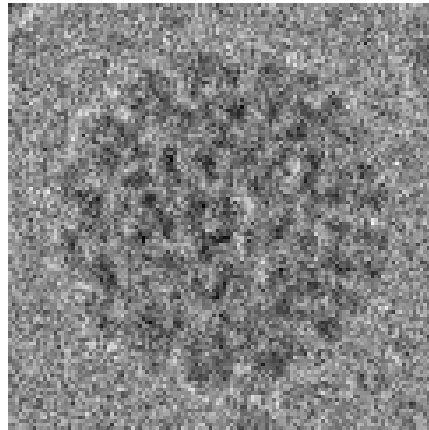
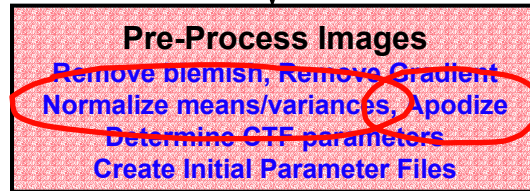
# Icosahedral Virus 3D Reconstruction Scheme



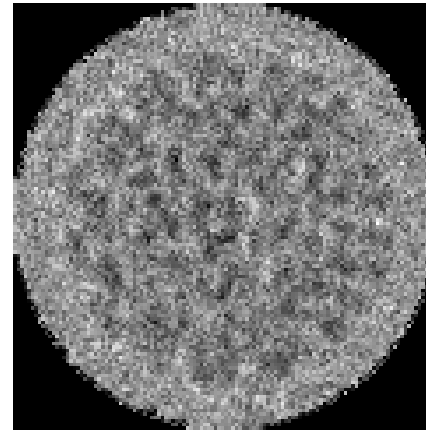
**Pre-Process Images**  
Remove blurriness, Remove Gradient  
Normalize means/variances, Apodize  
Determine CTF parameters  
Create Initial Parameter Files



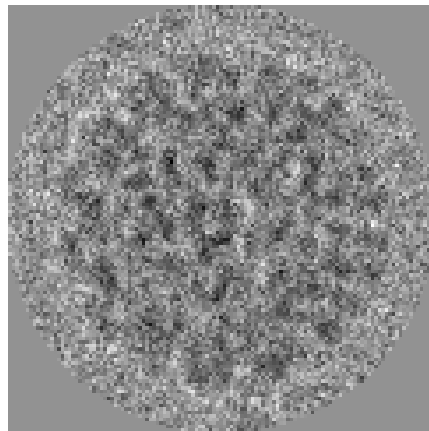
# Icosahedral Virus 3D Reconstruction Scheme



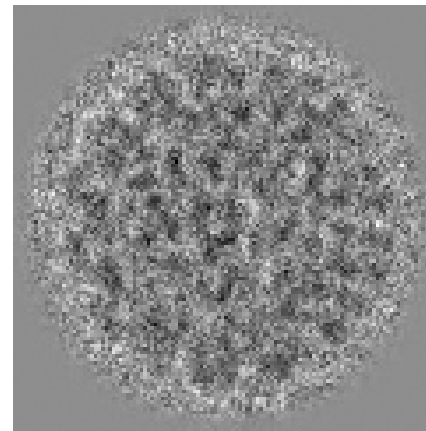
Extracted



Masked



Floated

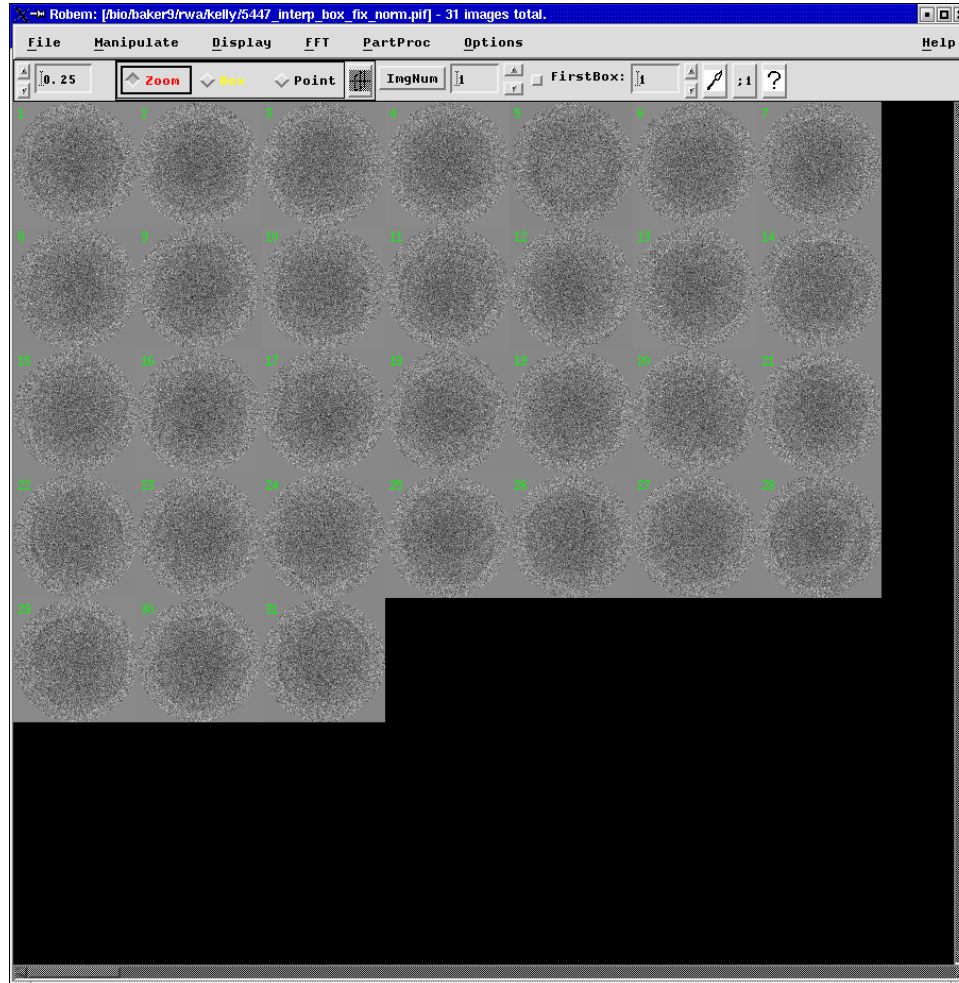


Apodized

# 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     1024 x 1024    Intensity  
 Log         512 x 512    Recalc FFT  
 Non-Linear     256 x 256  
                   128 x 128     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

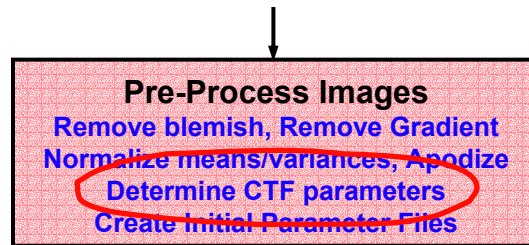
Overlay Intensity:  
 Zoom  
 Disp 1D CA     Contour 1D CircAvg

Pick CTF Pts    Flicker    Determine Image CTF  
Ang Major: \_\_\_\_\_  
Focus Major: \_\_\_\_\_  
Focus Minor: \_\_\_\_\_

CTF Node Num:   
FFT Average  
Average FFTs:  Inq#  
 Incoherent     Hvg

ScStp (um)     Mag   
PxSiz (nm)     Wiener   
AmpCont     AngMaj   
Volts (kV)     tFac   
Wave (nm)     Mode   
Cs (nm)     ctf   
FocMaj (um)     PixelSize in nanometers  
FocMin (um)

# Icosahedral Virus 3D Reconstruction Scheme



**FFT - CTF Estimation**

Linear     1024 x 1024    Intensity  
 Log         512 x 512    Recalc FFT  
 Non-linear     256 x 256  
                   128 x 128     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:   
FFT Average  
Average FFTs  Incoherent  Hvg

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

# Icosahedral Virus 3D Reconstruction Scheme



**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: 2.16  
Focus Minor: 3.31

CTF Node Num: 1

FFT Average  
Aver Ing#  
Average FFTs  
 Incoherent  Hvg

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

# Icosahedral Virus 3D Reconstruction Scheme

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

The screenshot displays two windows from a Cryo-EM software suite. The left window, titled "FFT - CTF Estimation", shows a 2D Fast Fourier Transform (FFT) image with concentric rings. A red circle highlights the central region. The right window, titled "Automatic Defocus Refinement", contains various input fields and controls. A red circle highlights the "Estimate the Defocus" button. Below the main windows, a panel shows estimated parameters for the CTF.

**Automatic Defocus Refinement Parameters:**

a0:	1	b0:	1
a1:	0	b1:	0
a2:	0	b2:	0
a3:	0	b3:	0
a4:	0	b4:	0

Highest Resolution (Angs): 8.480000

Estimated Defocus (um):

Chi squared:

Select different plots:

- Circular Average
- S
- S - B
- ctf^2
- D
- B

Zoom FFT image

Change Overlay Intensity to see CTF curves.

**Estimate the Defocus \*1**

Update FFT screen

**CTF Estimation Parameters:**

Ang Major	359.4
Focus Major	2.16
Focus Minor	3.31

CTF Node Num: 1

FFT Average: Aver Ing#

Average FFTs:  Incoherent hv9

**Global Parameters:**

AmpCont	0.07	AngMaj	359.4
Volts (kV)	300	tFac	0
Wave (nm)	0.001	Mode	1
Cs (nm)	2.0	ctf	1/ctf
FocMaj (um)	2.16	PixelSize 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 C

Pick CTF Pts  
Flicker Determine Image CTF

Ang Major 359.4  
Focus Major 2.204  
Focus Minor 2.204

CTF Node Num: 1

FFT Average  
Aver Ing#  
Average FFTs  
Incoherent hvv

**Automatic Defocus Refinement**

a0: 1.739071E+00  
a1: -6.500993E-01  
a2: 3.357709E-05  
a3: -1.871776E-01  
a4: 3.767466E-10

b0: 2.172054E-01  
b1: 2.077333E-03  
b2: -4.360161E-01  
b3: 2.043404E-07  
b4: -3.037543E-11

Highest Resolution (Angs): 8.480000

Estimated Defocus (um): 2.204652  
Chi squared: 0.111960

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

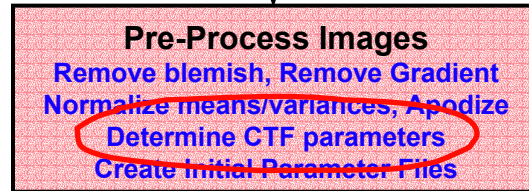
Zoom FFT image  
Change Overlay Intensity to see CTF curves.

Estimate the Defocus \*1  
Update FFT screen

AmpCont 0.07 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 i  
FocMin (um) 2.20 nanometers



# Icosahedral Virus 3D Reconstruction Scheme



**FFT - CTF Estimation**

Linear     1024 x 1024    Intensity  
 Log         512 x 512    Recalc FFT  
 Non-linear     256 x 256  
                   128 x 128     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

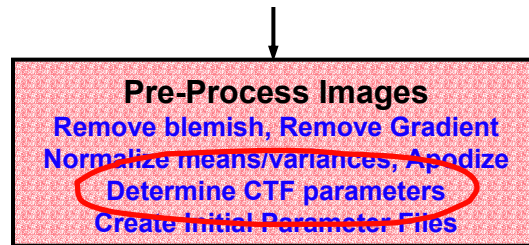
Overlay Intensity:  
 Zoom  
 Disp 1D CA     Contour 1D CircAvg

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  
Average FFTs:  Incoherent  Hvg

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

# Icosahedral Virus 3D Reconstruction Scheme



**FFT - CTF Estimation**

Linear     1024 x 1024    Intensity  
 Log         512 x 512    Recalc FFT  
 Non-linear     256 x 256  
                   128 x 128     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: [Slider]

Zoom     Disp 1D CA     Contour 1D CircAvg

Pick CTF Pts    **Flicker**    Determine Image CTF

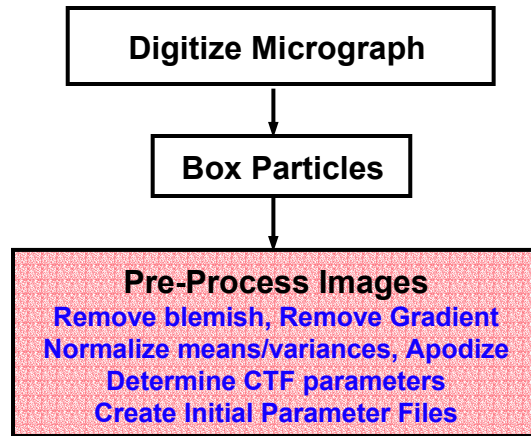
Ang Major: 359.4  
Focus Major: 2.204  
Focus Minor: 2.204

CTF Node Num: 1

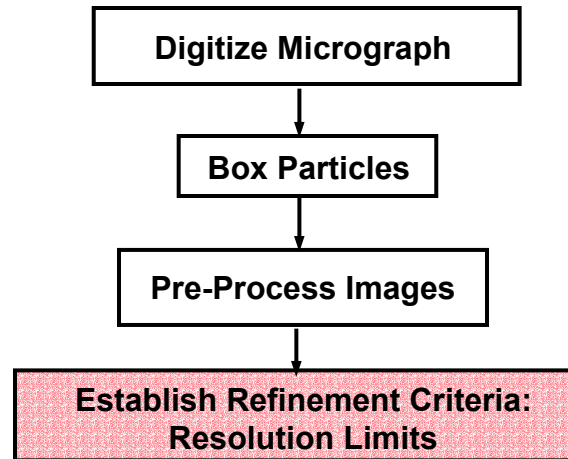
FFT Average  
Aver Ing#  
Average FFTs  
 Incoherent     Hvg

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

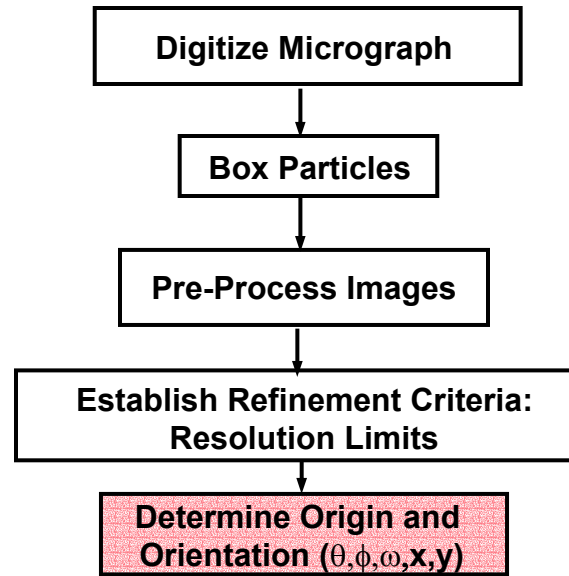
# Icosahedral Virus 3D Reconstruction Scheme



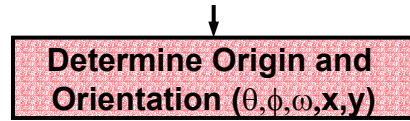
# Icosahedral Virus 3D Reconstruction Scheme



# Icosahedral Virus 3D Reconstruction Scheme



# Icosahedral Virus 3D Reconstruction Scheme

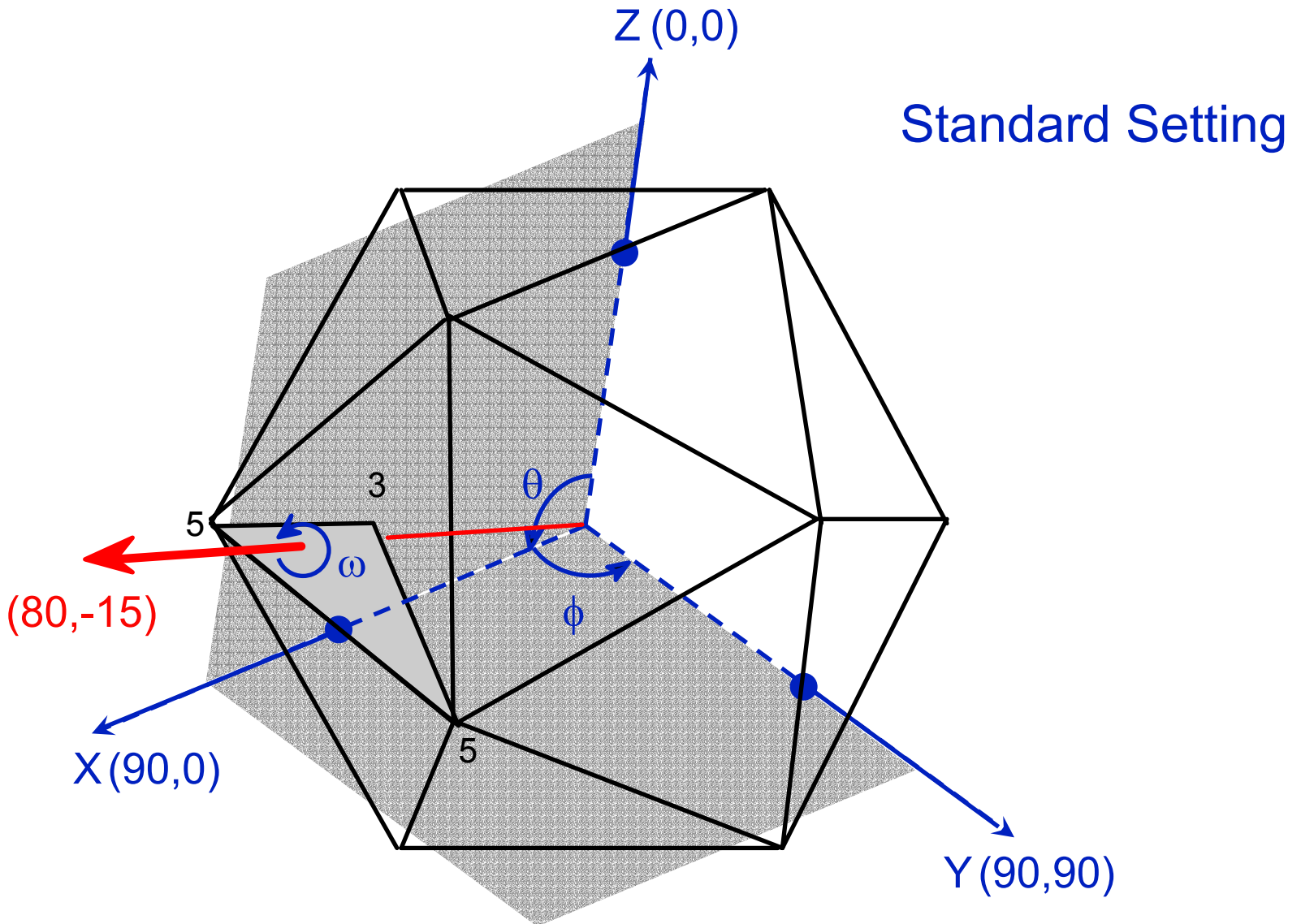


Goal: determine phase origin and view orientation for each boxed particle

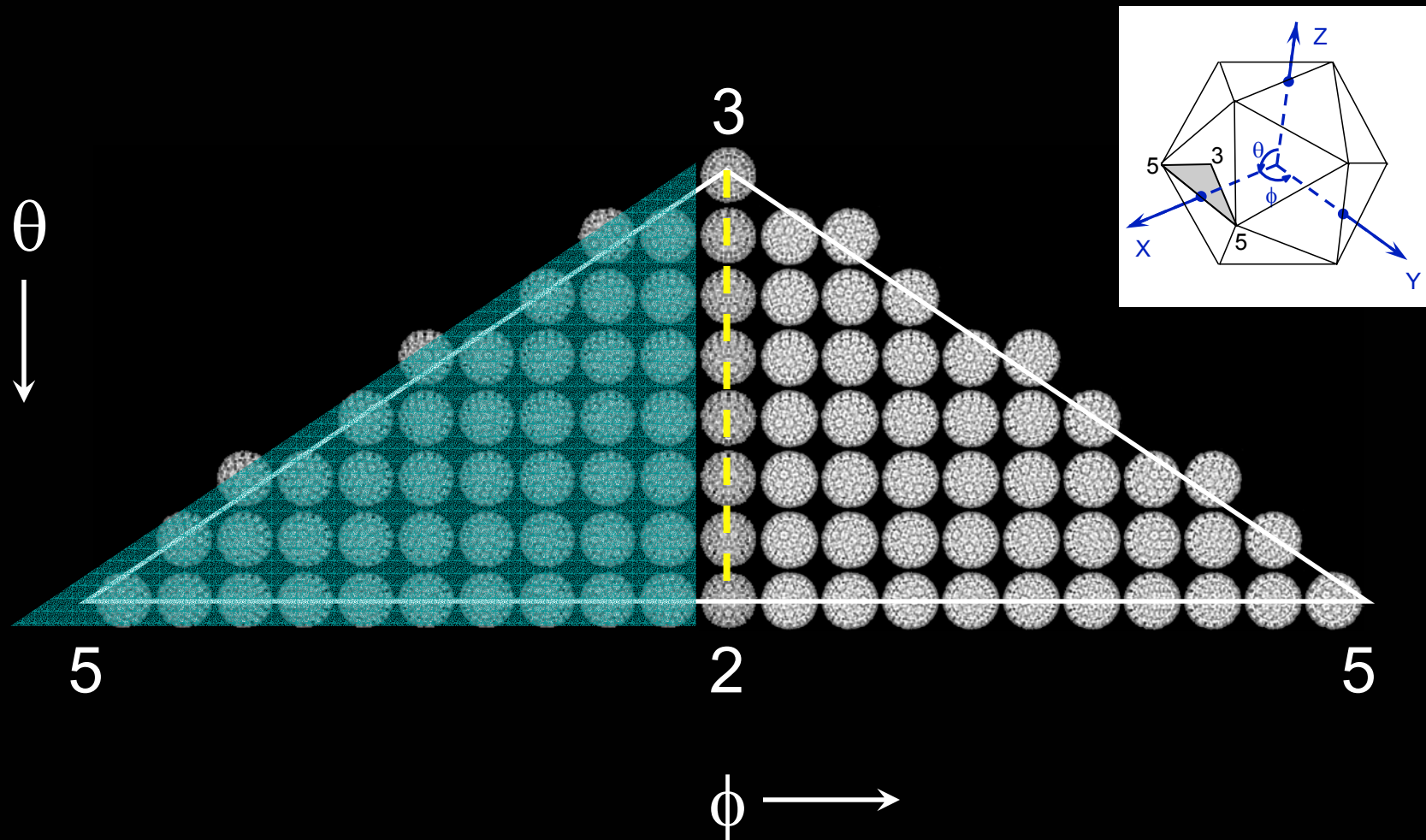
**MOST IMPORTANT STEP?**

*Garbage in -----> garbage out*

# Specifying Direction of View: $(\theta, \phi, \omega)$ Orientation



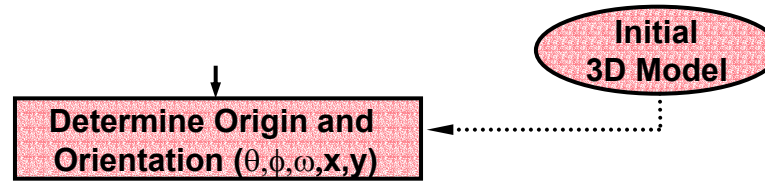
# BPV Projections: Icosahedral ASU







# Icosahedral Virus 3D Reconstruction Scheme



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

Two methods:

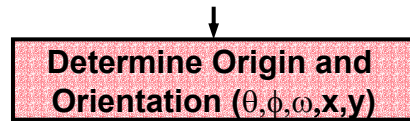
## 1. Common lines

New or unknown structure

## 2. Model-based (template) matching

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

# Icosahedral Virus 3D Reconstruction Scheme



## Common Lines

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

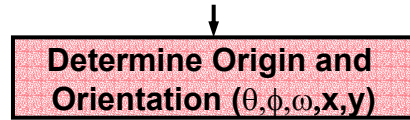
*“[Common lines] arise as follows:”*

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

*“The common line lies in the original section.”*

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

# Icosahedral Virus 3D Reconstruction Scheme



## Common Lines

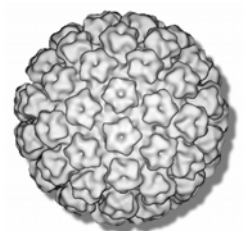
The 'gospel' continued:

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

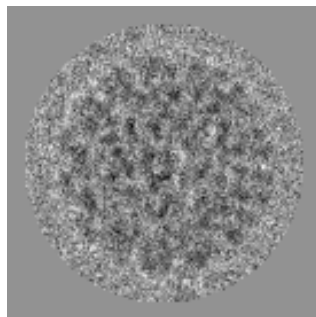
*“A similar pair of lines will be generated by each possible choice of pairs of symmetry operations”*

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

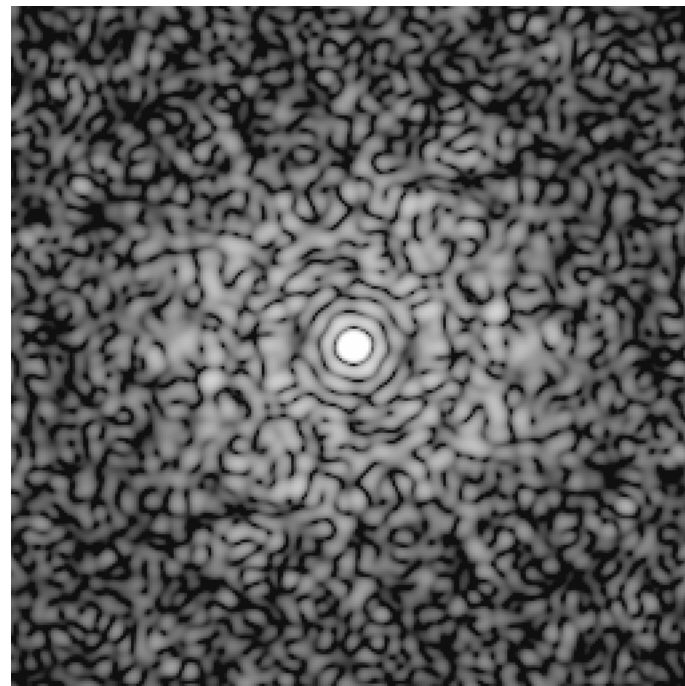
# Orientation Determination by Common Lines



3D Object



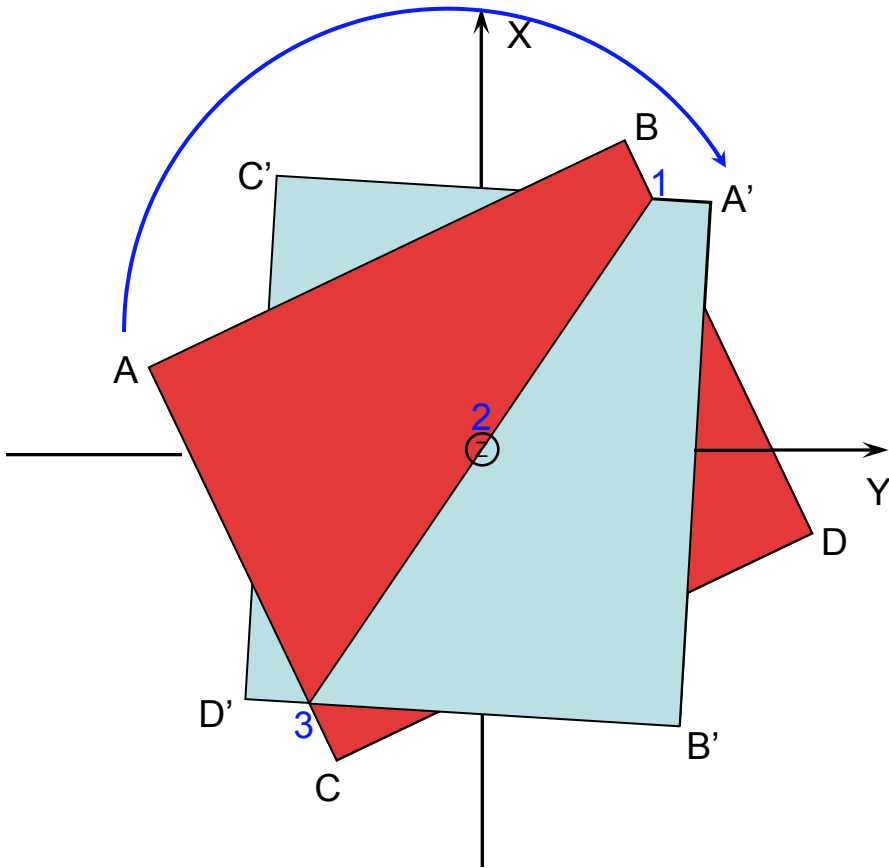
2D Projection  
( $\theta, \phi, \omega$ )



2D Fourier Transform

# Orientation Determination by Common Lines

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



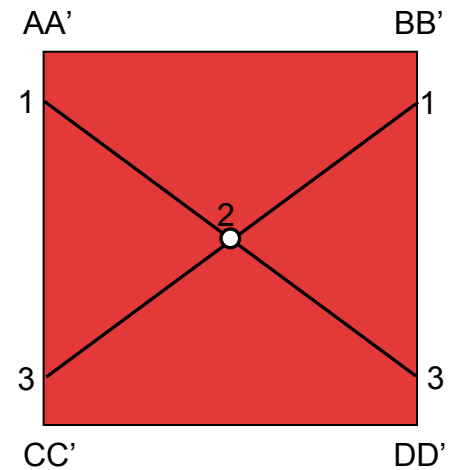
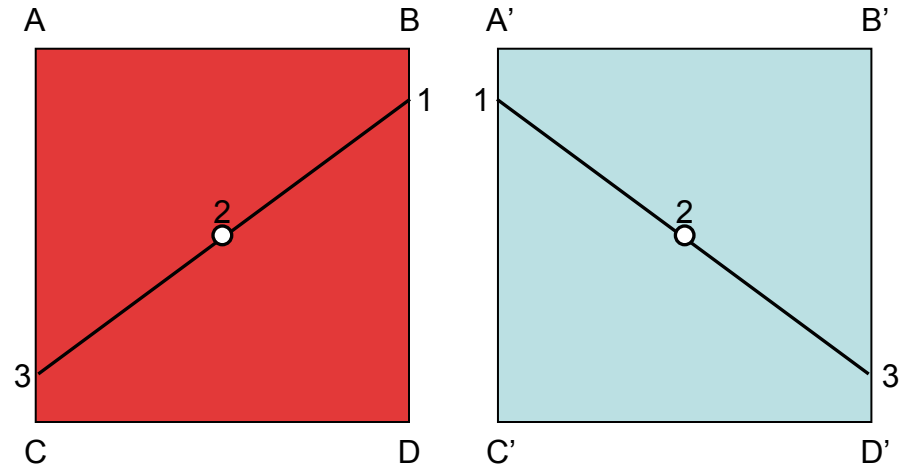
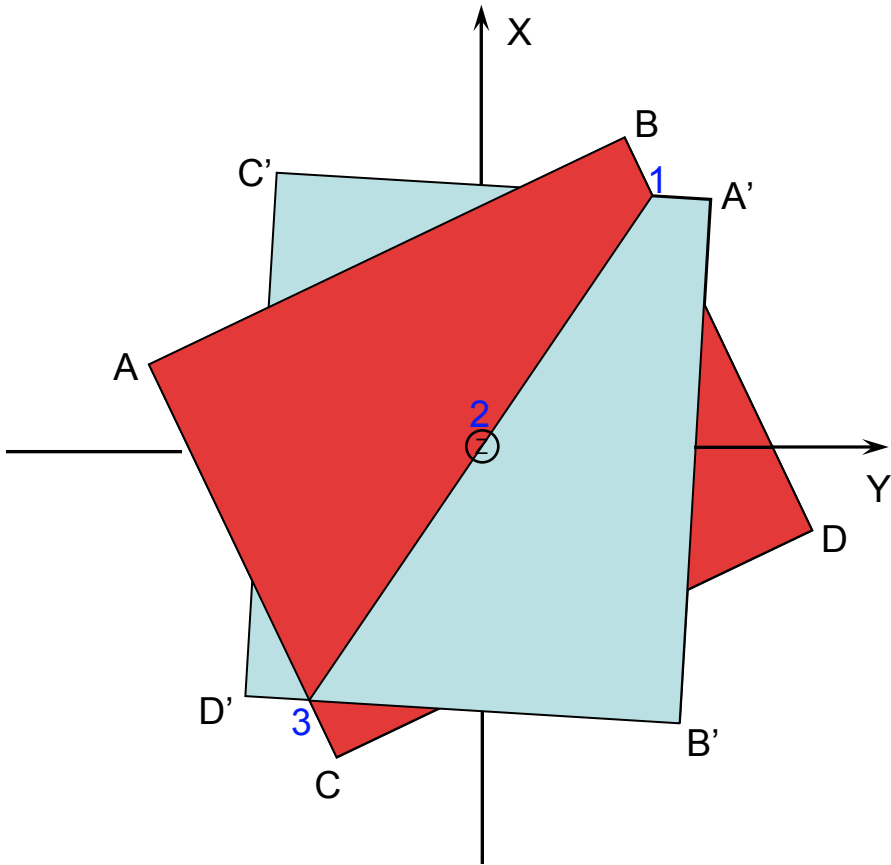
ABCD = 2D transform of image from particle **not** viewed along an axis of symmetry

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

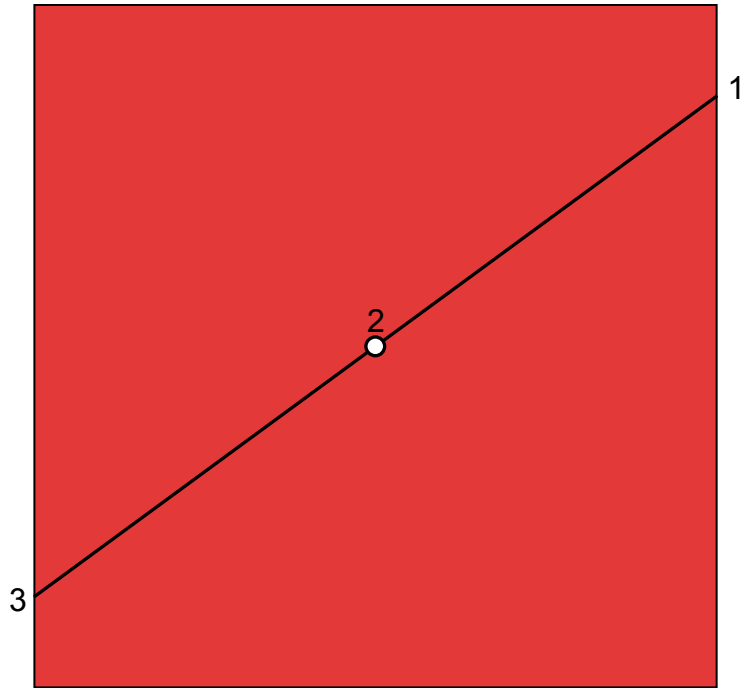
# Orientation Determination by Common Lines



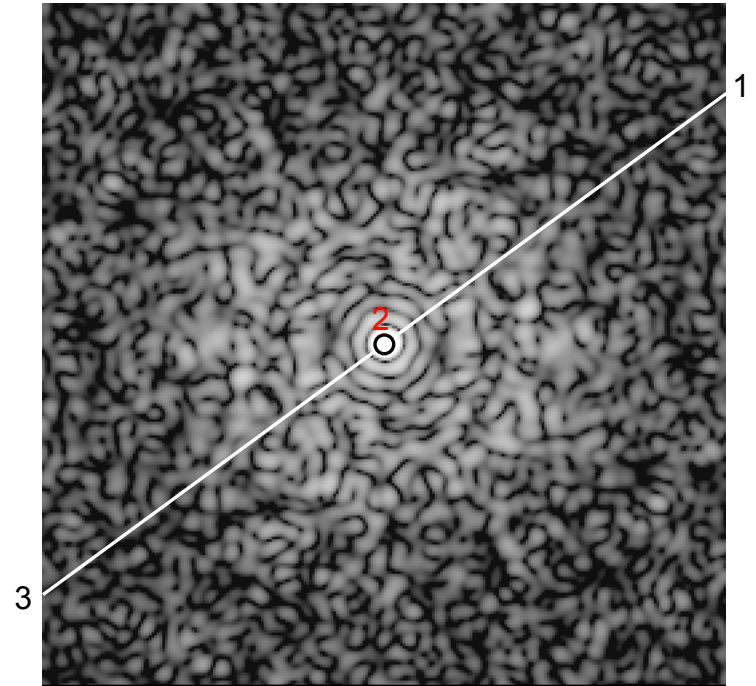
Adapted from Moody (1990) Fig. 7.68, p.245

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

# Orientation Determination by Common Lines

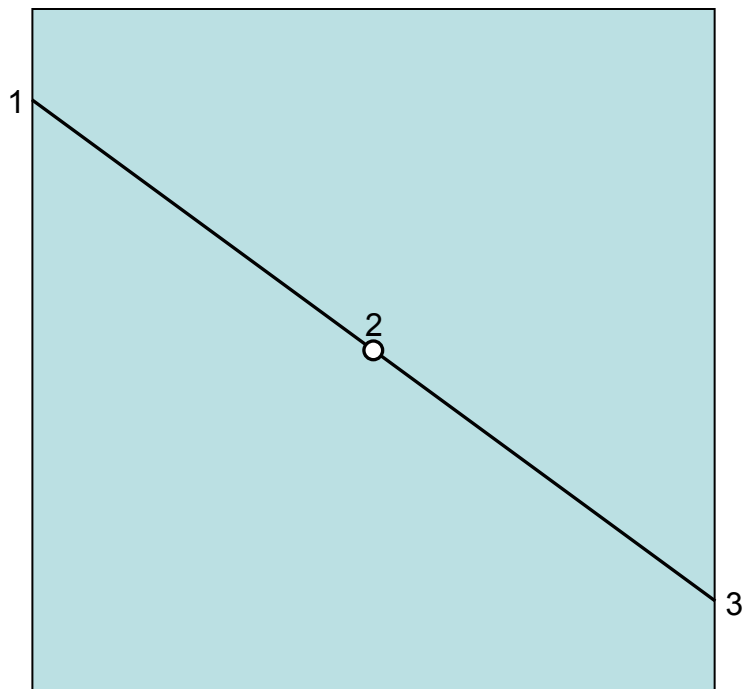


Original Transform Plane

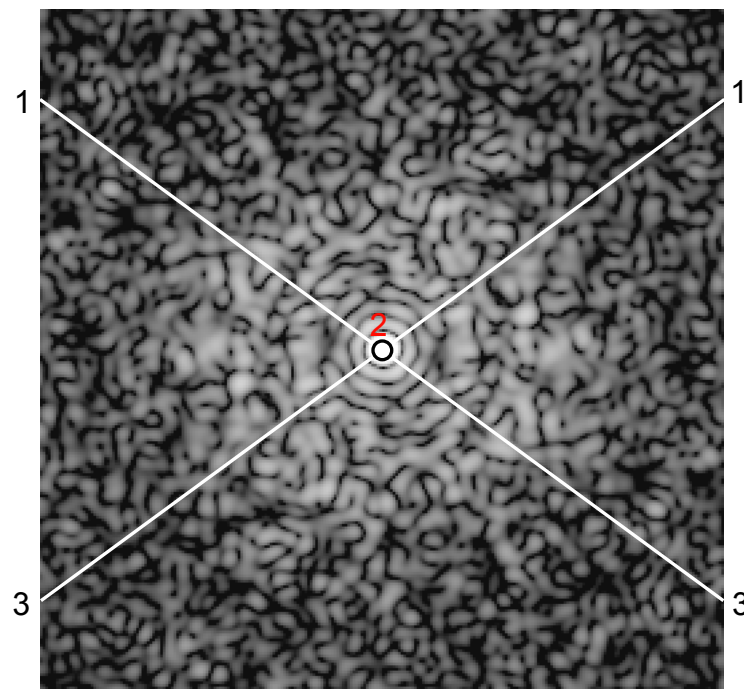




# Orientation Determination by Common Lines



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:

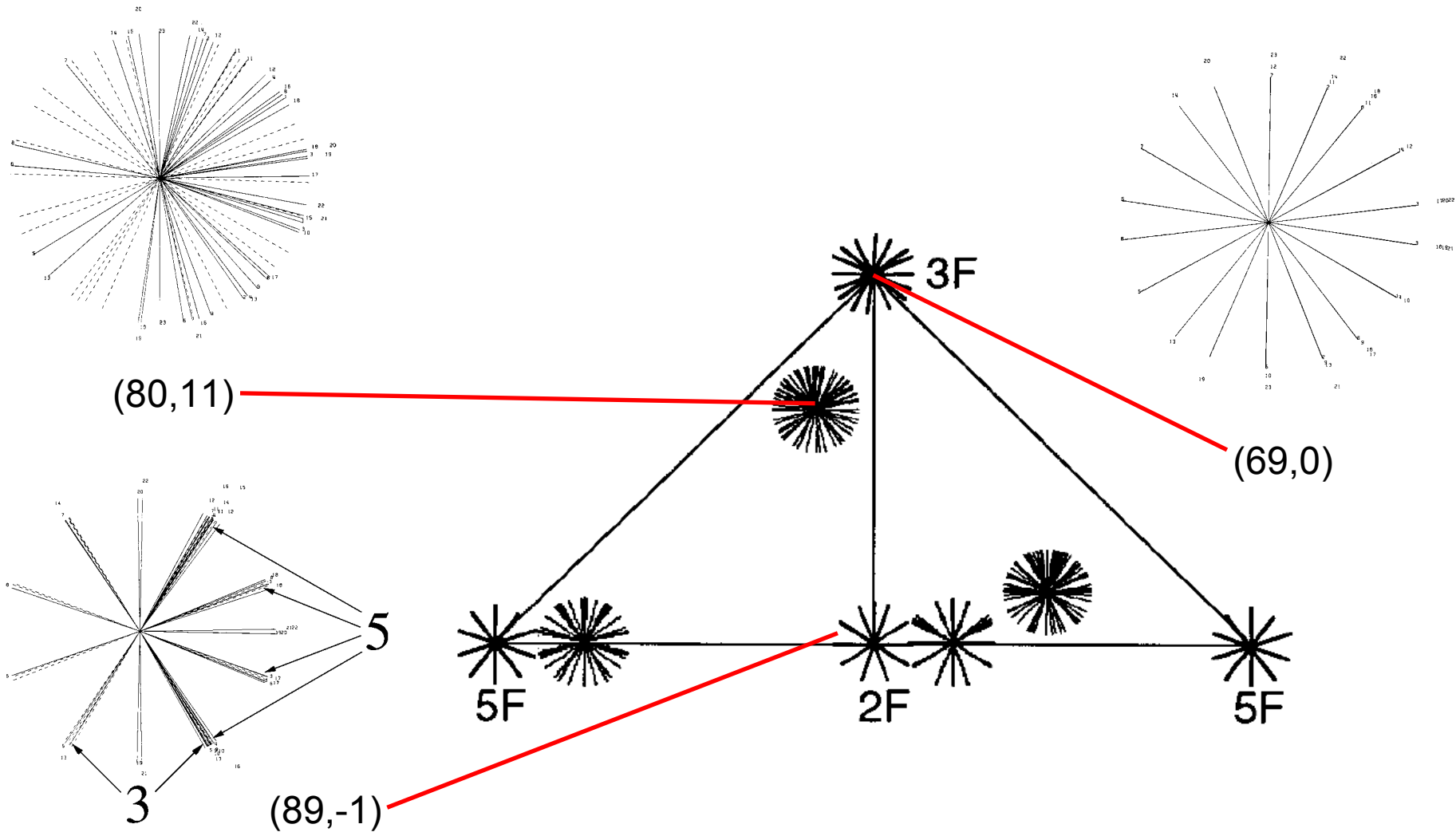
$$\text{5-folds: } \frac{12}{2} \times 2 = 12 \text{ pairs}$$

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

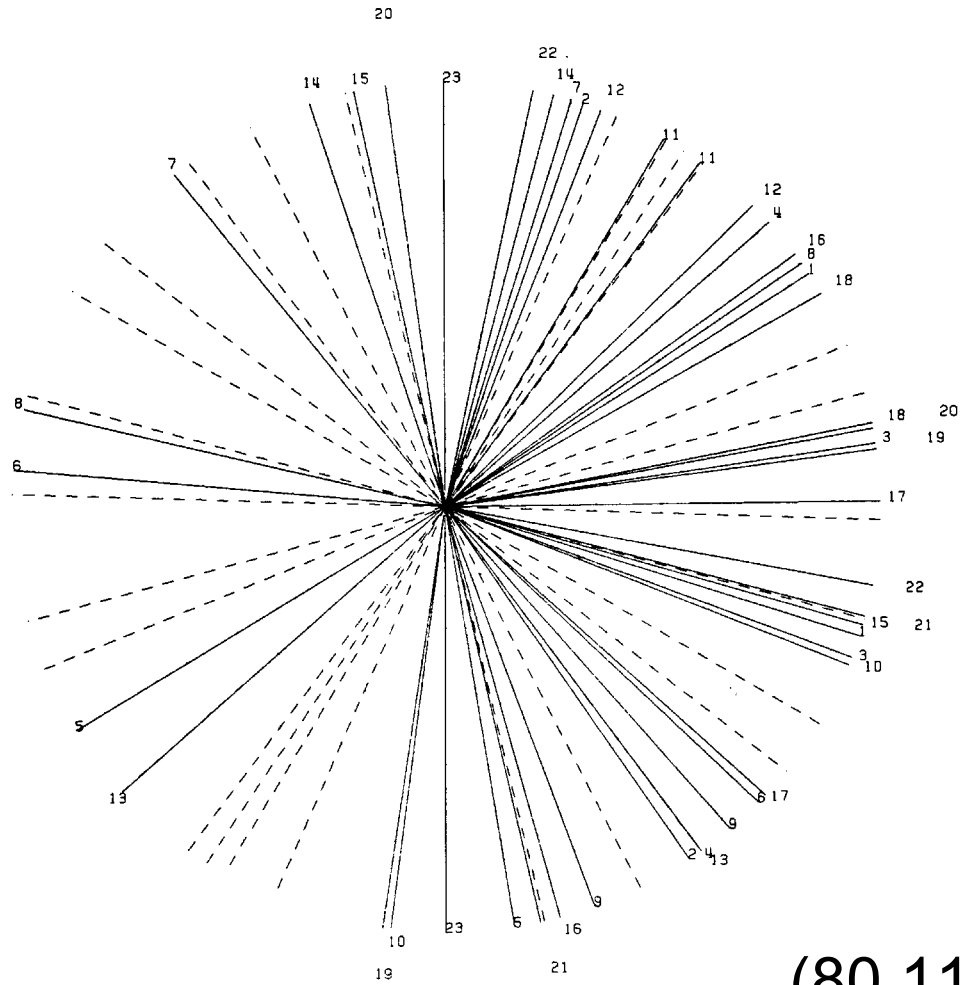
$$\text{2-folds: } \frac{30}{2} \times 1 = \underline{15} \text{ real lines}$$

37 common lines

# Orientation Determination by Common Lines

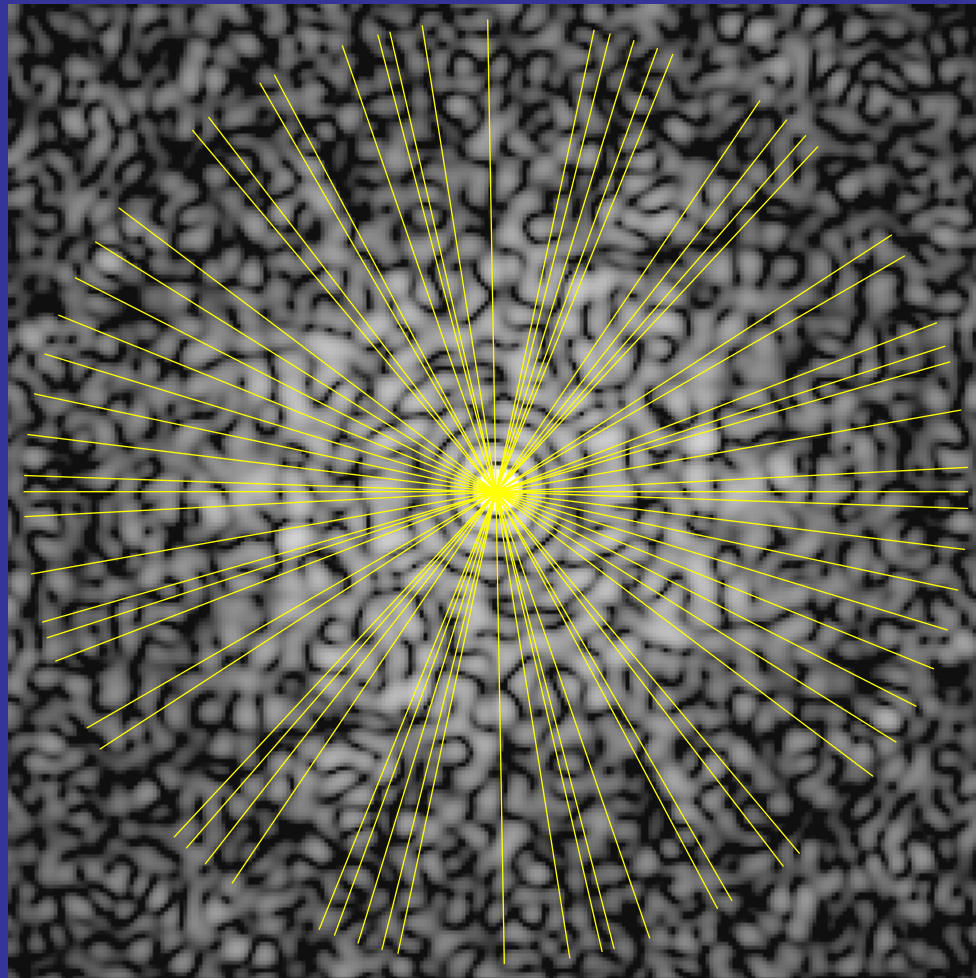


# Orientation Determination by Common Lines



(80,11)

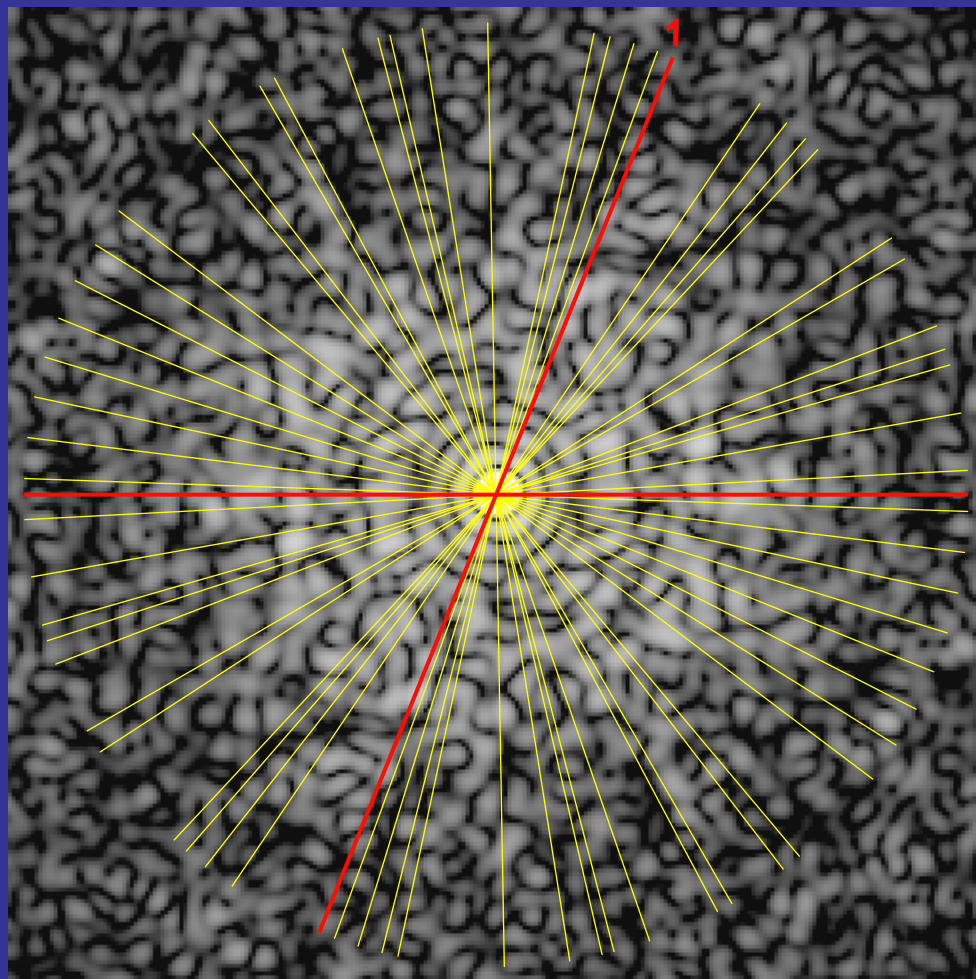
# Orientation Determination by Common Lines



(80,11)

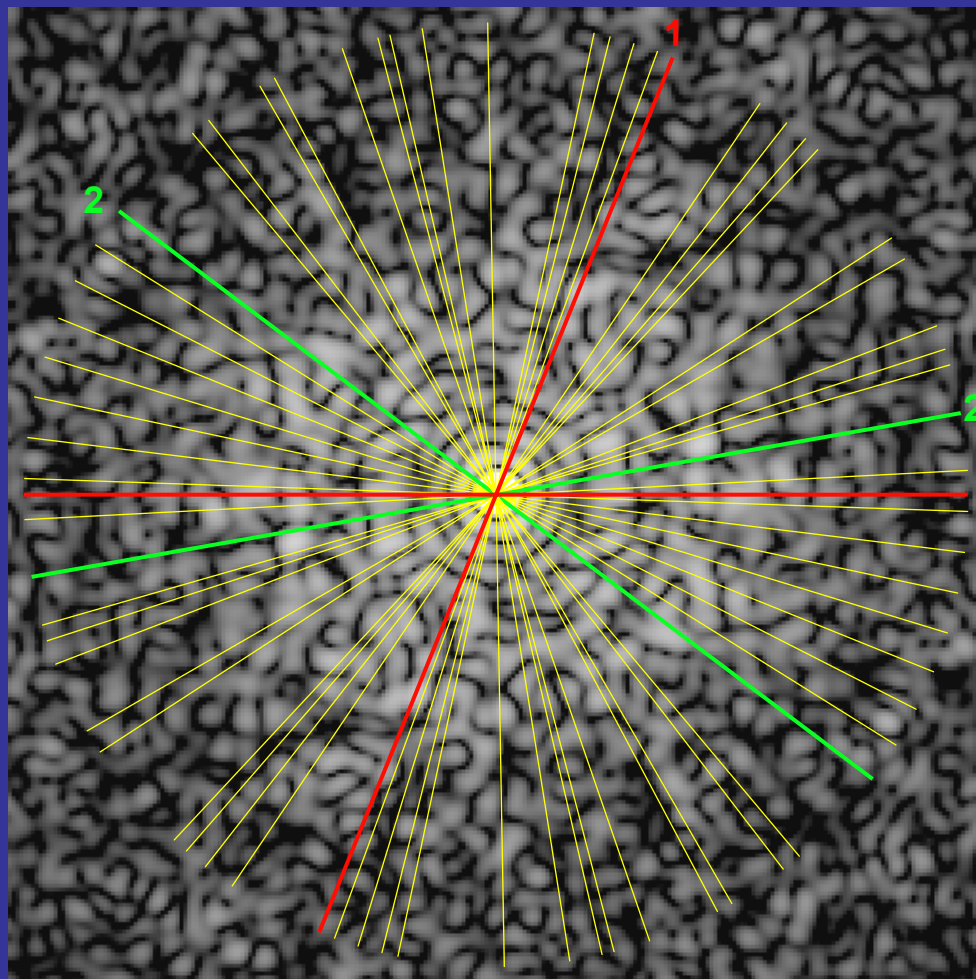
What is  $(\theta, \phi, \omega)$  for this particle?

# Orientation Determination by Common Lines

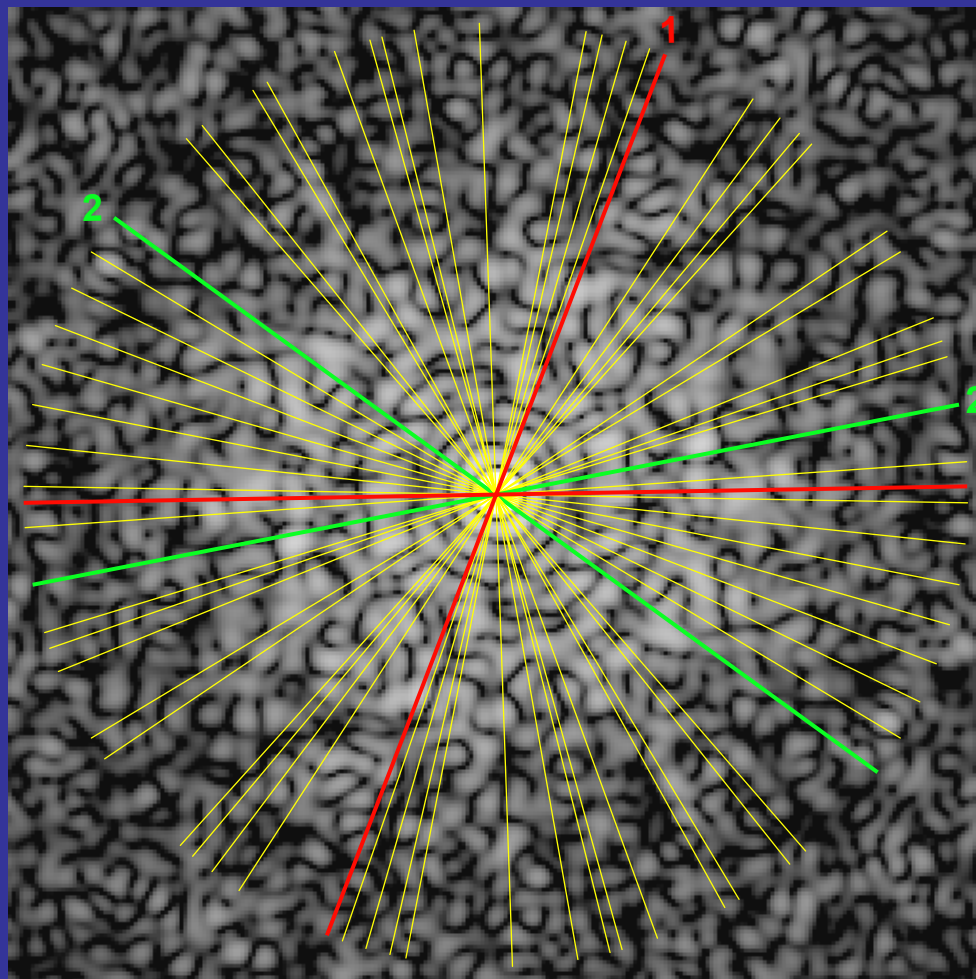


$\omega$   
↓  
(80, 11, 0)

# Orientation Determination by Common Lines

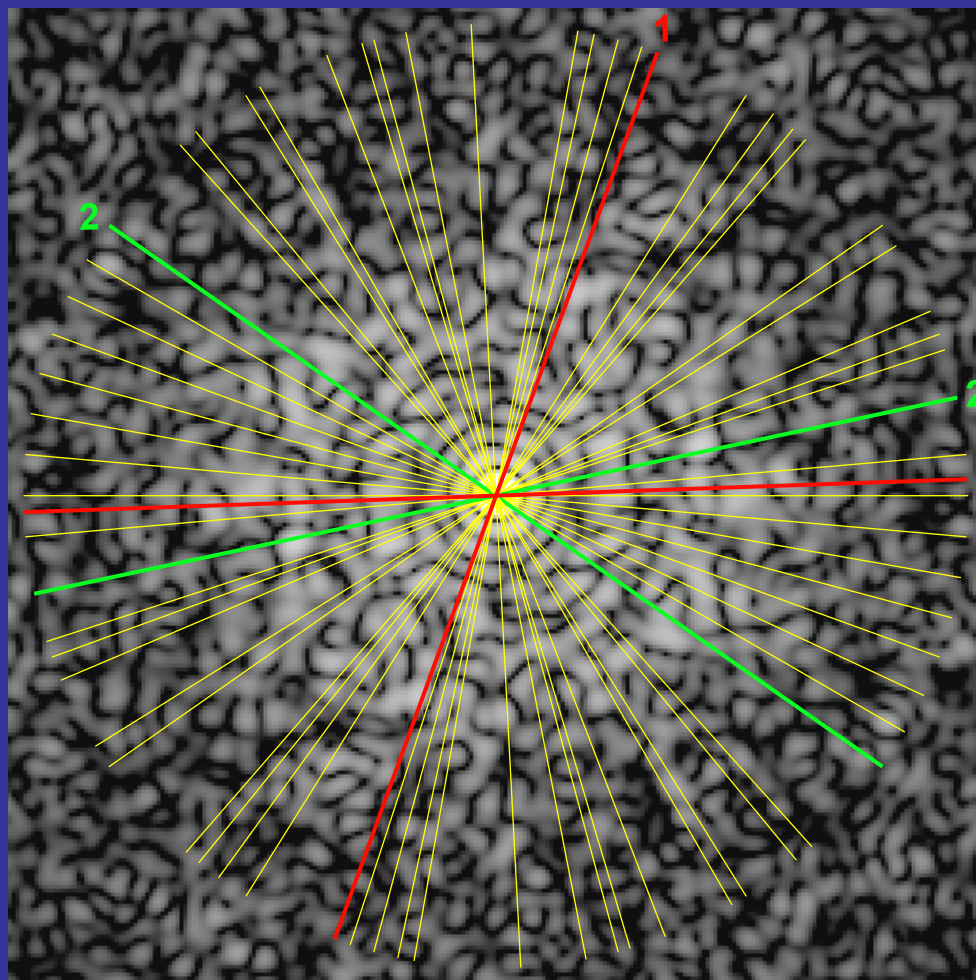


# Orientation Determination by Common Lines

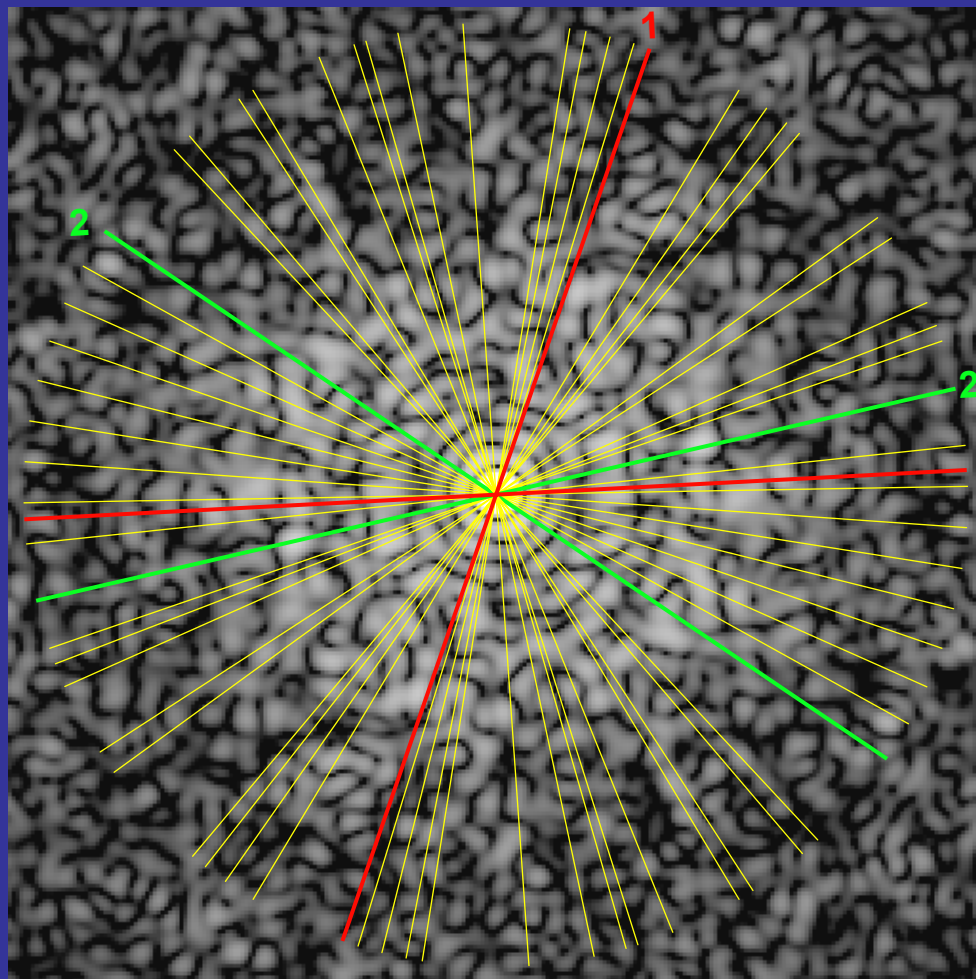




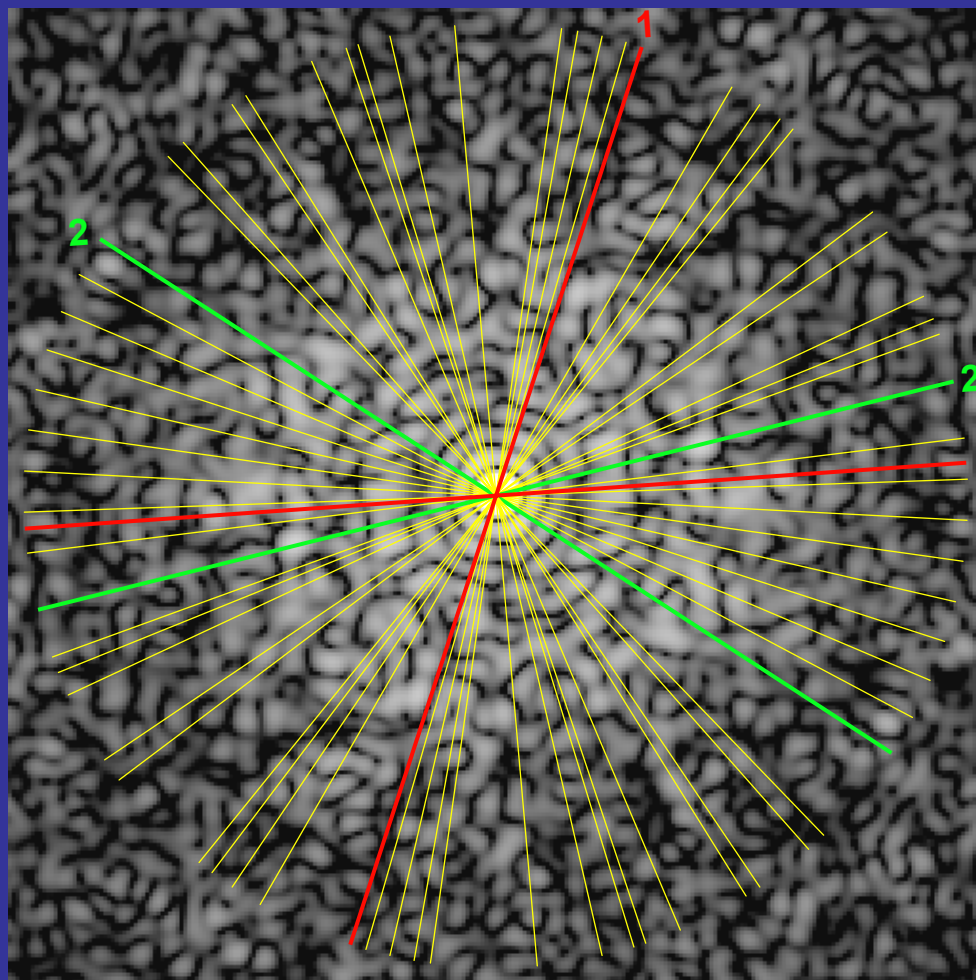
# Orientation Determination by Common Lines



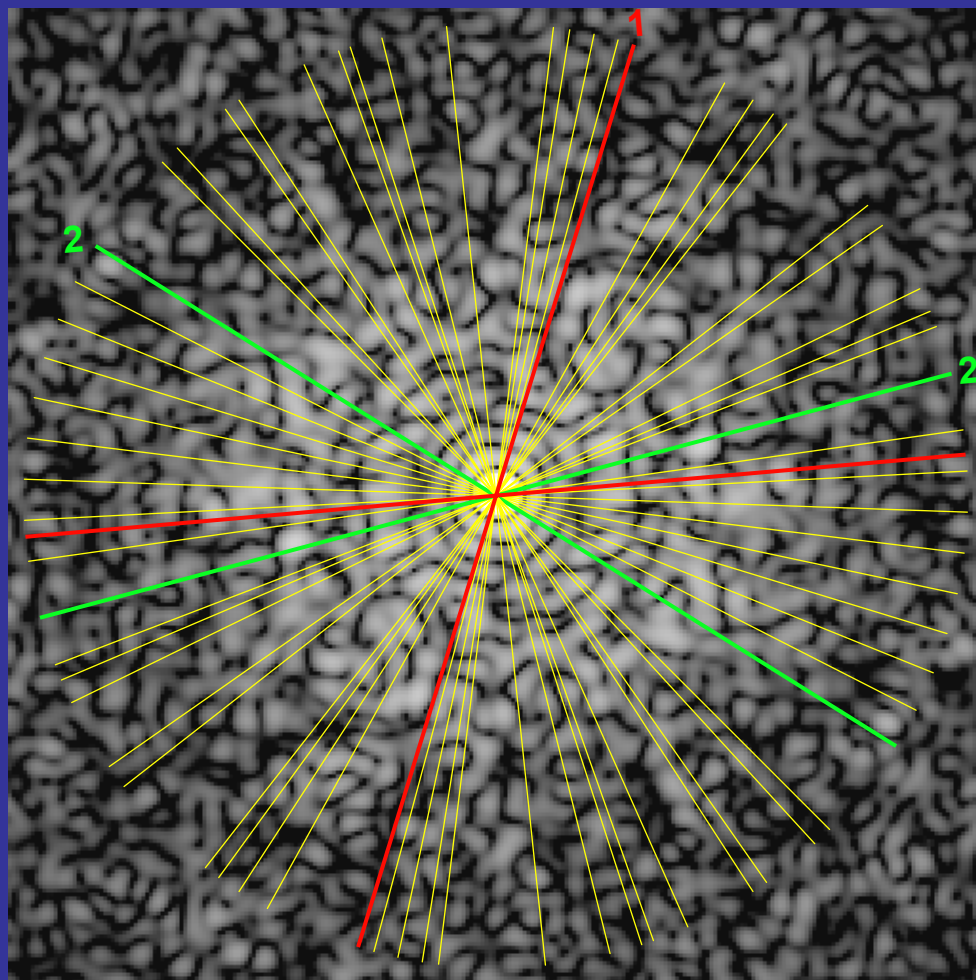
# Orientation Determination by Common Lines



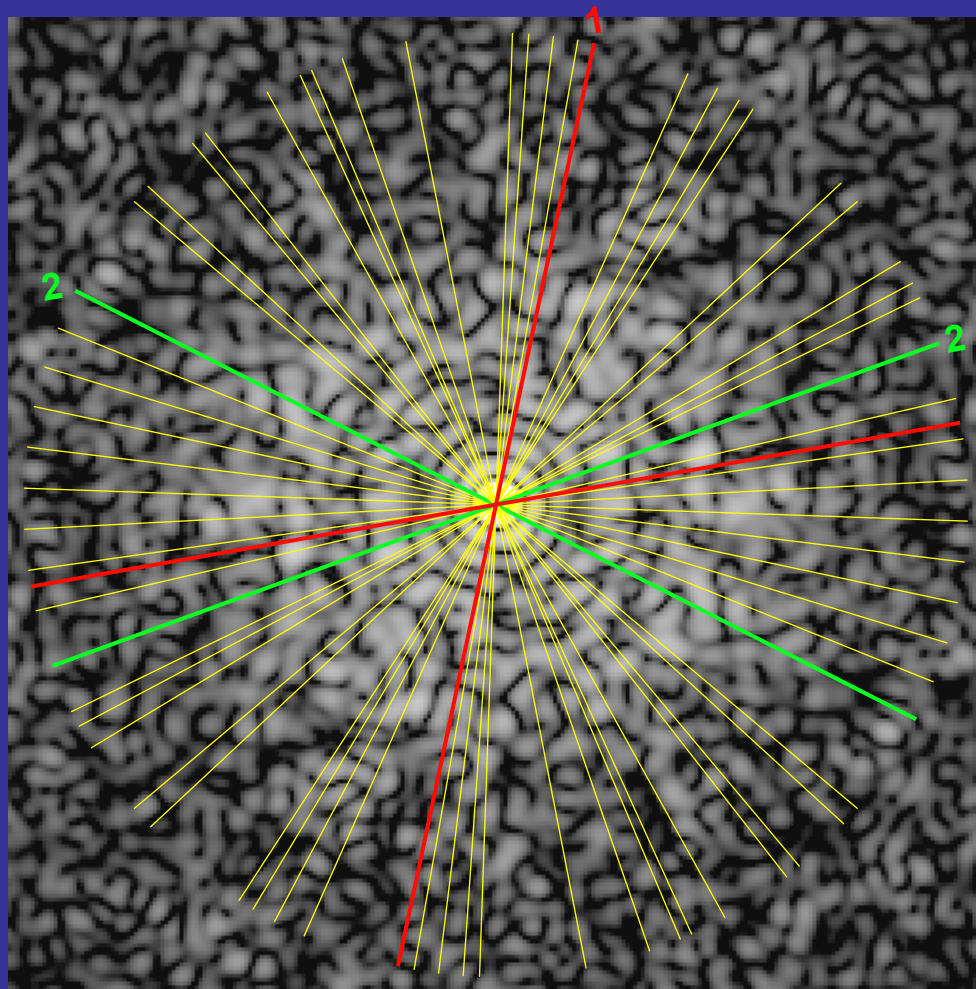
# Orientation Determination by Common Lines



# Orientation Determination by Common Lines

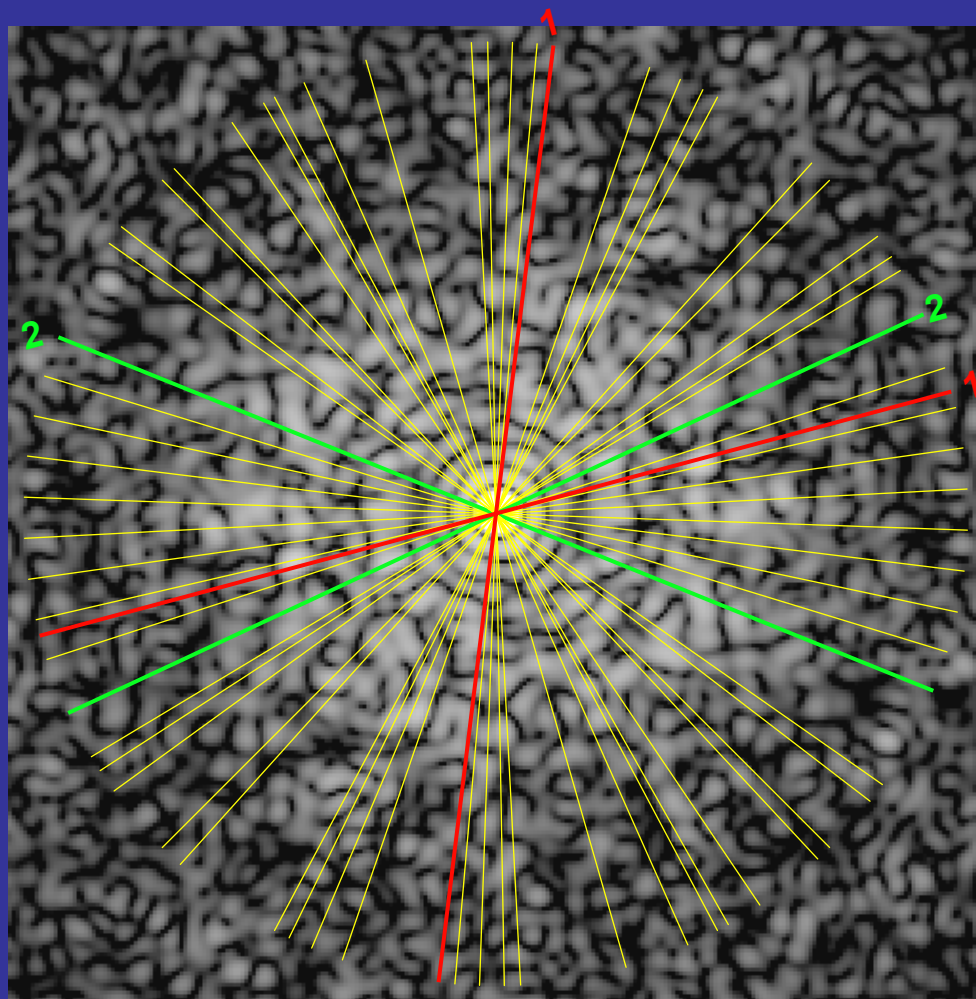


# Orientation Determination by Common Lines

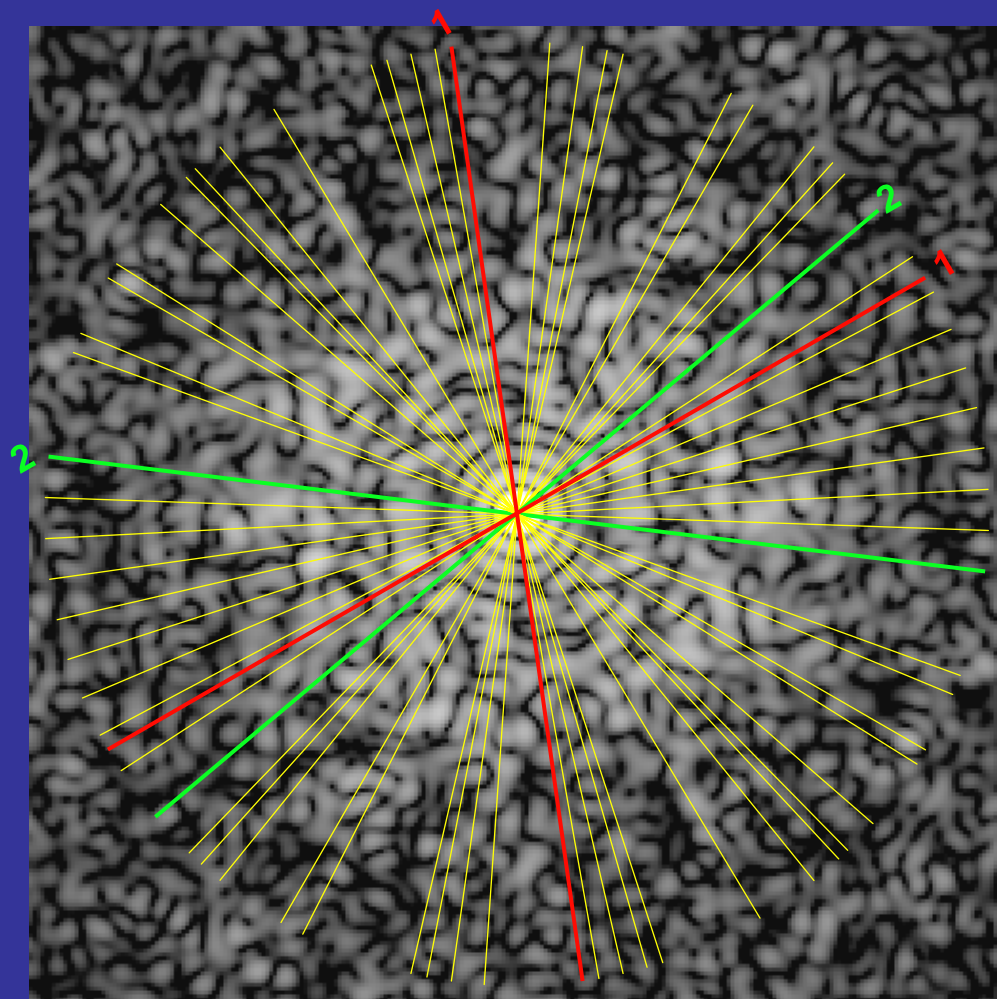


$\omega$   
↓  
(80, 11, 10)

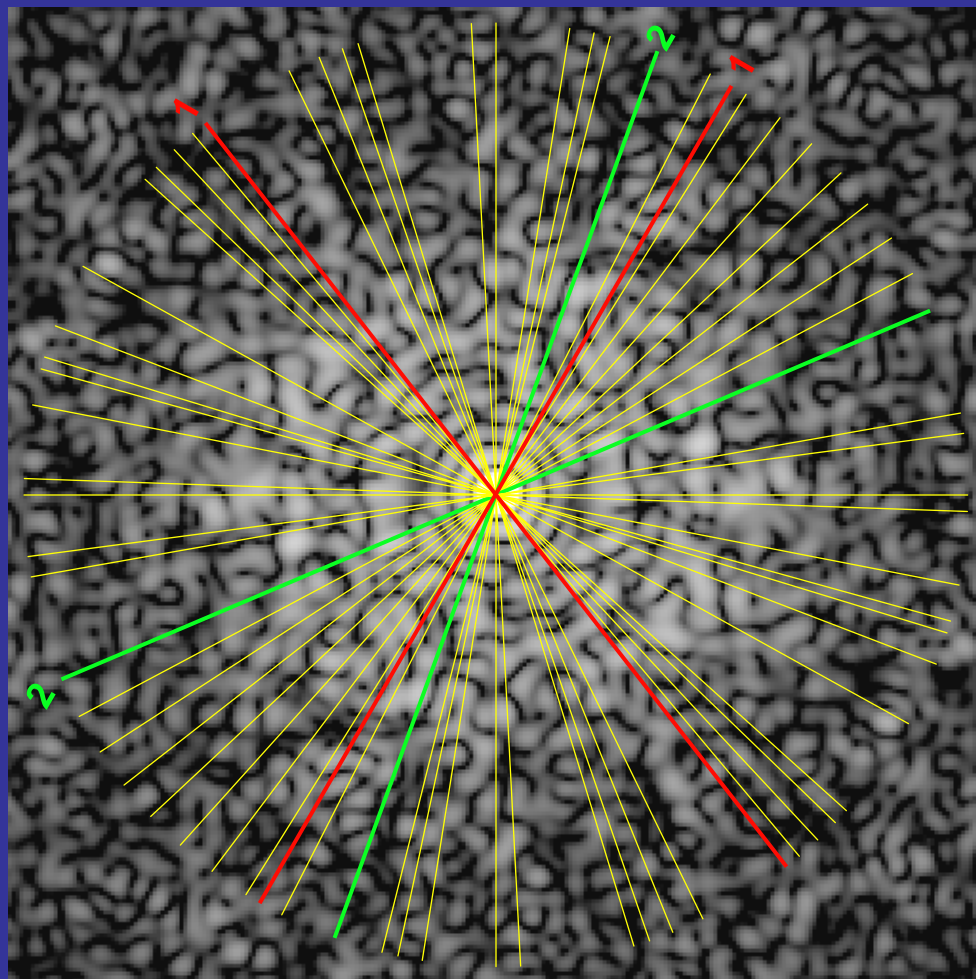
# Orientation Determination by Common Lines



# Orientation Determination by Common Lines

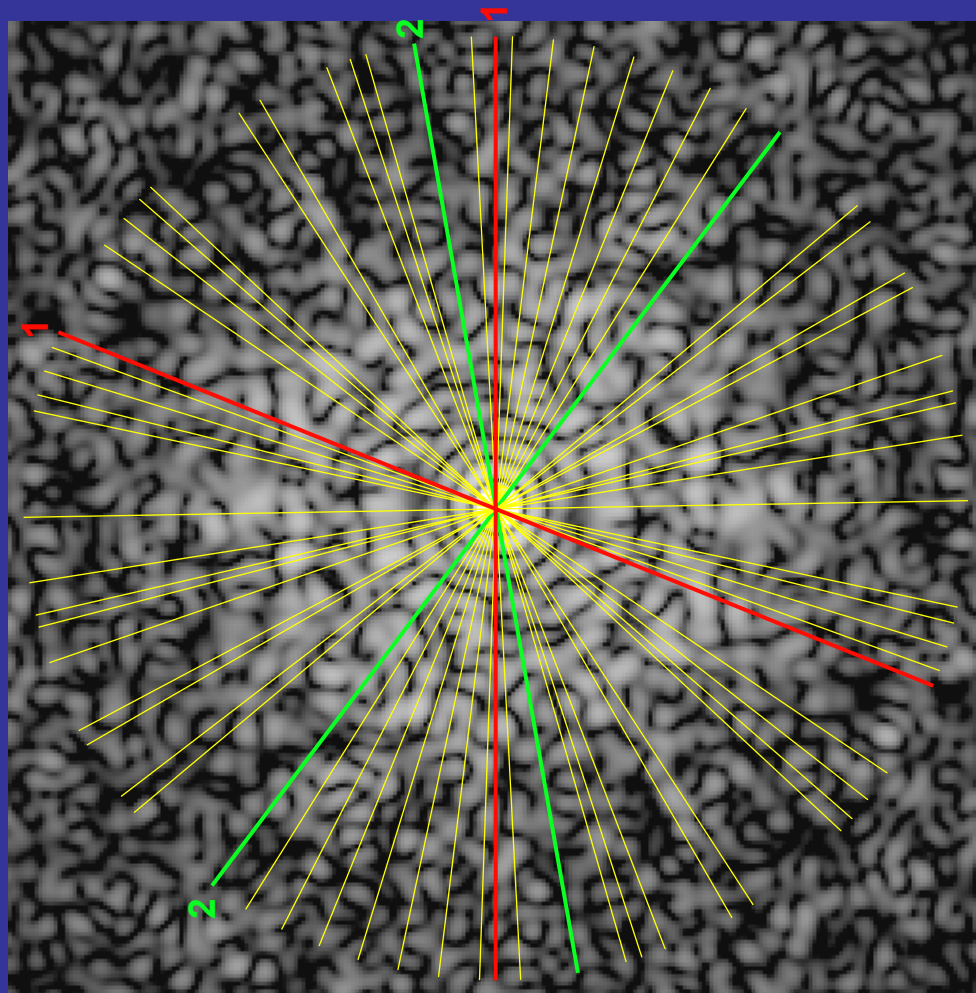


# Orientation Determination by Common Lines



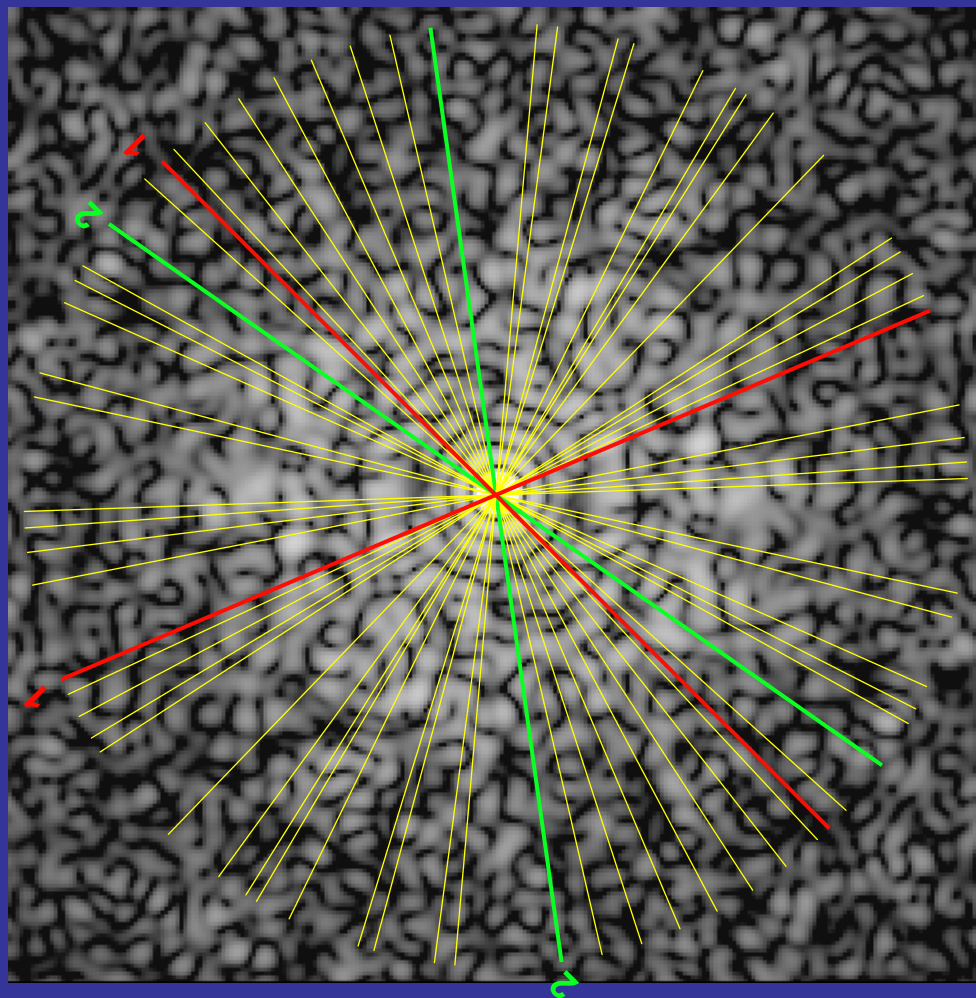


# Orientation Determination by Common Lines



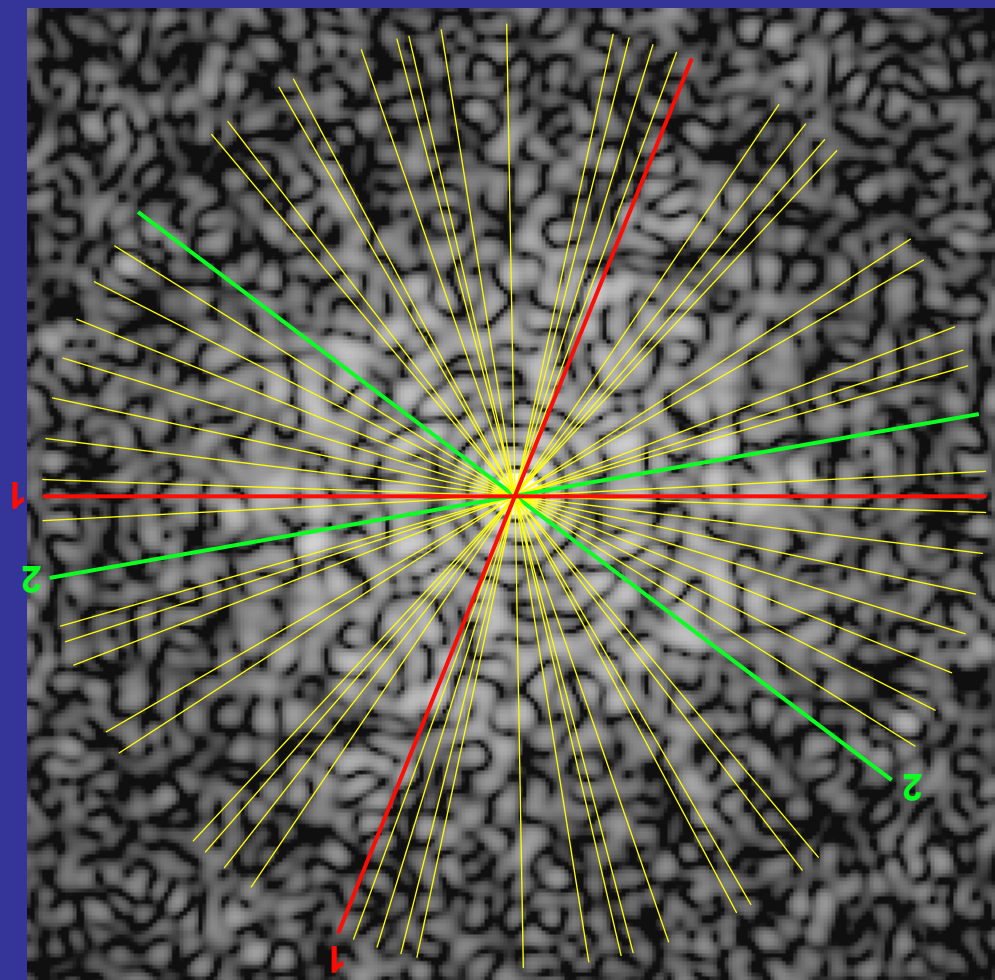
$\omega$   
 $(80, 11, 90)$

# Orientation Determination by Common Lines



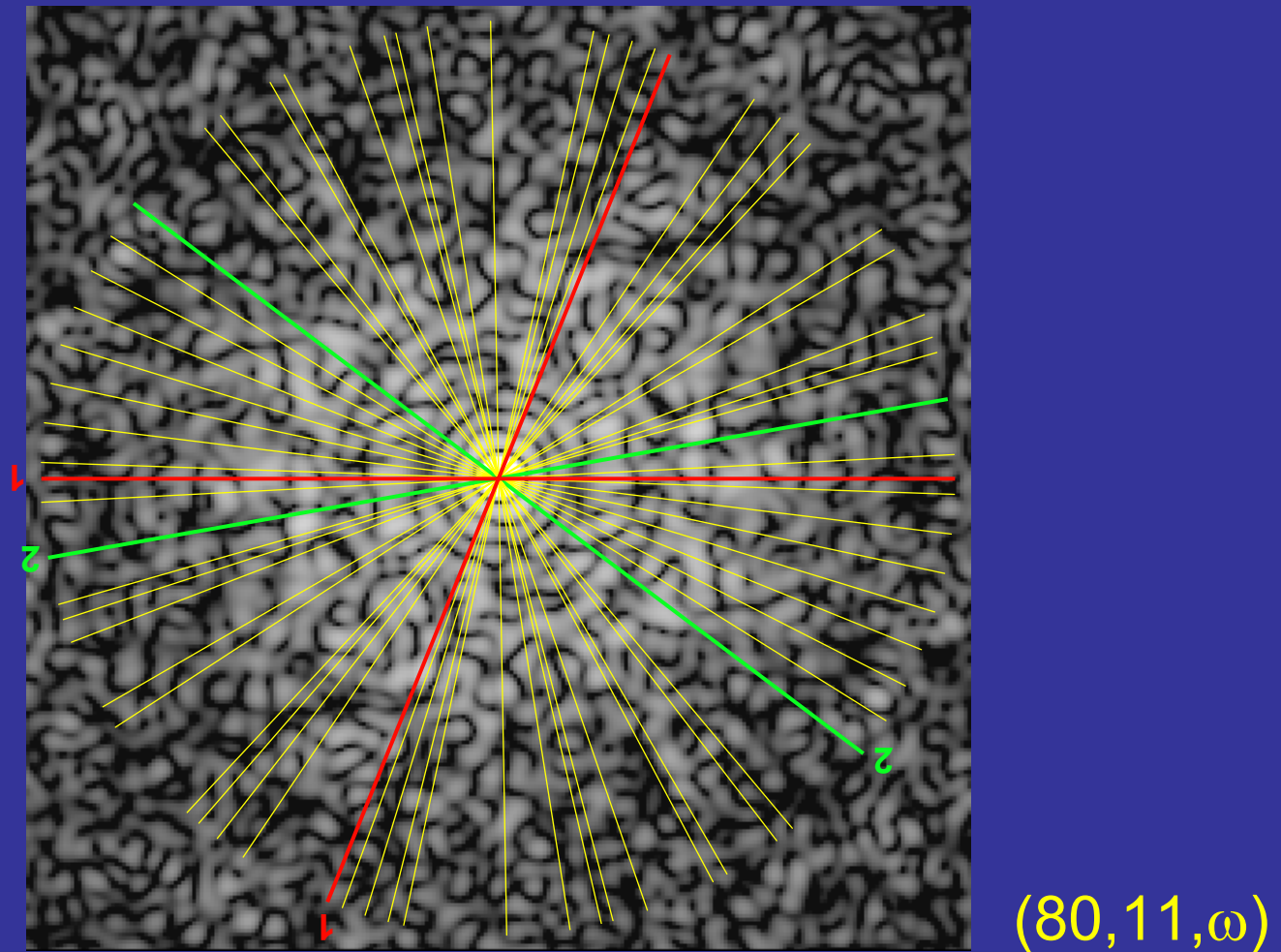
$\omega$   
↓  
(80, 11, 135)

# Orientation Determination by Common Lines



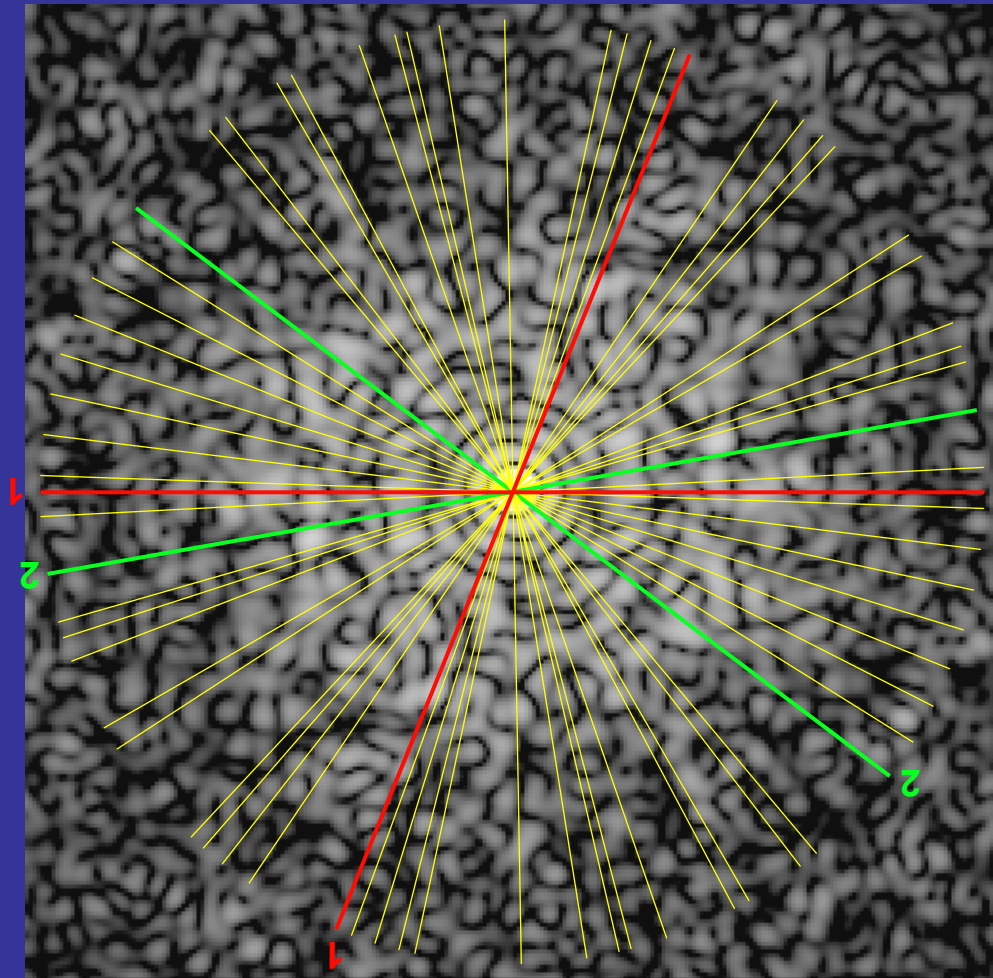
$\omega$   
↓  
(80, 11, 180)

# Orientation Determination by Common Lines



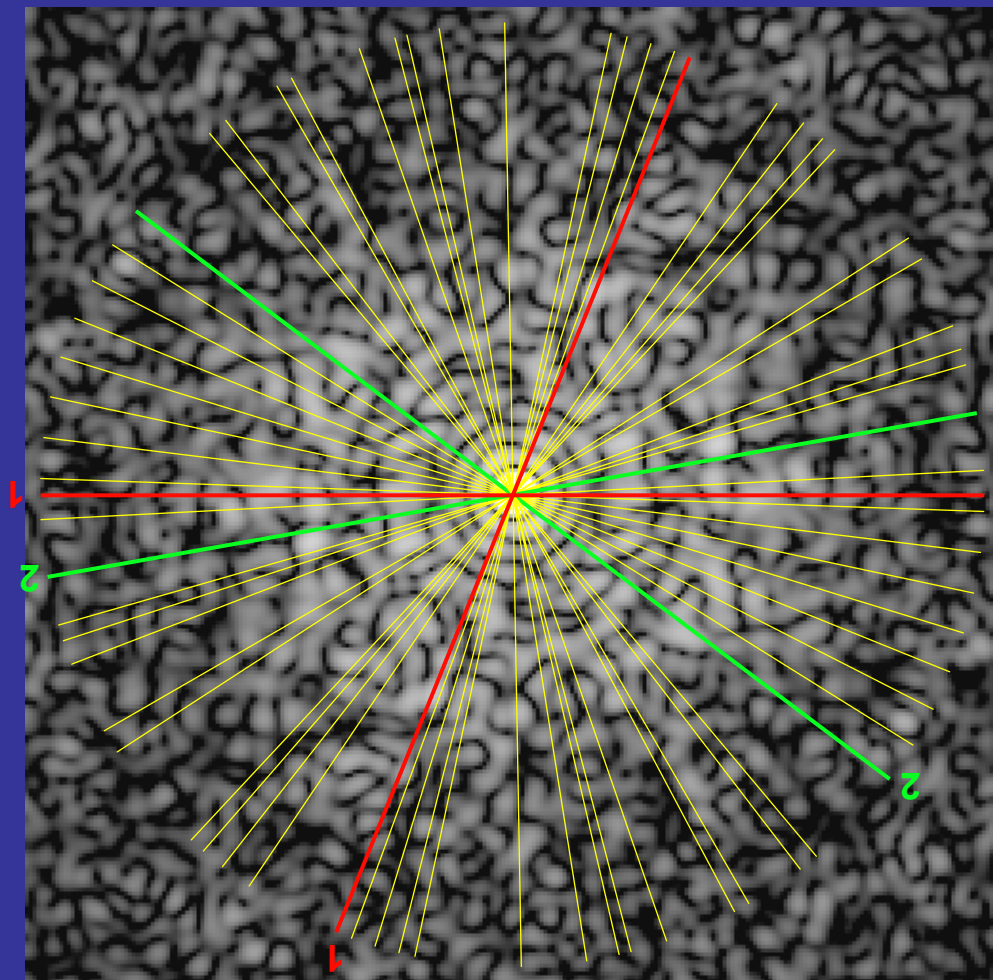
Metric: Identify  $\omega$  that gives lowest phase residual

# Orientation Determination by Common Lines



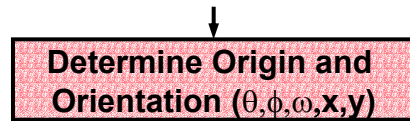
Repeat process for all possible  $(\theta, \phi, \omega)$  combinations

# Orientation Determination by Common Lines



> 250,000 combinations for  $1^\circ$  angular search intervals

# Icosahedral Virus 3D Reconstruction Scheme



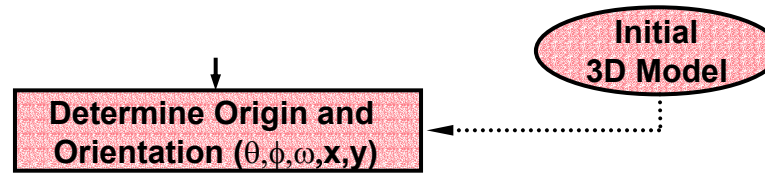
## Common Lines

The  $(\theta, \phi, \omega)$  that results in the lowest phase residual is selected as the best estimate for the particle view orientation

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

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

# Icosahedral Virus 3D Reconstruction Scheme



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

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

Bulk of structures now solved this way

Details discussed in practical session



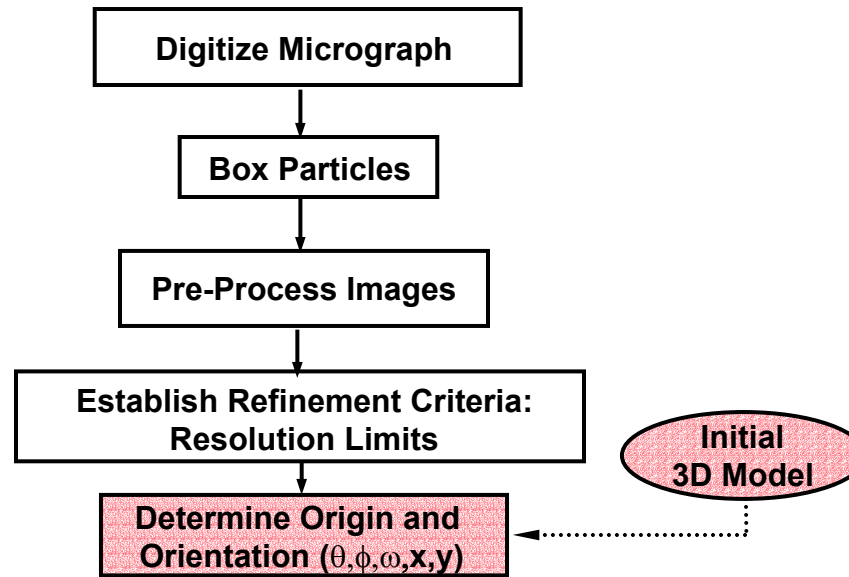
# Icosahedral Virus 3D Reconstruction Scheme

Initial  
3D Model

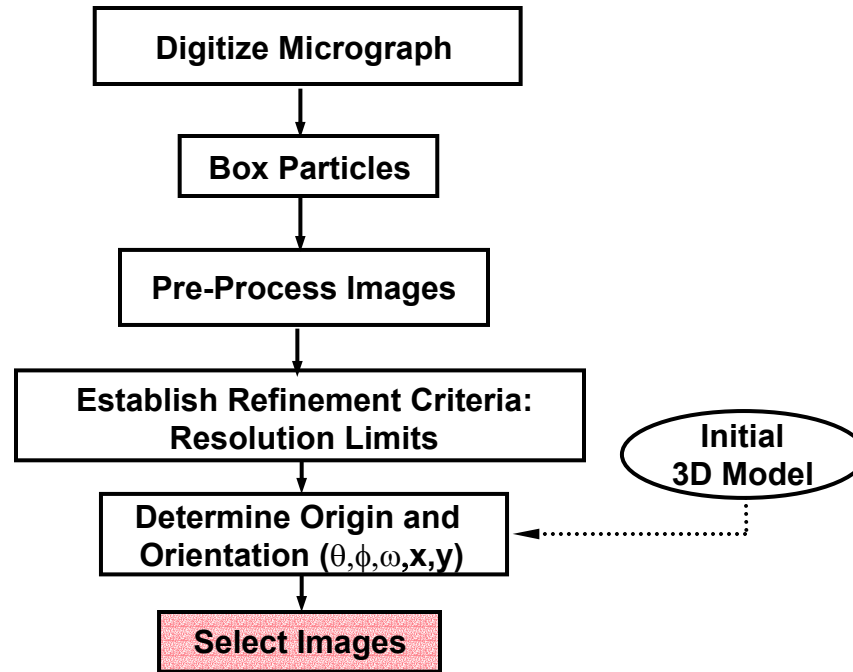
Determine Origin and  
Orientation ( $\theta, \phi, \omega, x, y$ )

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

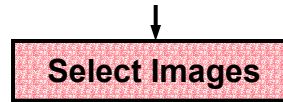
# Icosahedral Virus 3D Reconstruction Scheme



# Icosahedral Virus 3D Reconstruction Scheme



# Icosahedral Virus 3D Reconstruction Scheme



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

# Icosahedral Virus 3D Reconstruction Scheme

Select Images

ID	$\theta$	$\phi$	$\omega$	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

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,	0,667
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,	0,899,	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

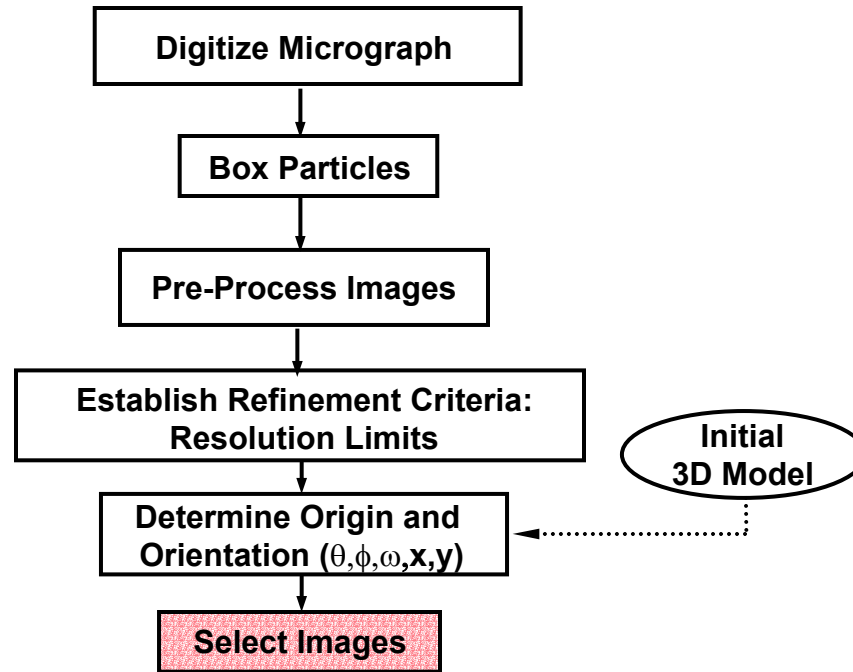
```
Shell - Konsole <2>
Session Edit View Settings Help
gandalf # more hsv_float.dat_001
1856_grad_float.pif
13.8800, 1, 100000.0, 0.0700, 1.5000, 1.5000, 0.0000, 2.00
1, 80.000, -13.201, 313.594, 48.751, 48.849, 1.015, 0.492, 0.713, 0.557
2, 85.000, 23.088, 49.922, 49.387, 48.429, 1.010, 0.598, 0.725, 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 0.005
PFT Coefficient 0.679 0.075
PRJ Coefficient 0.756 0.023
CMP Coefficient 0.609 0.038
PFT finished.
*****
gandalf #
```

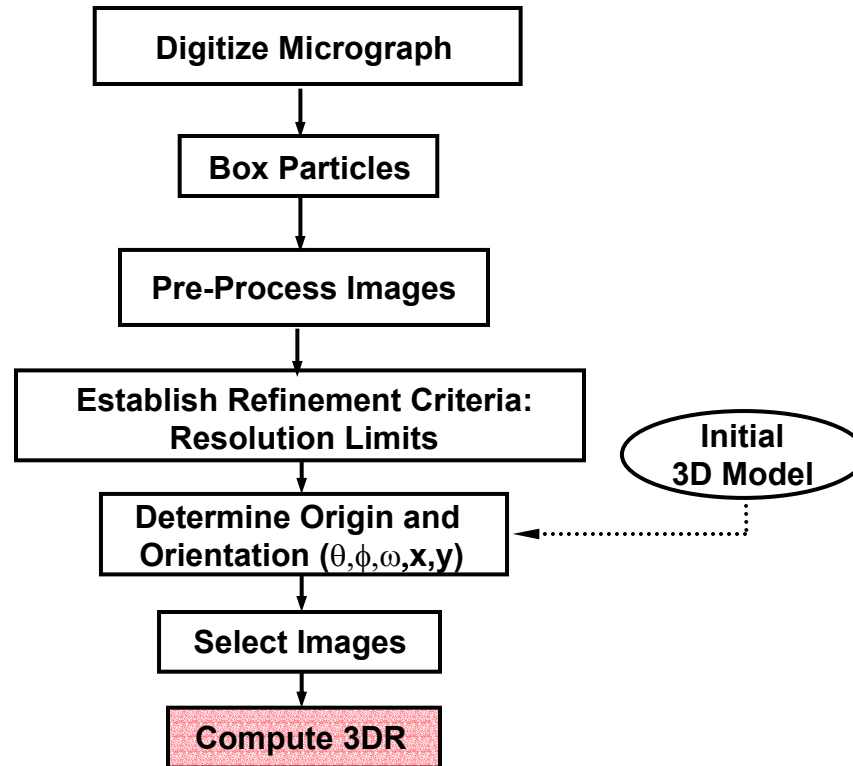
PFT Coefficient  $0.679 \pm 0.075$

# Icosahedral Virus 3D Reconstruction Scheme

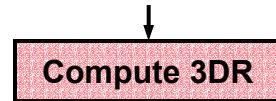




# Icosahedral Virus 3D Reconstruction Scheme



# Icosahedral Virus 3D Reconstruction Scheme

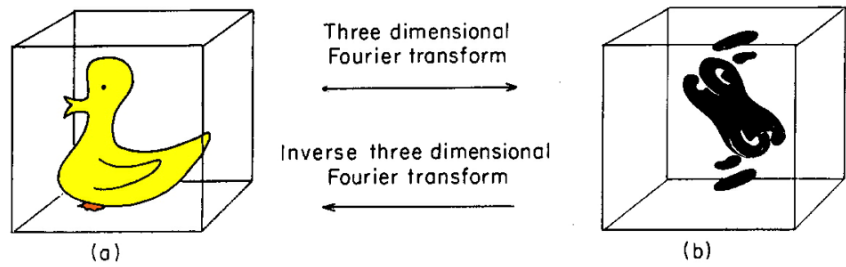


Goal: combine “good” particle images to compute a 3D density map

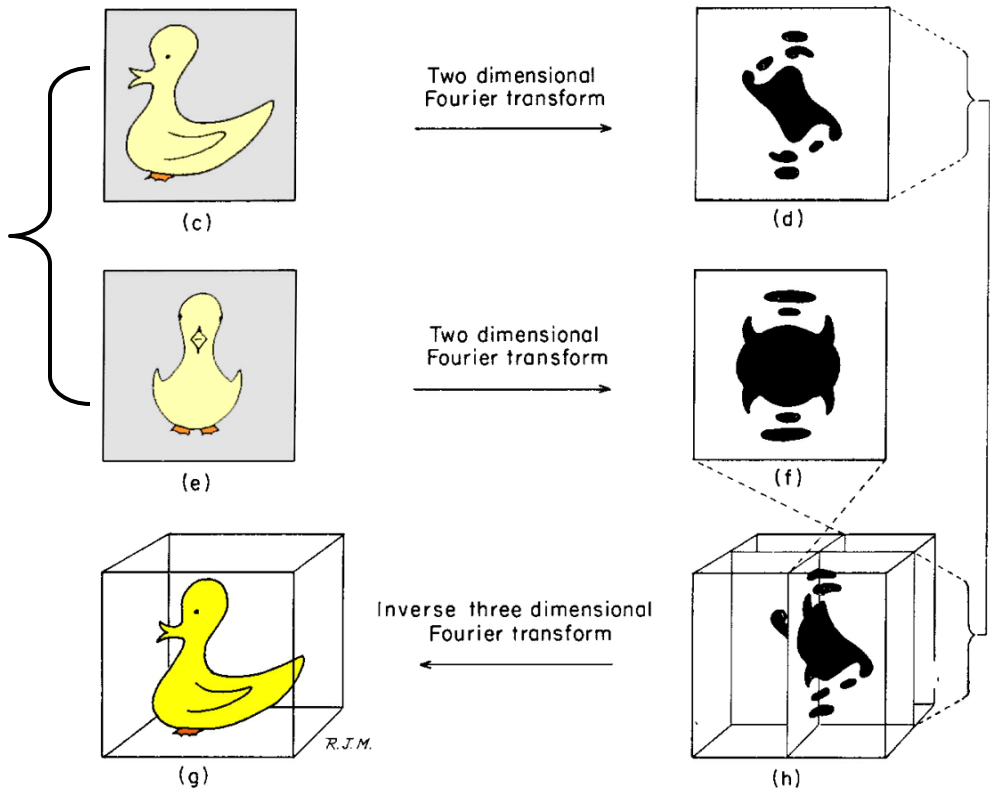
# Icosahedral Virus 3D Reconstruction Scheme

↓  
**Compute 3DR**

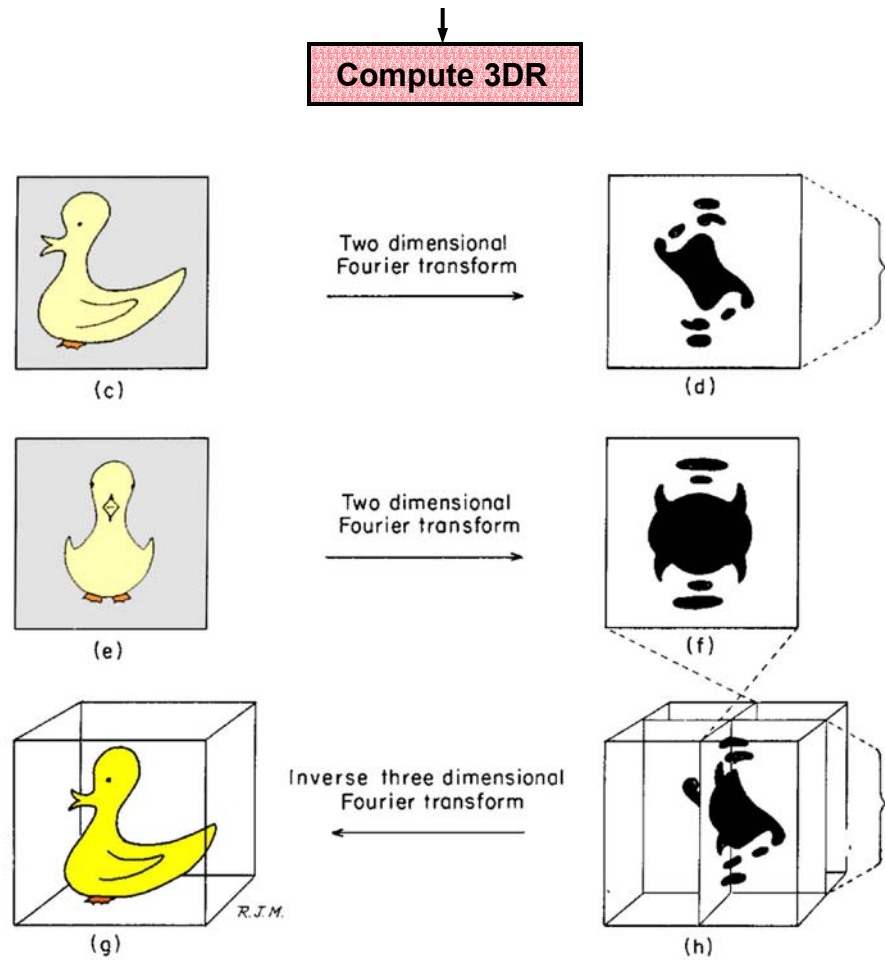
In theory



In practice



# Icosahedral Virus 3D Reconstruction Scheme



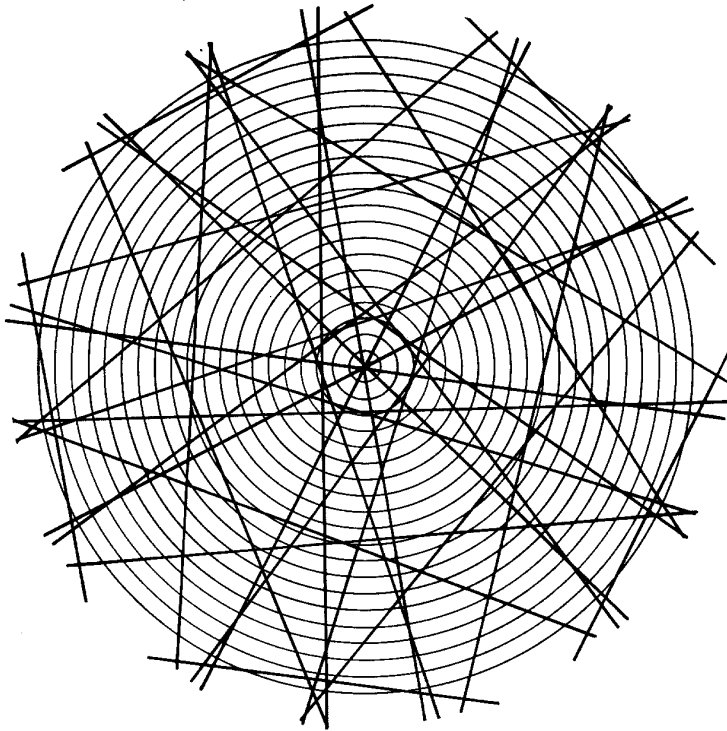
**Overall scheme:  $\rho \leftarrow g \leftarrow G \leftarrow F$**

# Icosahedral Virus 3D Reconstruction Scheme

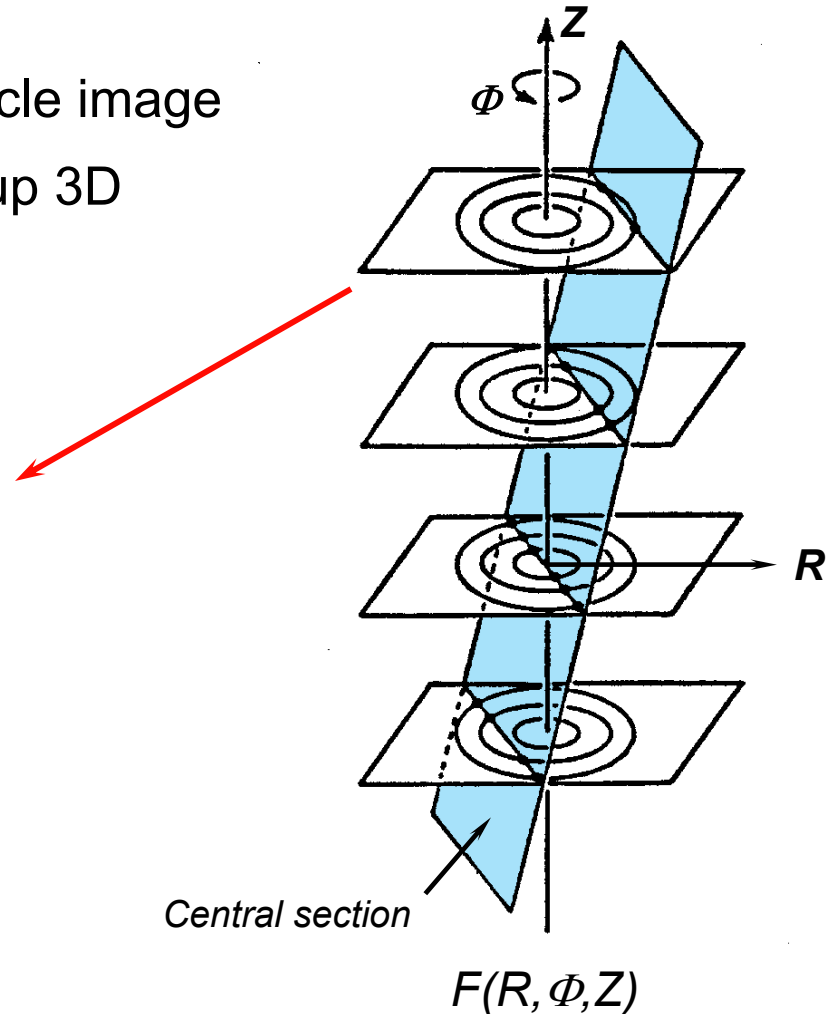
↓  
Compute 3DR

## Steps:

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

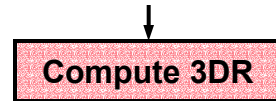


Crowther, DeRosier and Klug, 1970, p.329



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

# Icosahedral Virus 3D Reconstruction Scheme

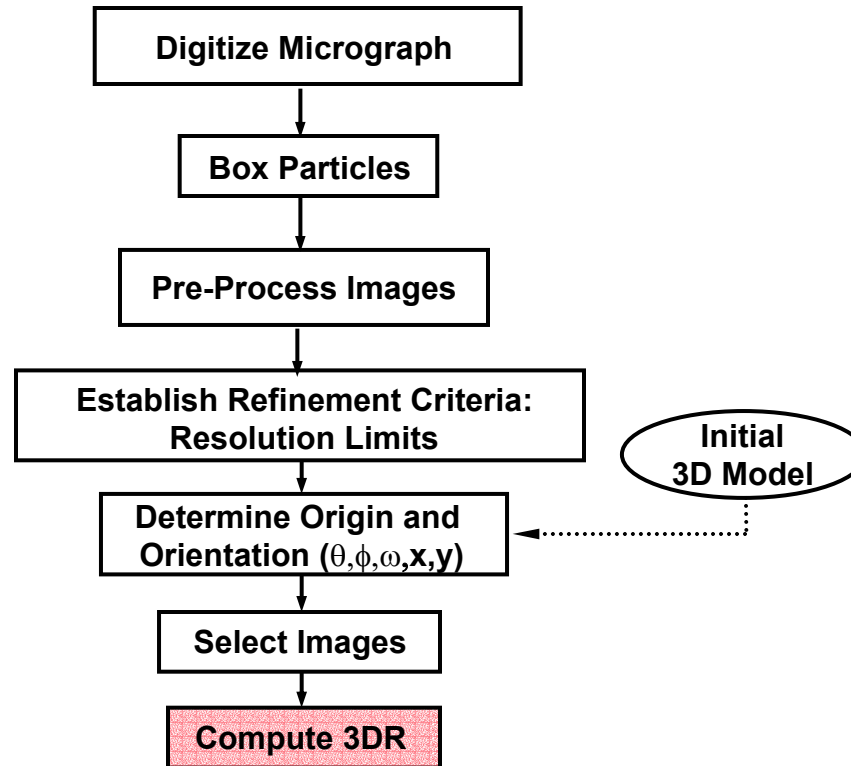


$$\rho \leftarrow \mathbf{g} \leftarrow \mathbf{G} \leftarrow \mathbf{F}$$

## Steps:

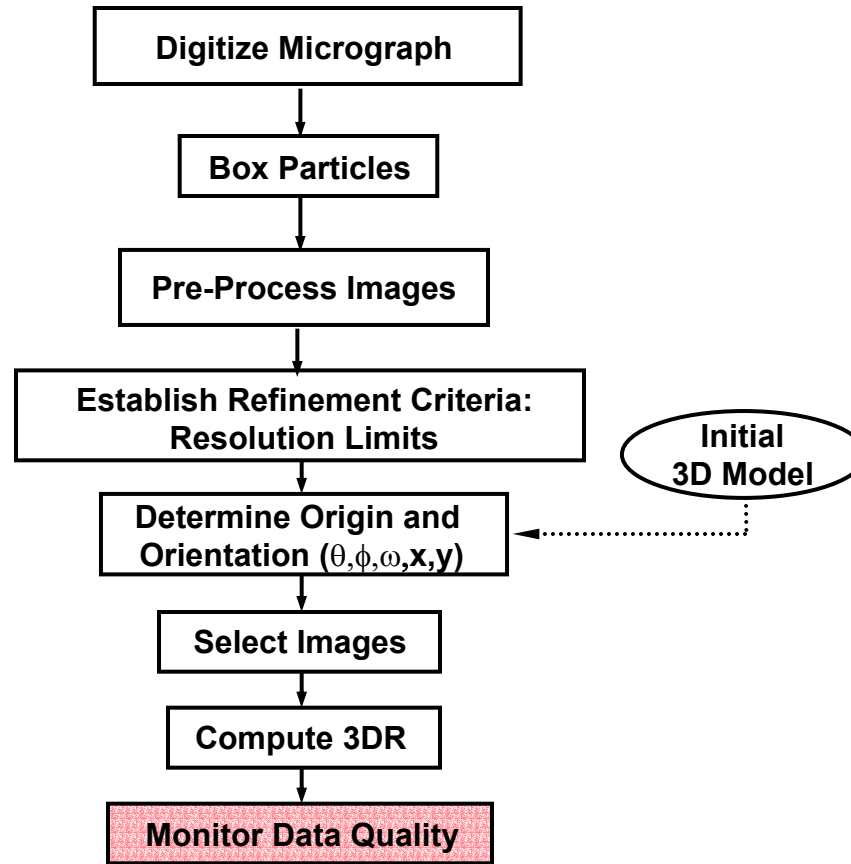
1. Compute 2D FFT of each particle image
2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform
3. Compute  $G_n$ 's on each annulus  $G = (B^\dagger B)^{-1} B^\dagger F$
4. Compute  $g_n$ 's from  $G_n$ 's (Fourier-Bessel transform)
5. Compute polar density map ( $\rho(r, \phi, z)$ ) from  $g_n$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





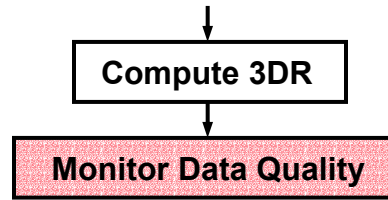
# Icosahedral Virus 3D Reconstruction Scheme



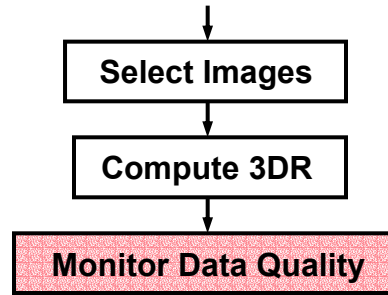
Monitor Data Quality

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

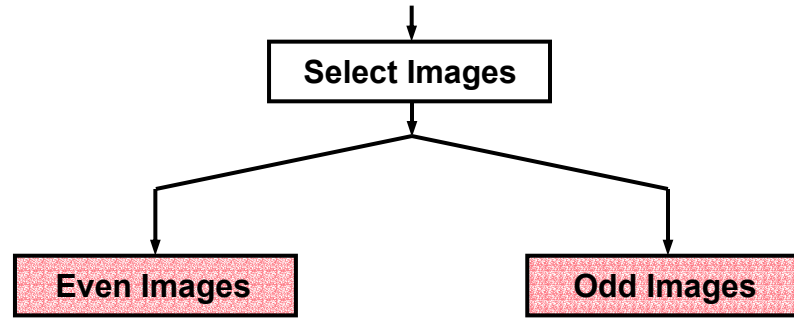
# Icosahedral Virus 3D Reconstruction Scheme



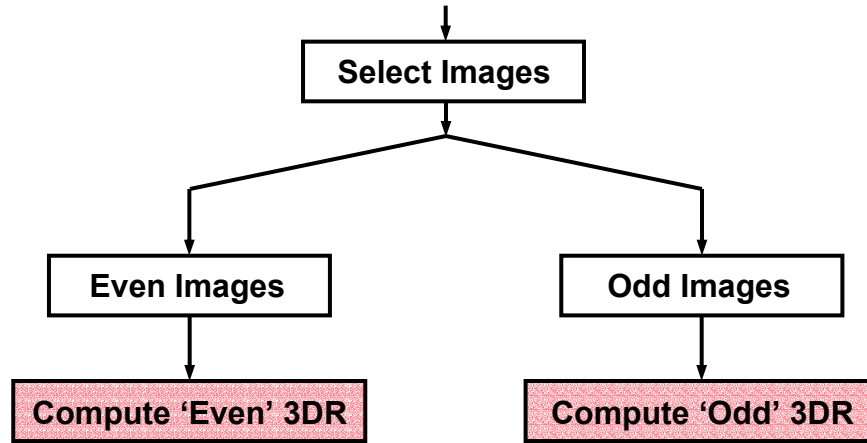
# Icosahedral Virus 3D Reconstruction Scheme



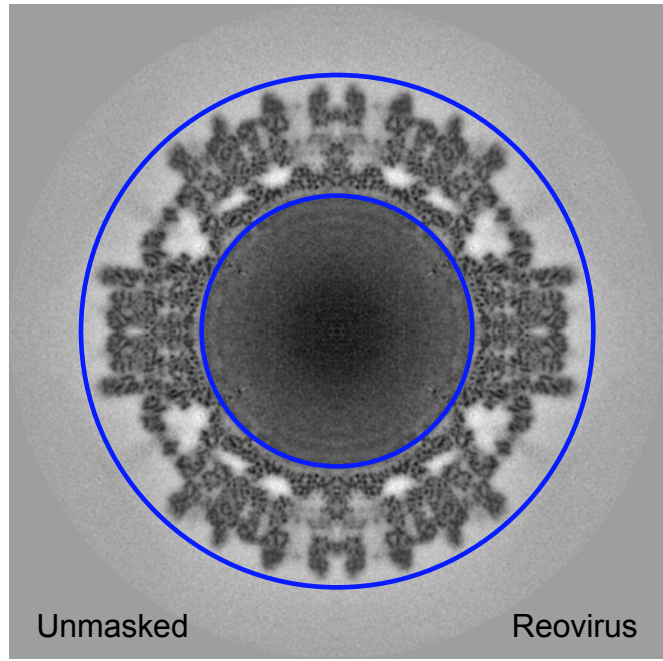
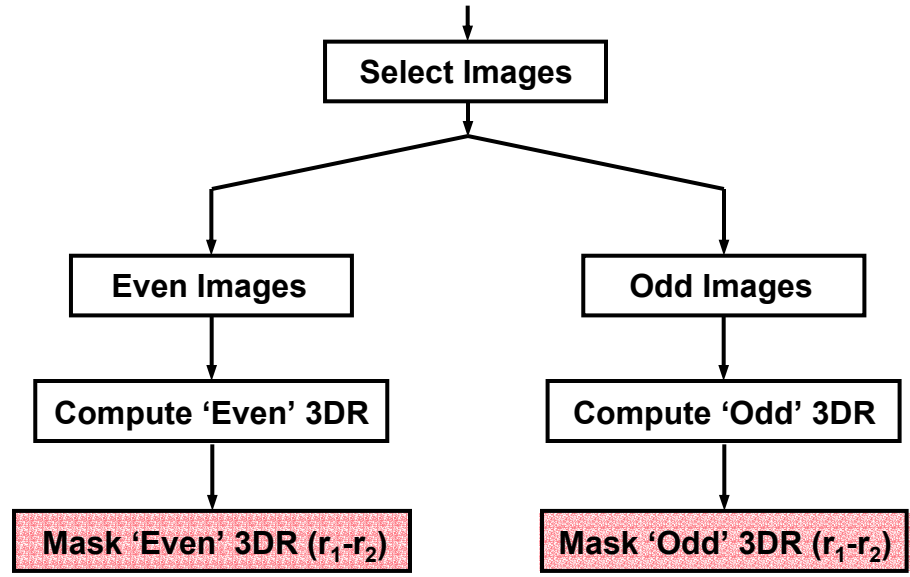
# Icosahedral Virus 3D Reconstruction Scheme



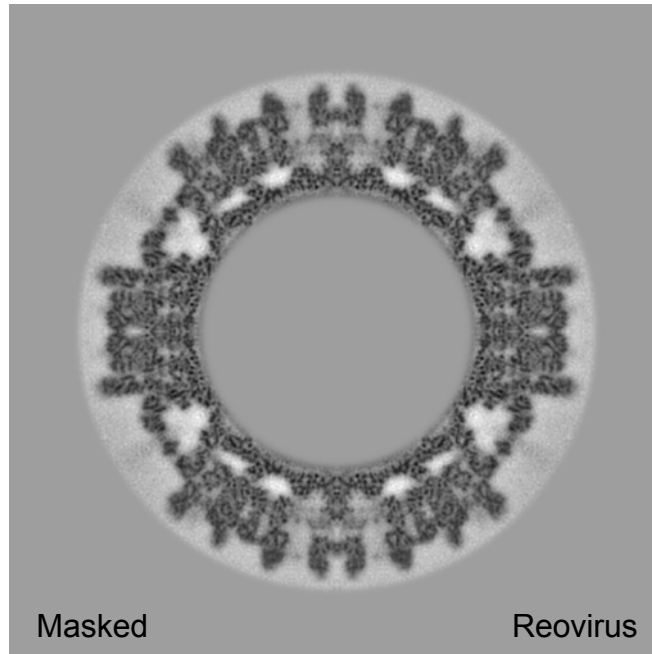
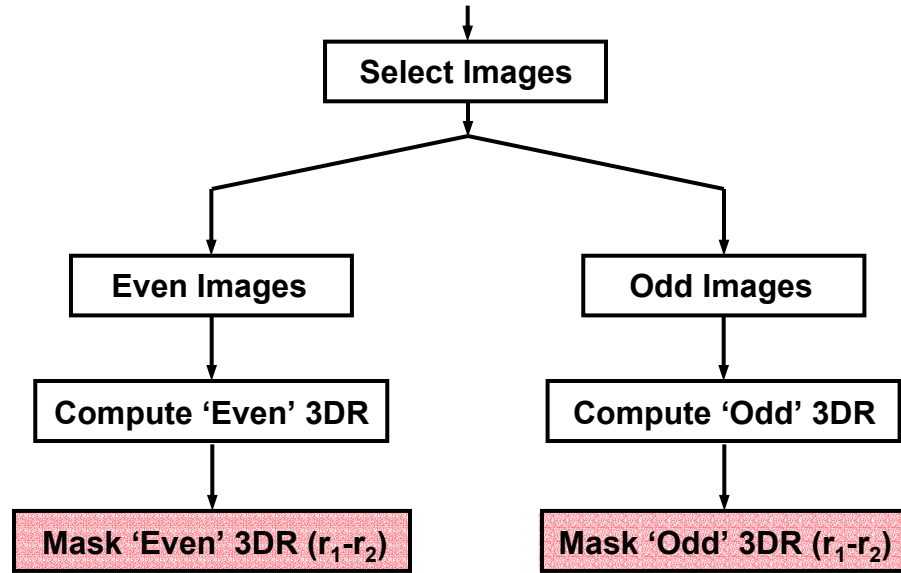
# Icosahedral Virus 3D Reconstruction Scheme



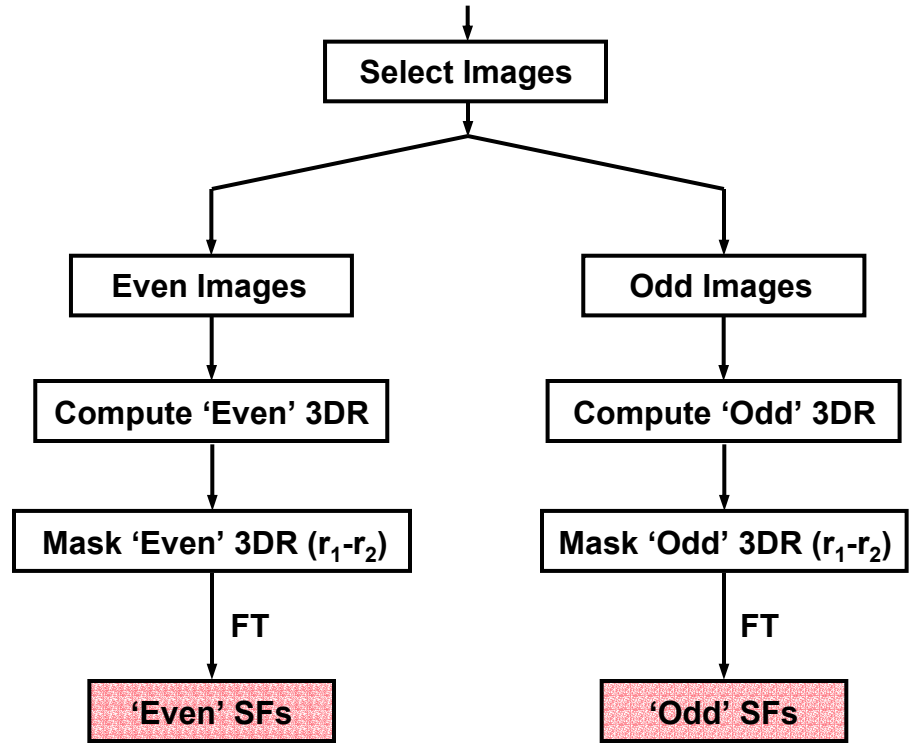
# Icosahedral Virus 3D Reconstruction Scheme



# Icosahedral Virus 3D Reconstruction Scheme

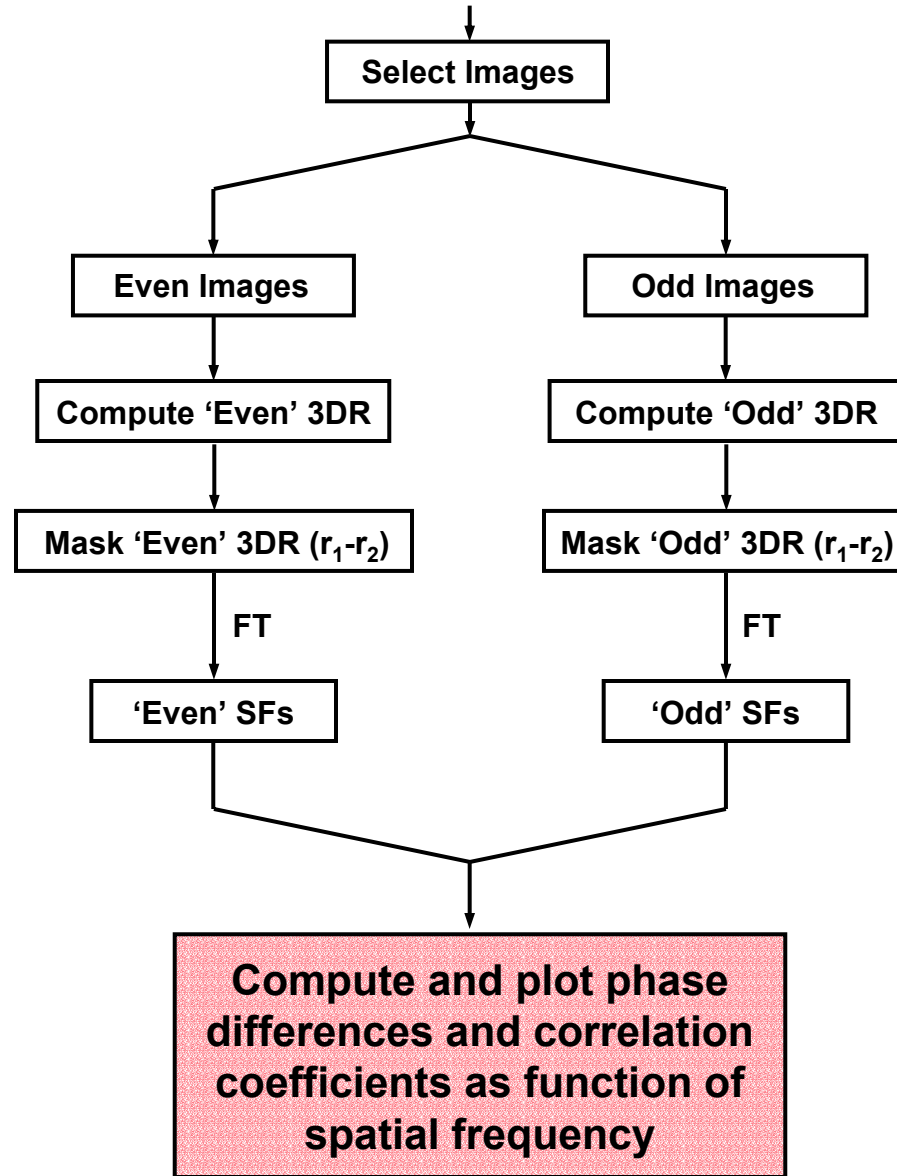


# Icosahedral Virus 3D Reconstruction Scheme



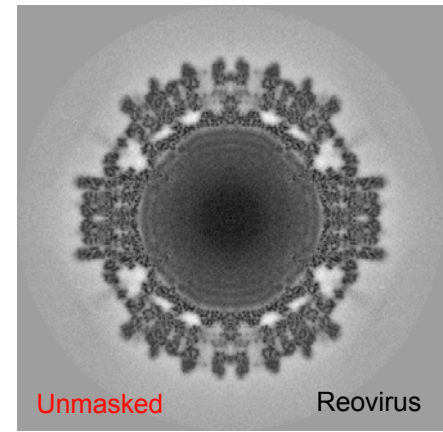
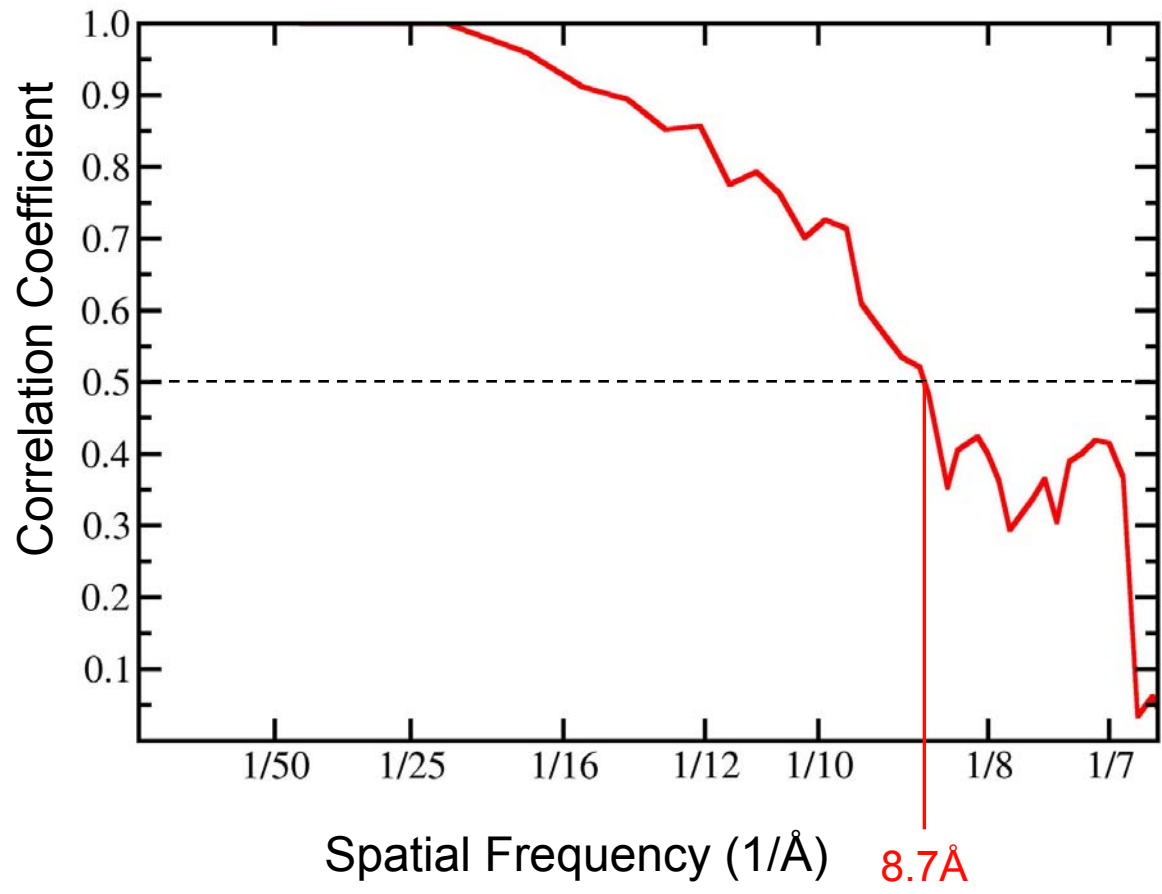


# Icosahedral Virus 3D Reconstruction Scheme



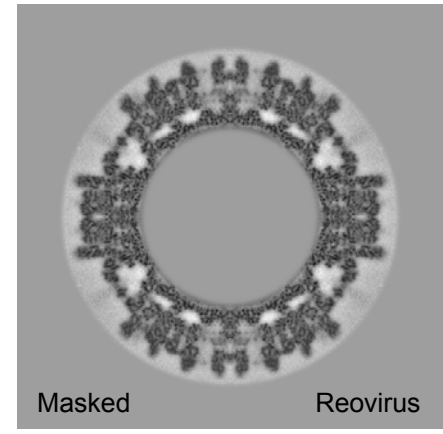
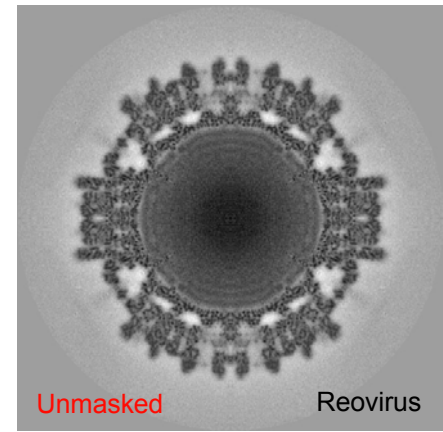
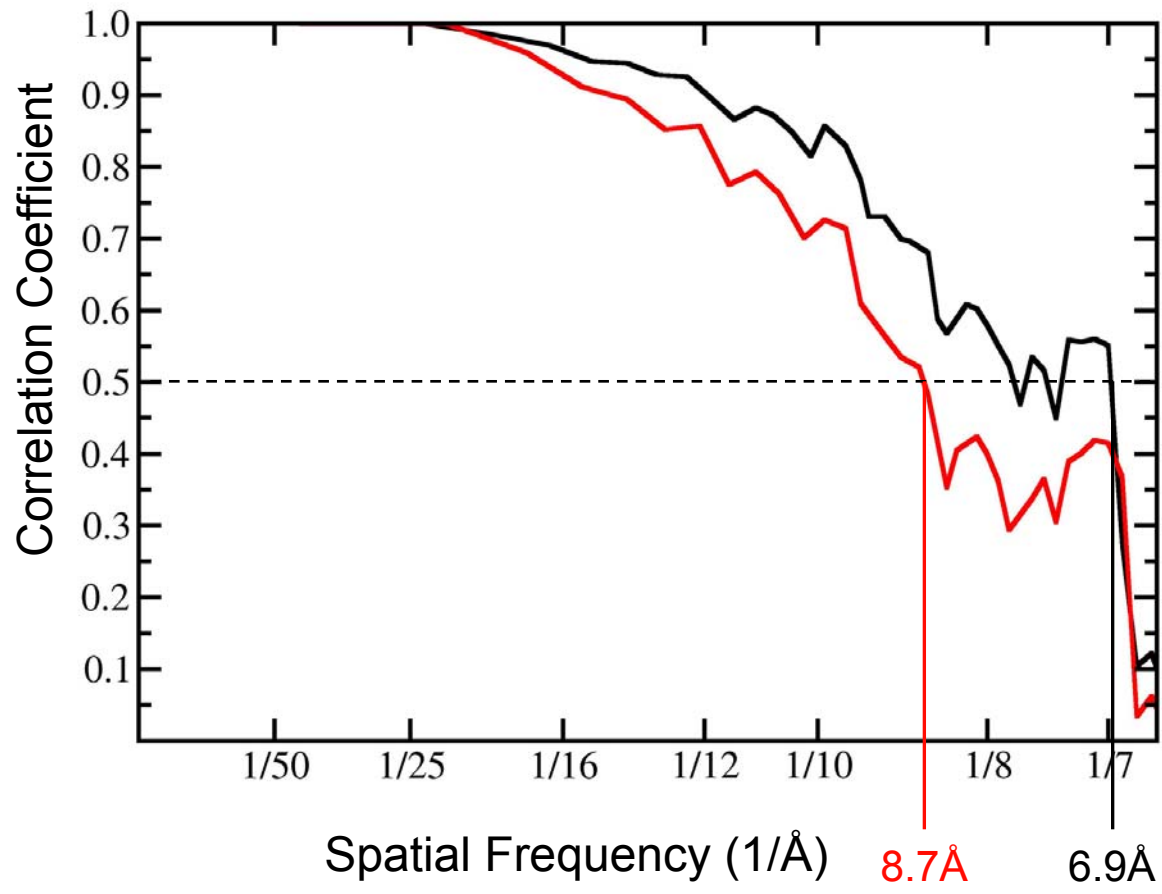
# Icosahedral Virus 3D Reconstruction Scheme

↓  
Monitor Data Quality

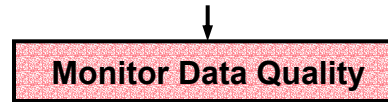


# Icosahedral Virus 3D Reconstruction Scheme

↓  
Monitor Data Quality



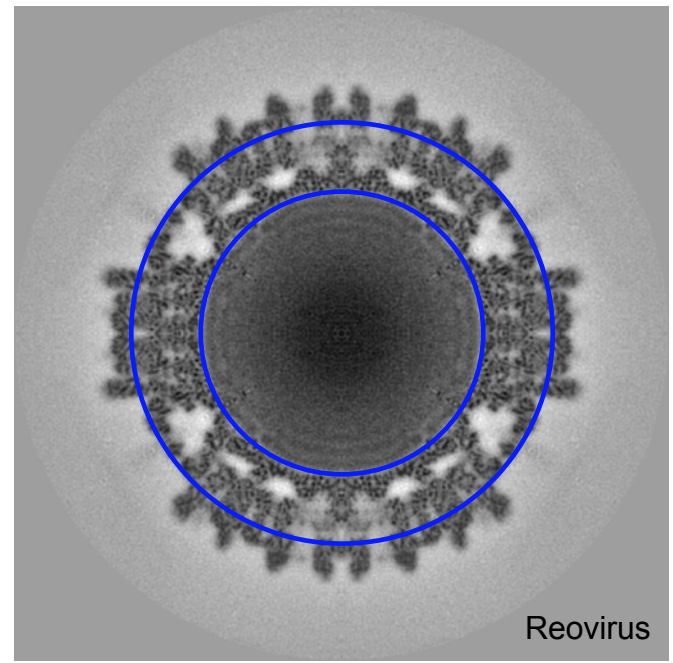
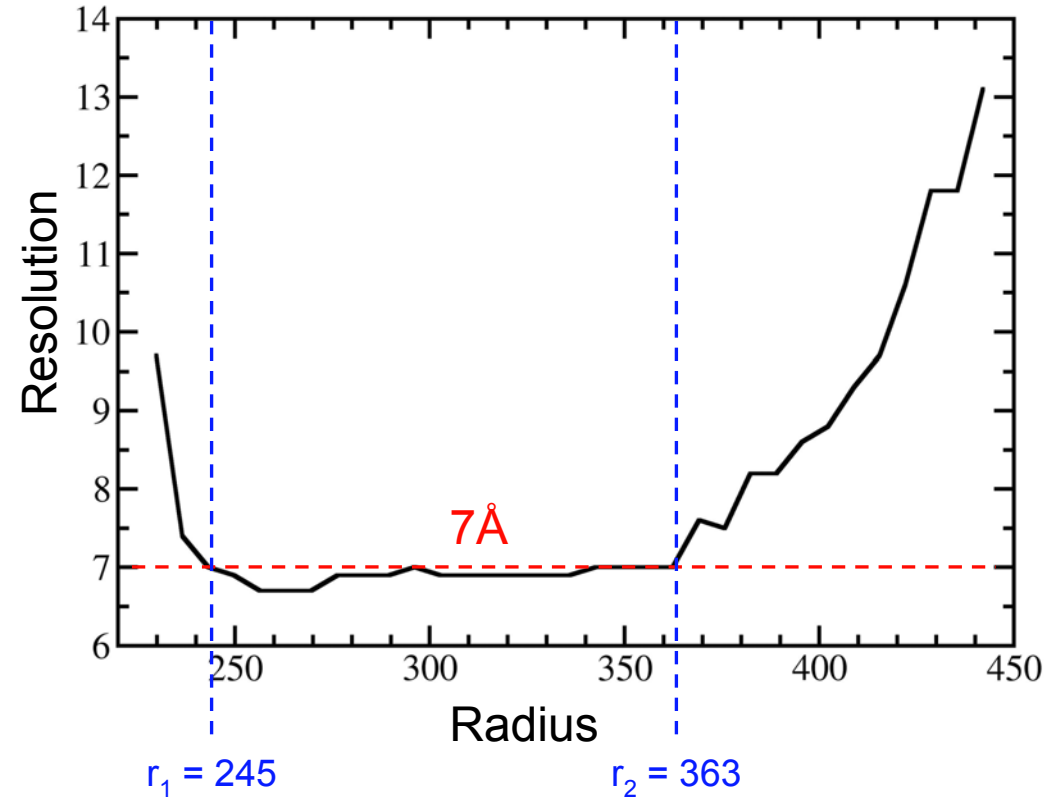
# Icosahedral Virus 3D Reconstruction Scheme



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

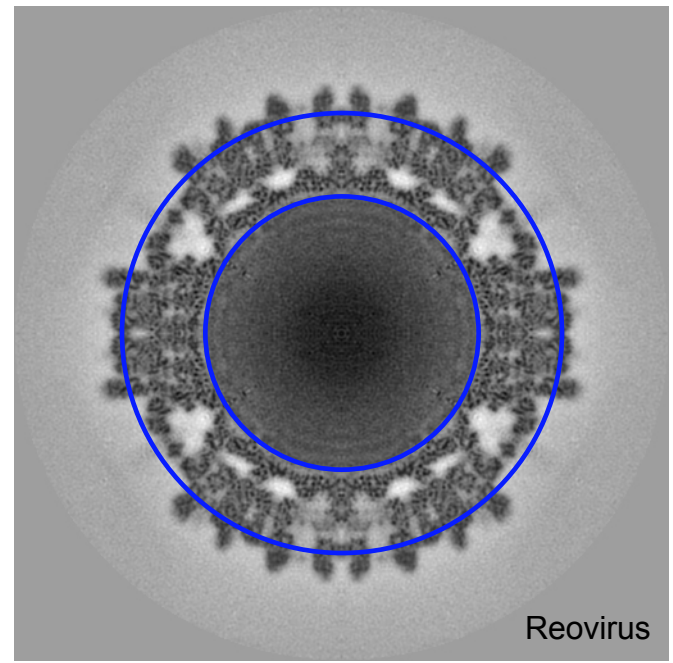
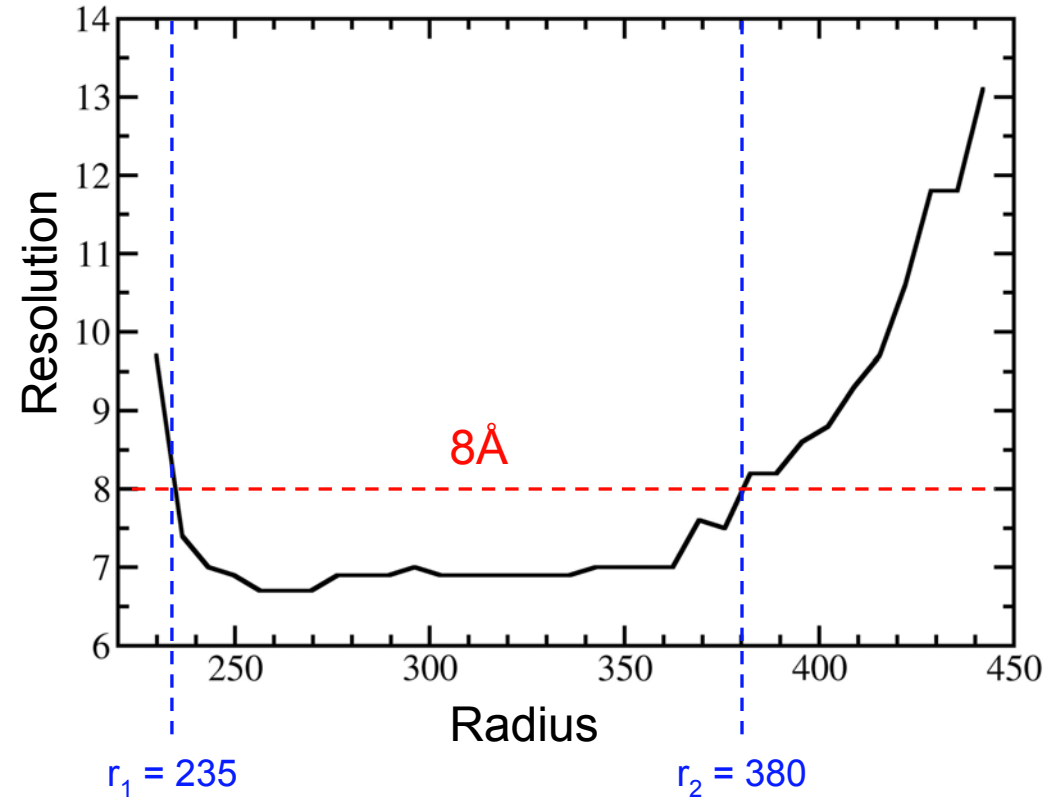
# Icosahedral Virus 3D Reconstruction Scheme

↓  
Monitor Data Quality



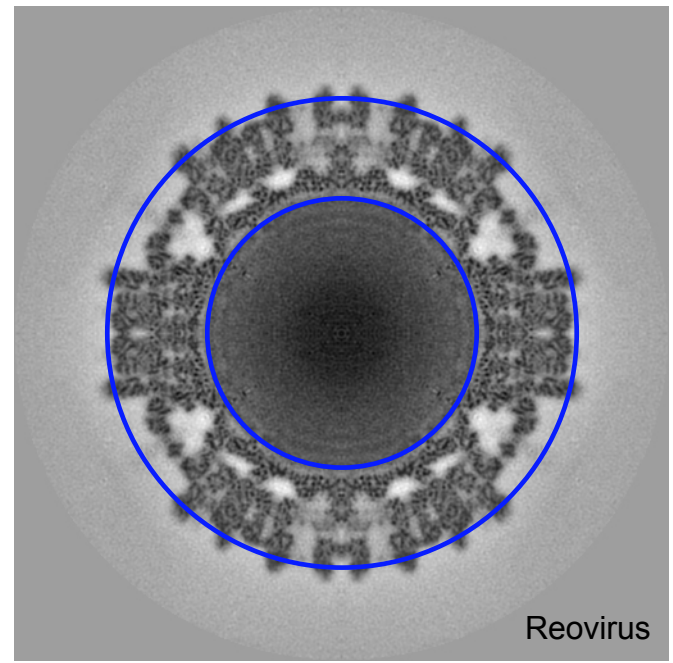
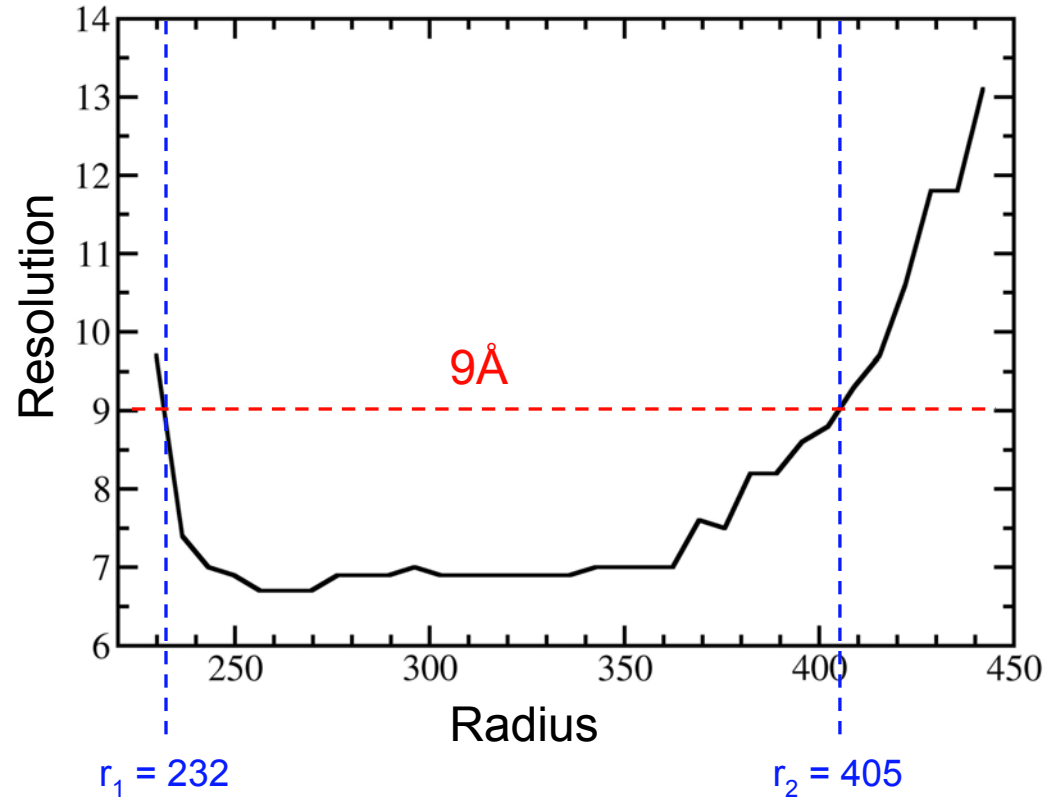
# Icosahedral Virus 3D Reconstruction Scheme

↓  
Monitor Data Quality



# Icosahedral Virus 3D Reconstruction Scheme

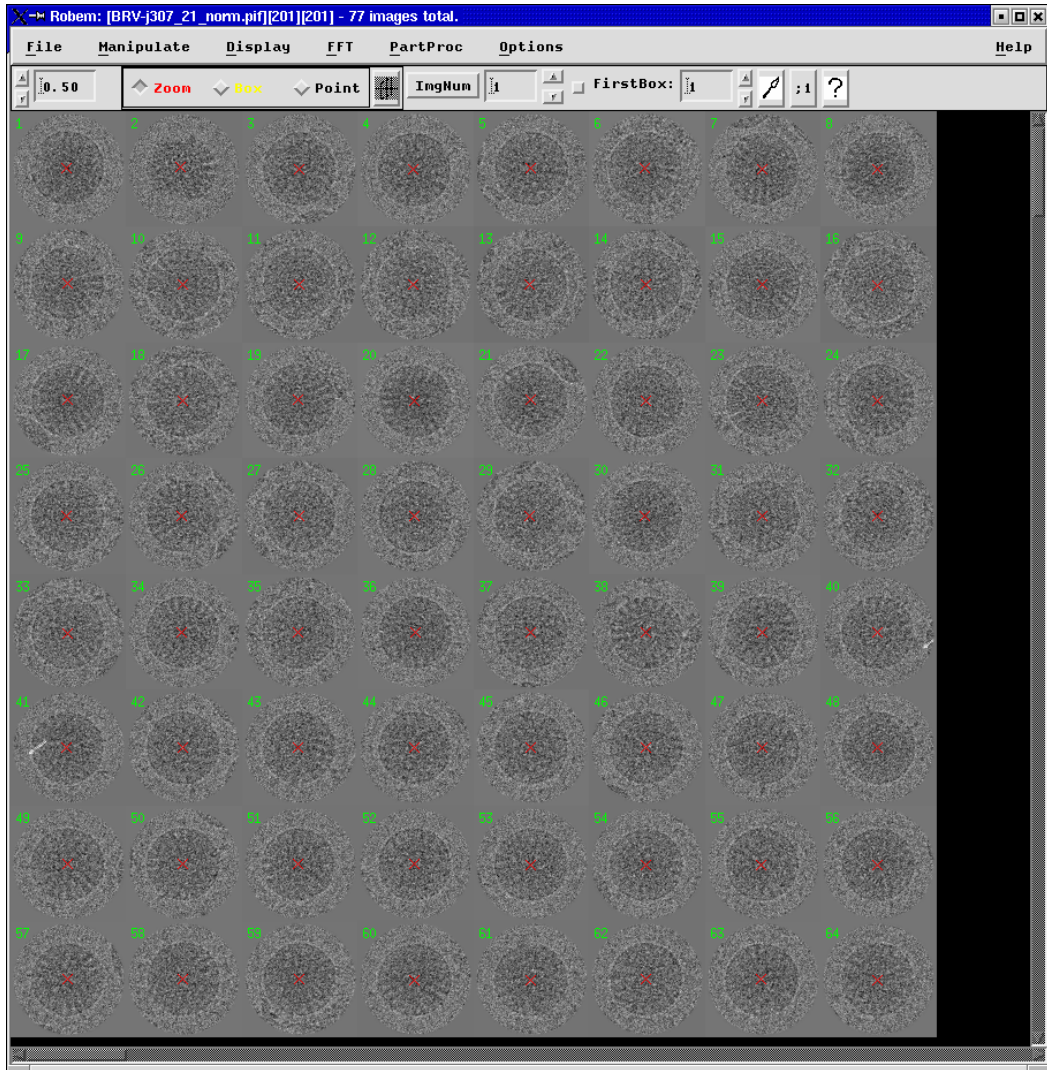
↓  
Monitor Data Quality



# Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

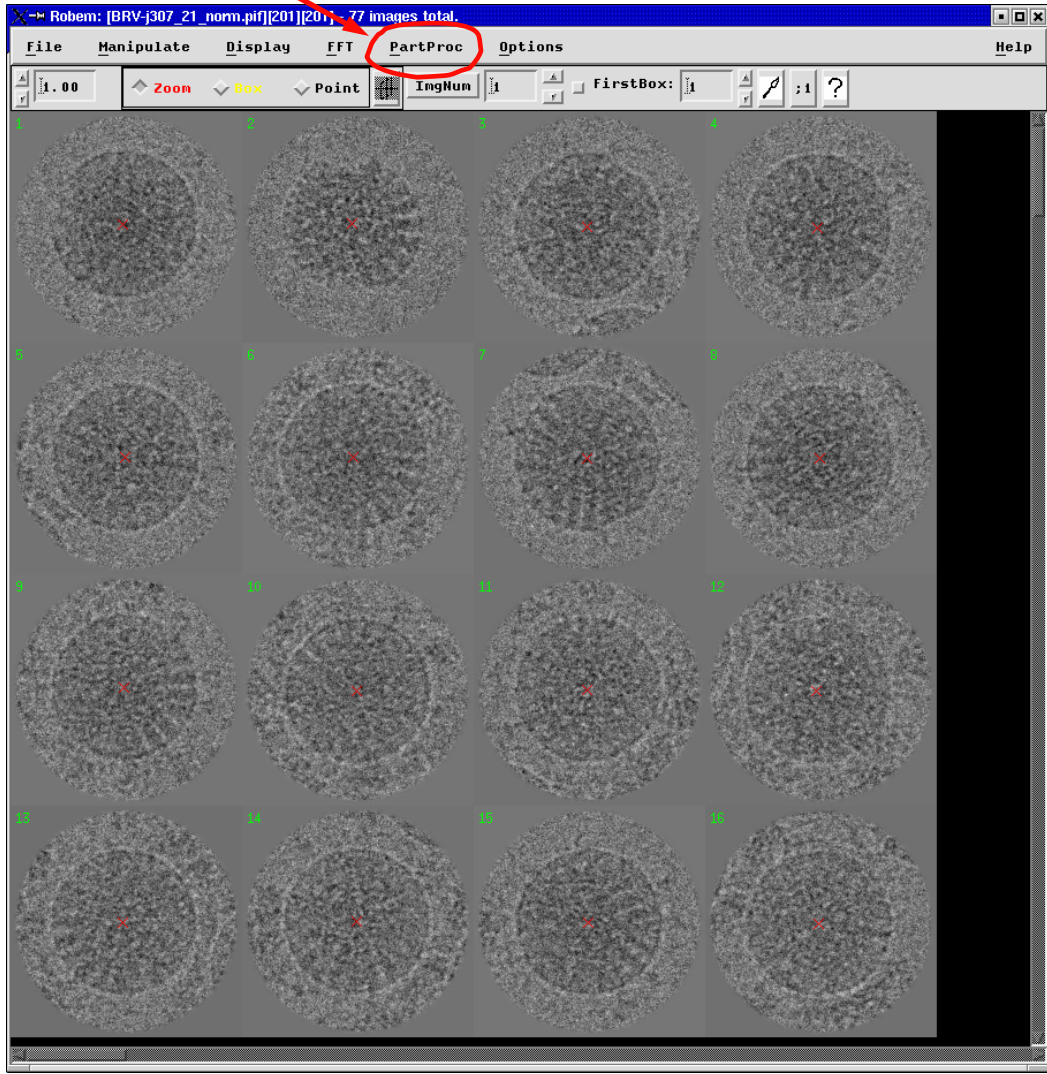




# Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality



# Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality

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

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

Parameter	Value	Units
PixSize	5.0000	1
Voltage	100000.0	
Ampl Factor	0.0700	
$\Delta f$ major	1.6910	
$\Delta f$ 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-hand window displays a triangular grid of red 'x' markers, representing the icosahedral symmetry. A red arrow points to a specific marker in the grid.

# Icosahedral Virus 3D Reconstruction Scheme



**Monitor Data Quality**

Robem: [BRV-j307\_21\_norm.pif][201][201] - 77 images total.

File Manipulate Display FFT PartProc Options Help

1.00 Zoom Box Point IngNum 44 FirstBox: 1

Partproc

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

Display centers  
Pick new particle center  
**MAP Projections** Projection Contour

PixSize 6.0000 Units 1  
Voltage 100000.0  
Amp Factor 0.0700  
 $\Delta f$  major 1.6910  
 $\Delta f$  minor 1.6910  
Ang major 0.0000  
Cs Coef 2.00

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

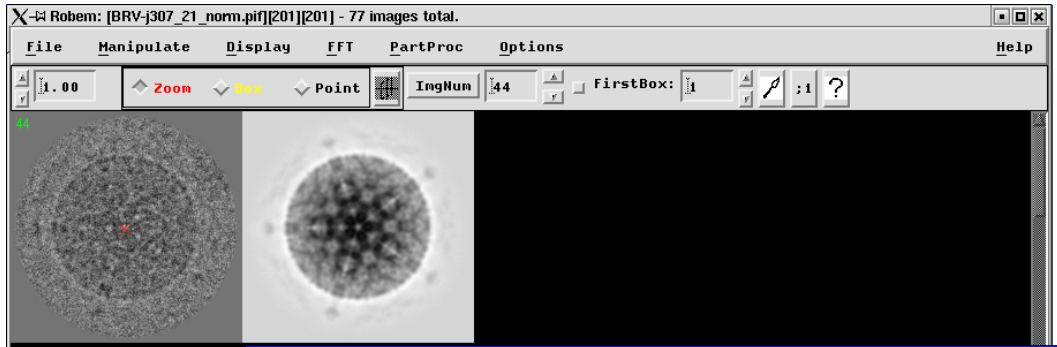
```
Particle[ 44] Theta [ 90.000] Phi [ 31.000] Omega [ 55.141]
X [ 98.694] Y [ 99.617] Mag [ 1.000]
pft_cc [ 0.536] proj_cc [ 0.559] cmp_cc [ 0.567]
deltaK [ -1.306] deltaY [ -0.383] distance [ 1.361]
state [ ON] rank [ 44]
```

90 69 31 0 -31

# Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality



**Partproc**

Parameter File:

Map File:

Scan File:

Display centers

Pick new particle center

MAP Projections  Projection Contour

PixSize	<input type="text" value="6.0000"/>	Units	<input type="text" value="1"/>
Voltage	<input type="text" value="100000.0"/>		
Amp Factor	<input type="text" value="0.0700"/>		
$\Delta f$ major	<input type="text" value="1.6910"/>		
$\Delta f$ minor	<input type="text" value="1.6910"/>		
Ang major	<input type="text" value="0.0000"/>		
Cs Coef	<input type="text" value="2.00"/>		

Particle[ 44]	Theta [ 90.000]	Phi [ 31.000]	Omega [ 55.141]
X[ 98.634]	Y[ 99.617]	Mag[ 1.000]	
pft_cc[ 0.536]	prj_cc[ 0.959]	cmp_cc[ 0.567]	
deltaK[ -1.306]	deltaY[ -0.383]	distance[ 1.361]	
state[ ON]	rank[44]		

# Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

The screenshot displays a software interface for 3D reconstruction. The main window, titled "Robem: [BRV-j307\_21\_norm.tif][201][201] - 77 images total.", contains two image thumbnails: a noisy circular image on the left and a clearer circular image on the right. Below these is a "Partproc" sub-window with the following sections:

- 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
- Parameters:**
  - PixSize: `6.0000` Units: `1`
  - Voltage: `100000.0`
  - Amp Factor: `0.0700`
  - $\Delta f$  major: `1.6910`
  - $\Delta f$  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 "Partproc" window also features a table of particle parameters and a plot of particle positions:

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

The plot shows a triangular grid of red 'x' marks representing particle positions. A red arrow points to a specific particle at coordinates (76.7, 12.0). Two icosahedral virus models are shown in the top right of the plot area: one in blue and one in red.

# Icosahedral Virus 3D Reconstruction Scheme



Monitor Data Quality

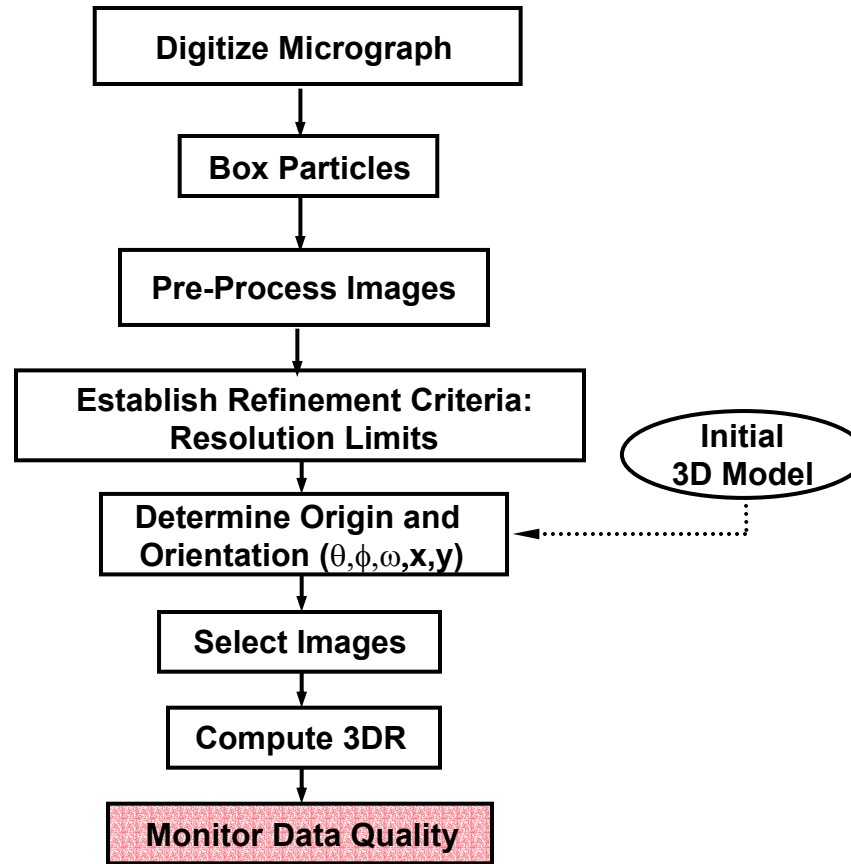
The screenshot displays a software interface for 3D reconstruction, divided into several windows:

- Main Window:** Shows two images of a virus particle. The left image is a noisy reconstruction, and the right image is a clearer reconstruction. The title bar reads: "X Robem: [BRV-j307\_21\_norm.tif][201][201] - 77 images total." The menu bar includes: File, Manipulate, Display, FFT, PartProc, Options, Help. The toolbar shows: 1.00, Zoom, Box, Point, IngNum: 20, FirstBox: 1.
- Partproc Window:** Contains parameters for particle processing. The title bar reads: "X Partproc".
  - Parameter File: /bio/gandalf/tsb/NRAHM/B
  - Map File: /bio/gandalf/tsb/NRAHM/B
  - Scan File: (empty)
  - Buttons: Browse, Batch Job, Calc, Symmetry, Display
  - Table of particle parameters:

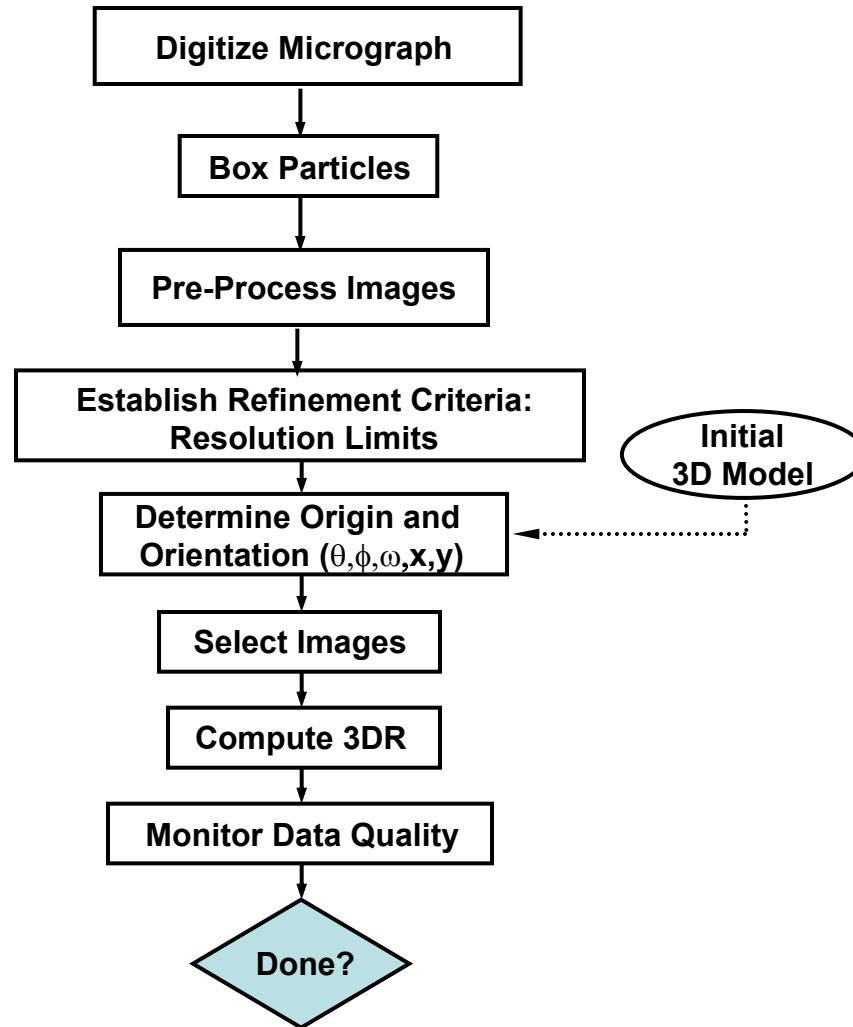
Particle[	20]	Theta [	70.000]	Phi[	-1.064]	Omega[	181.813]
X[	99.732]	Y[	99.592]	Mag[	1.000]		
pft_cc[	0.560]	prj_cc[	0.623]	cmp_cc[	0.577]		
deltaK[	-0.268]	deltaY[	-0.408]	distance[	0.488]		
state[	ON]	rank[20]					

  - Display centers
  - Pick new particle center
  - MAP Projections  Projection Contour
  - Parameters:
    - PixSize: 6.0000 Units: 1
    - Voltage: 100000.0
    - Amp Factor: 0.0700
    - $\Delta f$  major: 1.6910
    - $\Delta f$  minor: 1.6910
    - Ang major: 0.0000
    - Cs Coef: 2.00
  - Buttons: Compute Circular Avg, Add delta omega, Manipulate Particle Params, Add delta X,Y to centers, Rebox ALL Particles, Rebox THIS Particle, Write New Parm File
- Plot Window:** Shows a triangular grid of red 'x' marks representing particle positions. A red arrow points to a specific particle at coordinates (70.0, -1.0). Two icosahedral virus models are shown in the top right: one blue and one red.

# Icosahedral Virus 3D Reconstruction Scheme

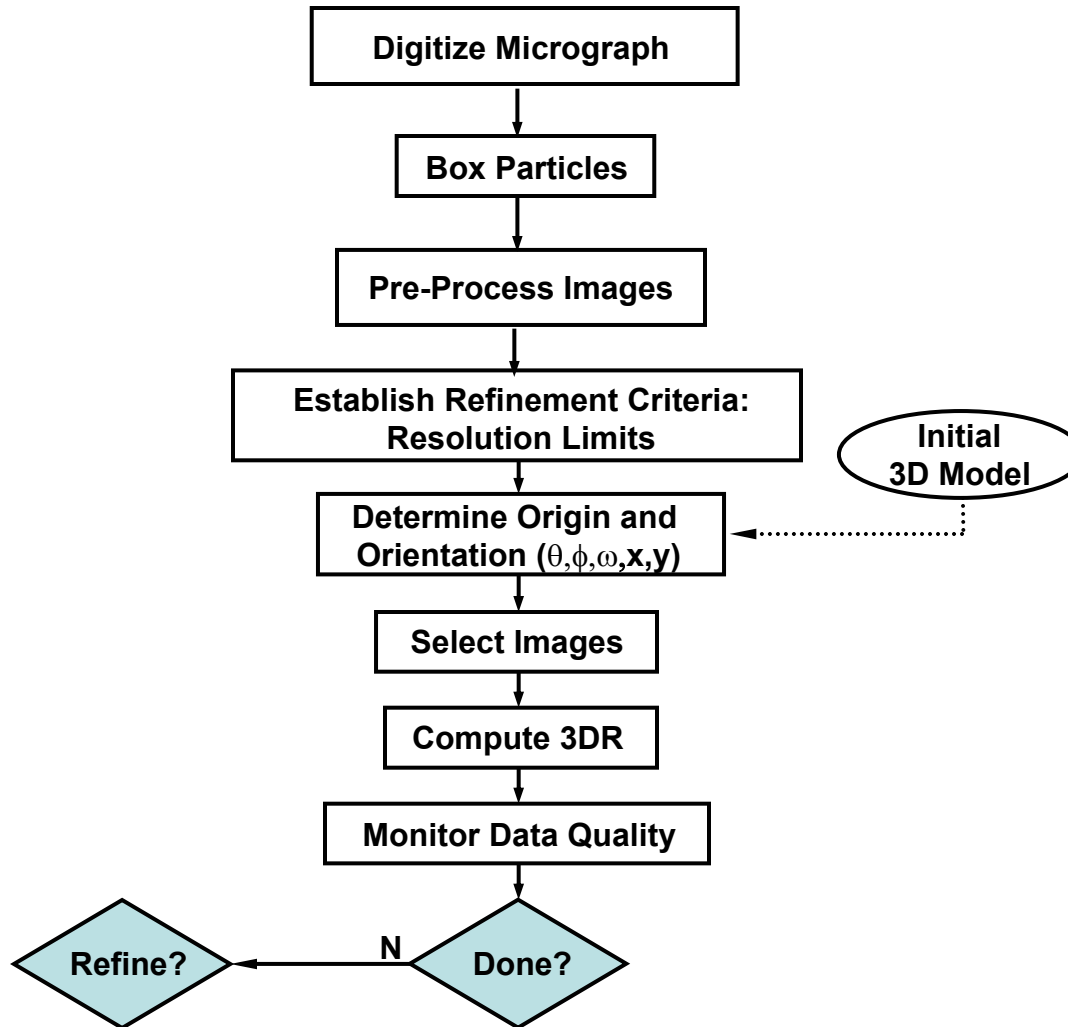


# Icosahedral Virus 3D Reconstruction Scheme

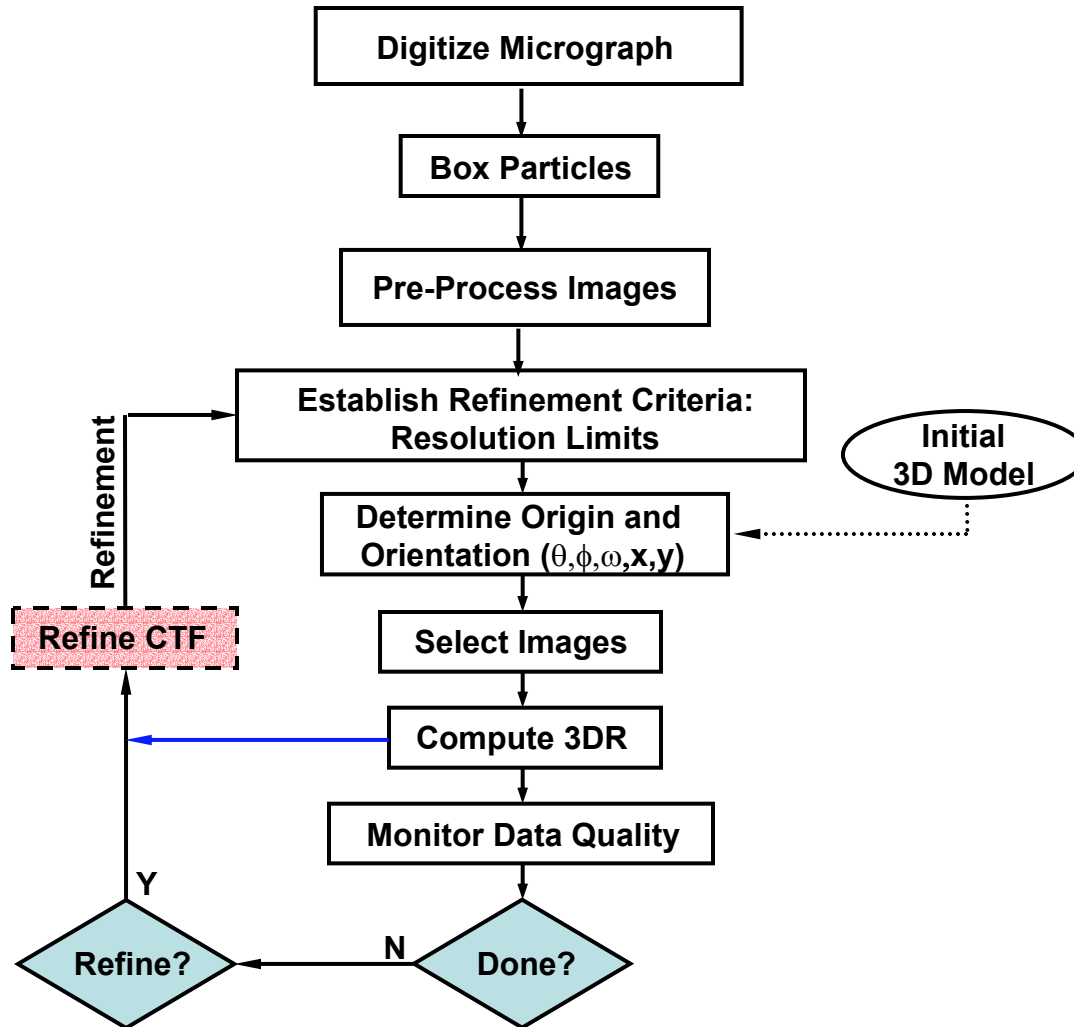




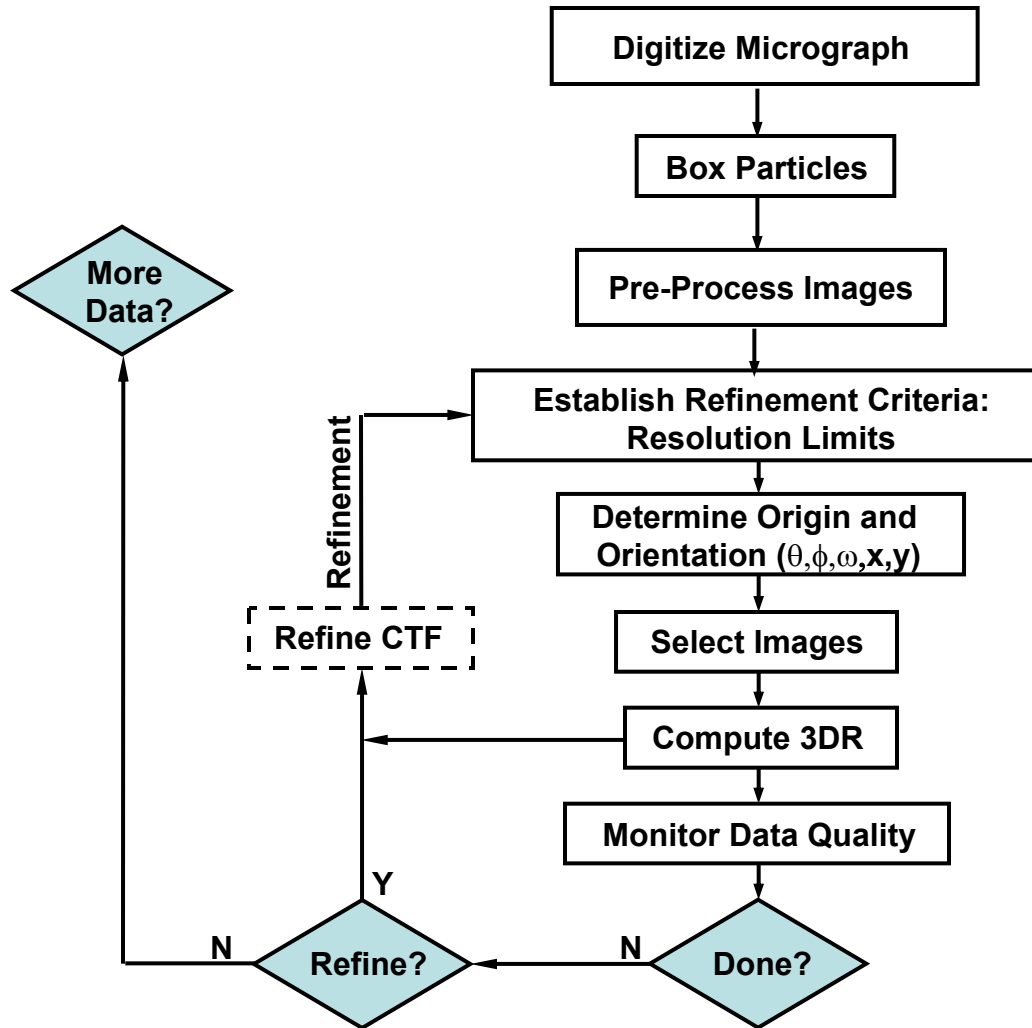
# Icosahedral Virus 3D Reconstruction Scheme



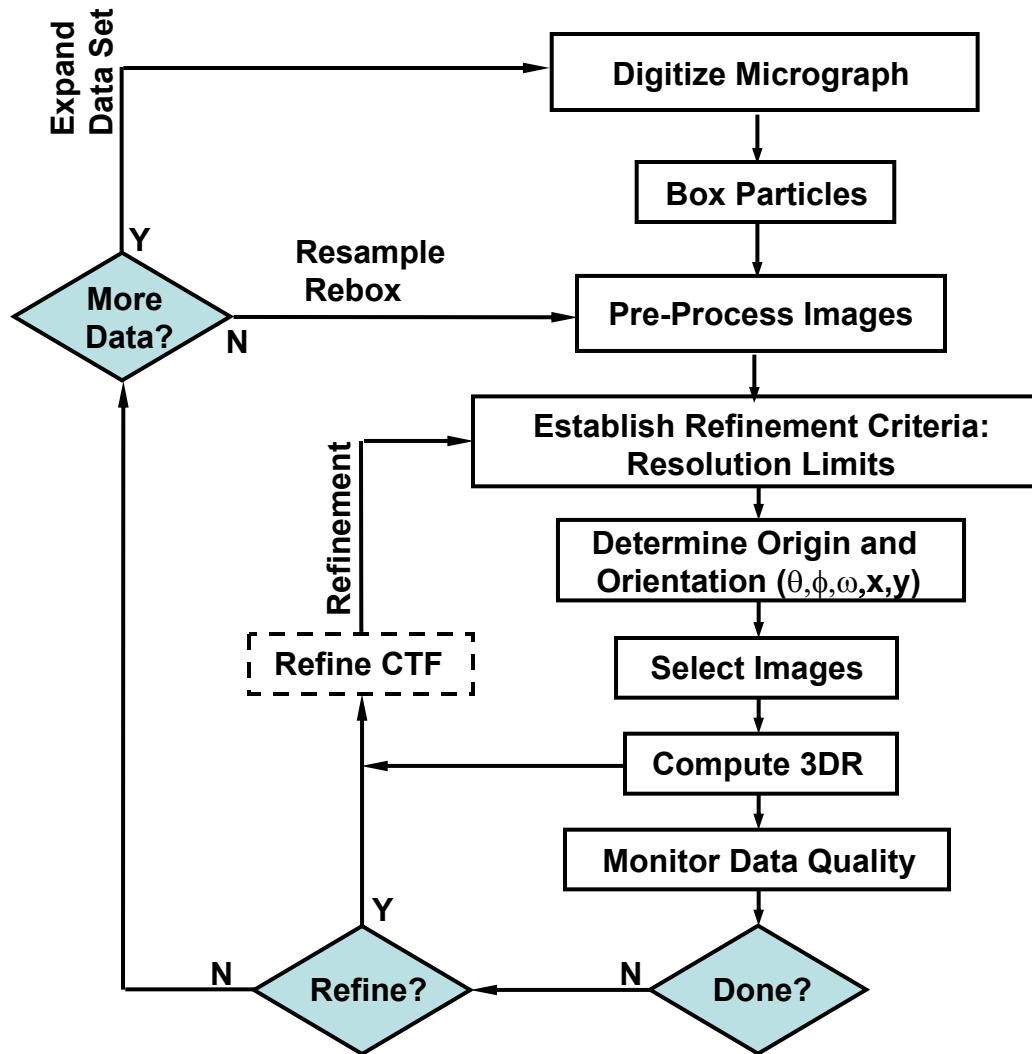
# Icosahedral Virus 3D Reconstruction Scheme



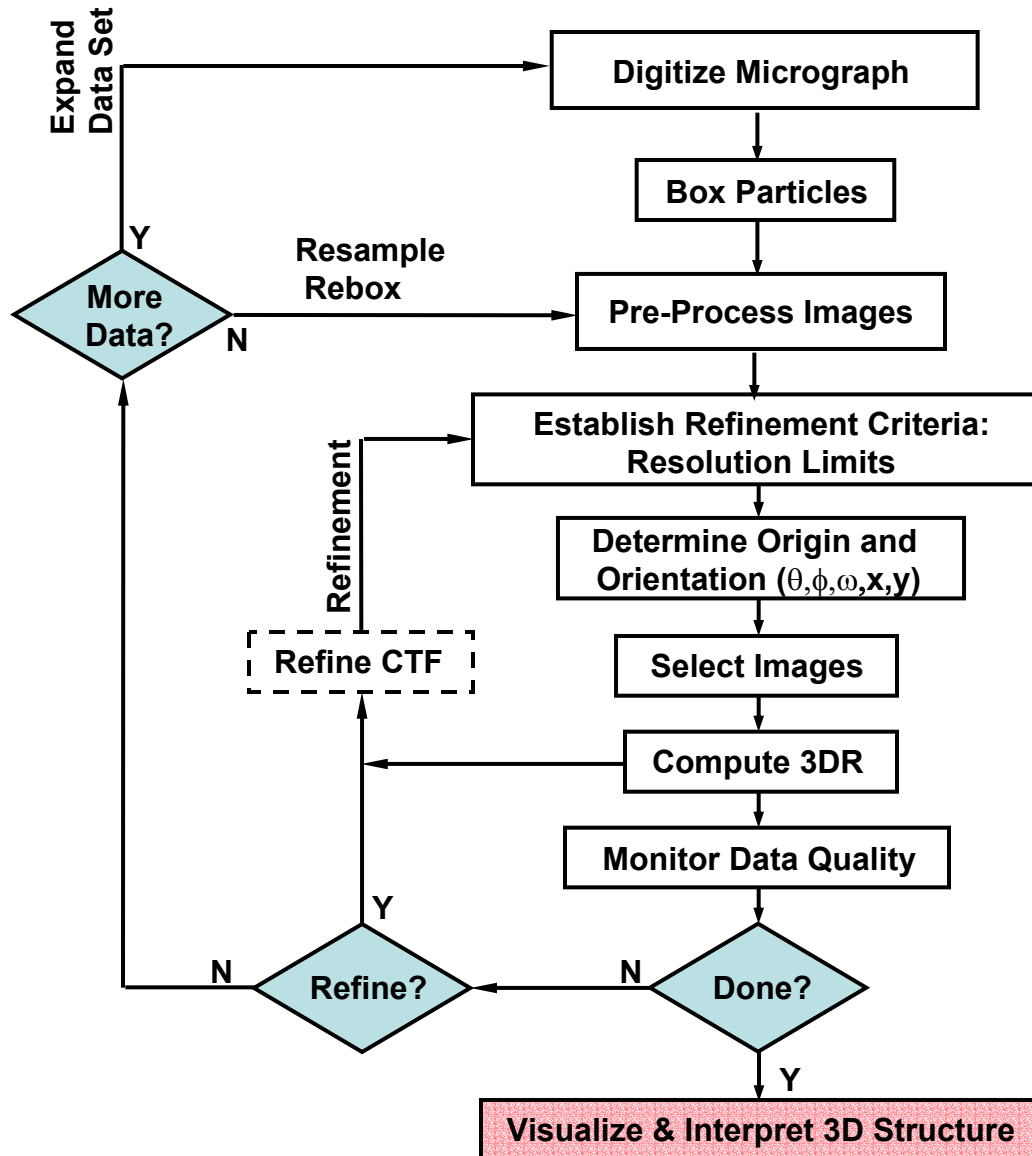
# Icosahedral Virus 3D Reconstruction Scheme



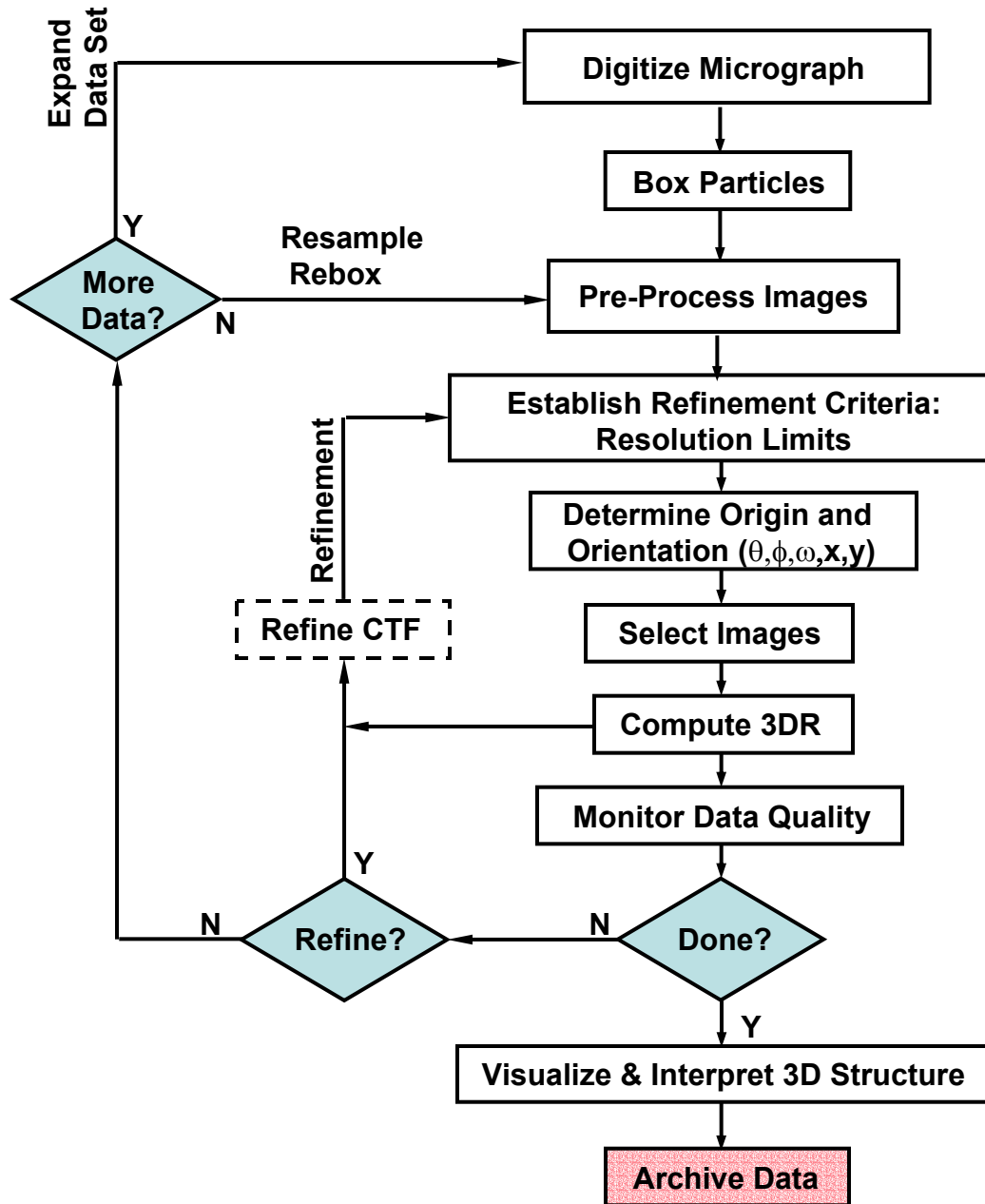
# Icosahedral Virus 3D Reconstruction Scheme



# 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



# 3D Reconstruction of Icosahedral Particles

Time for lunch???