THE UNIVERSITY *of* TEXAS

SCHOOL OF HEALTH INFORMATION SCIENCES AT HOUSTON

Virus Capsids and Icosahedral Reconstruction

For students of HI 6001-125

"Computational Structural Biology"

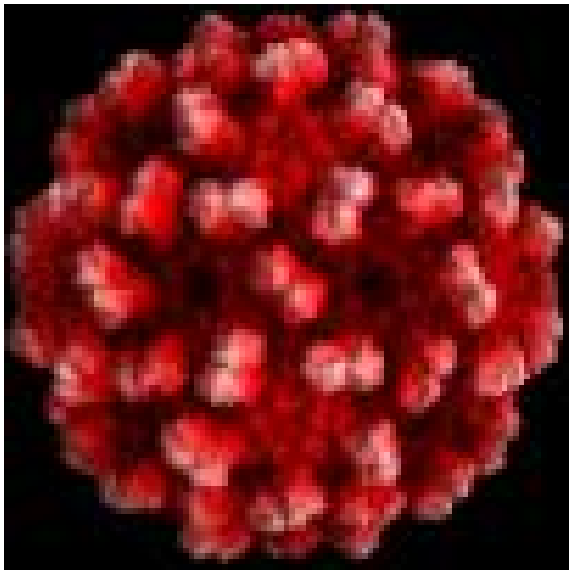
Willy Wriggers, Ph.D.

http://biomachina.org/courses/structures/10.html

Overview and Biological Relevance

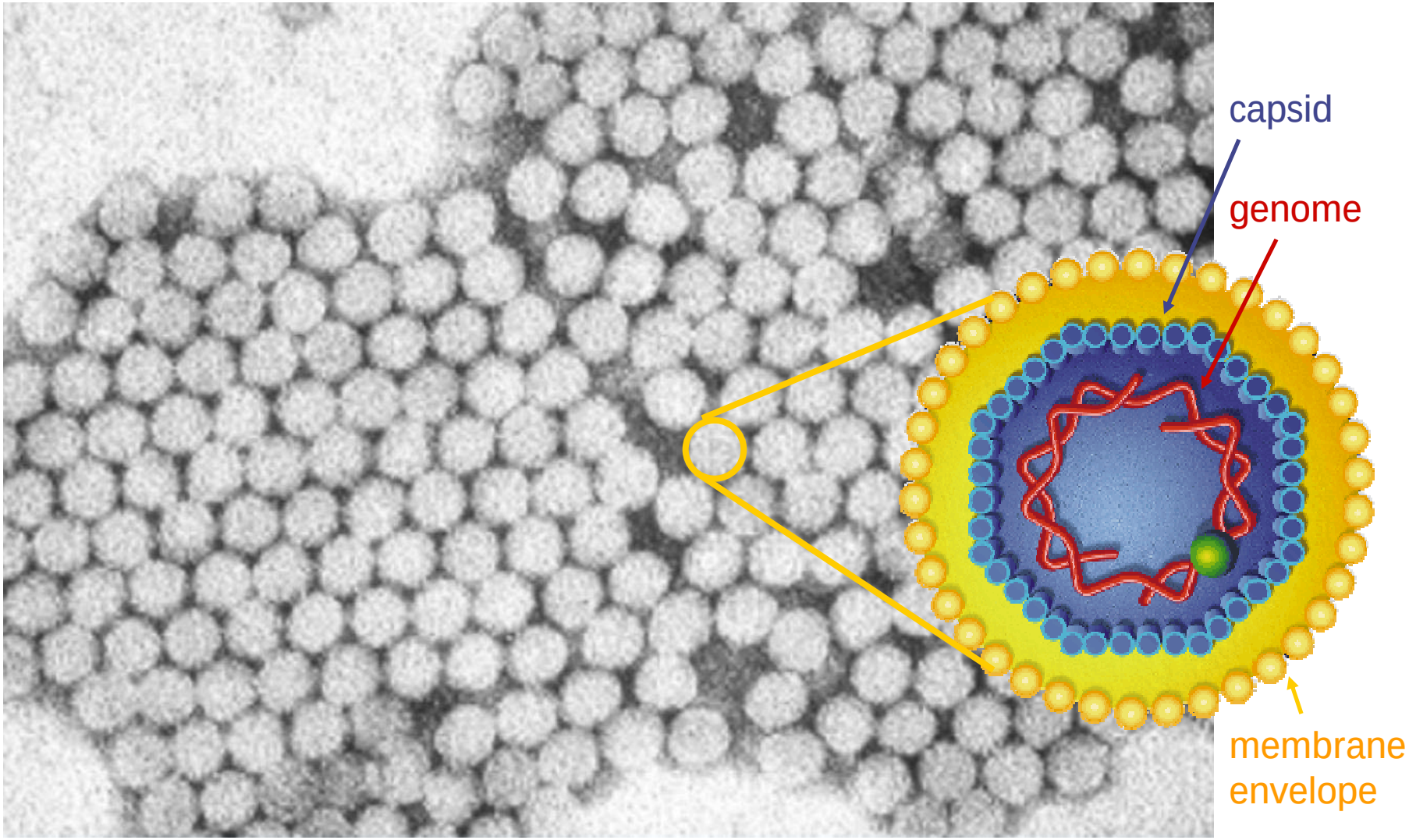
Virus Structure

- crystallization of a virus was first reported in the 1930s.
- first atomic resolution structure of a virus was 1978, tomato bushy stunt virus (Stephen Harrison):



Example

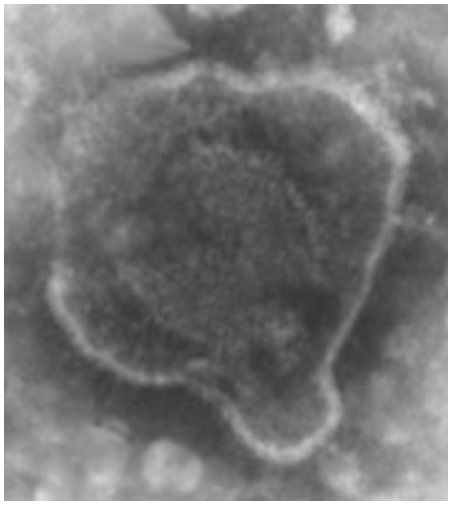
Hepatitis A virus.

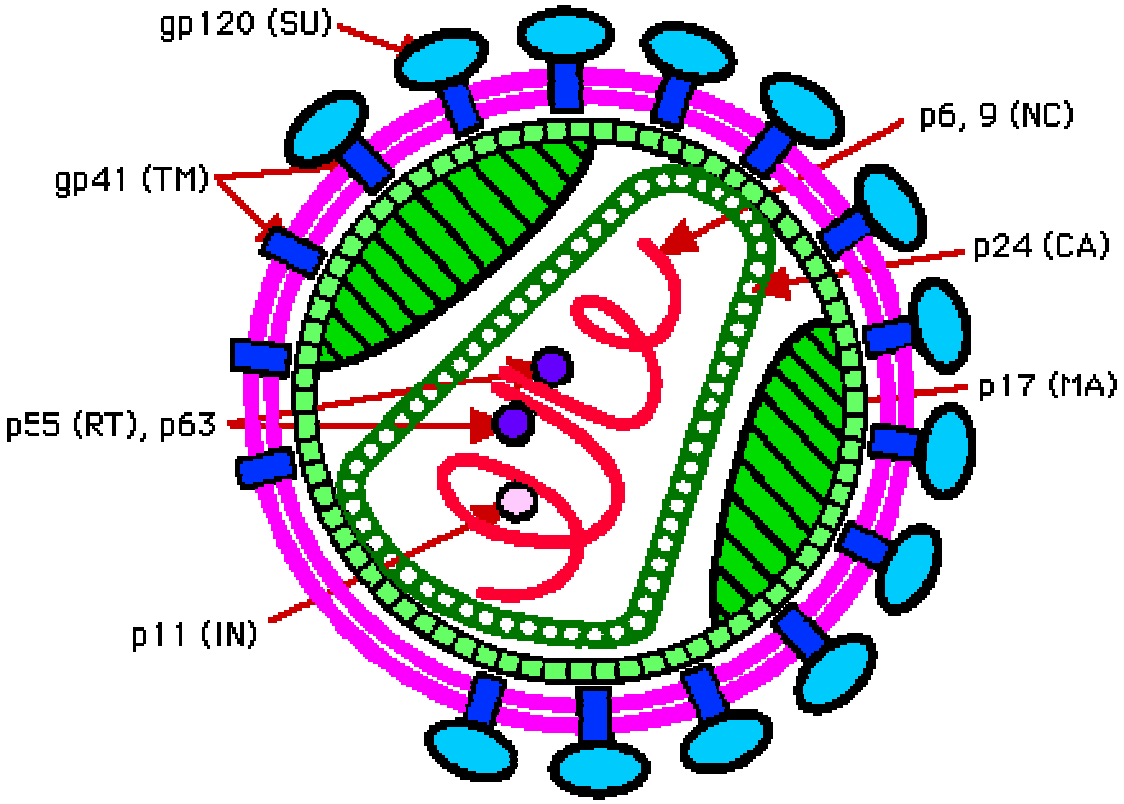


Role of Virus Capsids

- Function of the outer shell (**capsid**) of a virus particle is to protect the fragile nucleic acid genome from:
- **Physical damage** - Shearing by mechanical forces.
- **Chemical damage**- UV irradiation (from sunlight) leading to chemical modification. **Enzymatic damage** - Nucleases derived from dead or leaky cells or deliberately secreted by vertebrates as defence against infection.
- Protein subunits in a virus capsid are **multiply redundant**, i.e. present in many copies per particle. Damage to one subunit may render that subunit non-functional, but does not destroy the infectivity of the whole particle.

Membrane Envelopes





Membrane envelopes acquired from a cellular structure during release. Membranes are modified by proteins. Matrix proteins are found inside the envelope. Glycoproteins traverse the envelope.

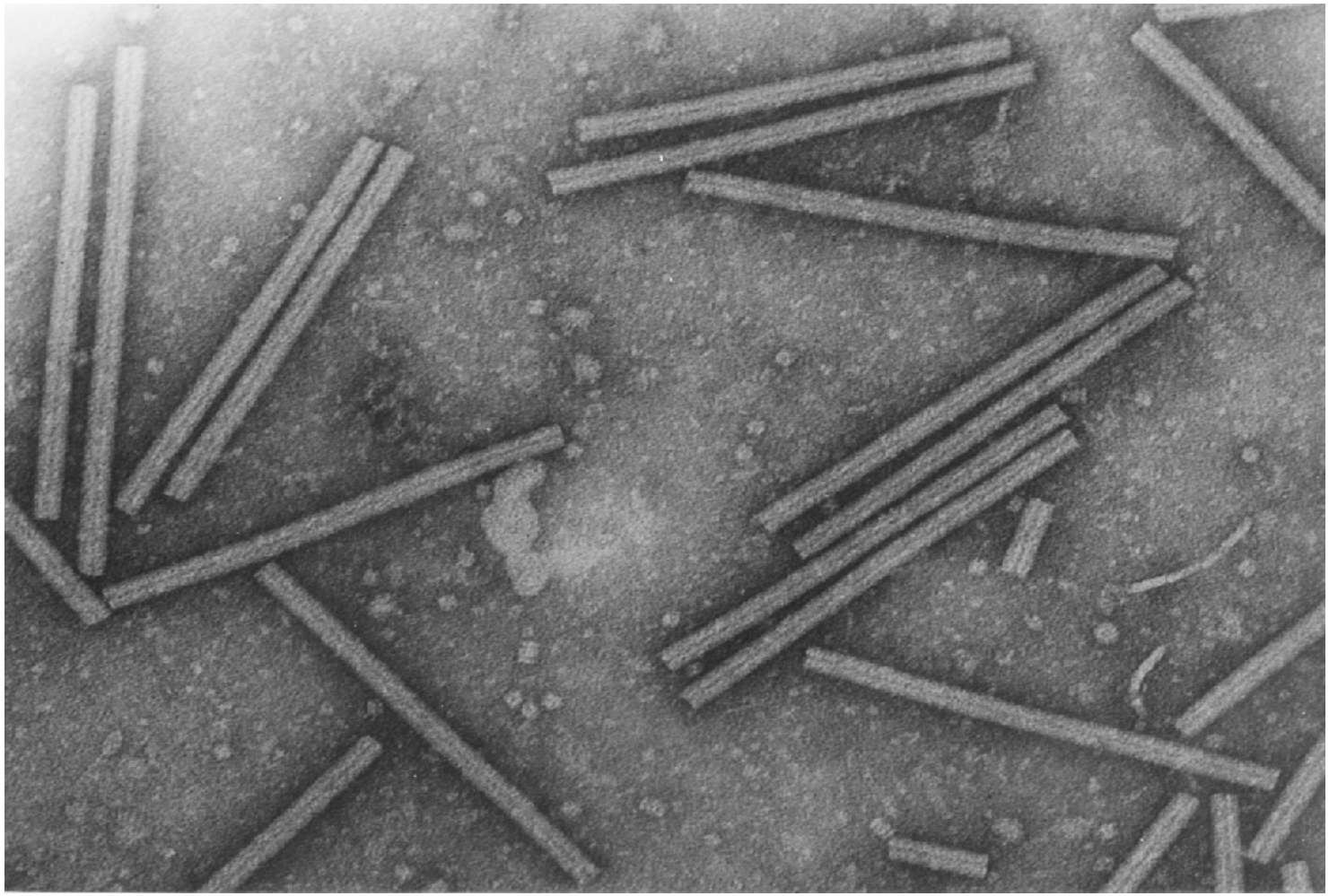
Infection

- The outer surface of the virus is responsible for **recognition of the host cell**. Initially, this takes the form of binding of a specific **virus-attachment protein** to a **cellular receptor molecule**. The capsid also has a role to play in initiating infection by delivering the genome from its protective shell in a form in which it can interact with the host cell.
- To form an infectious particle, a virus must overcome two fundamental problems:

1. assemble the particle utilizing only the information available from the components which make up the particle itself (capsid + genome).
2. Form regular geometric shapes, even though the proteins from which they are made are irregularly shaped.

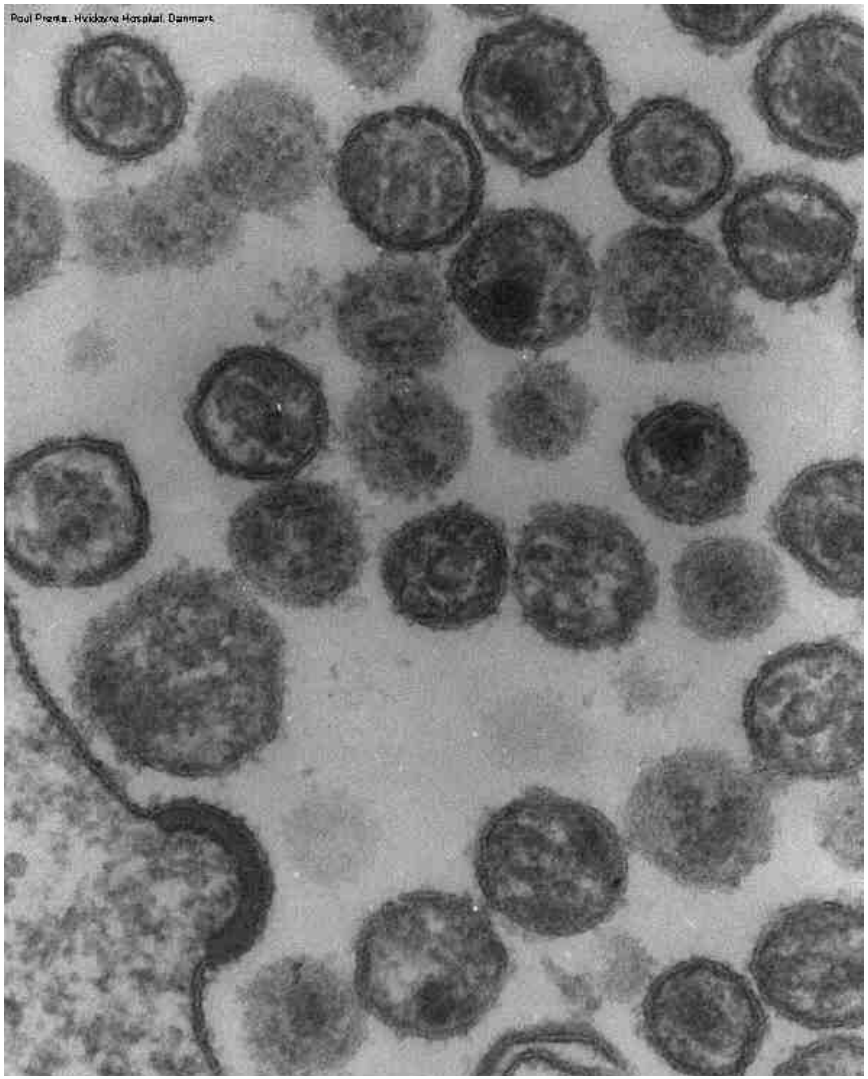
Virus Shapes

Tobacco mosaic virus (helical).



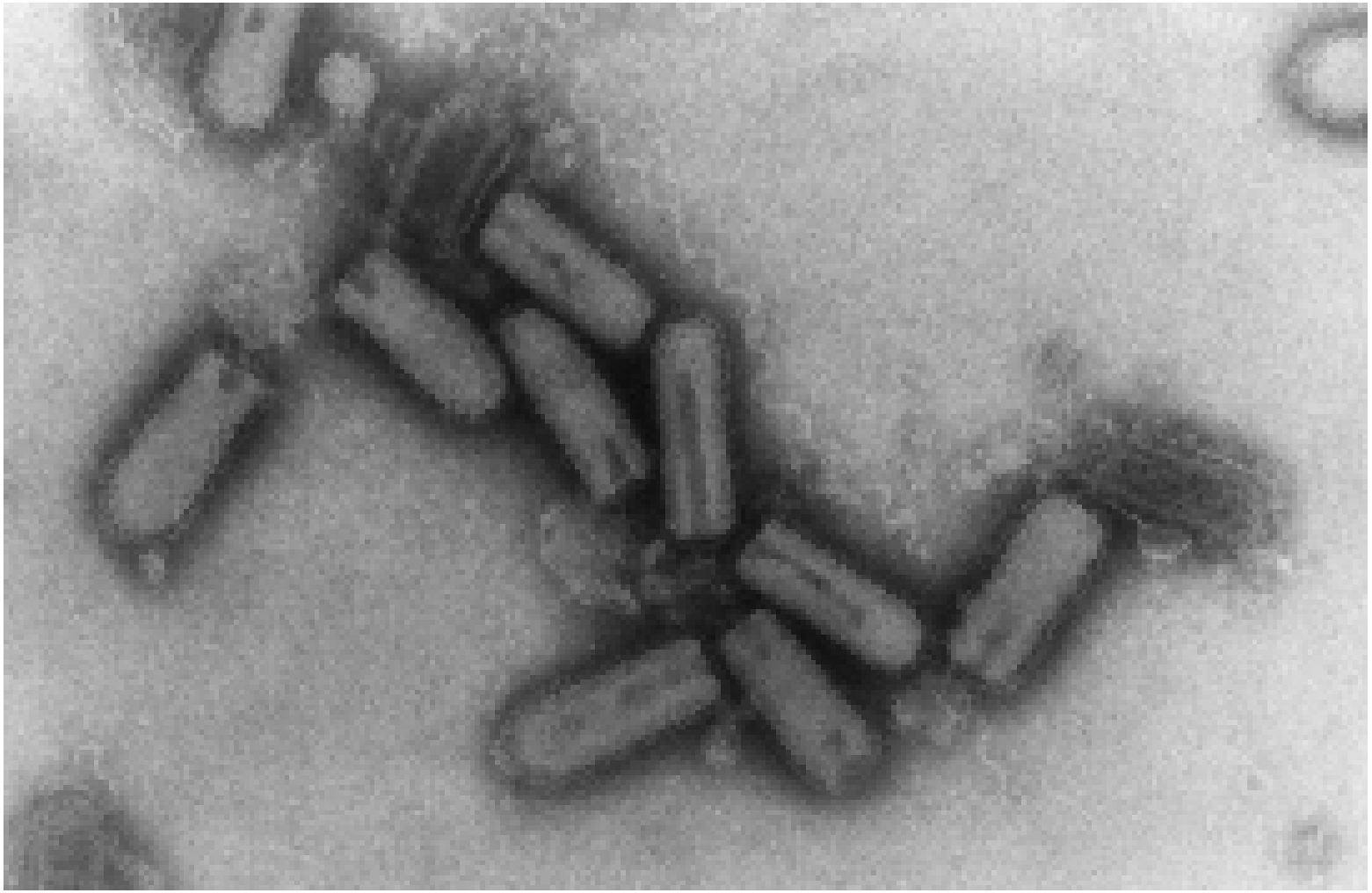
Virus Shapes

HIV (complex globular, enveloped)



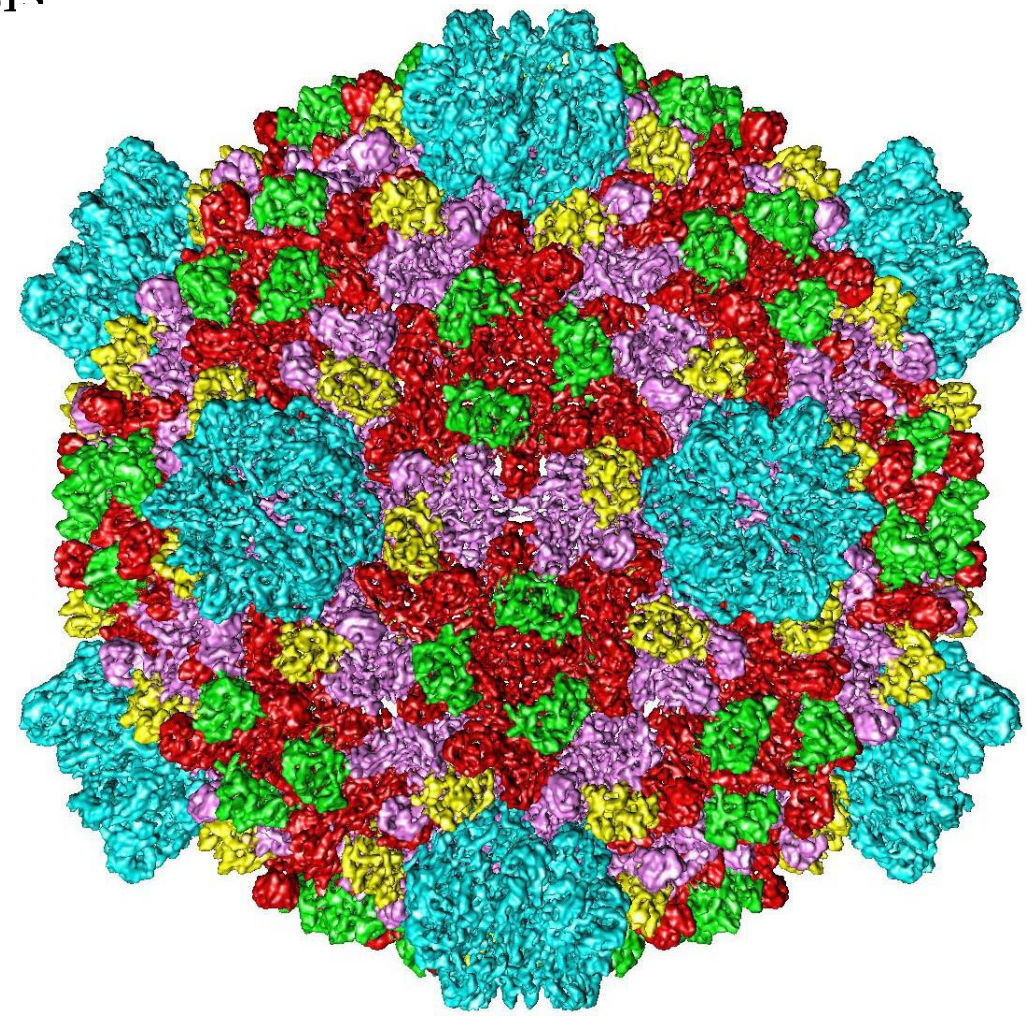
Virus Shapes

Vesicular stomatitis virus: bullet shaped



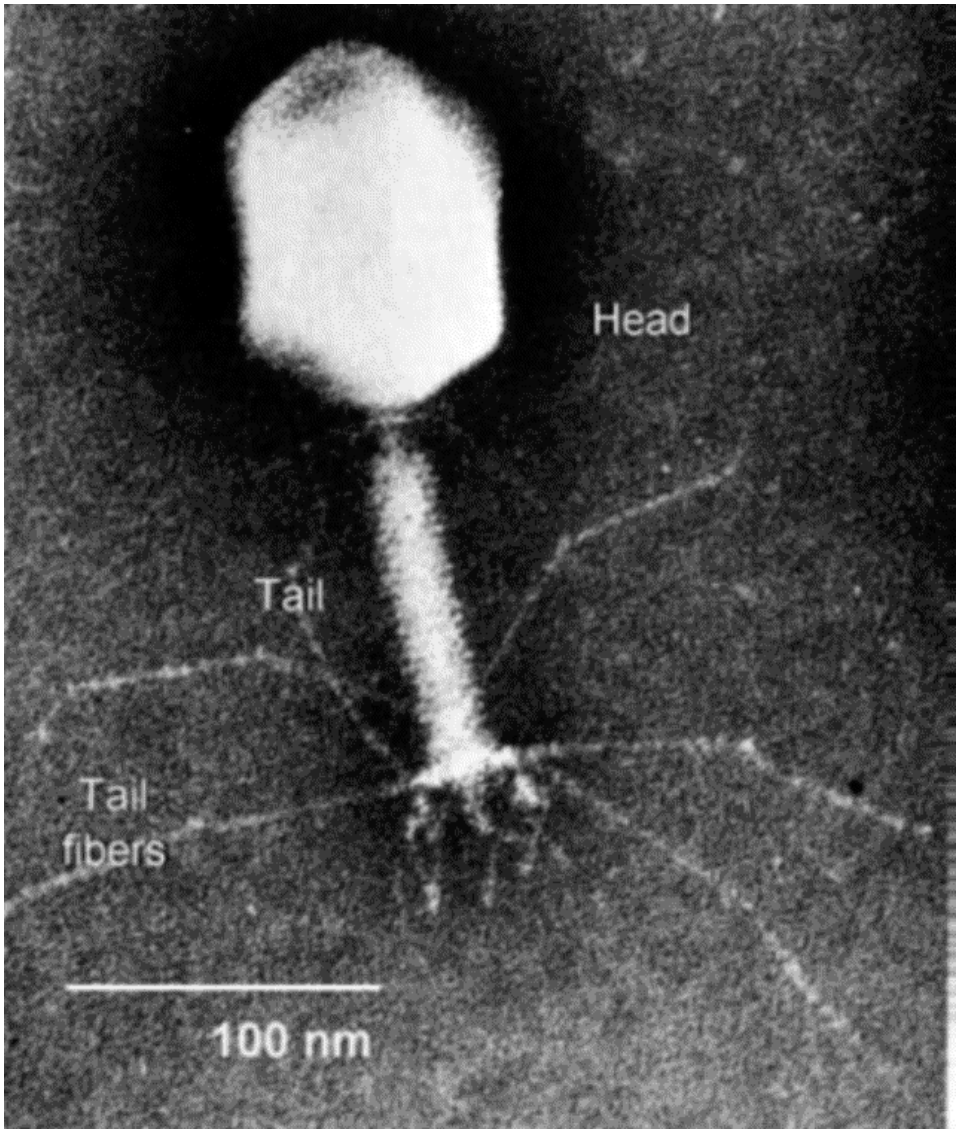
Virus Shapes

Cytoplasmic polyhedrosis virus (icosahedral)



Virus Shapes

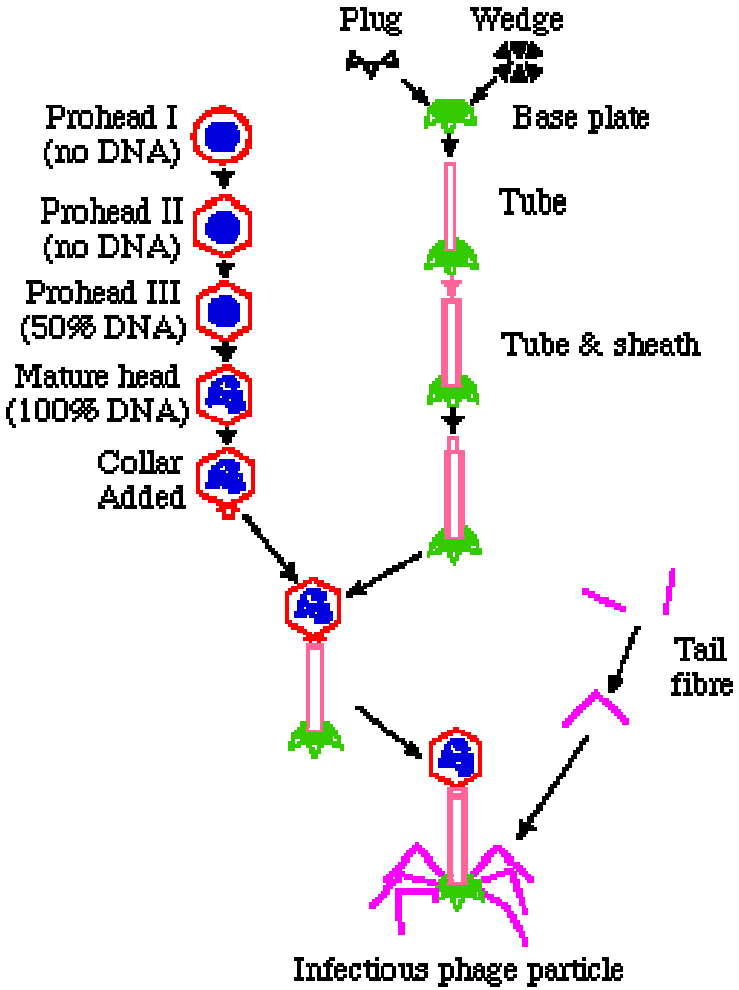
Bacteriophage T4
(icosahedral and helical)

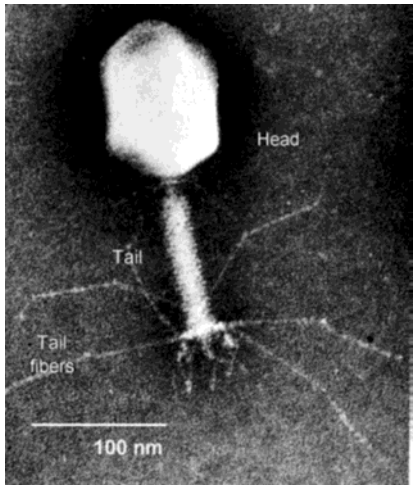


Virus Shapes

Bacteriophage T4

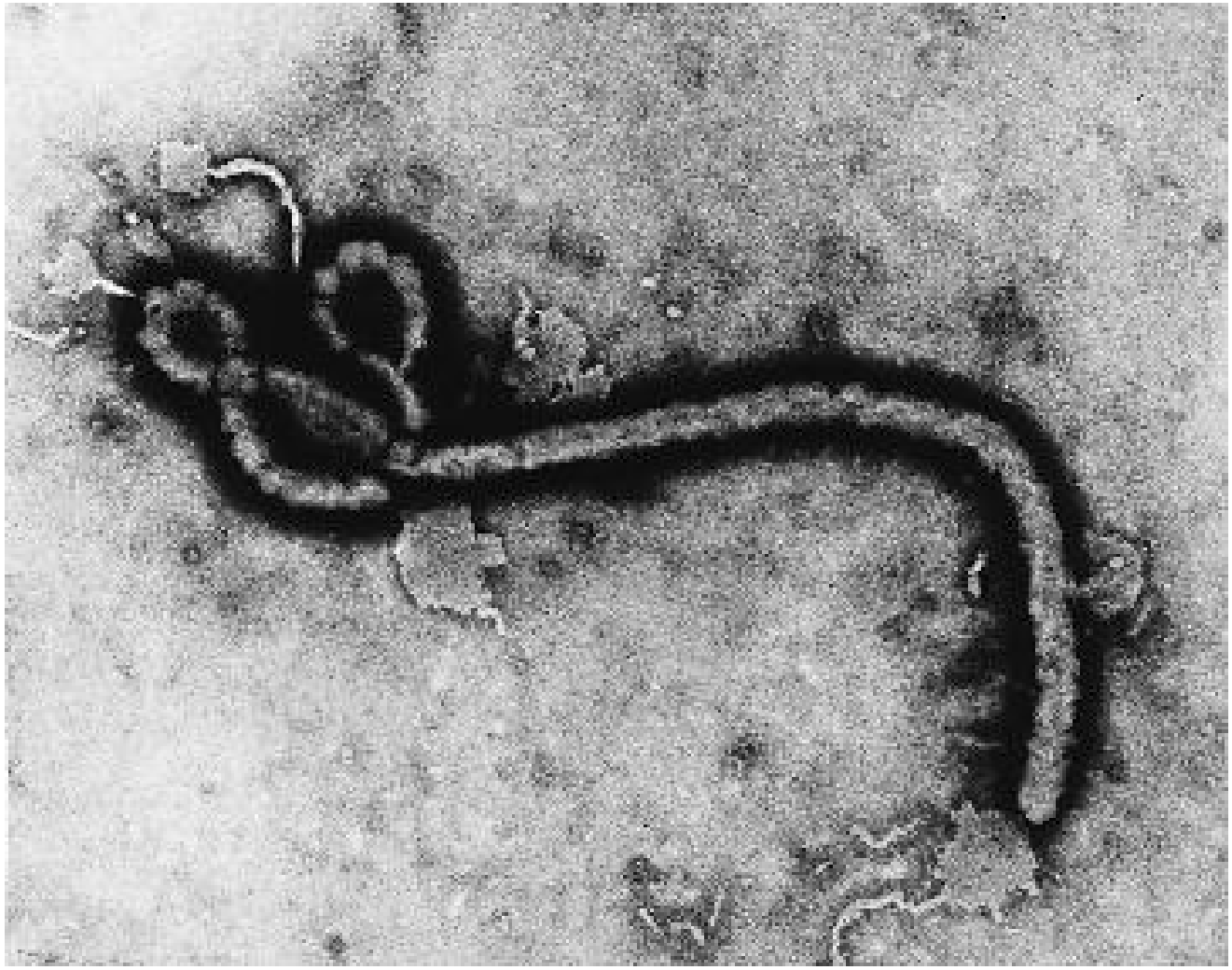
Head consists of an icosahedral shell attached via a collar to a helical tail. At the end of the tail is a plate which functions in attachment to the bacterial host.In addition thin protein fibres are attached to the plate, again involved in binding to host.





Virus Shapes

Ebola (irregular)

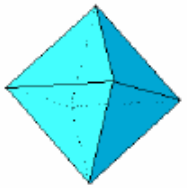


Icosahedral Symmetry

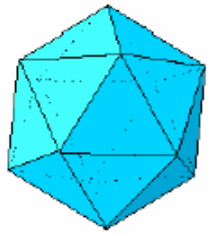
The Five Platonic Solids

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

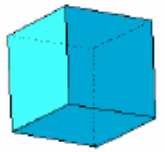
with 4 faces at each vertex, an **octahedron**



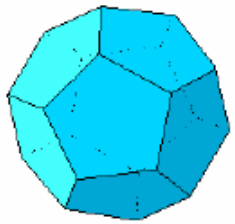
with 5 faces at each vertex, an **icosahedron**



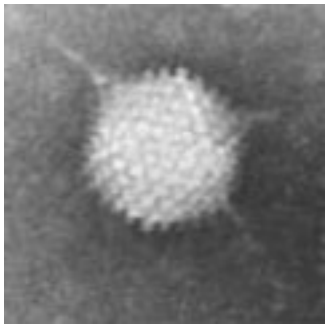
From **squares** you can make:
with 3 faces at each vertex, a **cube**



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



Virus Structure: Icosahedra

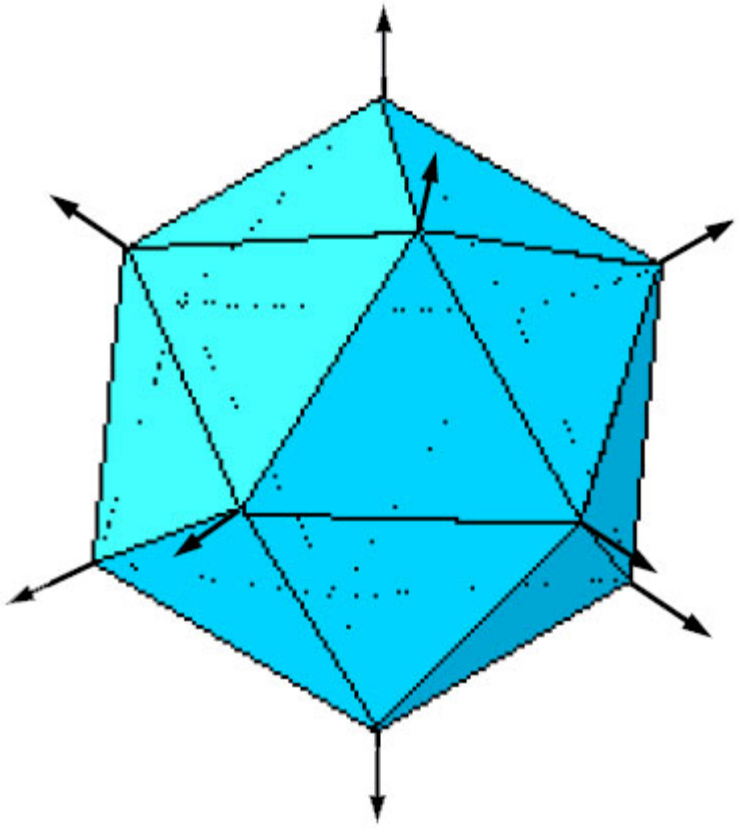


- A common way of building a virus capsid is to arrange protein subunits in the form of a hollow quasi-spherical structure, enclosing the genome within.

Crick &Watson (1956), after seeing electron microcraphs, were the first to suggest that virus capsids are composed of numerous identical protein sub-units arranged either in helical or icosahedral symmetry.

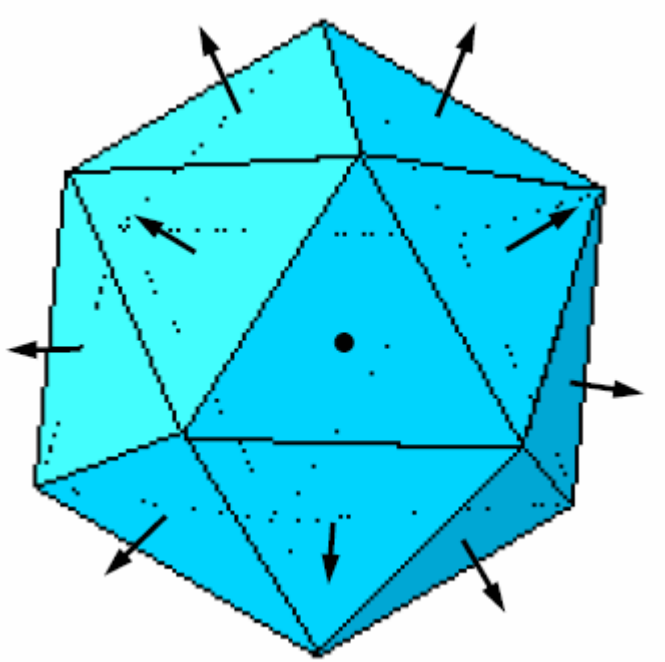
In order to construct a capsid from repeated subunits, a virus must 'know the rules' which dictate how these are arranged. For an icosahedron, the rules are based on 2-3-5 rotational symmetry.

The Icosahedron



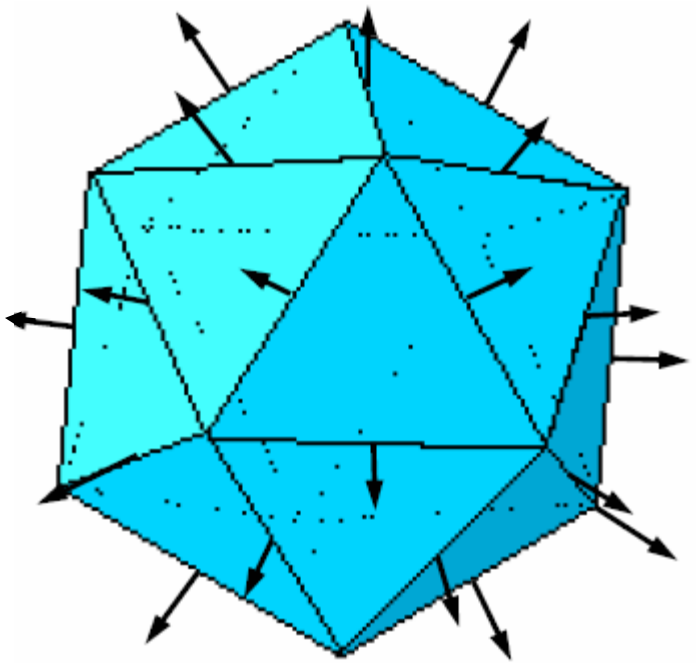
No of vertices : 12 (5-fold symmetry)

The Icosahedron



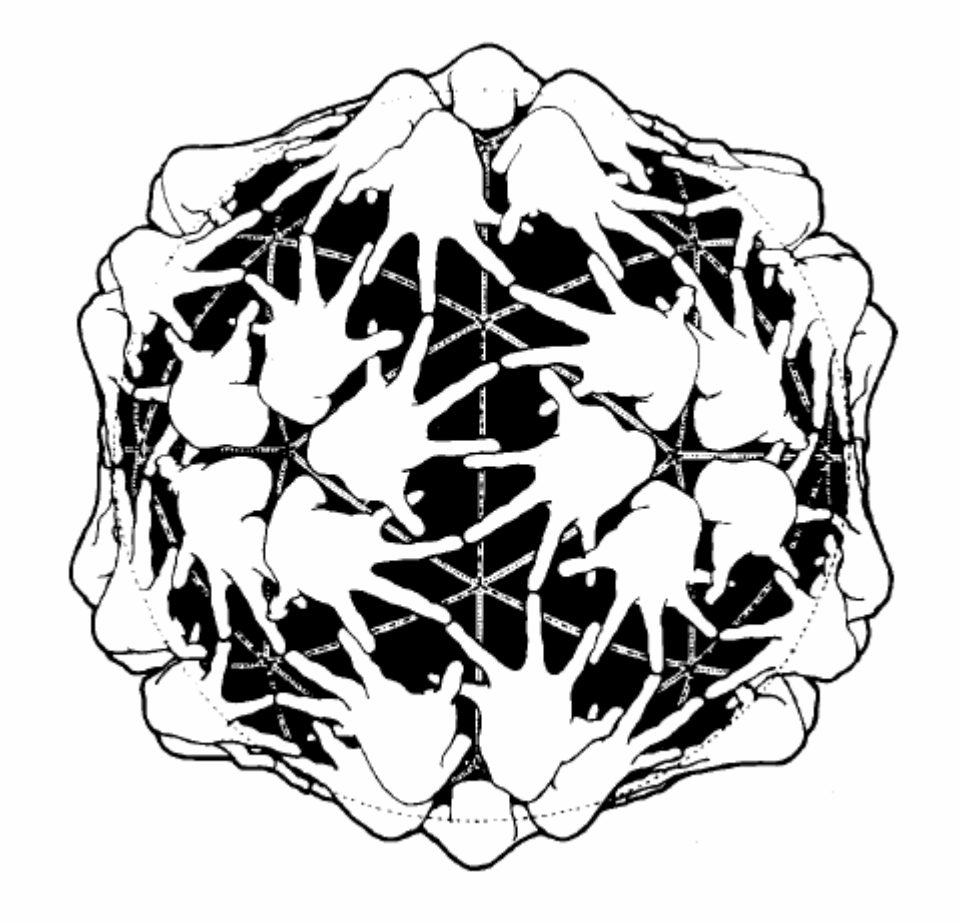
No of faces : 20 (3-fold symmetry)

The Icosahedron

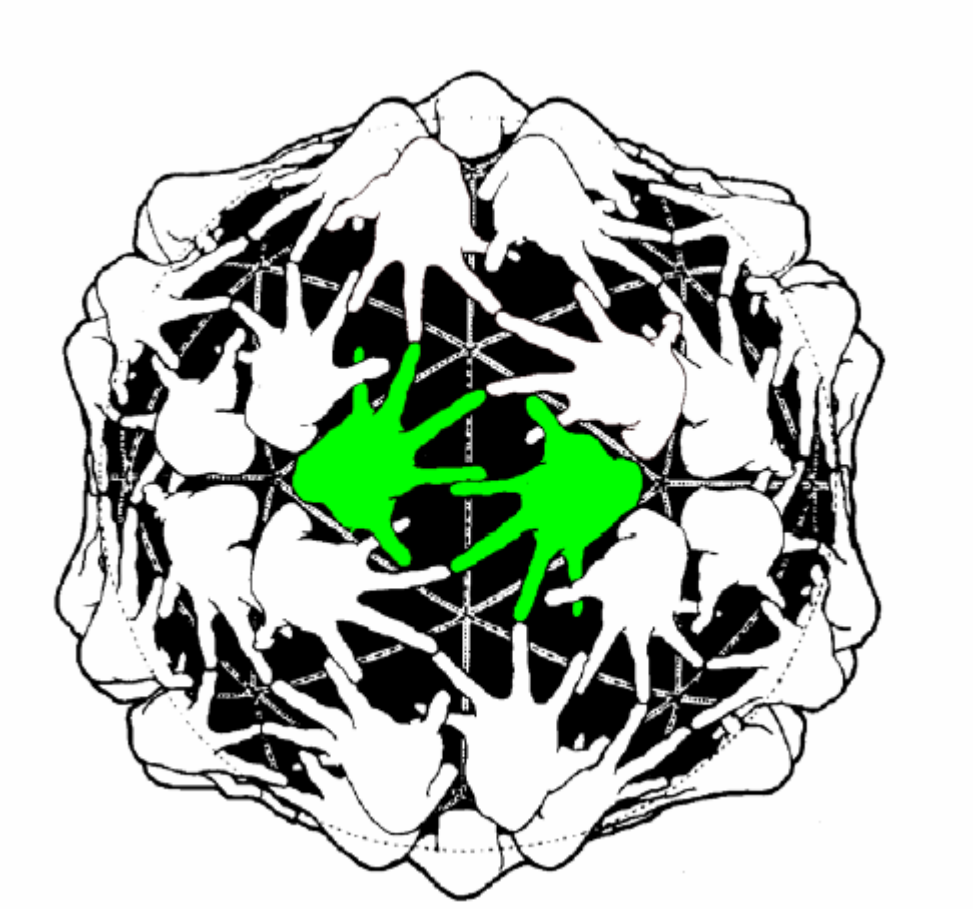


No of edges : 30 (2-fold symmetry)

532 Point Group Symmetry

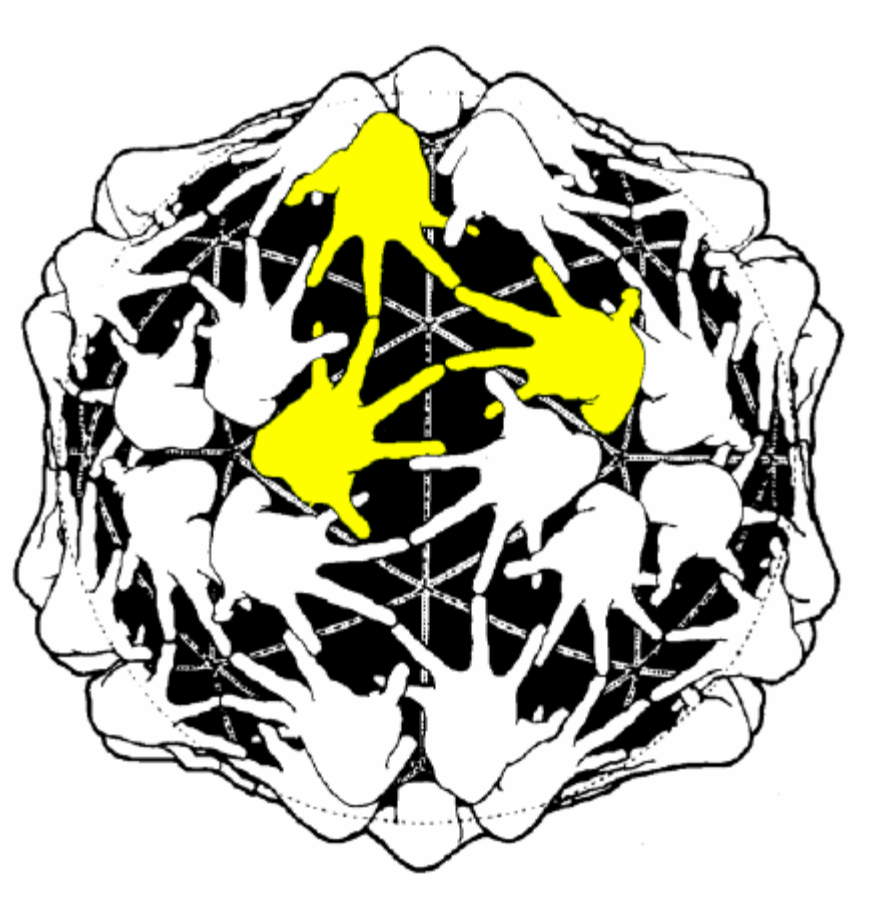


532 Point Group Symmetry



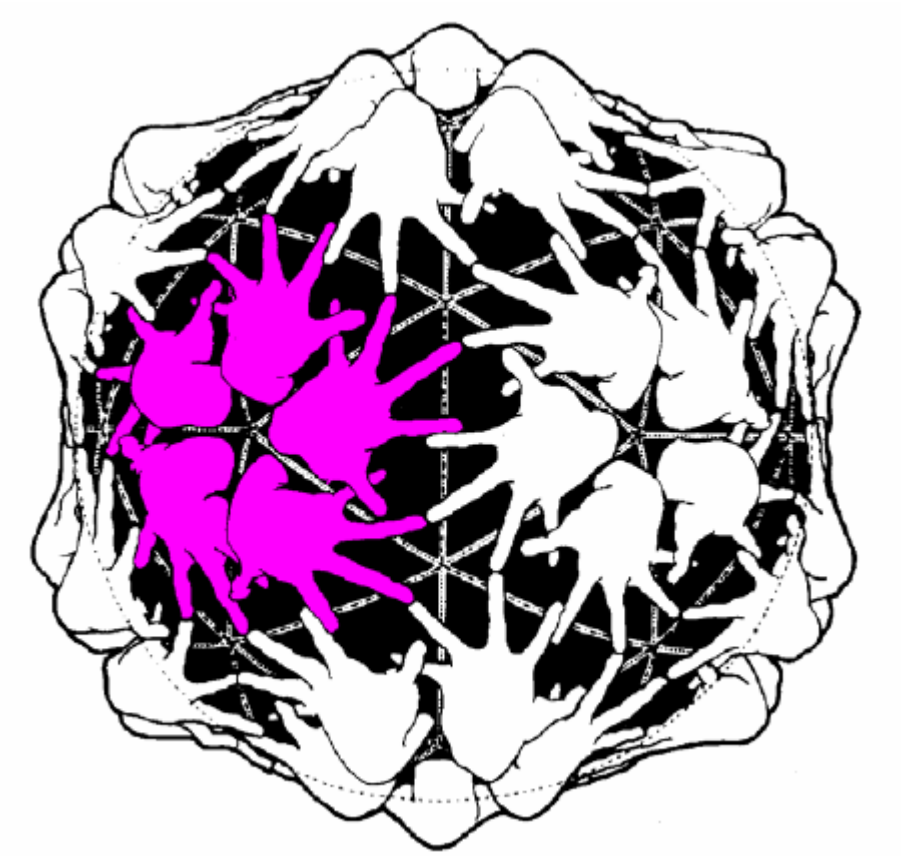
30 dimers

532 Point Group Symmetry



20 trimers

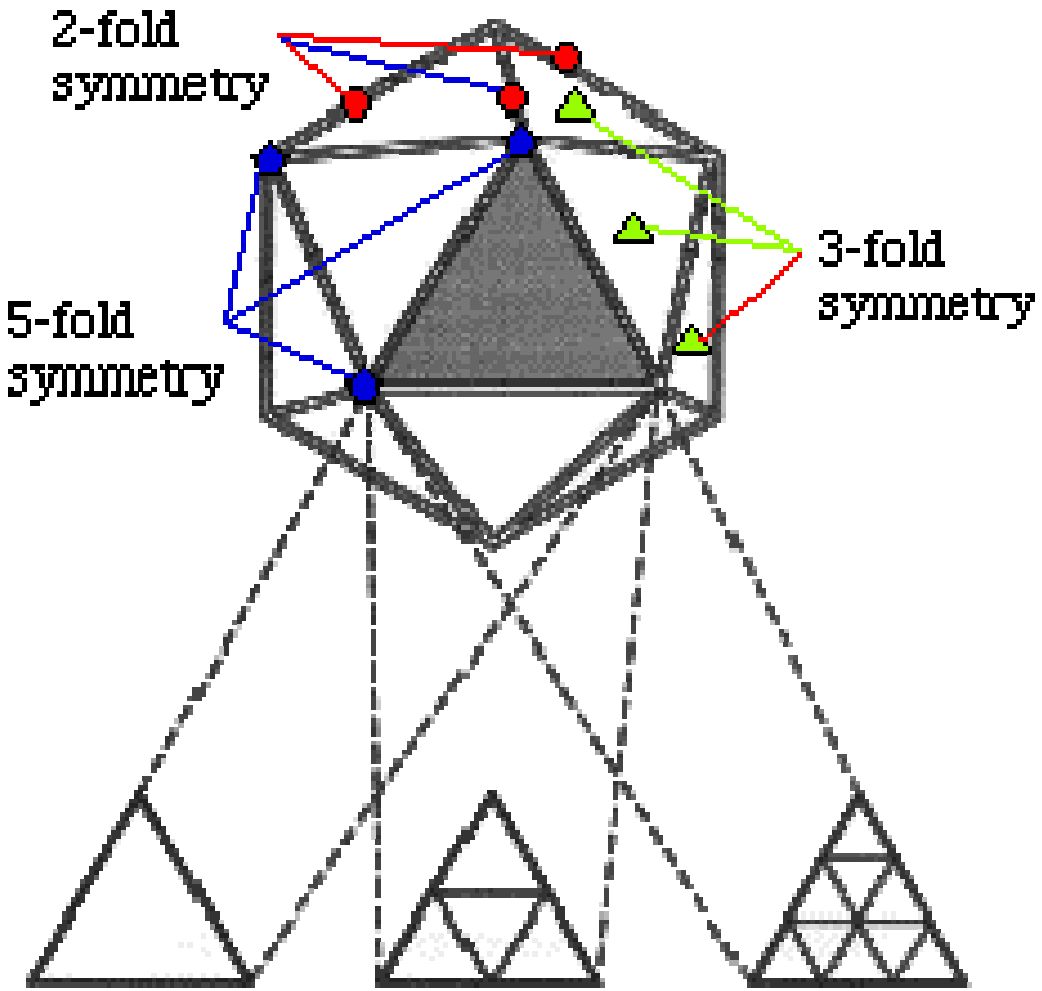
532 Point Group Symmetry



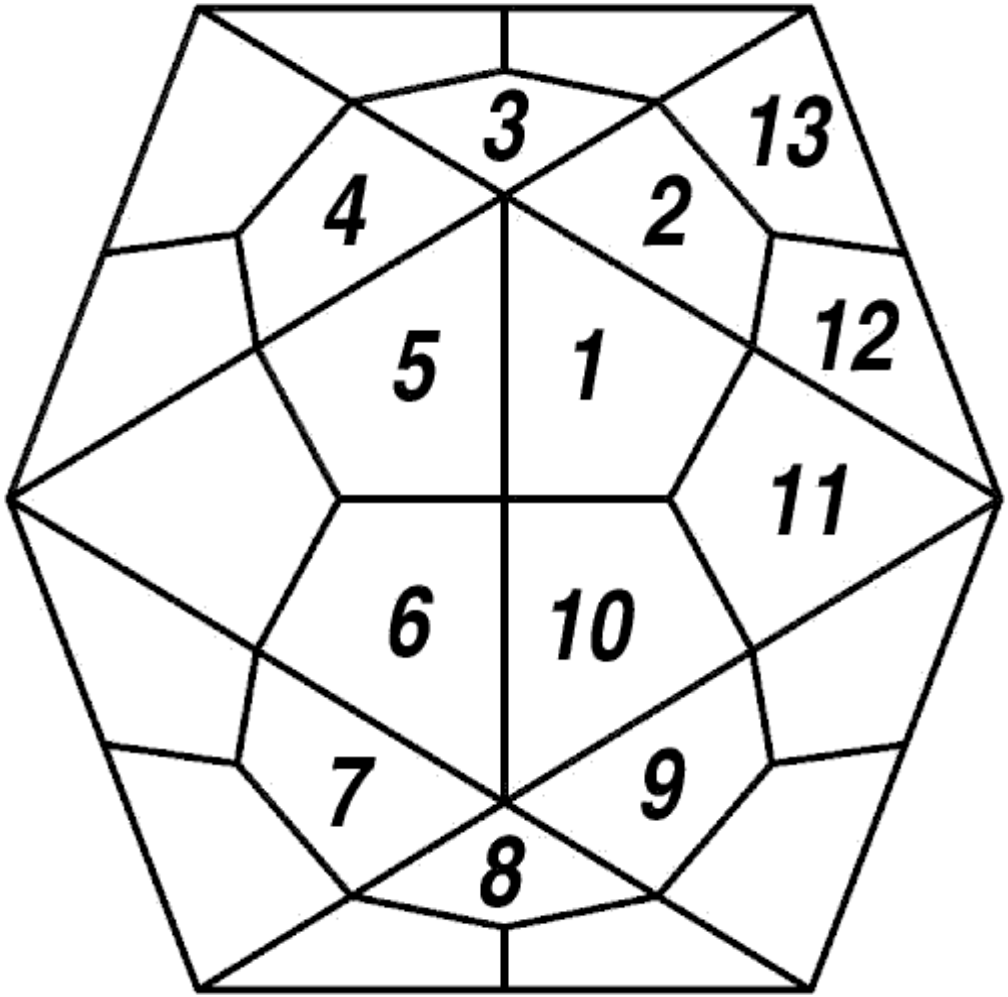
12 pentamers

Virus Structure: Icosahedra

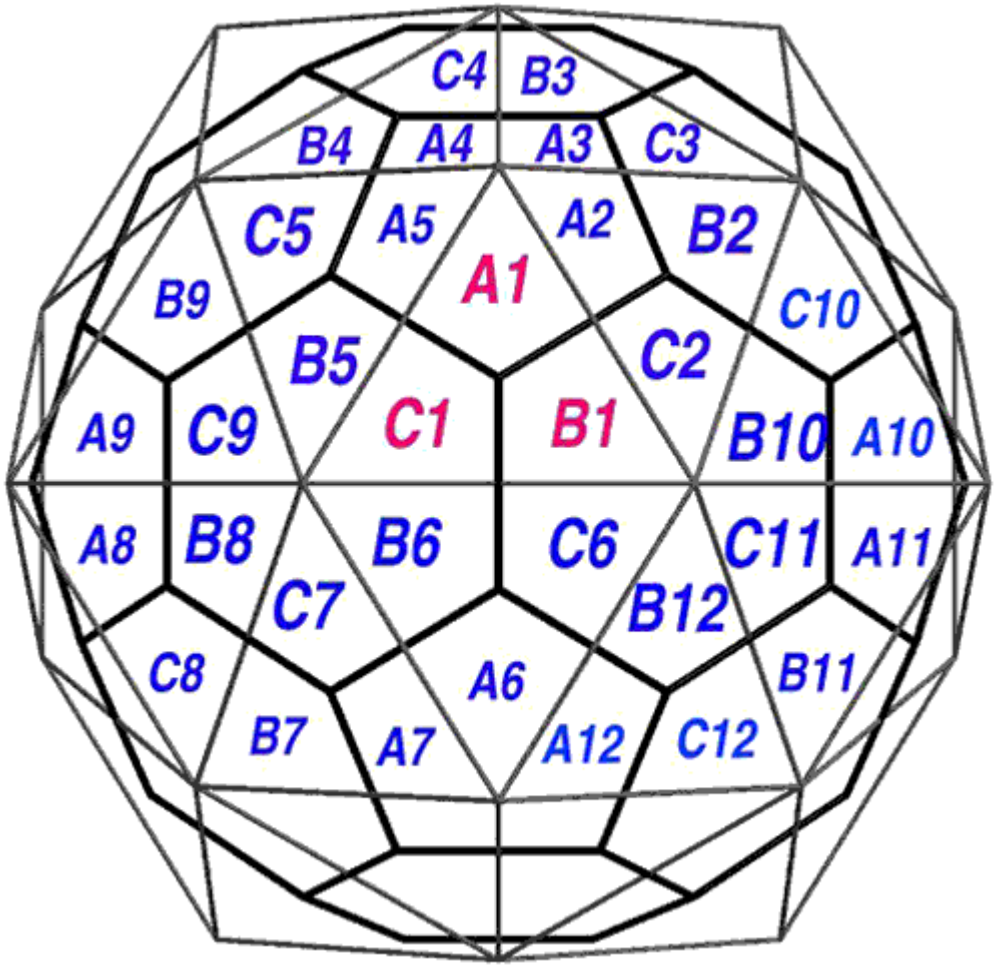
- 20 equilateral triangles arranged into a sphere.
- **bacteriophage ØX174. 60 identical subunits** form a capsid. 3 protein subunits per triangular face (T=1). This is the simplest case; most viruses have more subunits per face (higher T number).



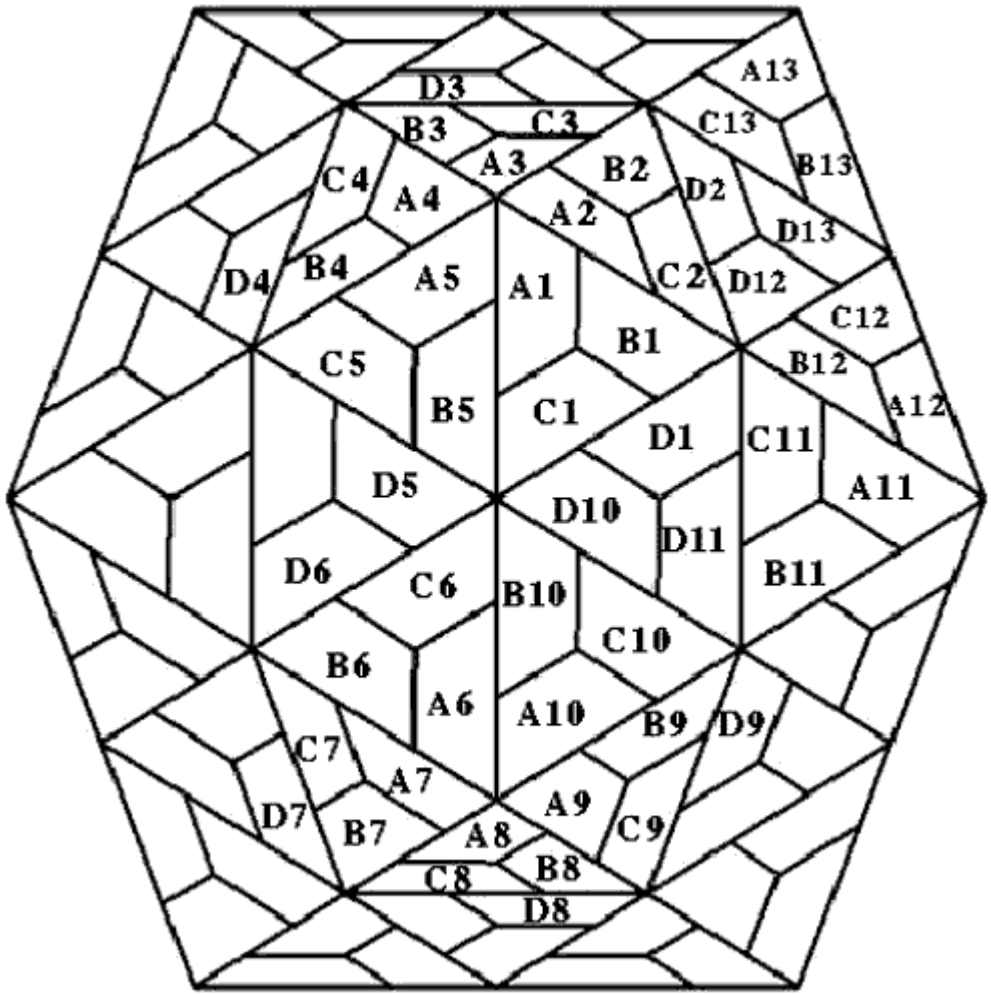
T=1 Triangulation



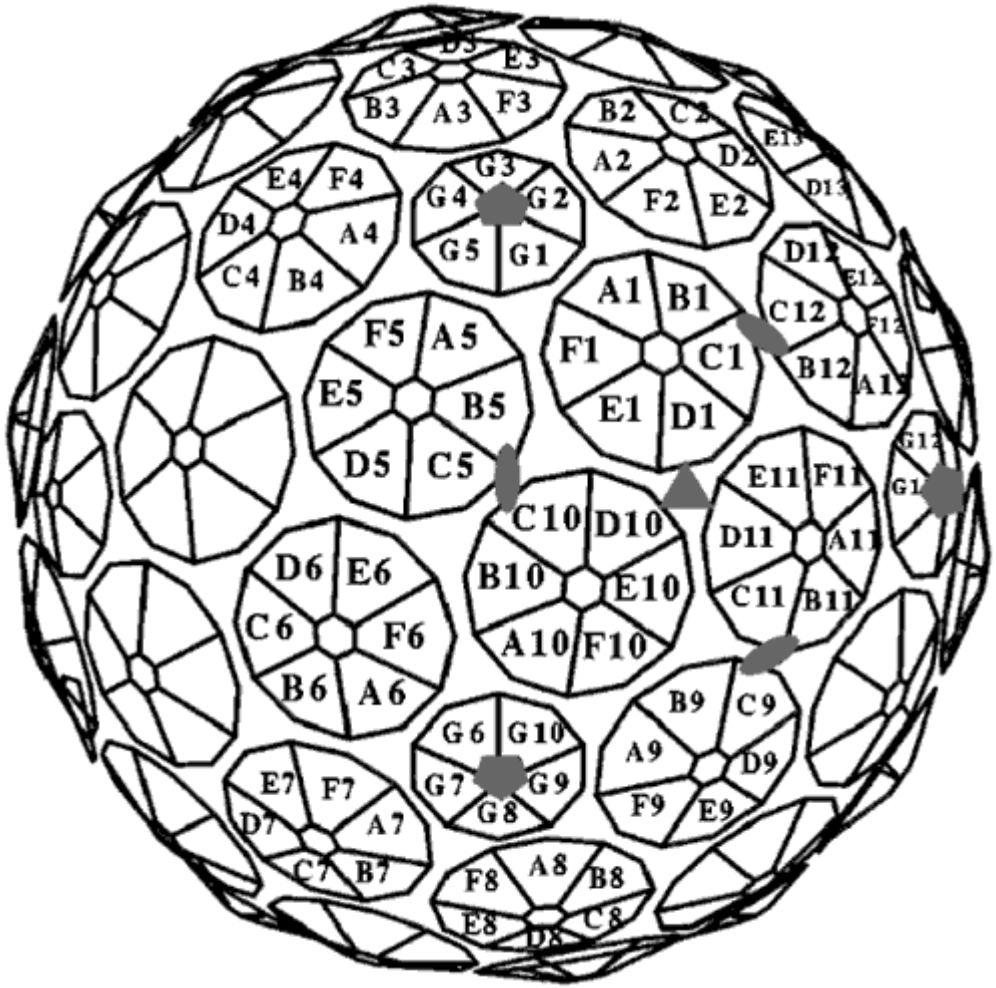
T=3 Triangulation



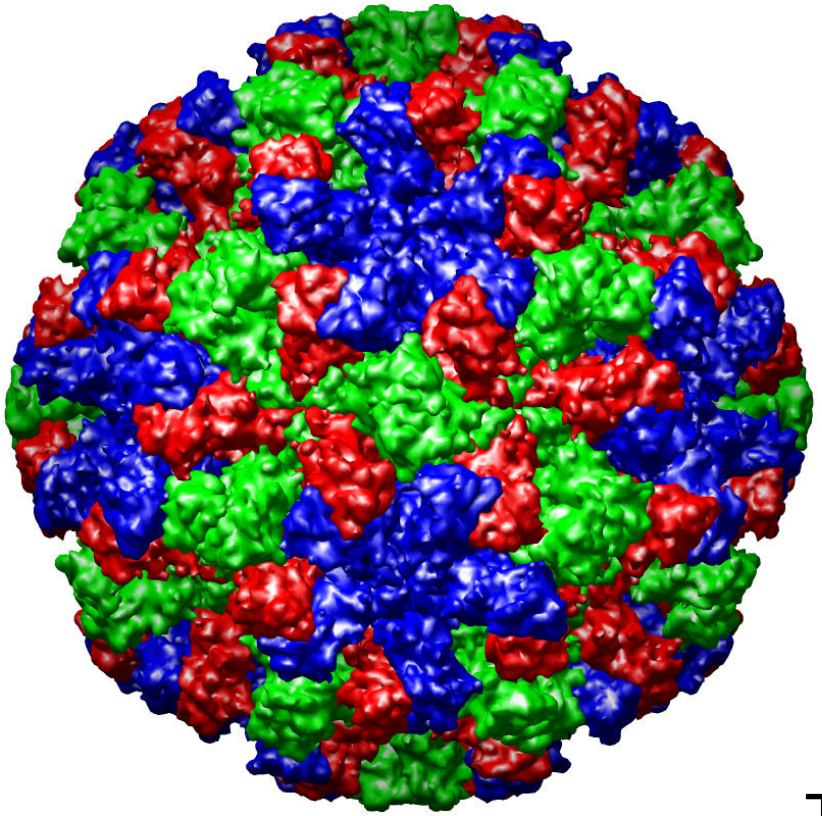
T=4 Triangulation

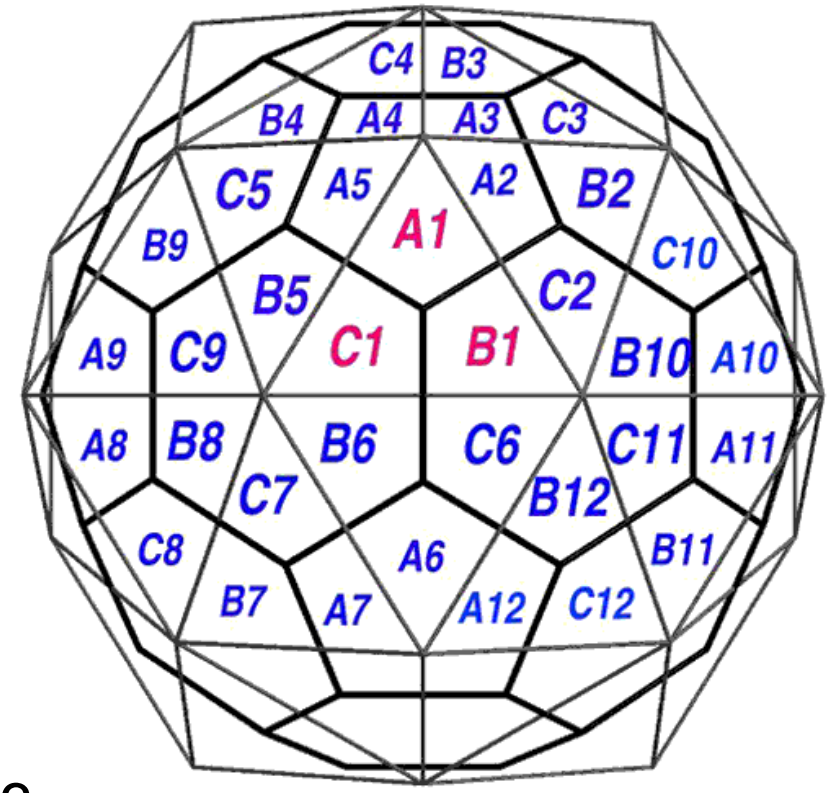


T=7 Triangulation



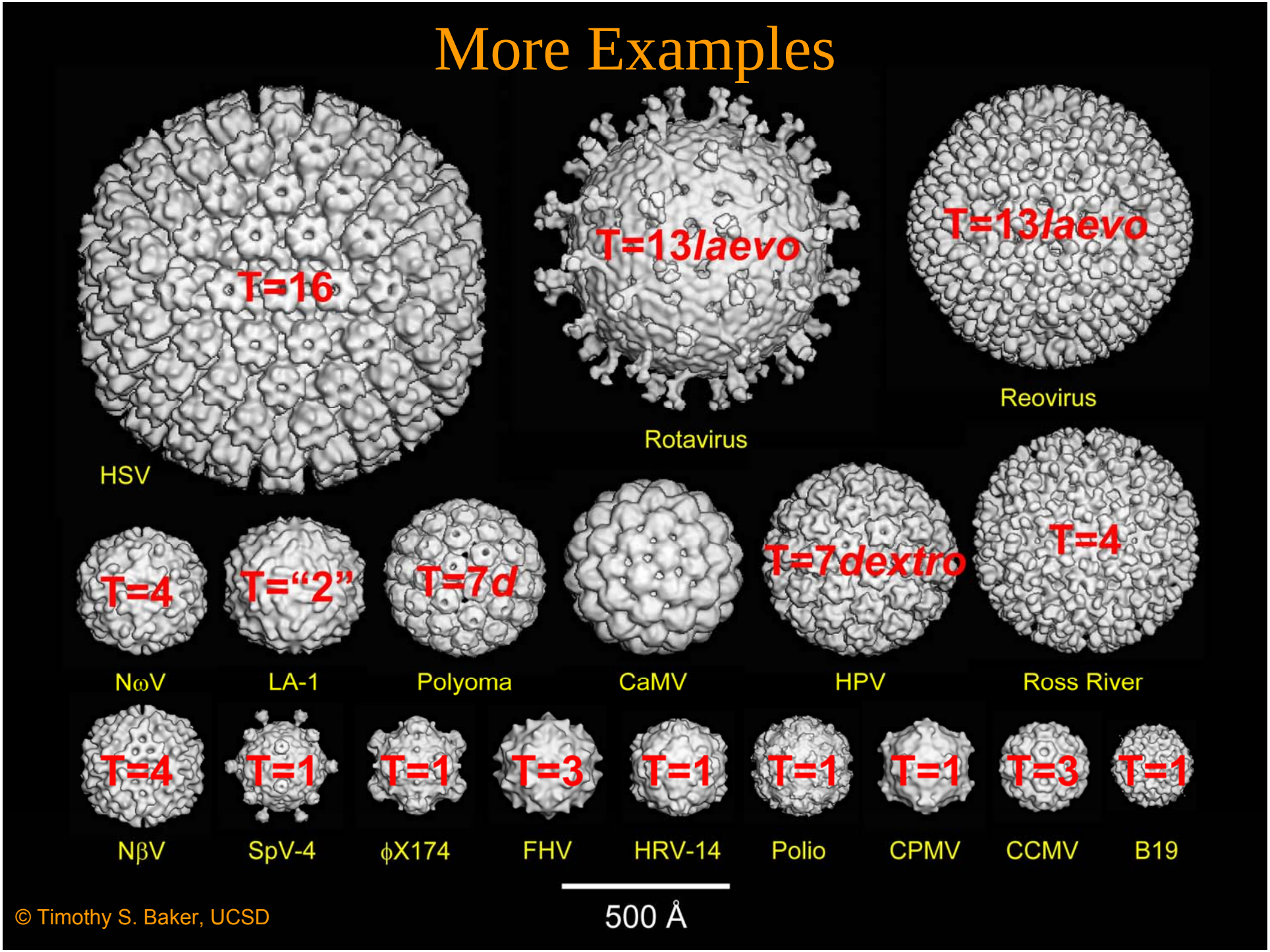
Example: Norwalk Virus Structure



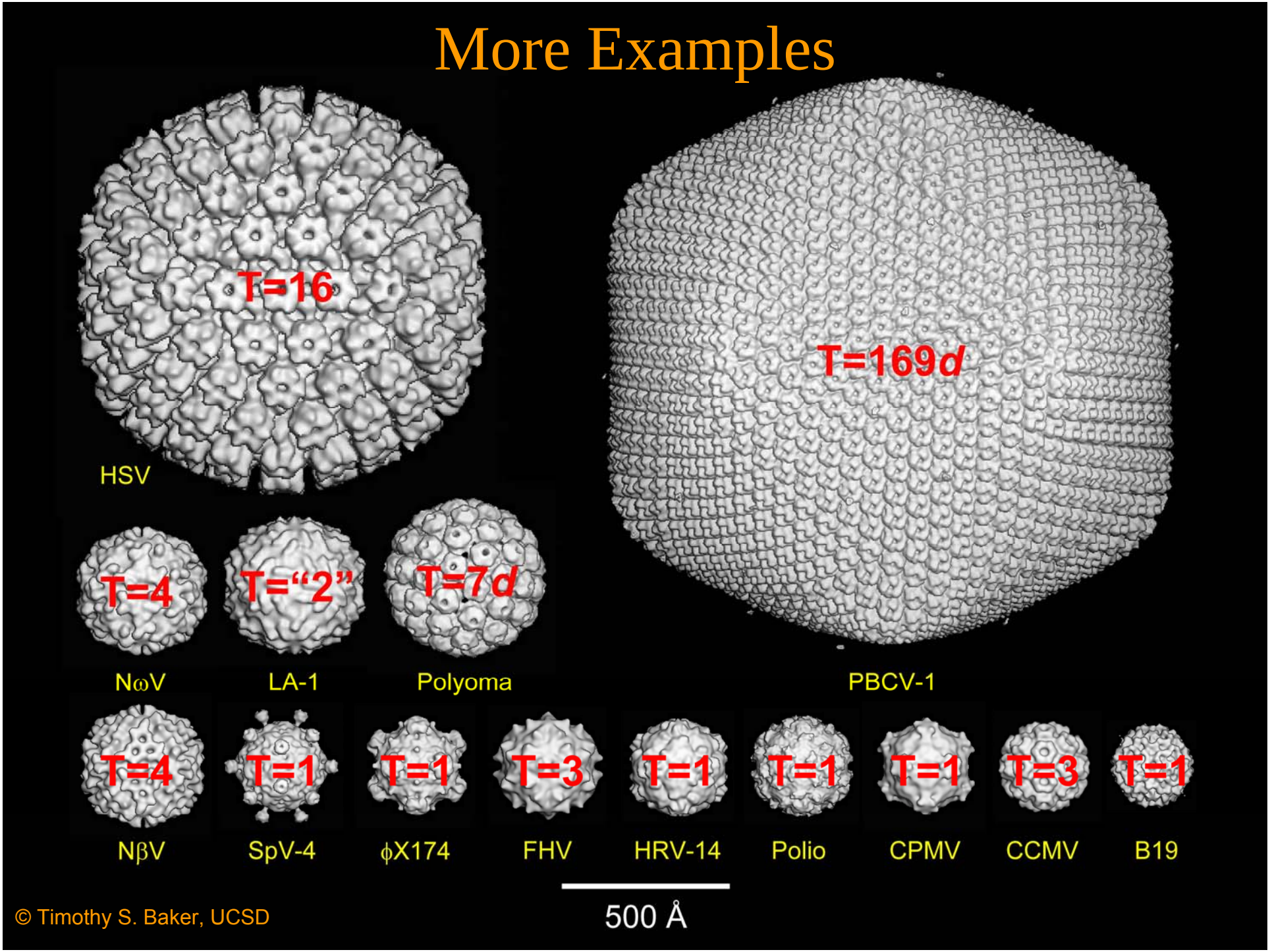


T=3

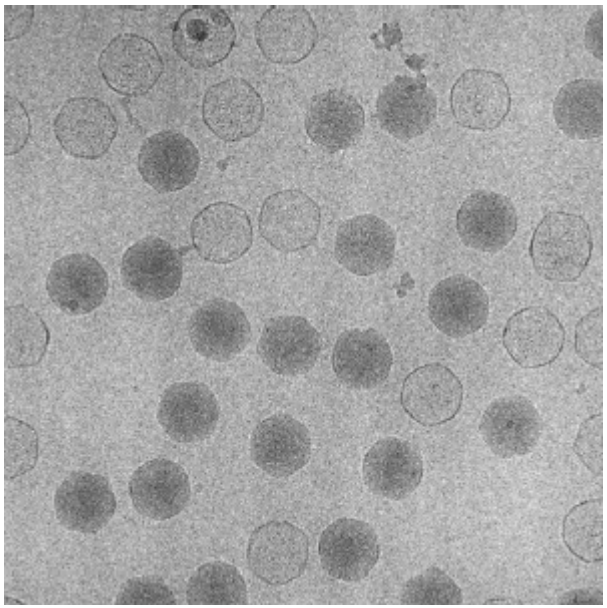
More Examples



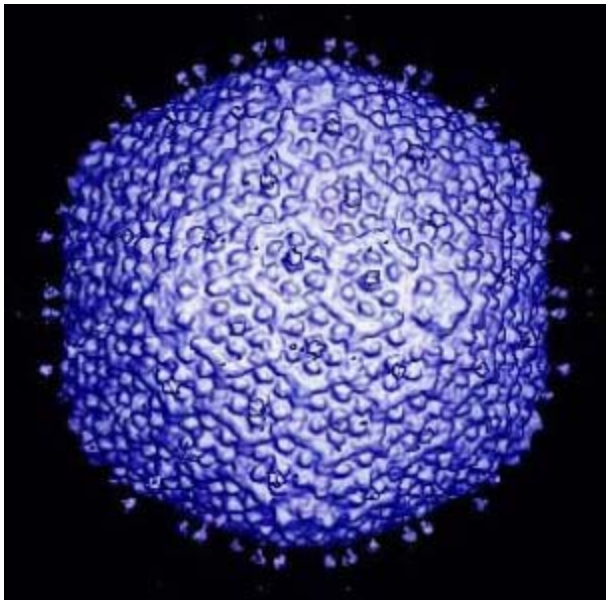
More Examples



3D Reconstruction



2D



3D

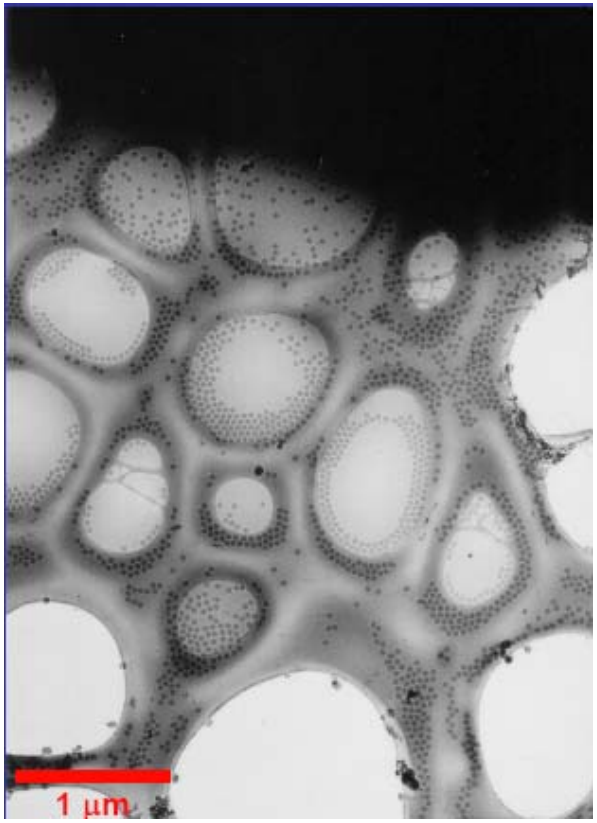
Why CryoEM?

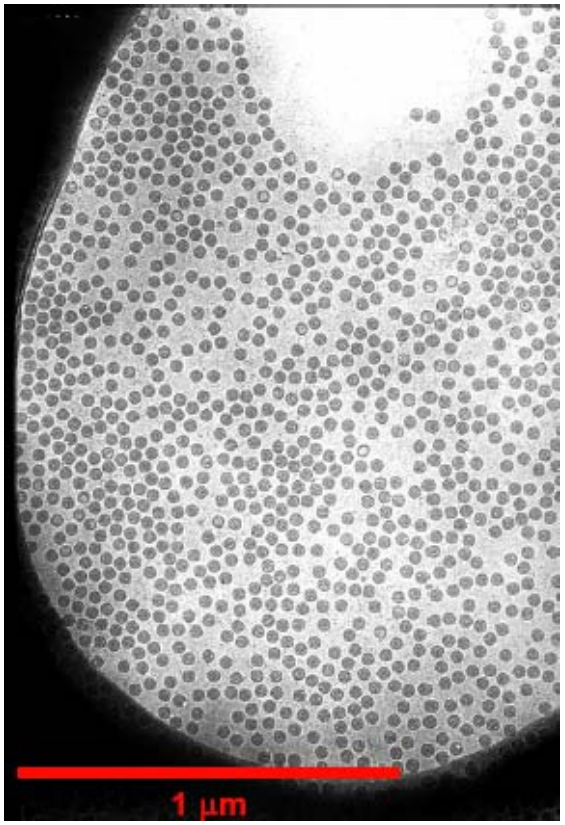
- Well suited for large macromolecules
- Resolution limit near 5Å
- Sample is frozen in vitreous ice and imaged at liquid nitrogen temperatures
- Imaging thousands of individual particle randomly orientated on a thin substrate
- Computer reconstructions to generate 3D structure

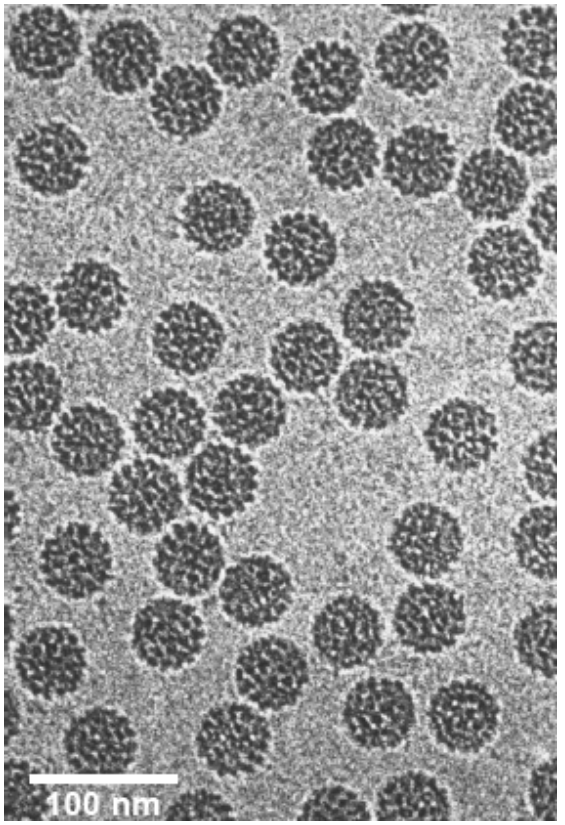
CryoEM

Sample : ~2-3 µl at 1-5 mg/ml
Specimen support: holey carbon film (1-2 µm)
Microscope: 200-300 keV with FEG
Defocus range: 1-3 µm underfocus
Dose: 10-20 $e^-/Å^2$

Film: SO-163 (12 min, full strength)
Micrographs: 25-100
Particles: 10^3-10^4
Target resolution: 12 - 6 Å







Basic Assumptions

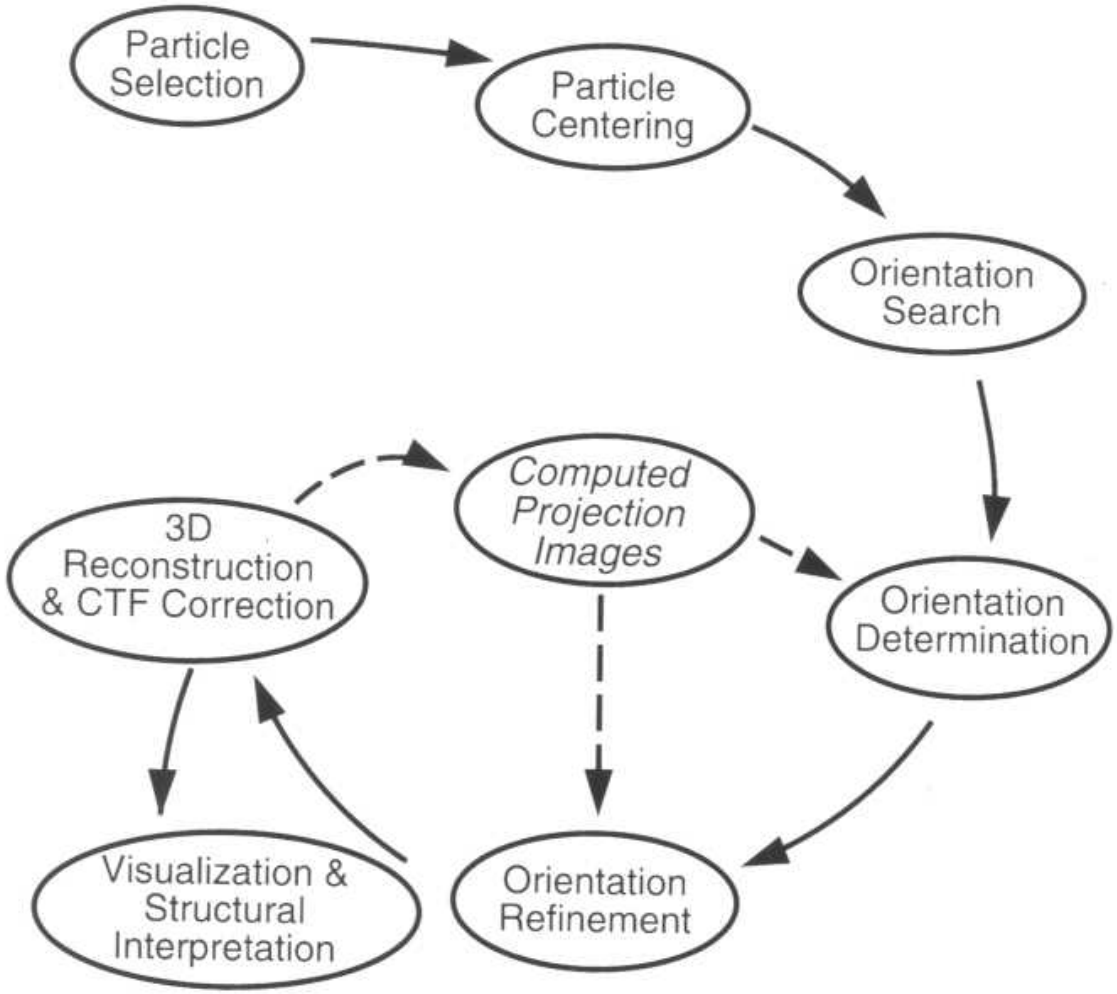
Specimen consists of stable particles with ‘identical’ structures (else averaging is invalid)

Programs test for and *assume* presence of icosahedral (532) symmetry

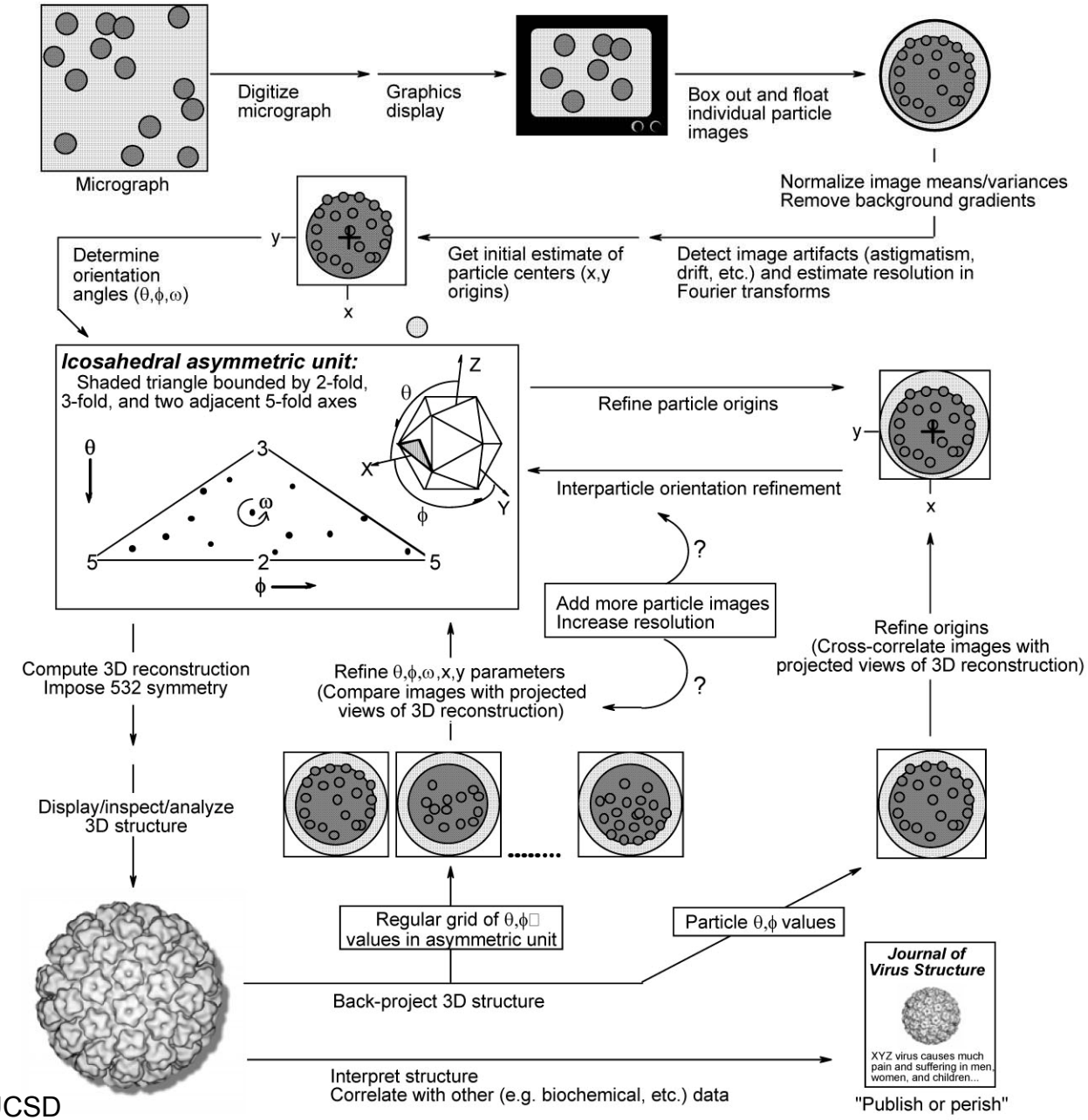
T (triangulation) symmetry is not incorporated into or enforced by the 3D reconstruction algorithms

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

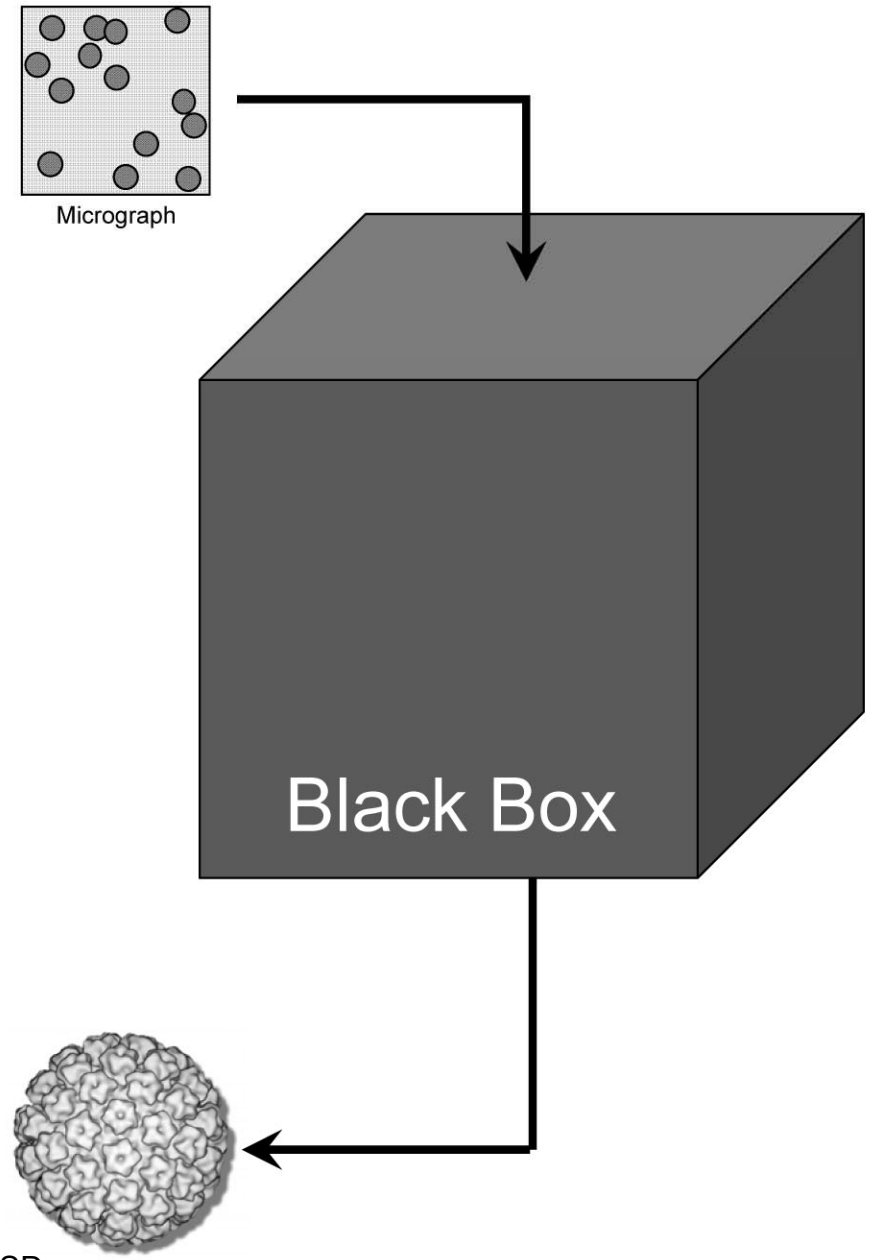
Overview of Reconstruction Scheme



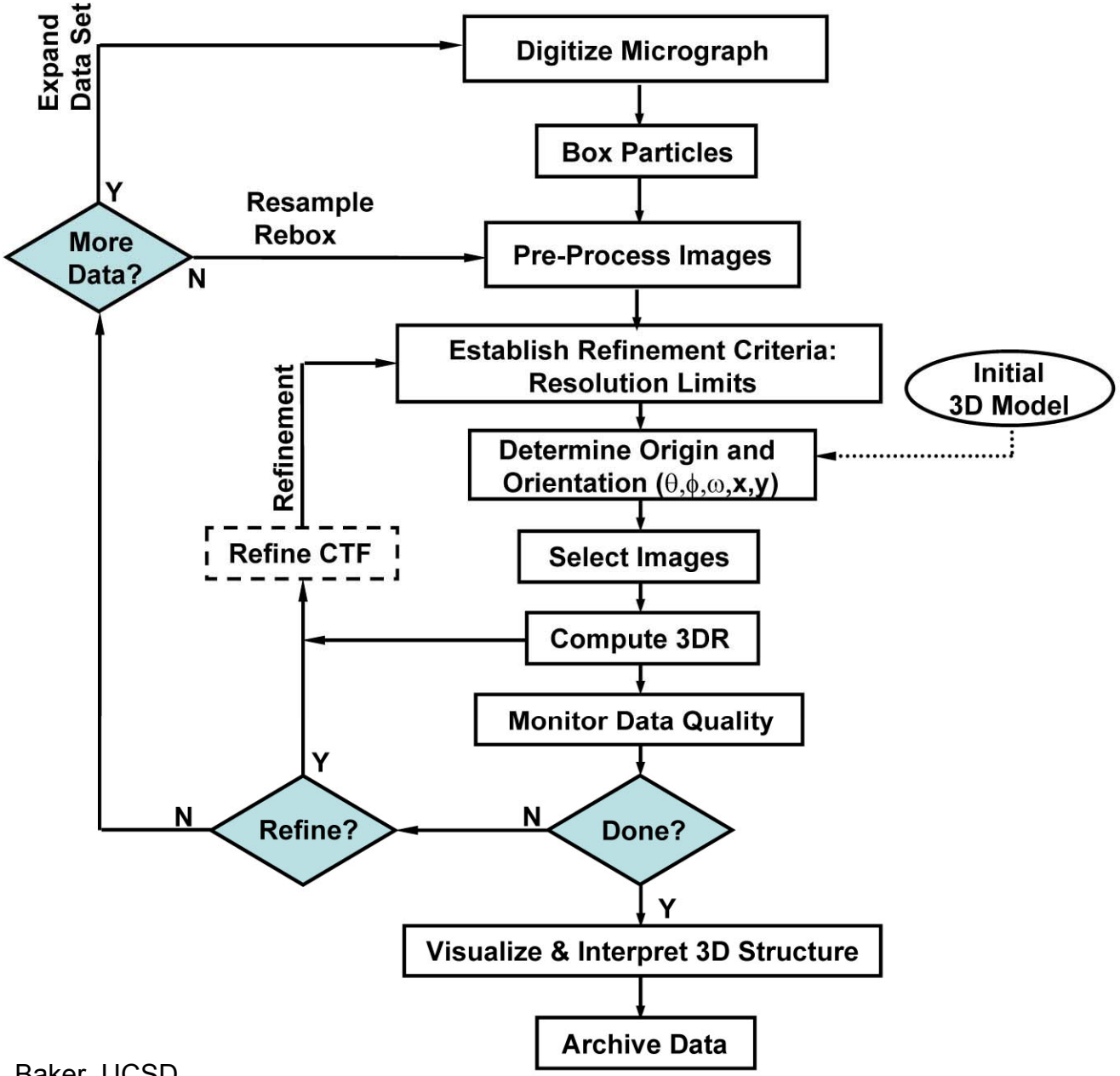
Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme



PSC, PhotoScan
TId2pif, Uniconv
RobEM

RobEM

RobEM
AutoPPI

PFTSEARCH,
OOR, POR

EMPFT-select
AutoSel

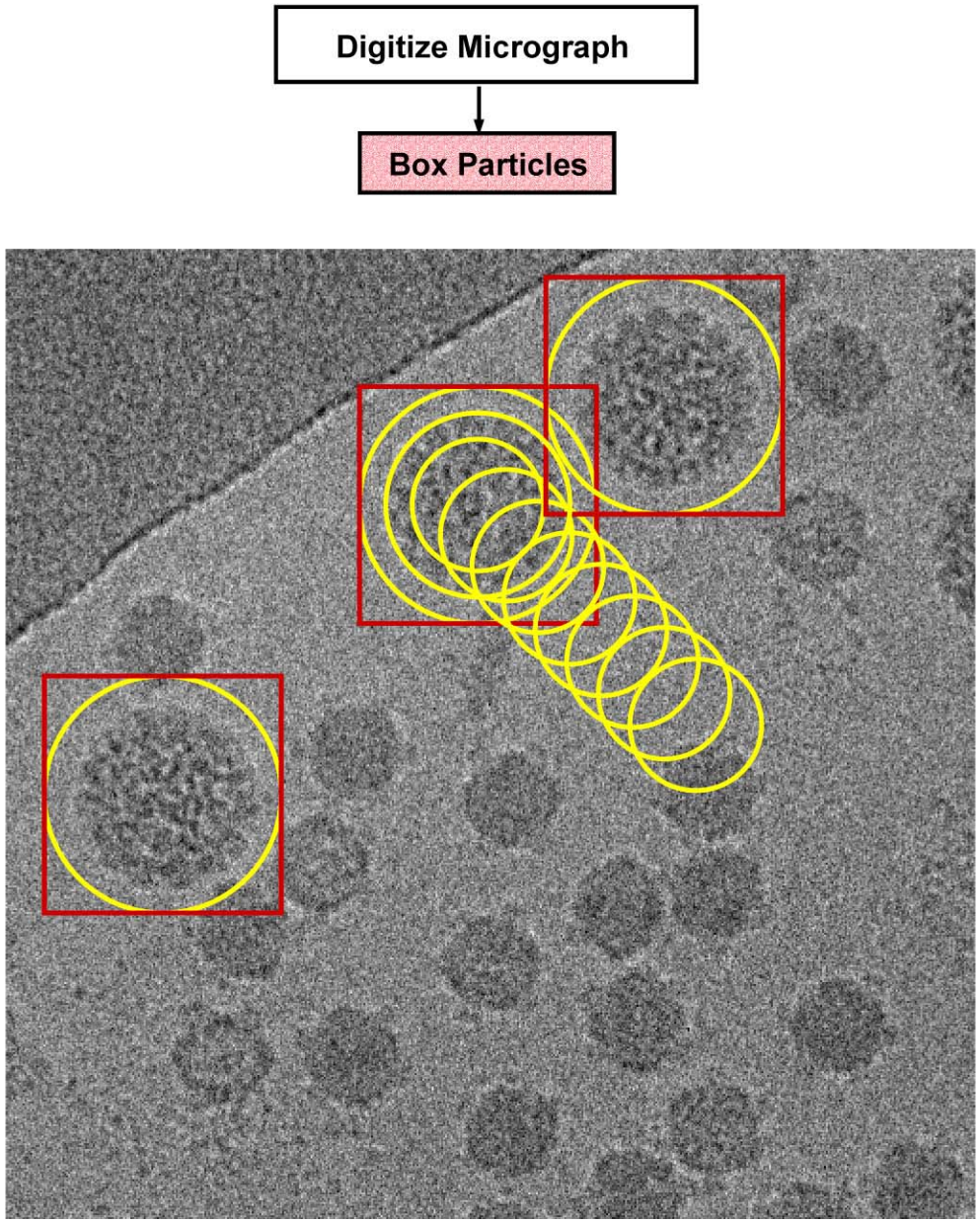
EM3DR, P3DR

RobEM
EMMAP3DT, EMSF
UNICONV, PSF
CUTSHELL
MAPFFT, EMRES

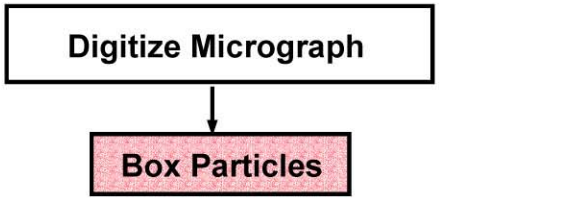
RobEM, O
EMfit, Situs
Explorer
PIF2ASC
Uniconv
MAPMAN
Etc.

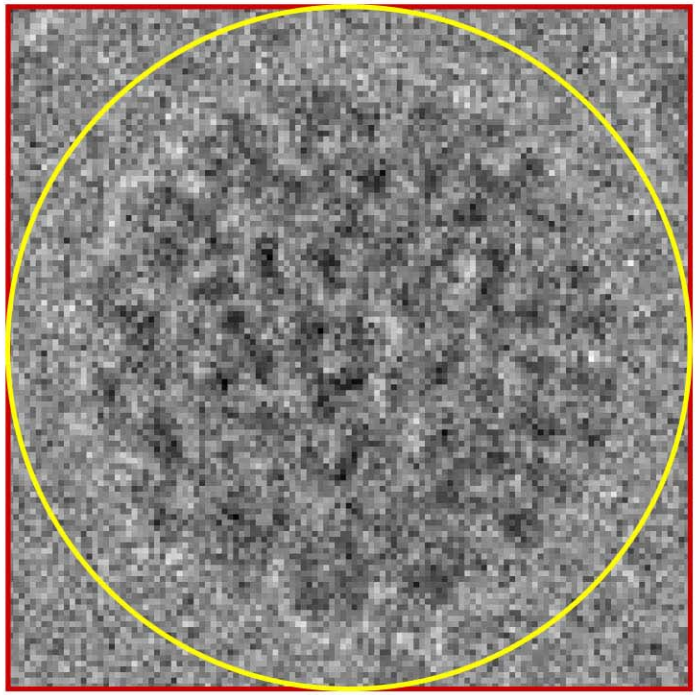
Icosahedral Particle Reconstruction Scheme

Icosahedral Particle Reconstruction Scheme



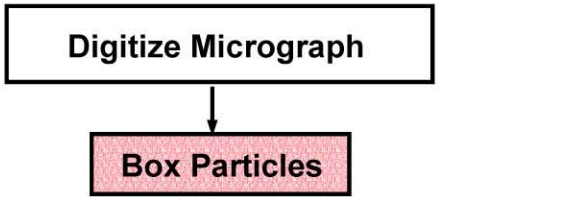
Icosahedral Particle Reconstruction Scheme

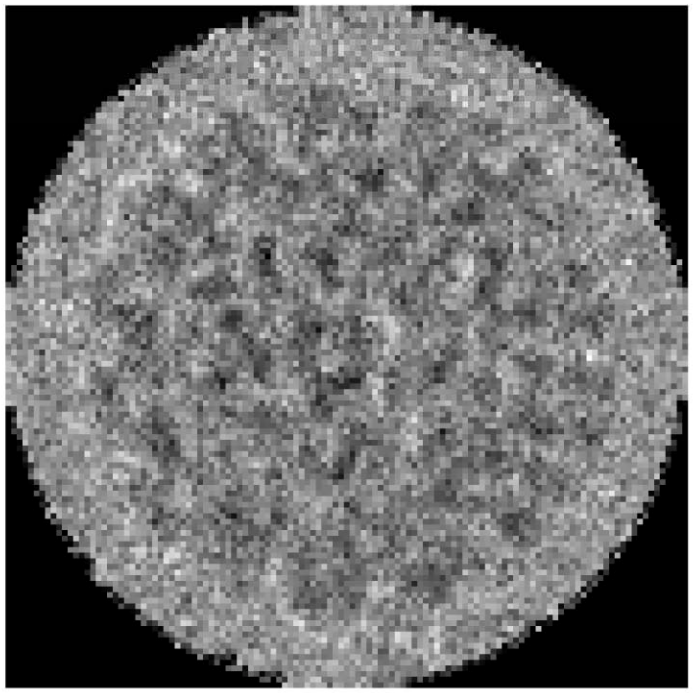




Extracted

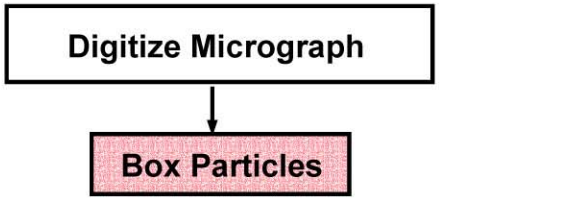
Icosahedral Particle Reconstruction Scheme

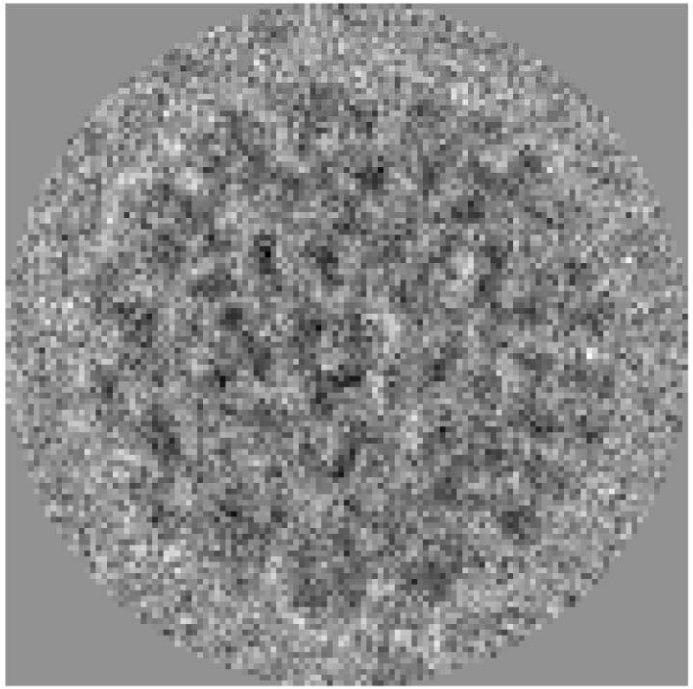




Masked

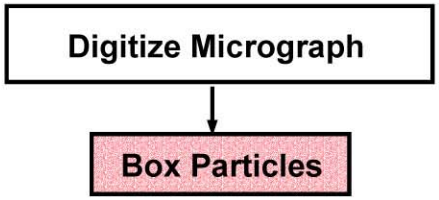
Icosahedral Particle Reconstruction Scheme

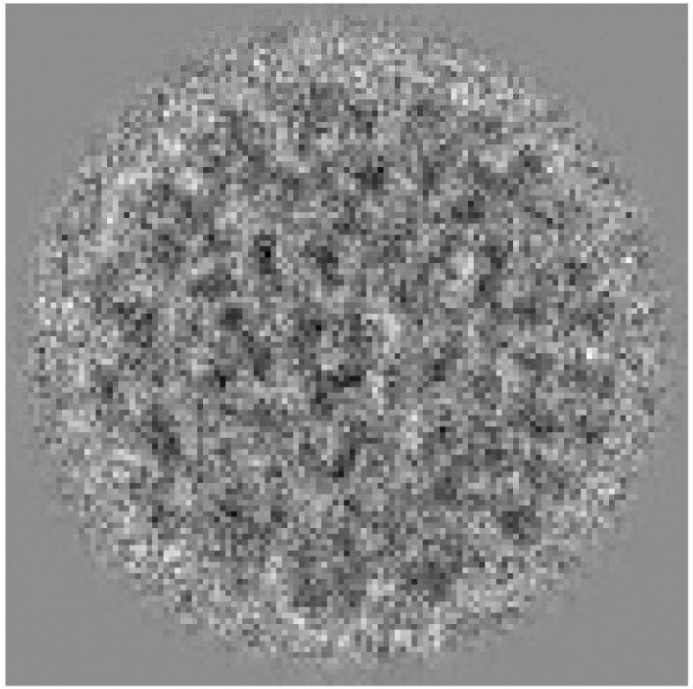




Floated

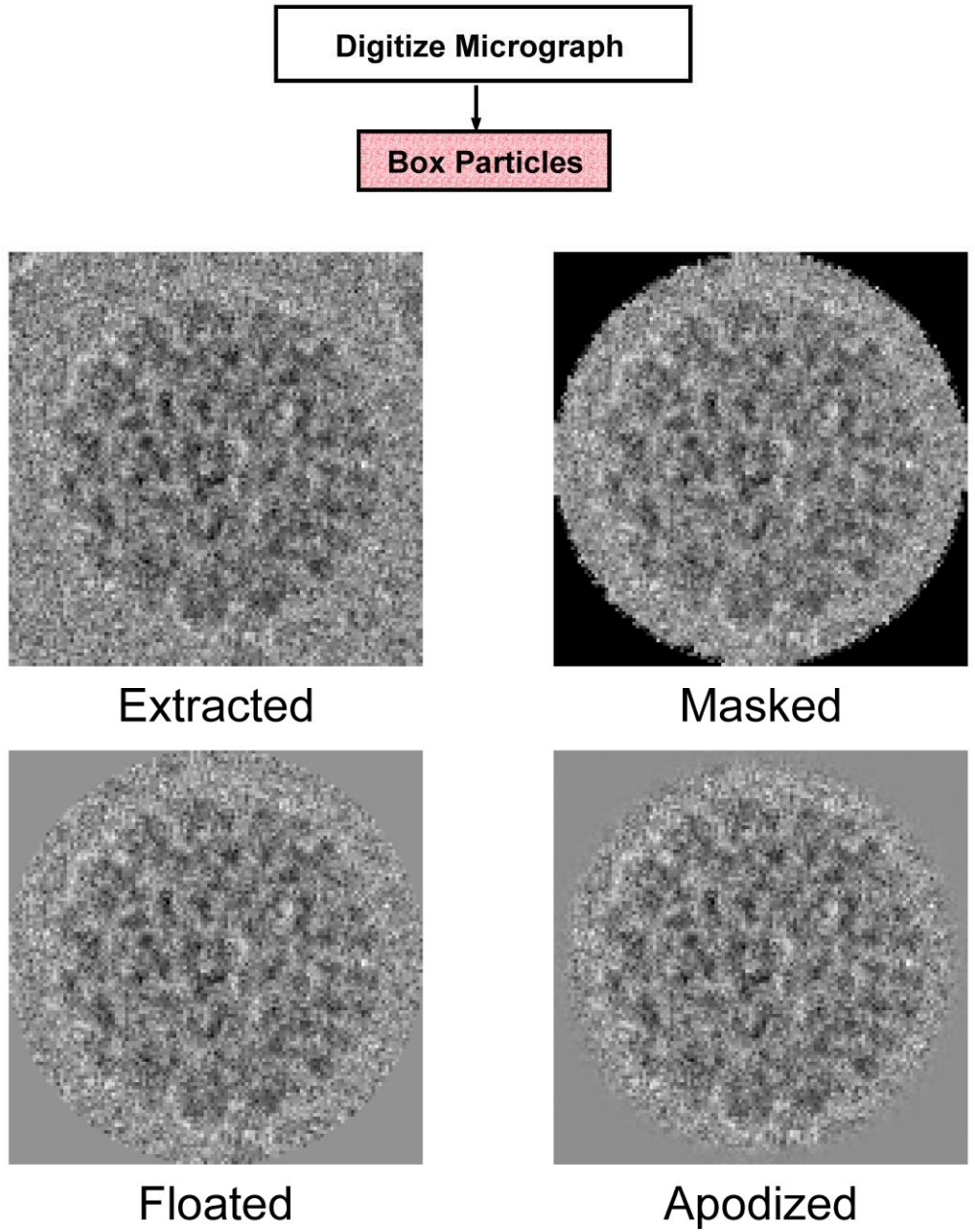
Icosahedral Particle Reconstruction Scheme



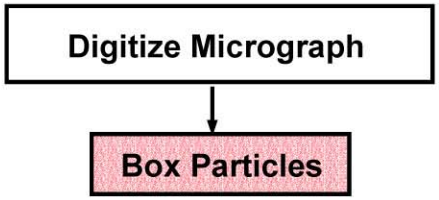


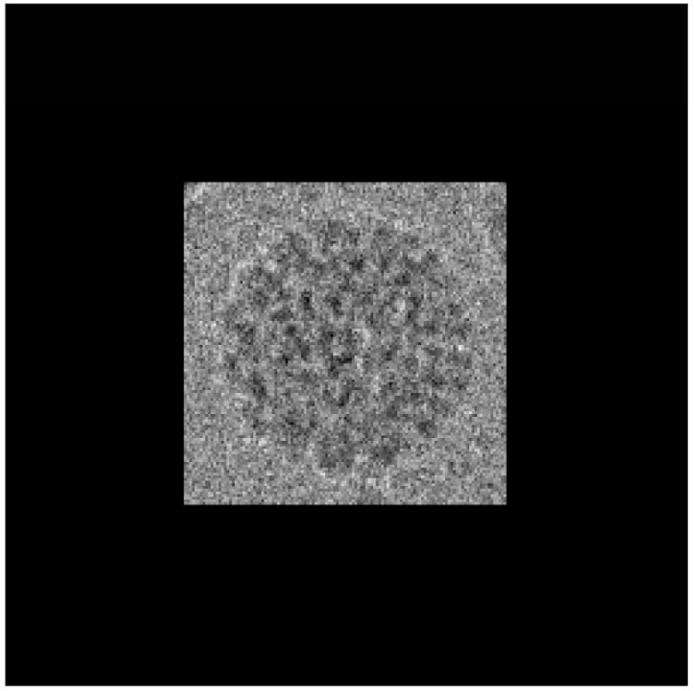
Apodized

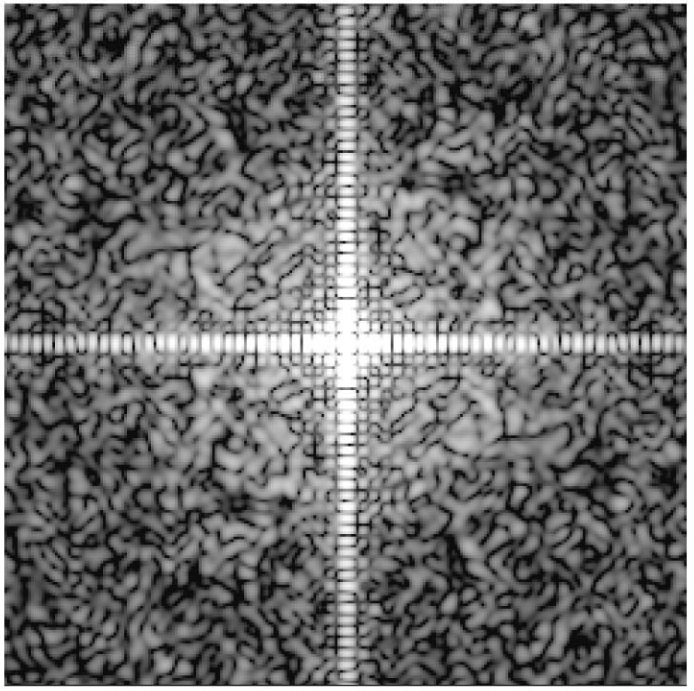
Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme

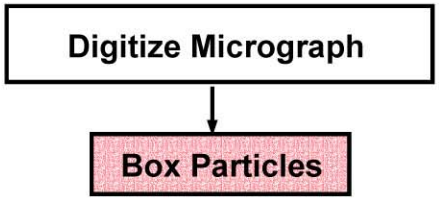


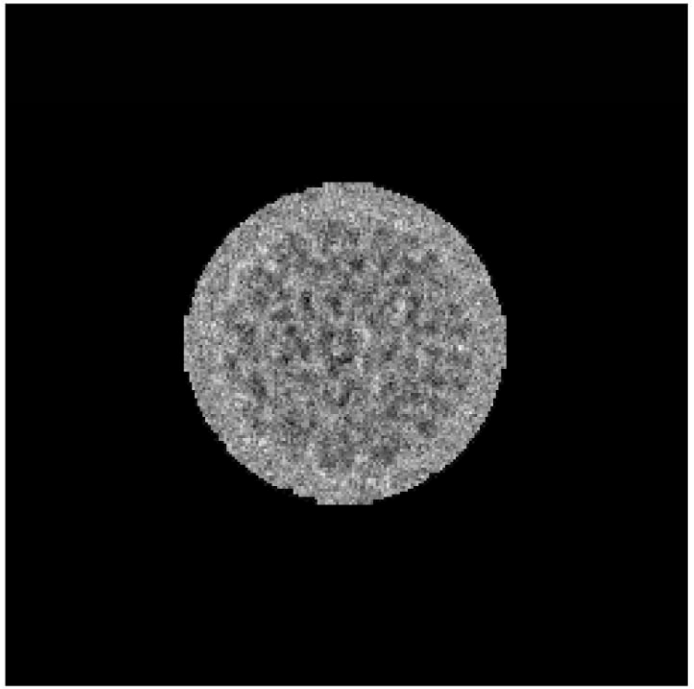


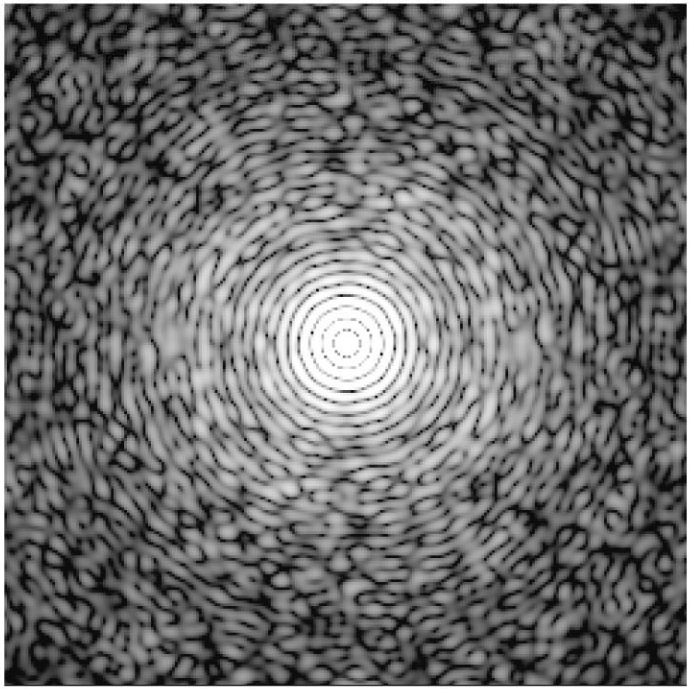


Square mask; **un**floated

Icosahedral Particle Reconstruction Scheme

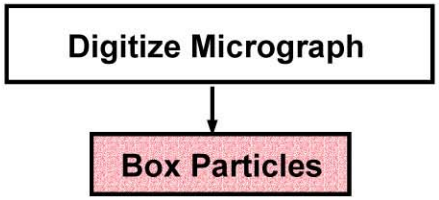


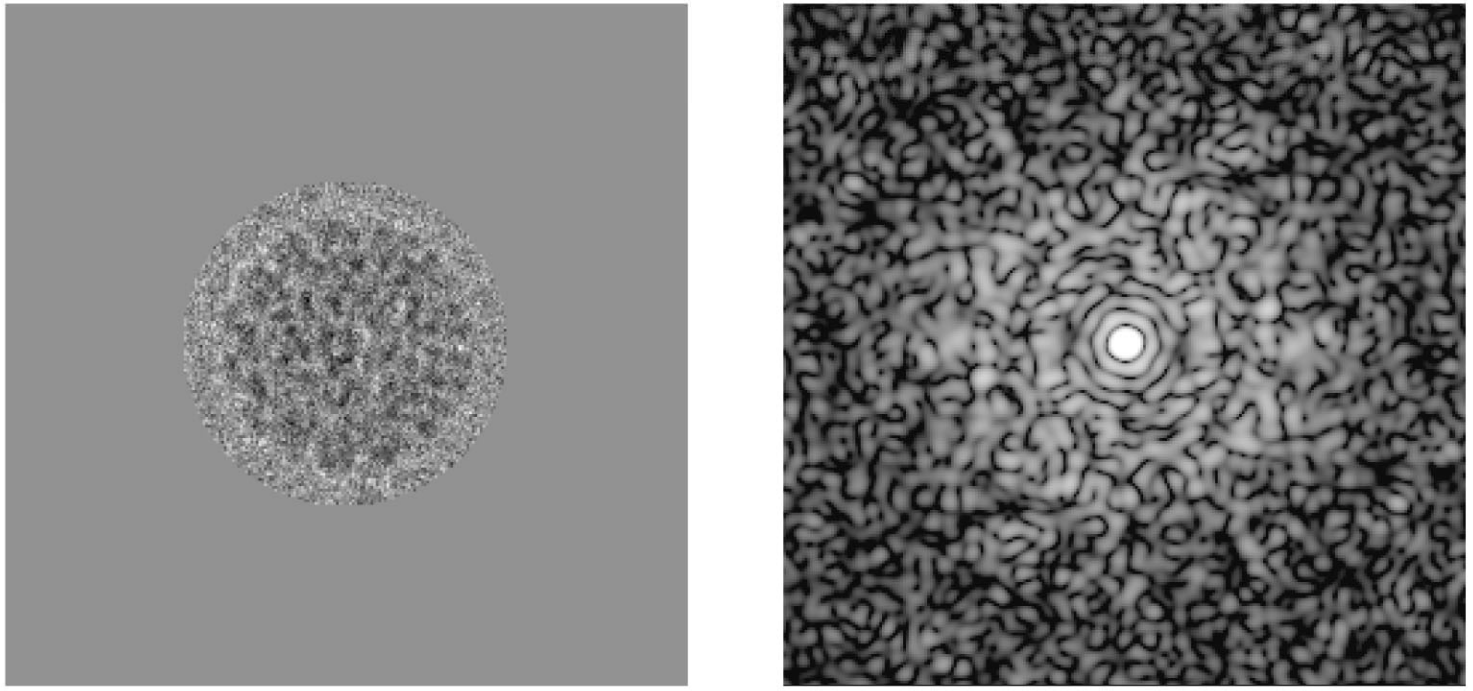




Circular mask; **un**floated

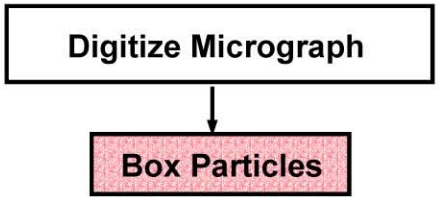
Icosahedral Particle Reconstruction Scheme

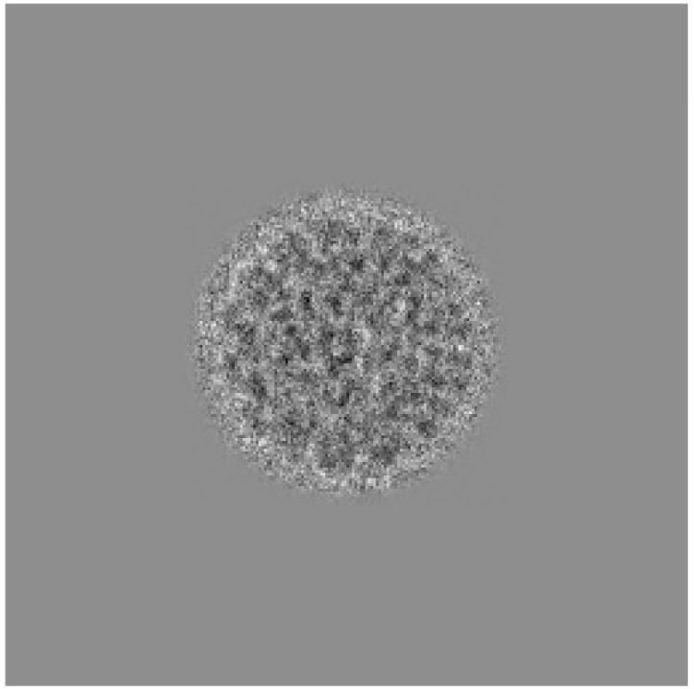


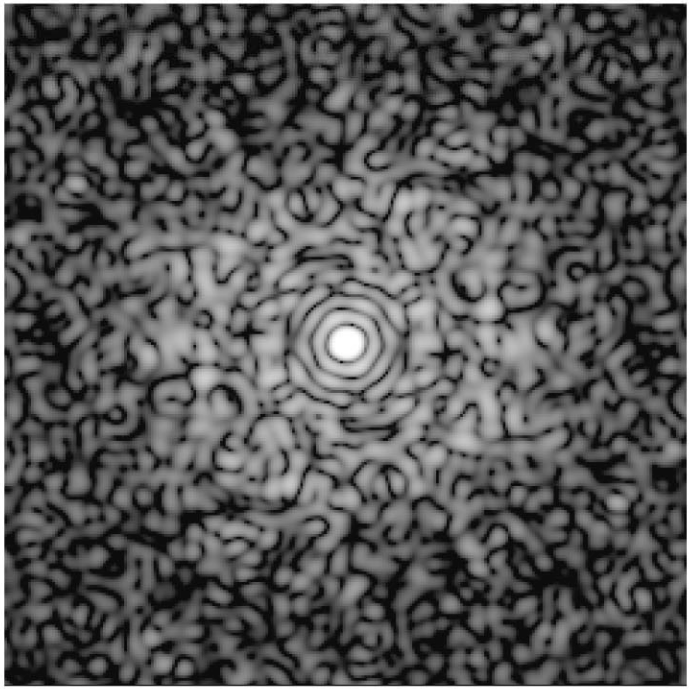


Circular mask; floated

Icosahedral Particle Reconstruction Scheme

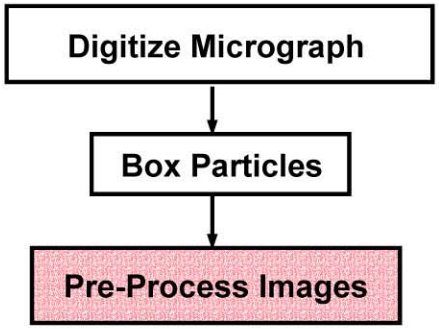




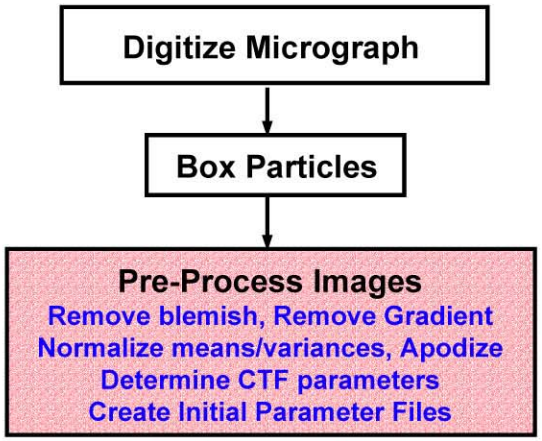


Circular mask; floated & apodized

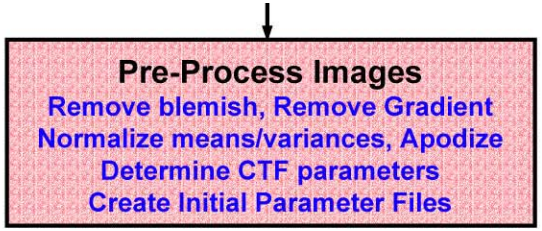
Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme

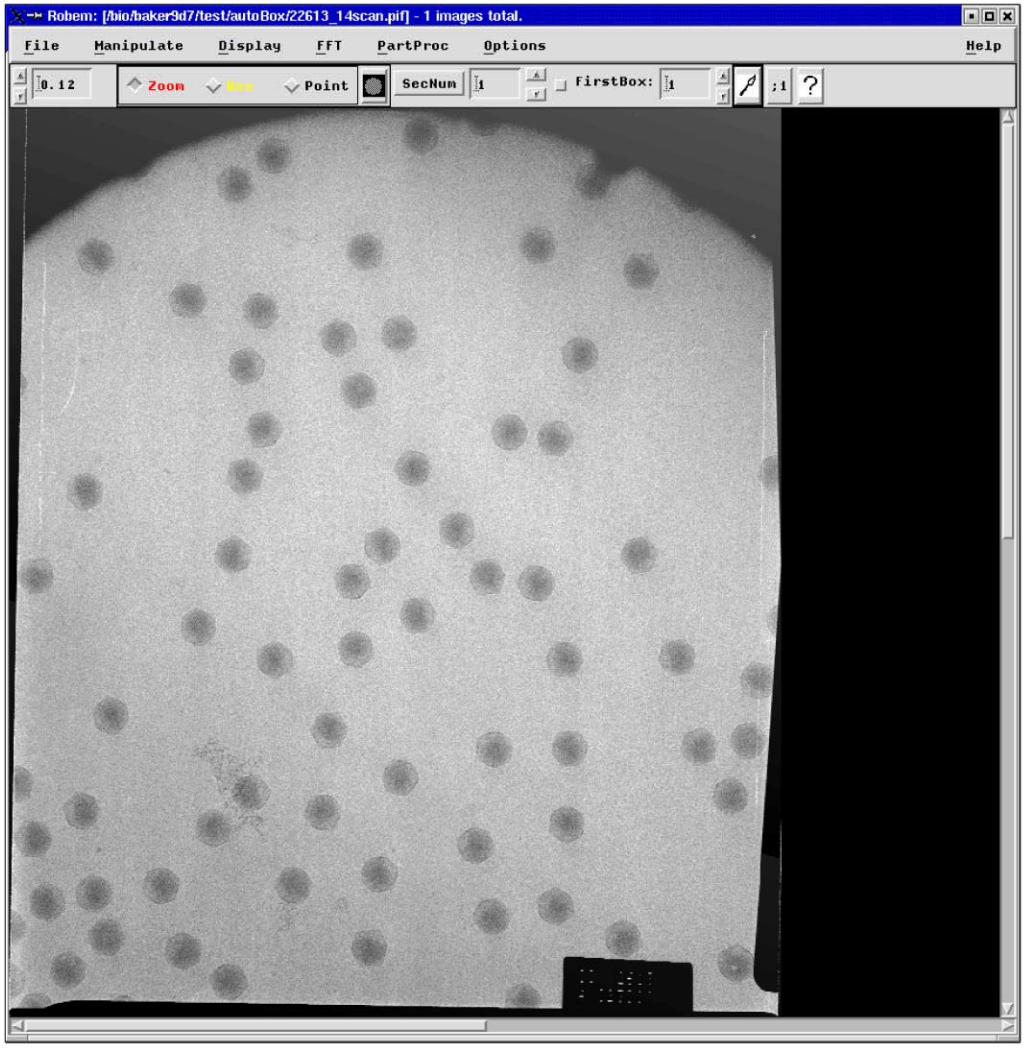


Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme

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



Gradient removed

Icosahedral Particle Reconstruction Scheme

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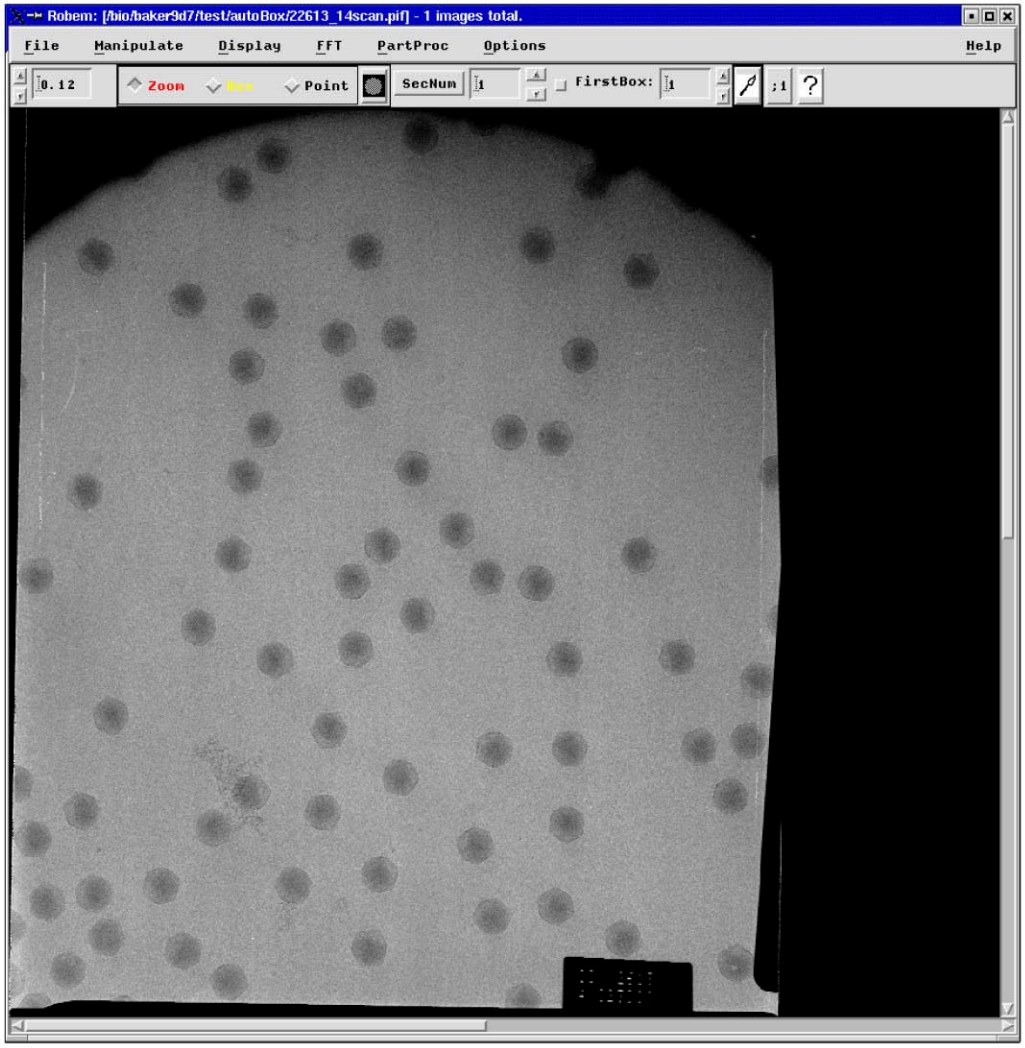
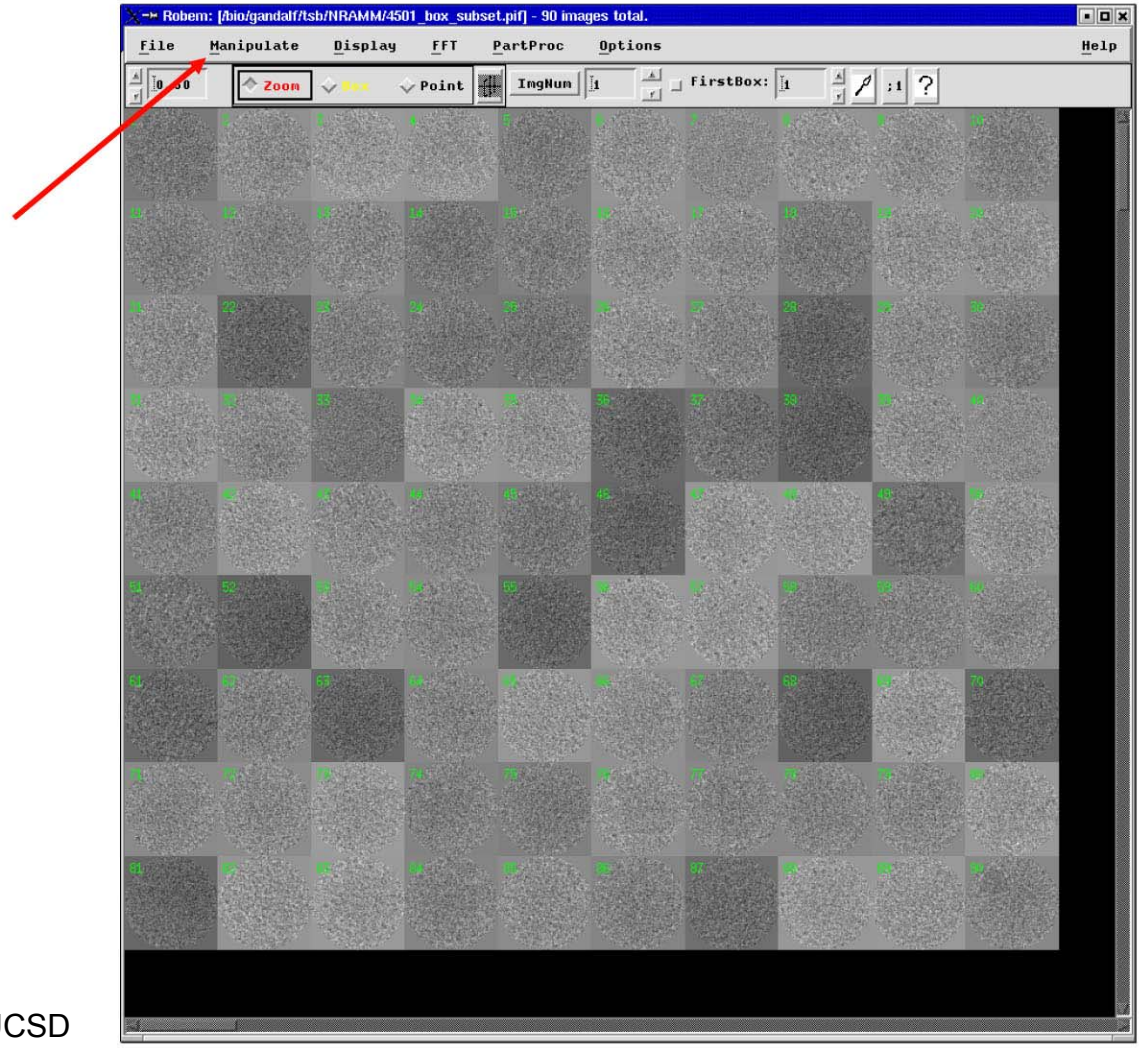
Remove blemish, Remove Gradient
Normalize means/variances, Apodize
Determine CTF parameters
Create Initial Parameter Files



Gradient
not removed

Icosahedral Particle Reconstruction Scheme

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



Icosahedral Particle Reconstruction Scheme

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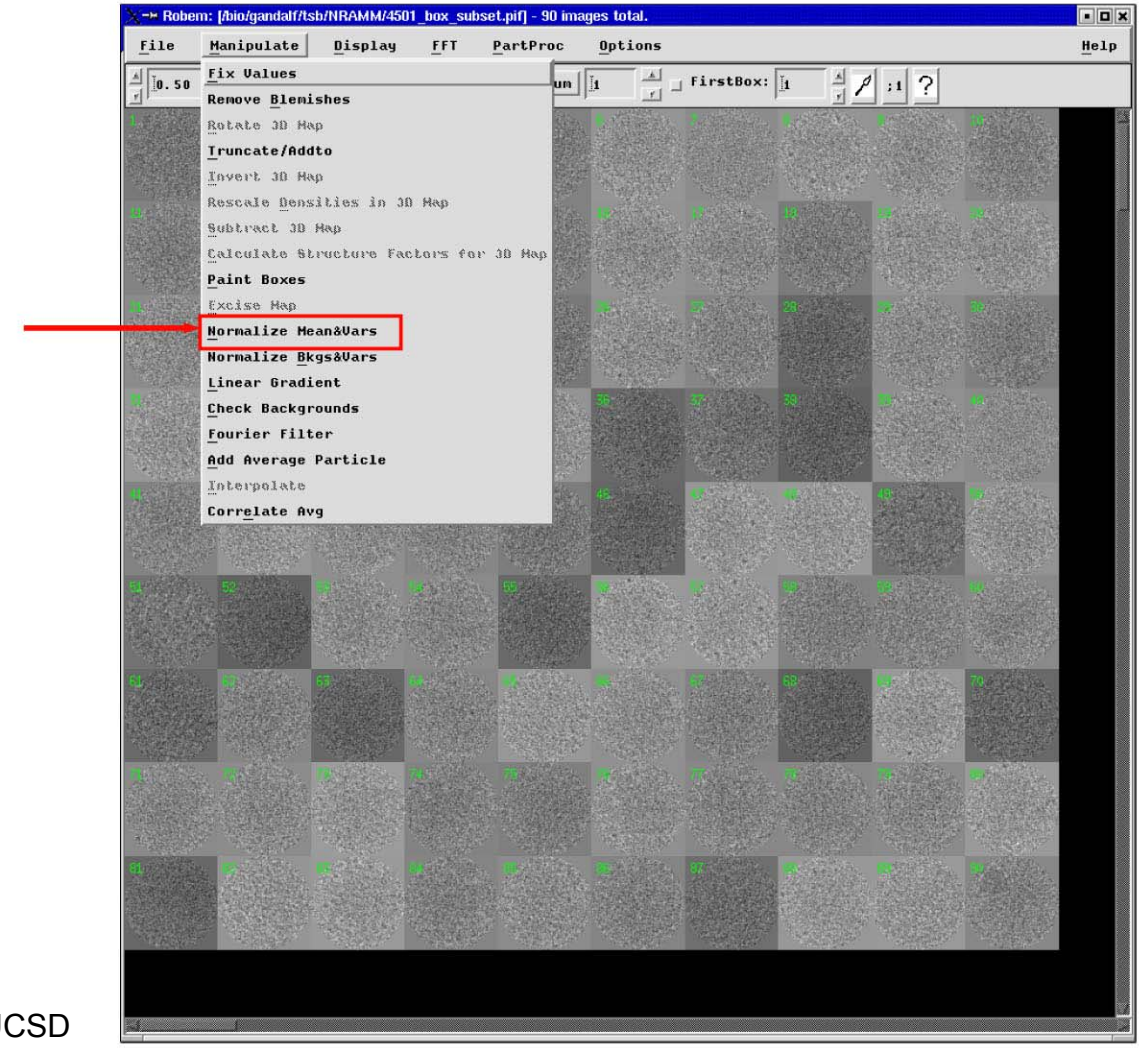

Icosahedral Particle Reconstruction Scheme

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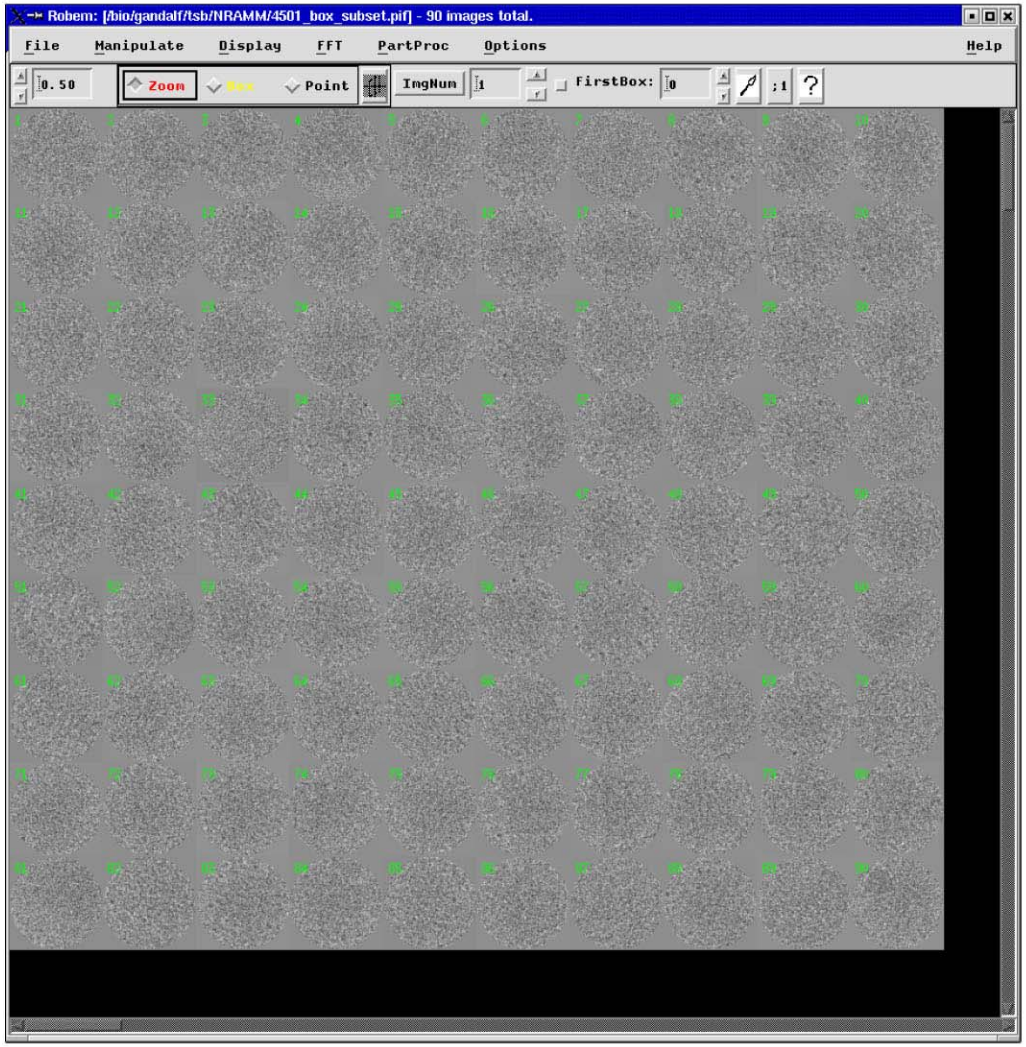
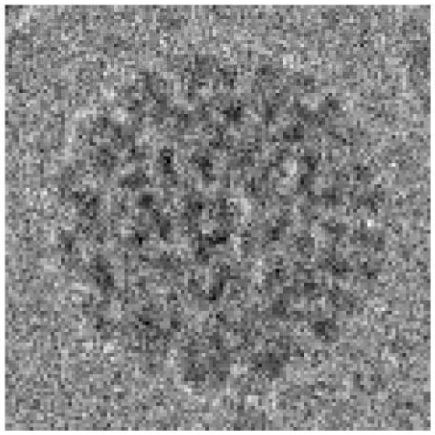
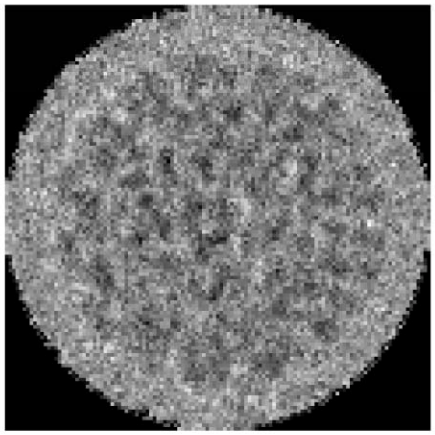


Icosahedral Particle Reconstruction Scheme

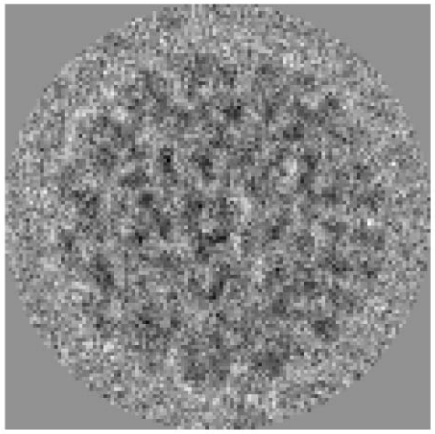
Pre-Process Images
~~Remove blemish, Remove Gradient~~
Normalize means/variances, Apodize
~~Determine CTF parameters~~
Create Initial Parameter Files



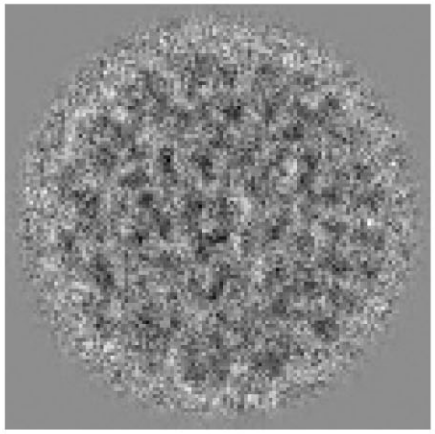
Extracted



Masked



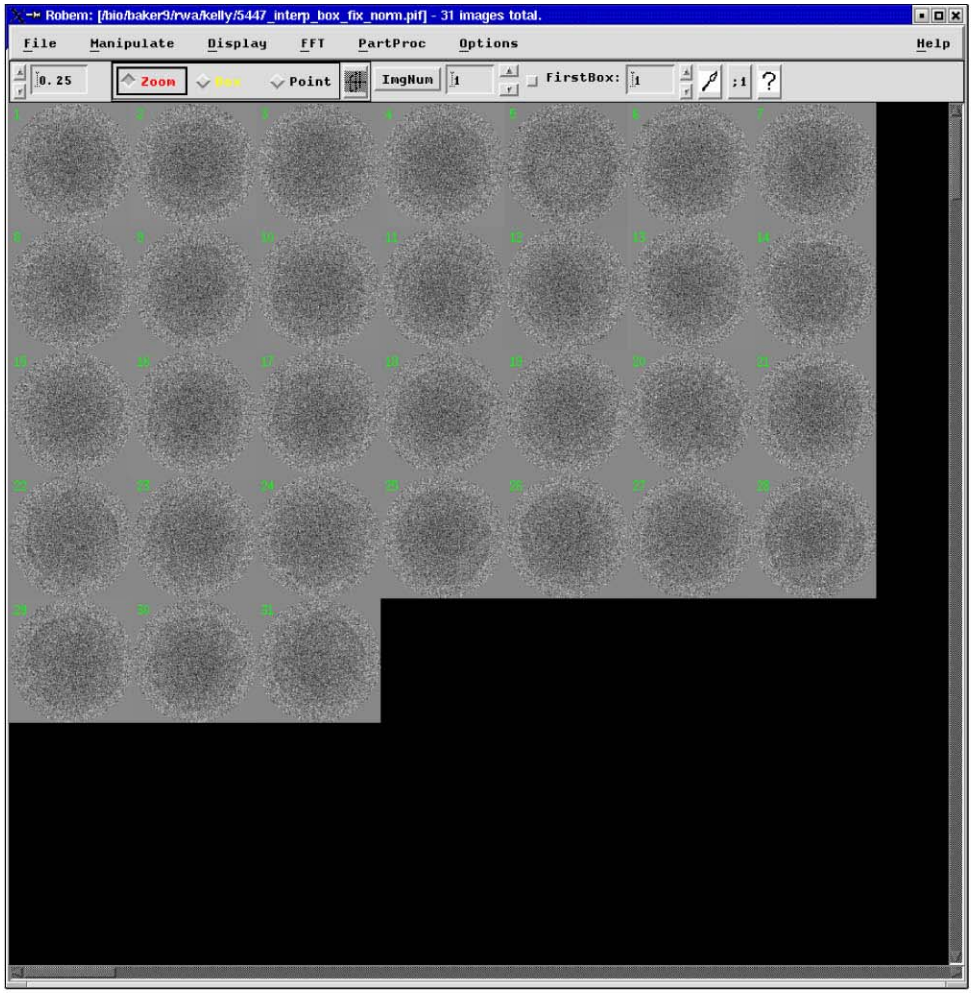
Floated



Apodized

Icosahedral Particle Reconstruction Scheme

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



Icosahedral Particle Reconstruction Scheme

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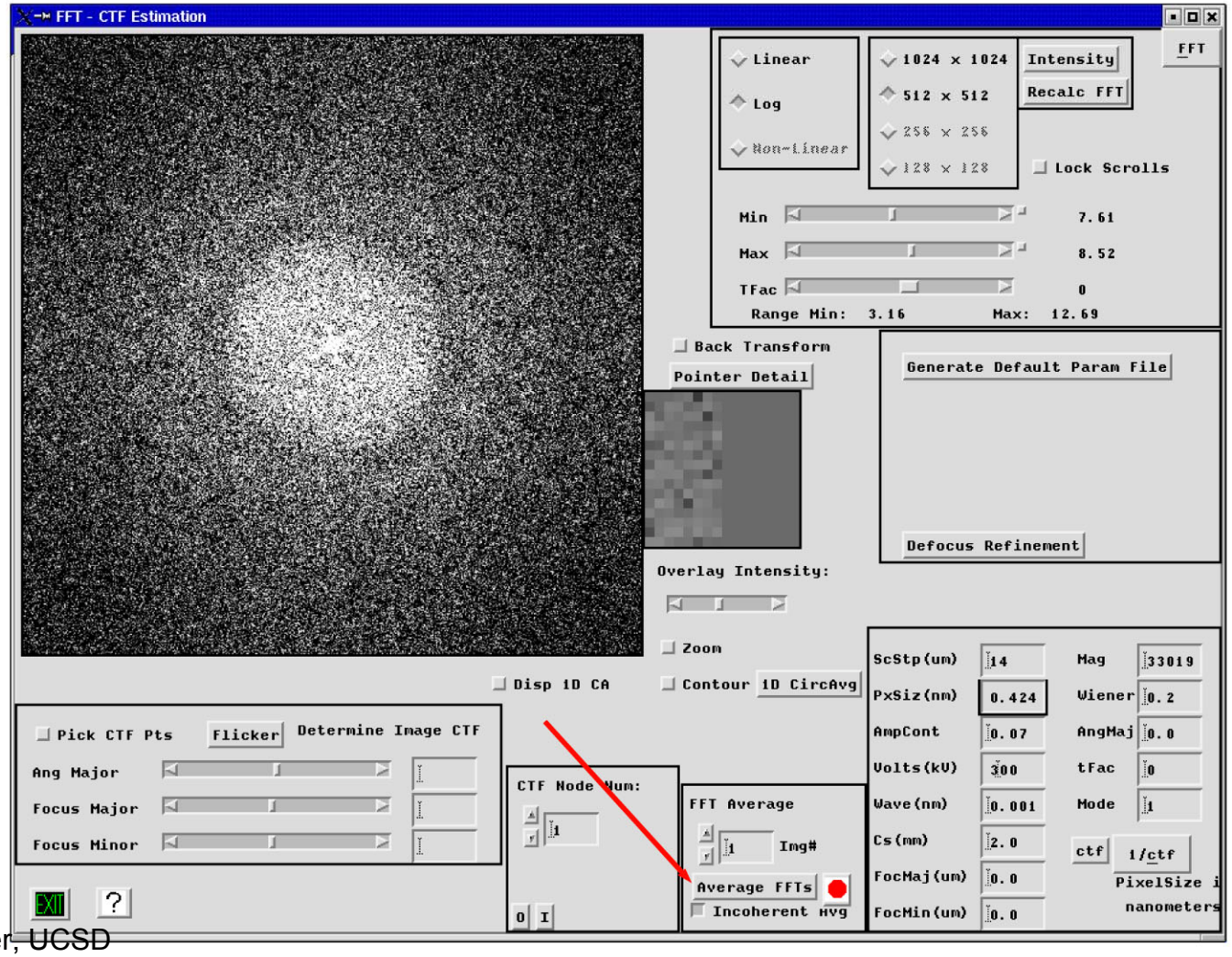
Create Initial Parameter Files



Icosahedral Particle Reconstruction Scheme

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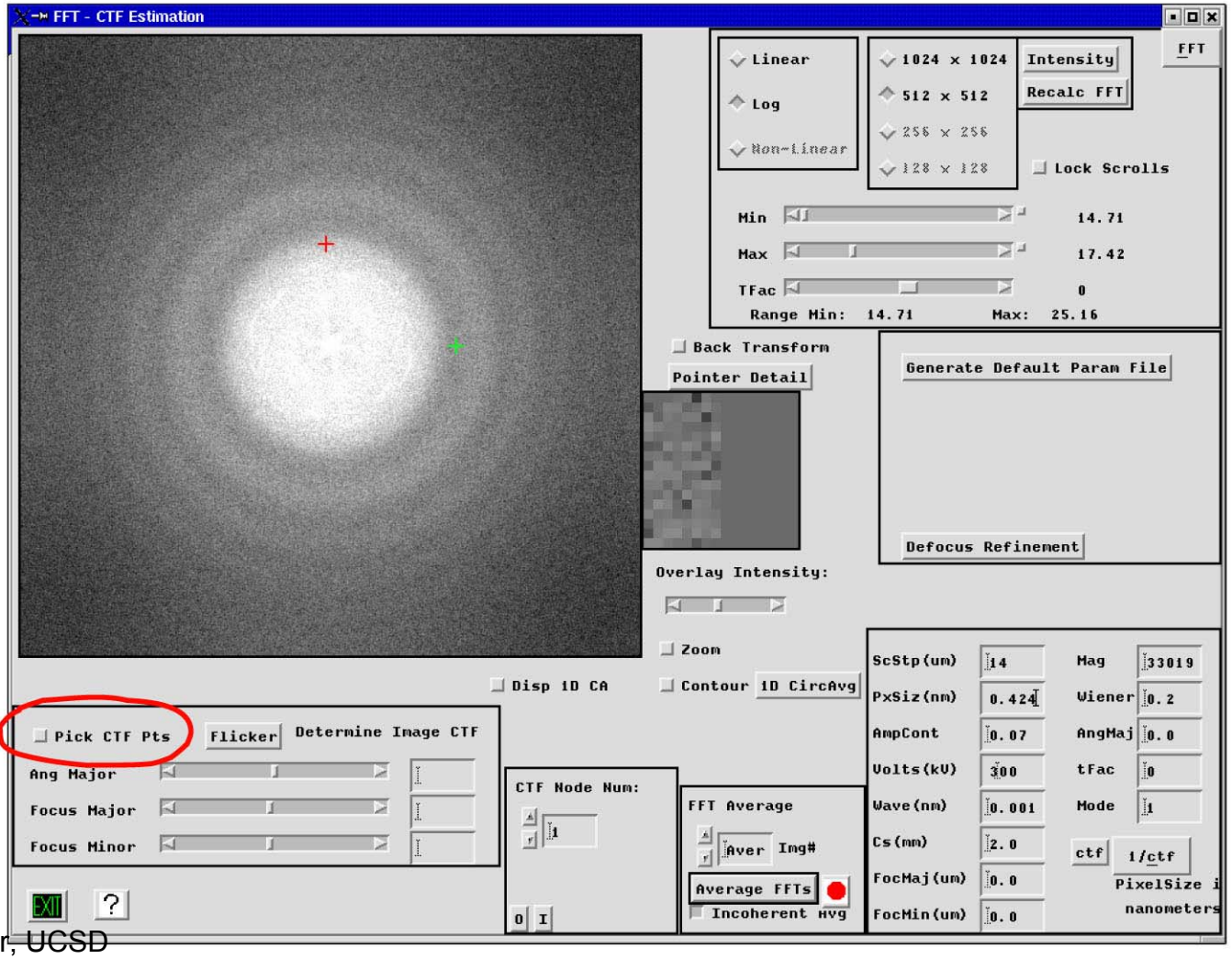

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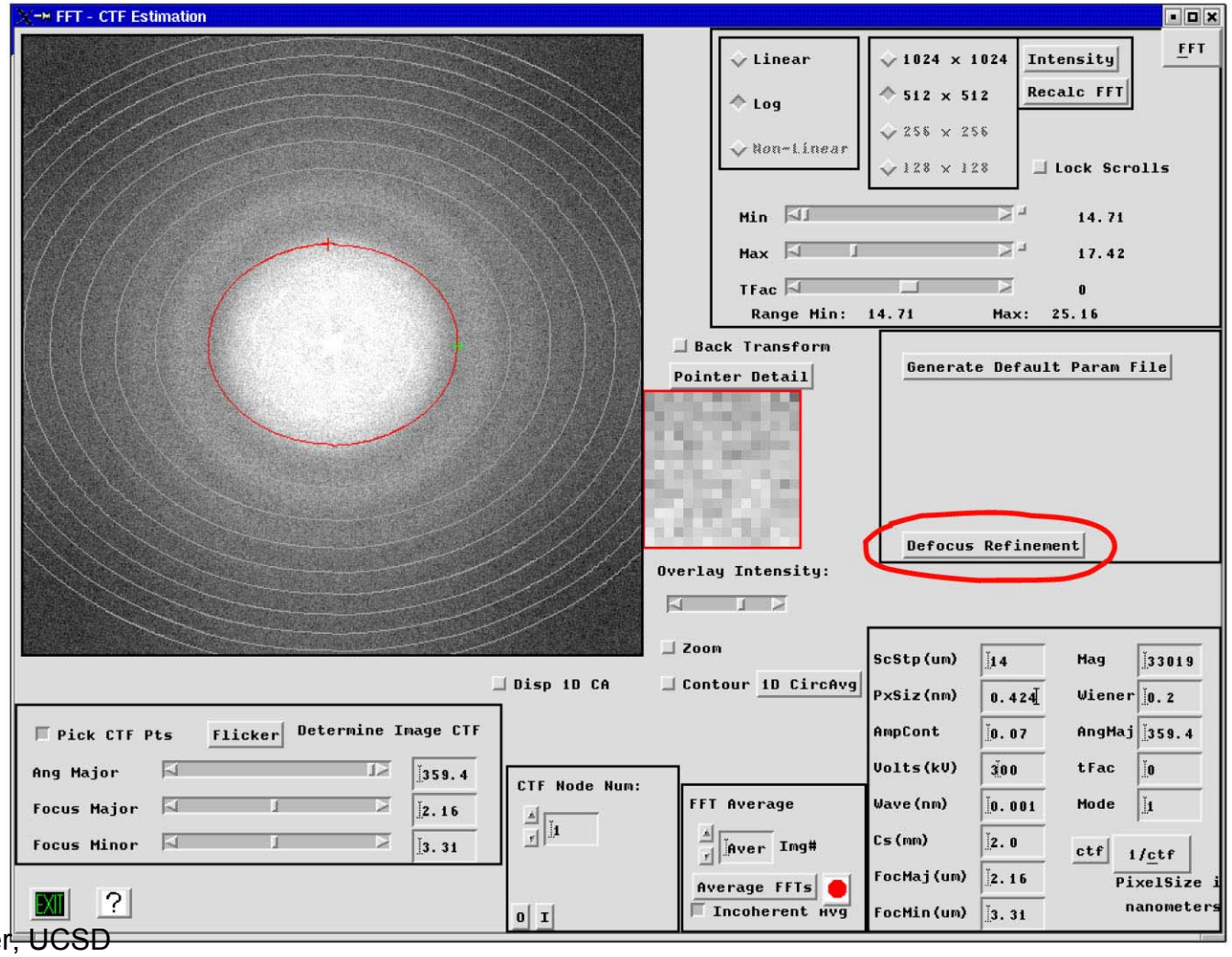

Icosahedral Particle Reconstruction Scheme

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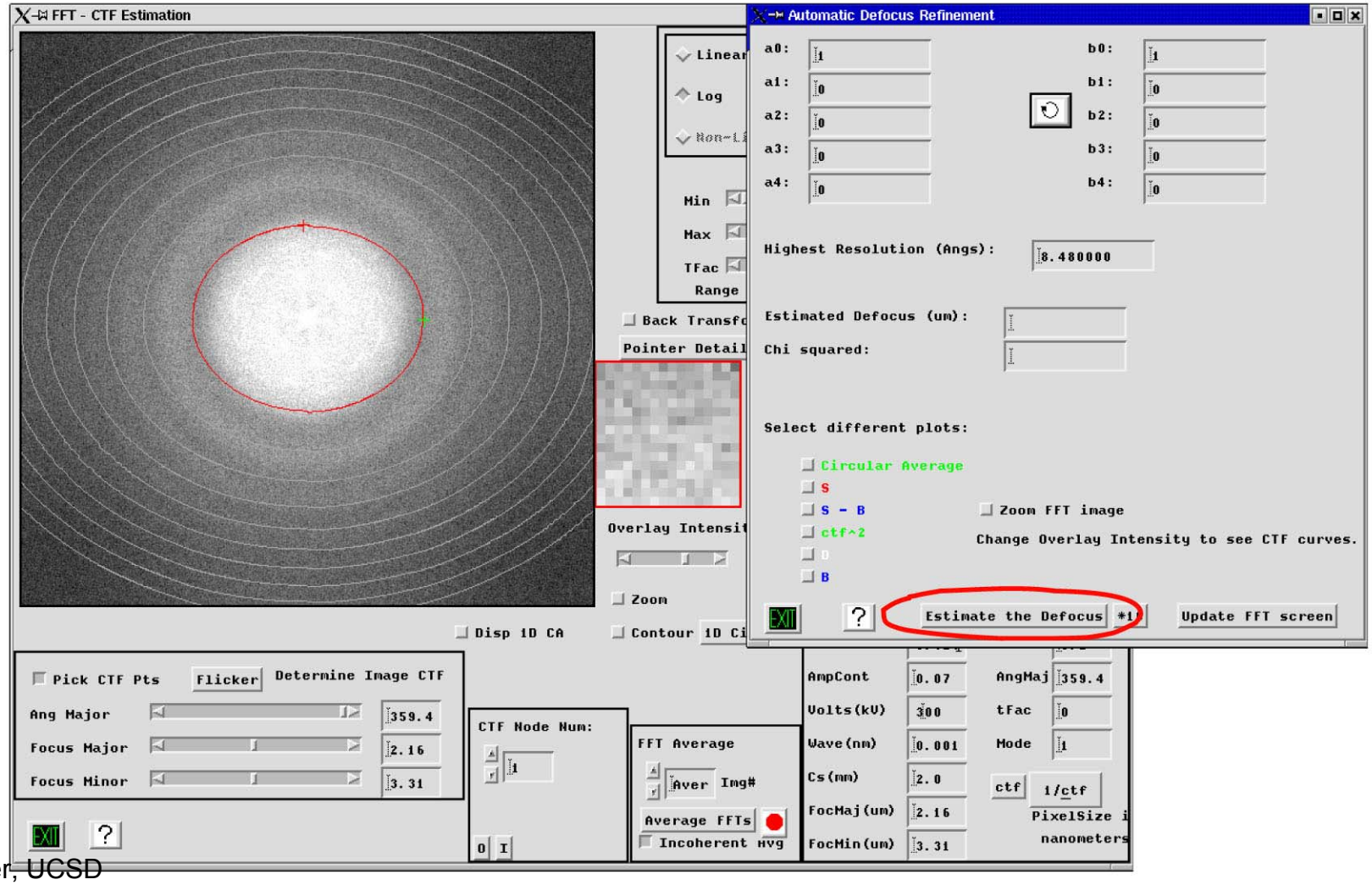

Icosahedral Particle Reconstruction Scheme

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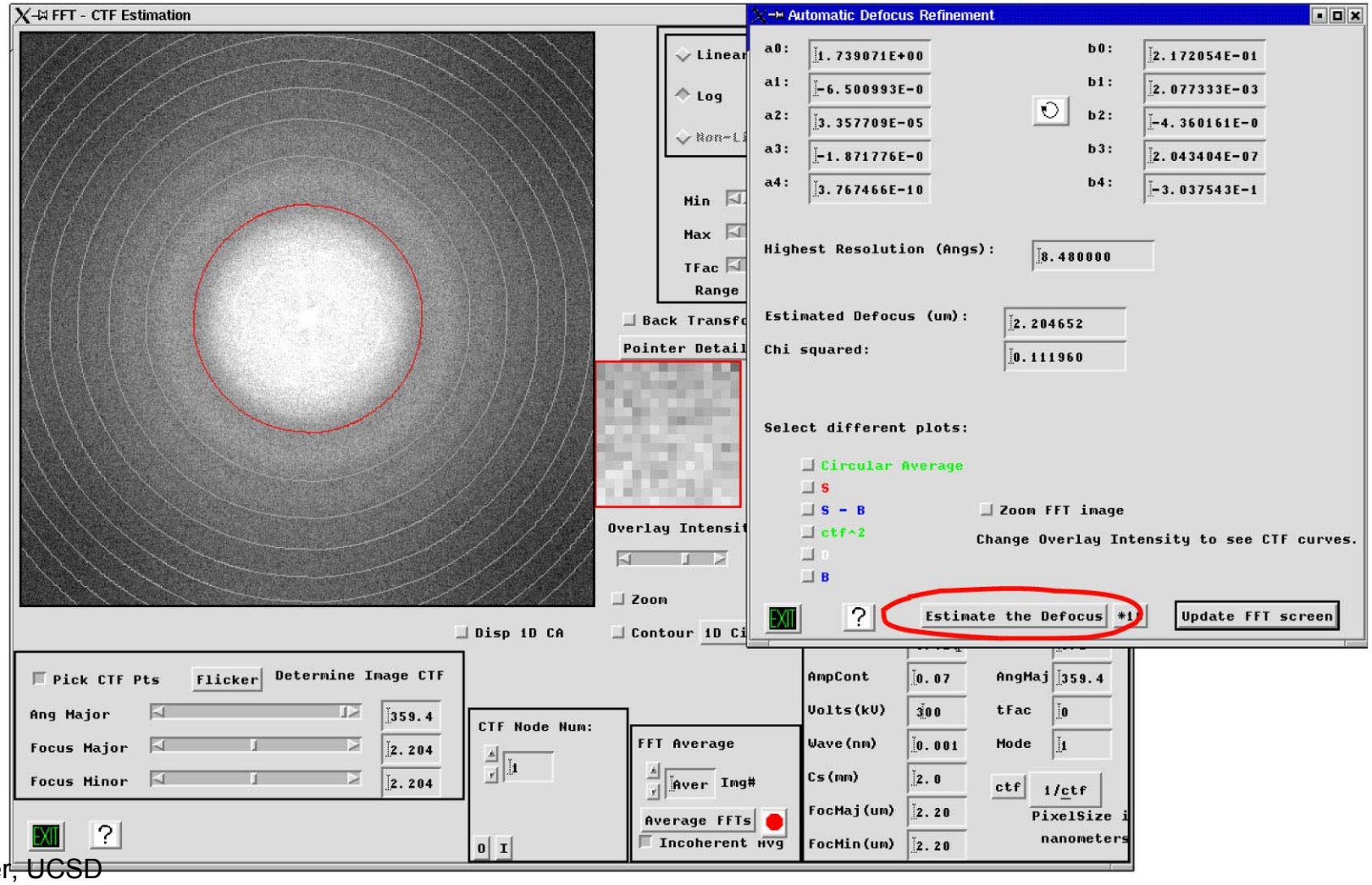
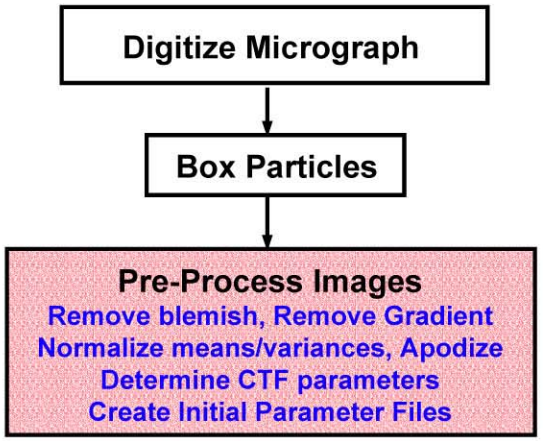
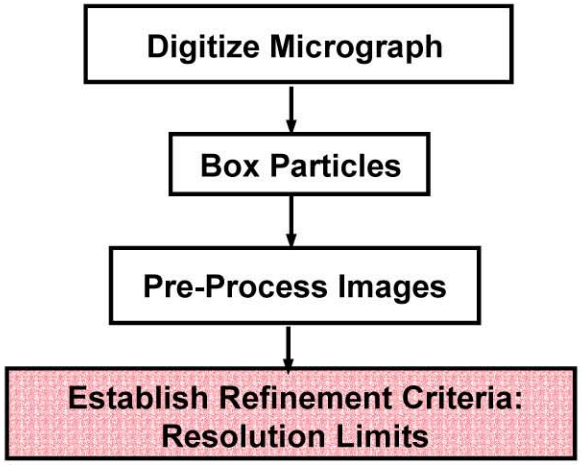


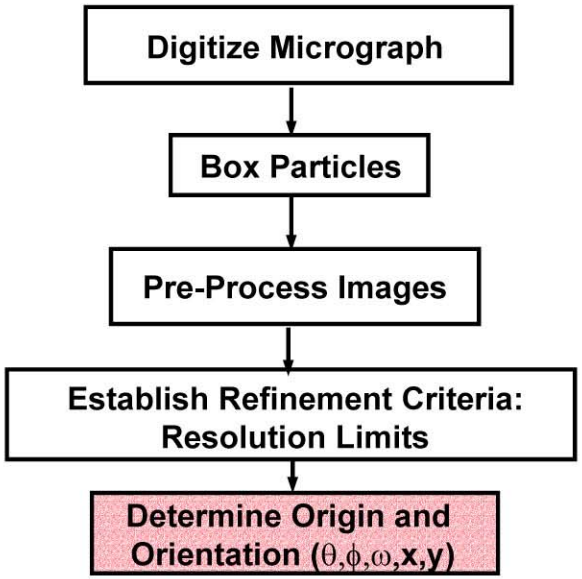
Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme

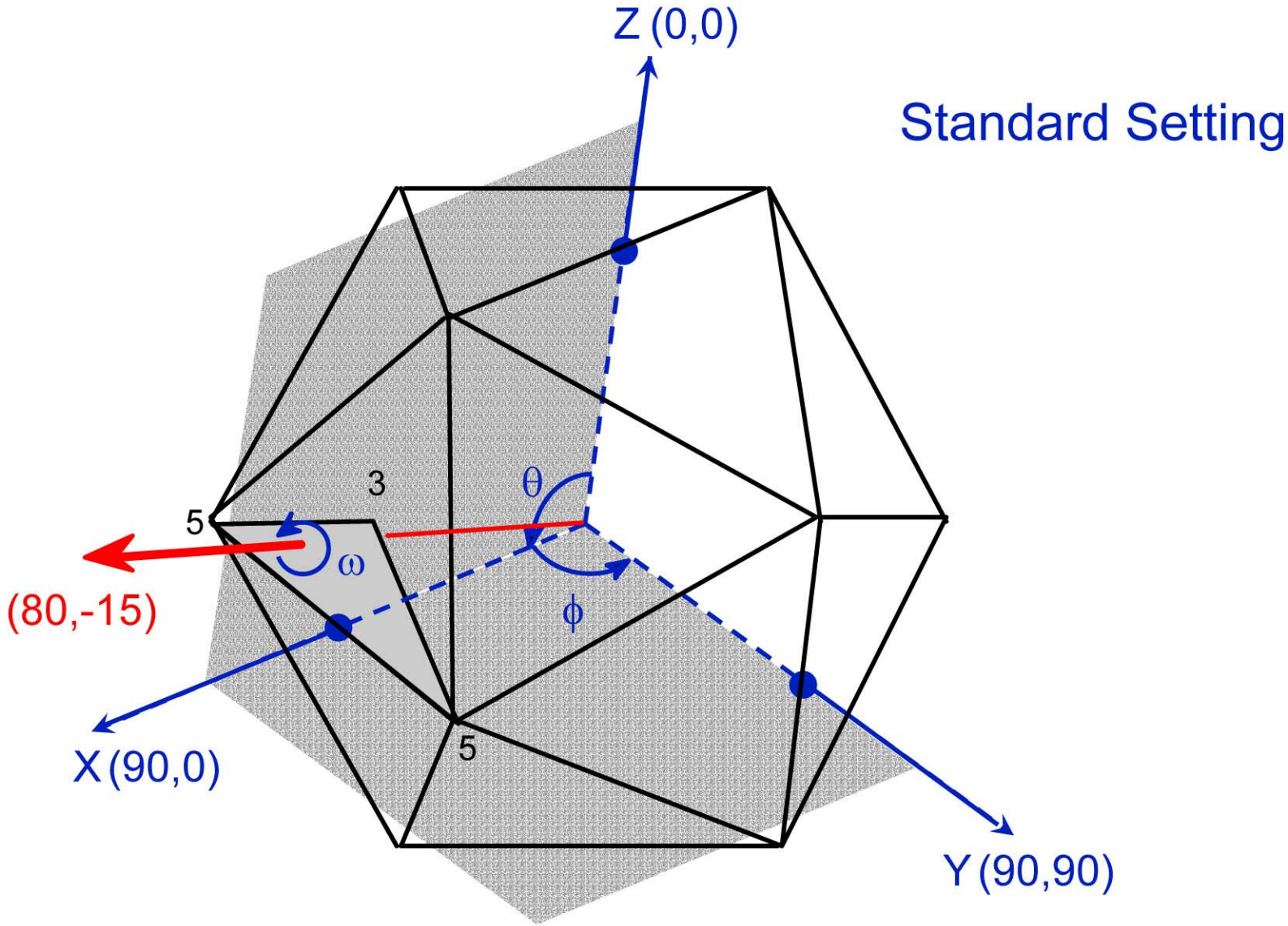


Icosahedral Particle Reconstruction Scheme

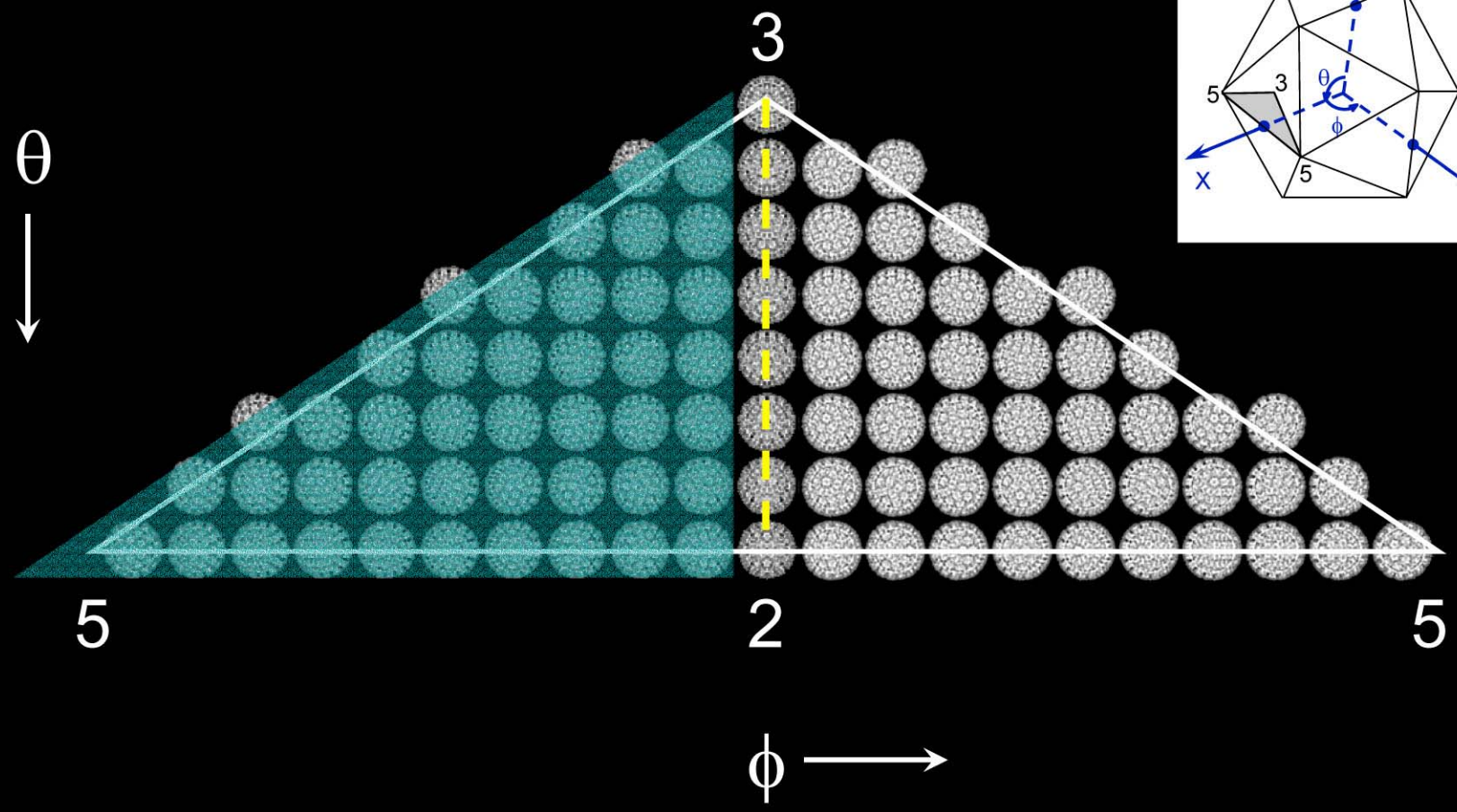


Determining Origin and Orientation

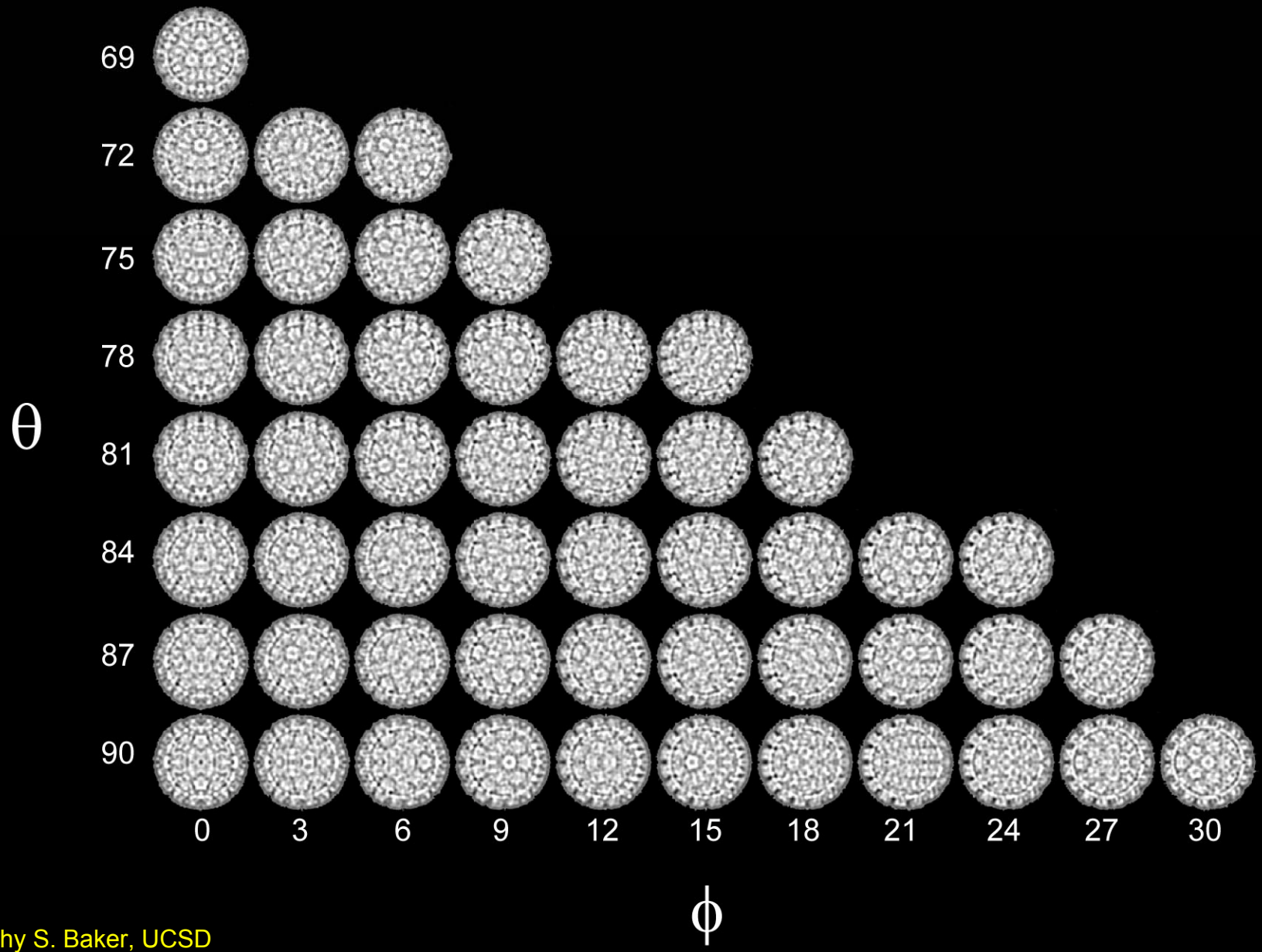
Convention of Coordinate System



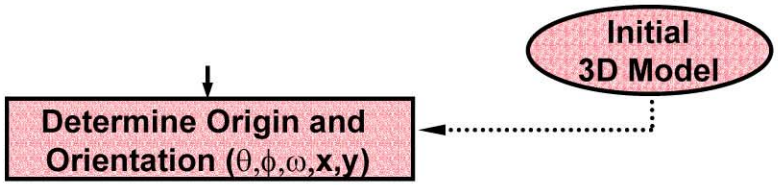
BPV Projections: Icosahedral ASU



BPV Projections: ½ Icosahedral ASU



Icosahedral Particle Reconstruction Scheme



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

Two methods:

1. Common lines

   New or unknown structure

2. Model-based (template) matching

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

Icosahedral Particle Reconstruction Scheme

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

Common Lines

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

"[Common lines] arise as follows:"

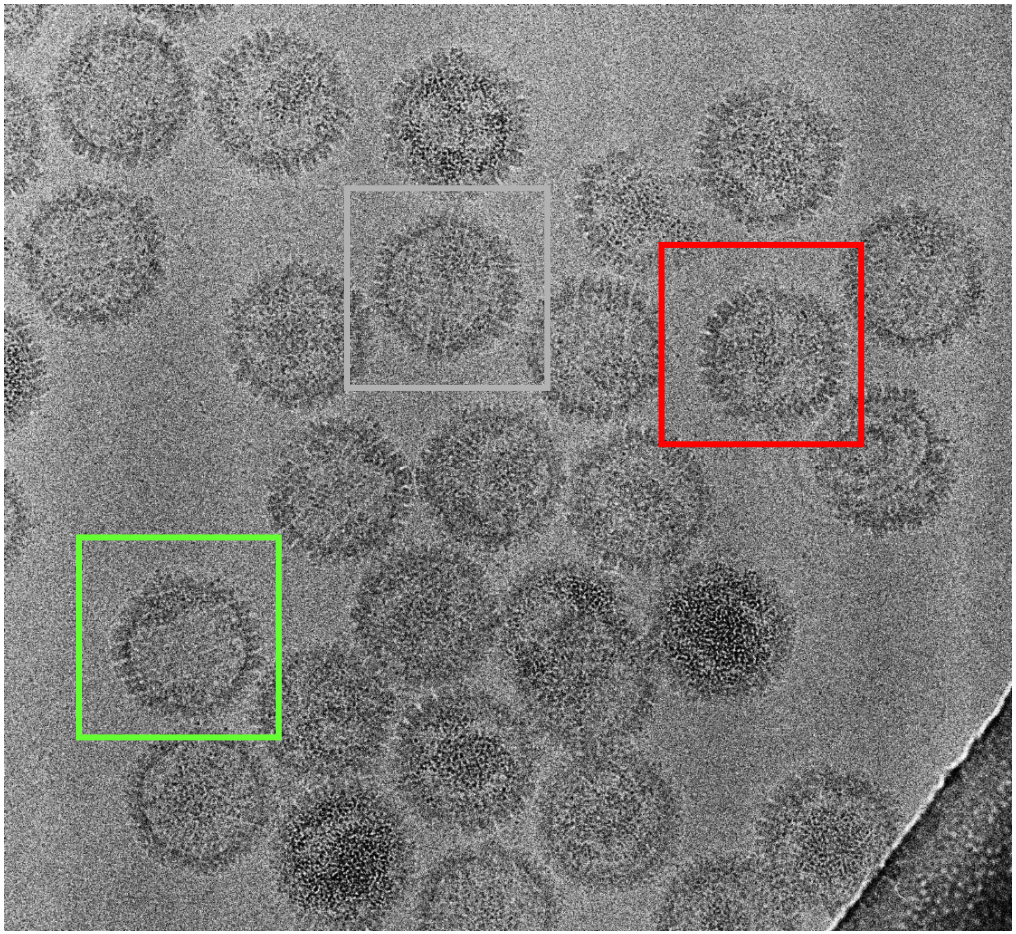
"An observed section of the transform intersects an identical symmetry-related section in a line, along which the transform must have the same value in both sections"

"The common line lies in the original section."

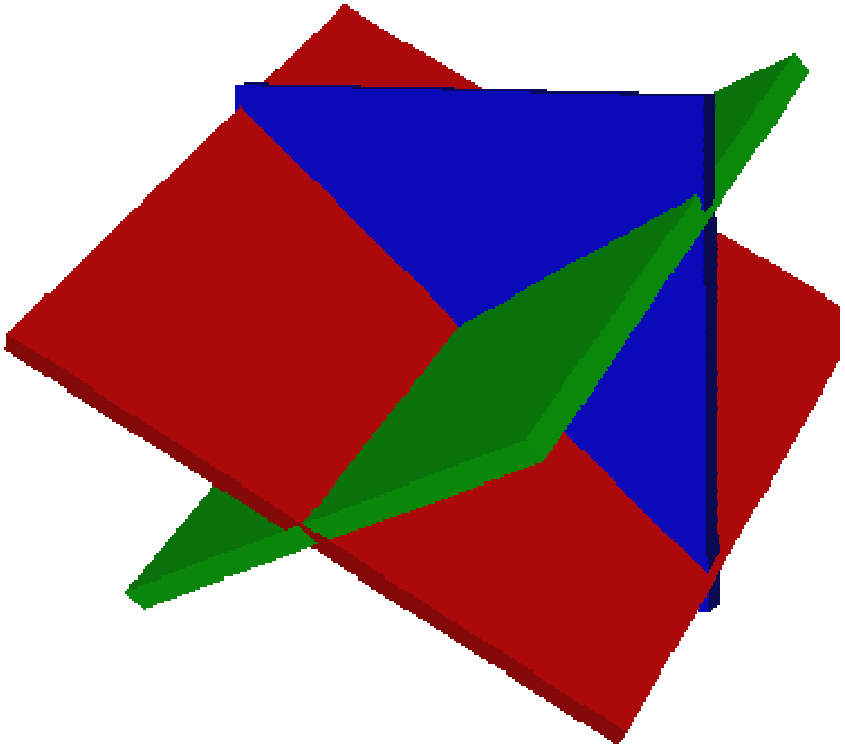
"However, regarded as lying in the symmetry-related section it must have been generated by the symmetry operation from some other line in the original section."

Common Lines

Electron Images of Virus Particles



Equivalent data in Fourier space

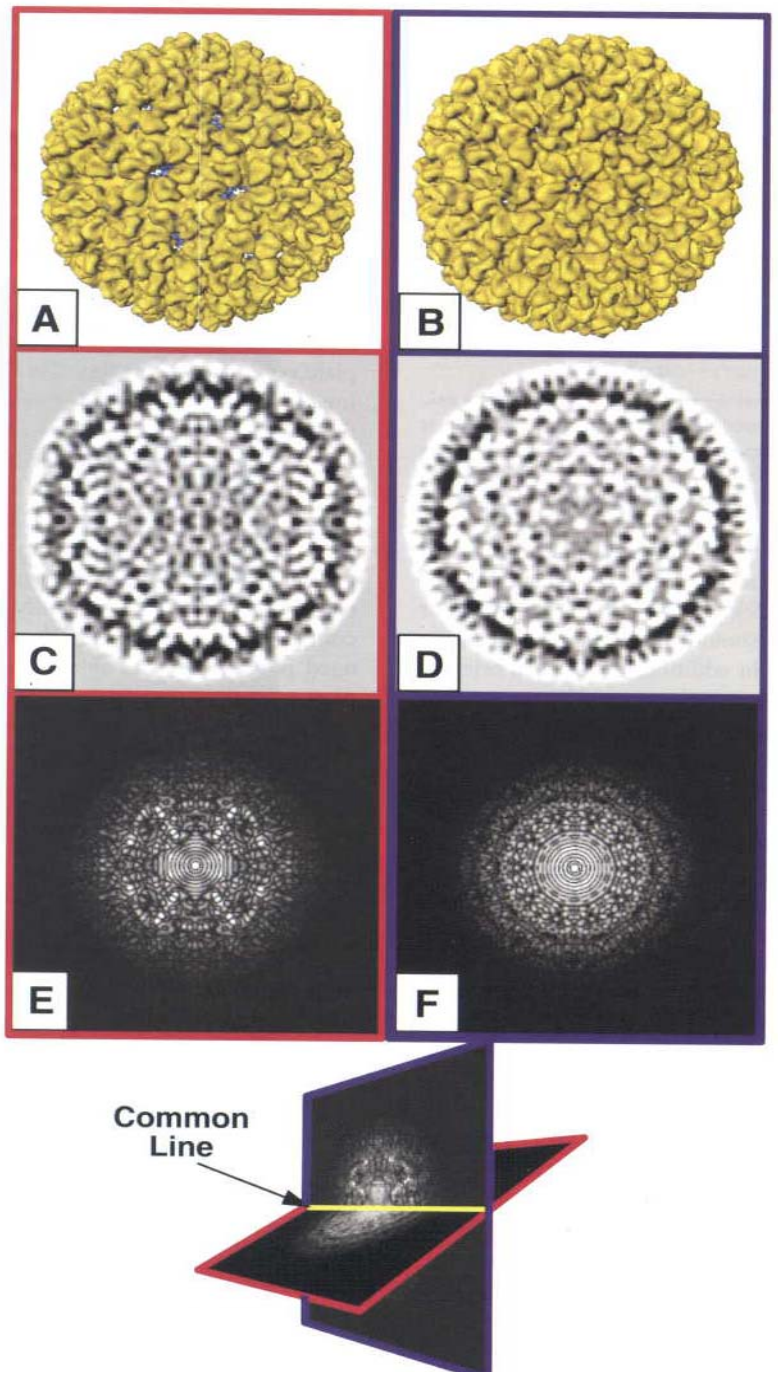


Common Lines

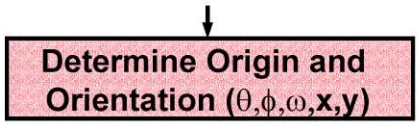
3D Object

Projection Image

Fourier Transform



Icosahedral Particle Reconstruction Scheme



Common Lines

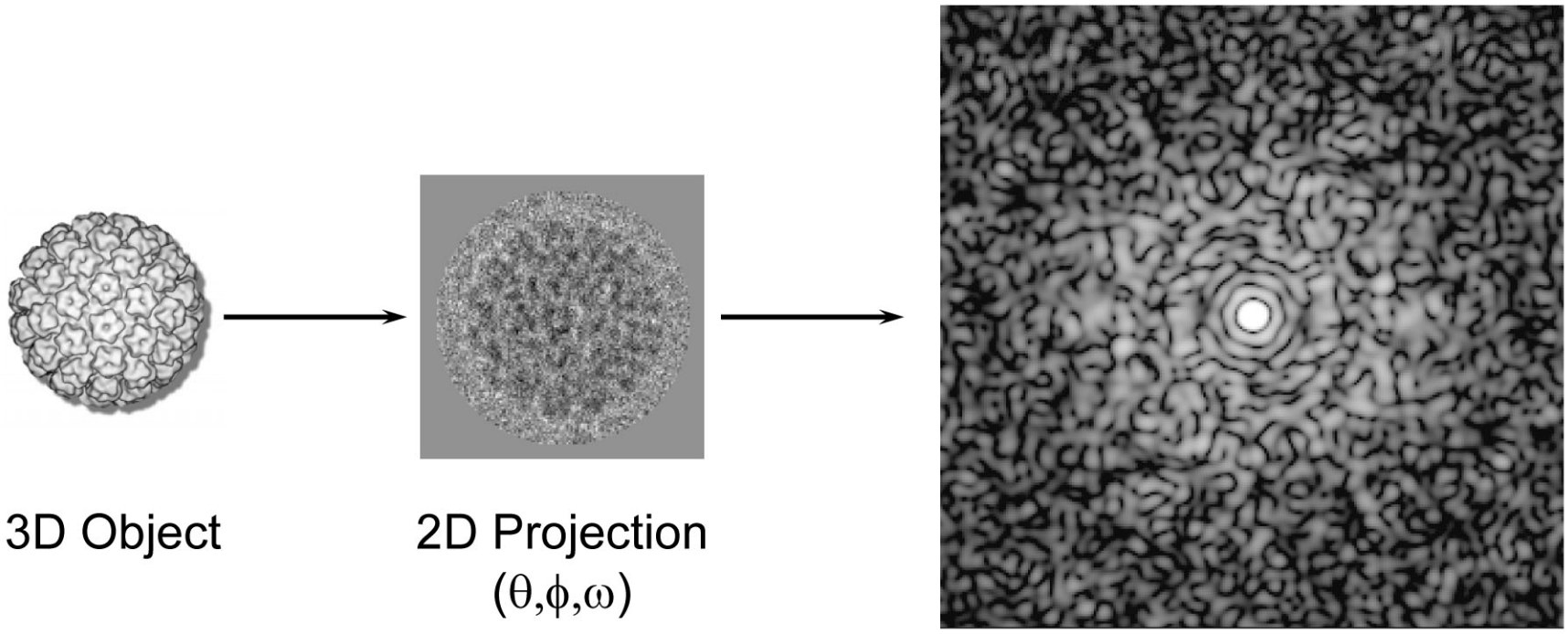
The ‘gospel’ continued:

“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”

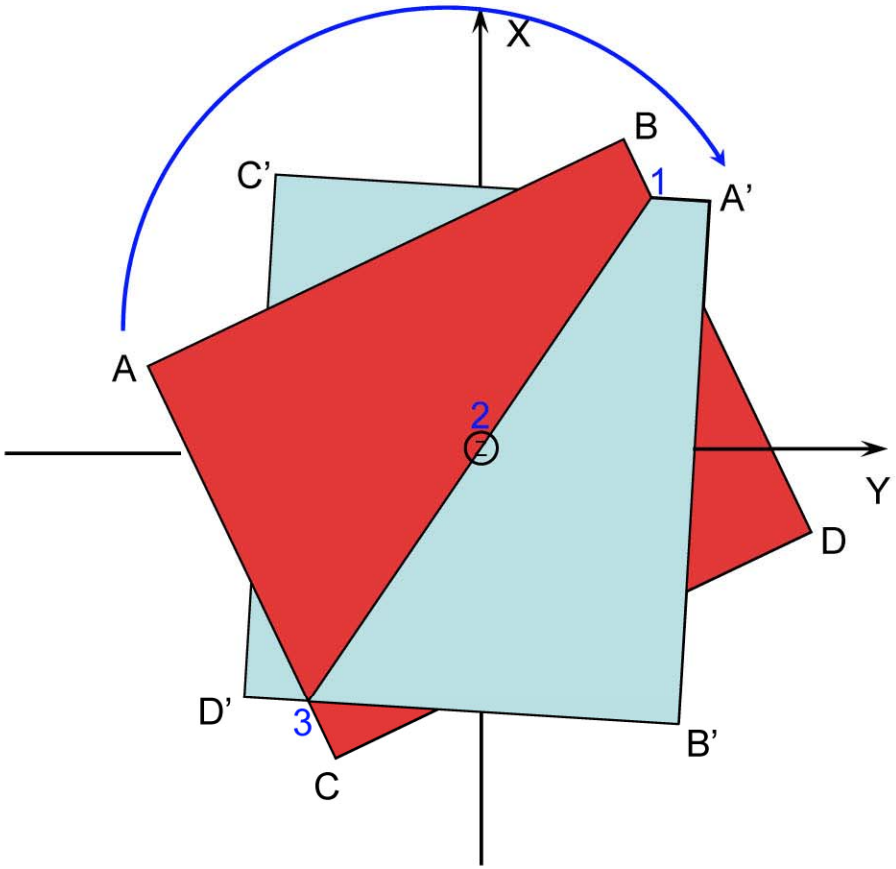
“The angular positions of these lines are dependent on the orientation of the particle.”

Orientation Determination by Common Lines



Orientation Determination by Common Lines

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



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

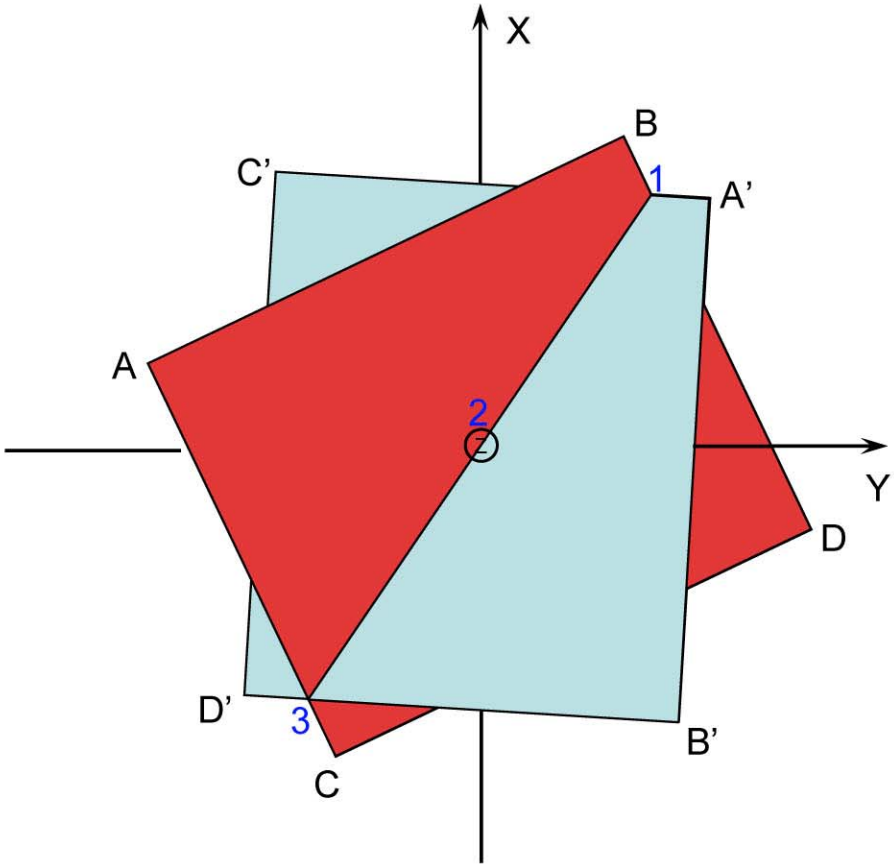
Let z-direction coincide with **3-fold** axis of symmetry

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

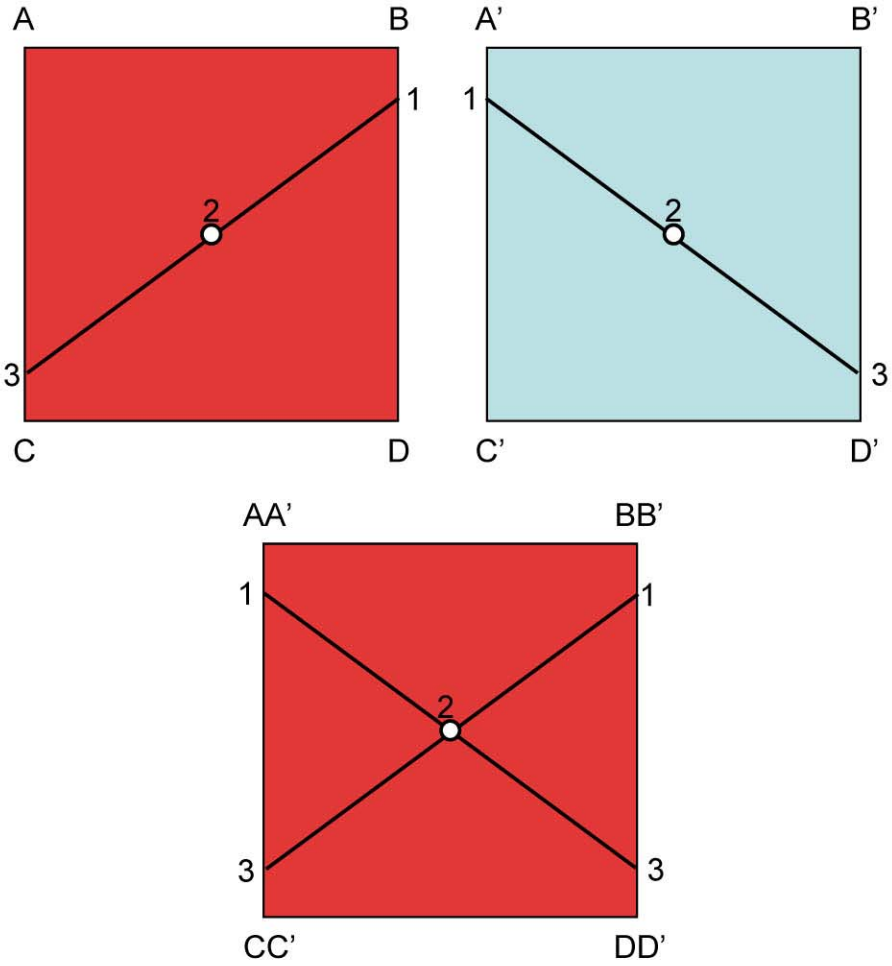
Both planes have **common values** along the **line** (1,2,3) of their intersection

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

Orientation Determination by Common Lines

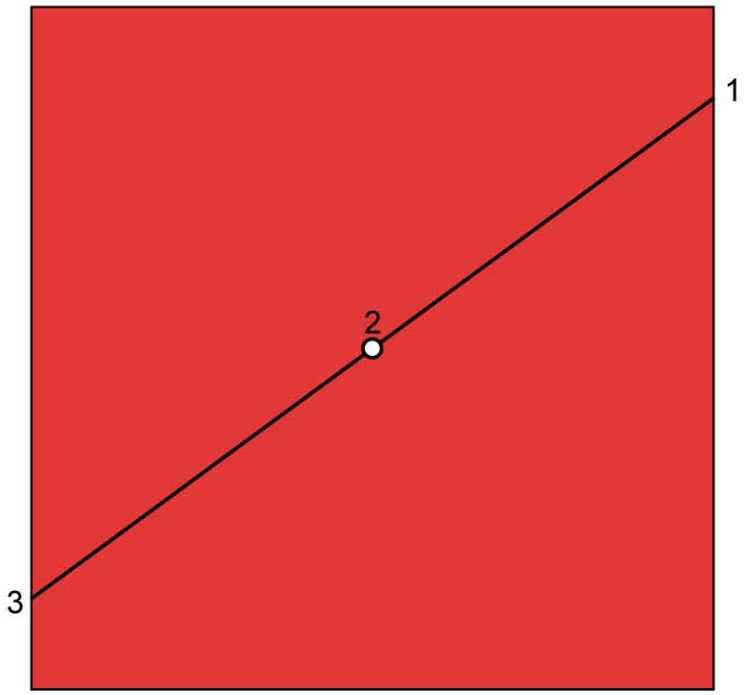


Adapted from Moody (1990) Fig. 7.68, p.245

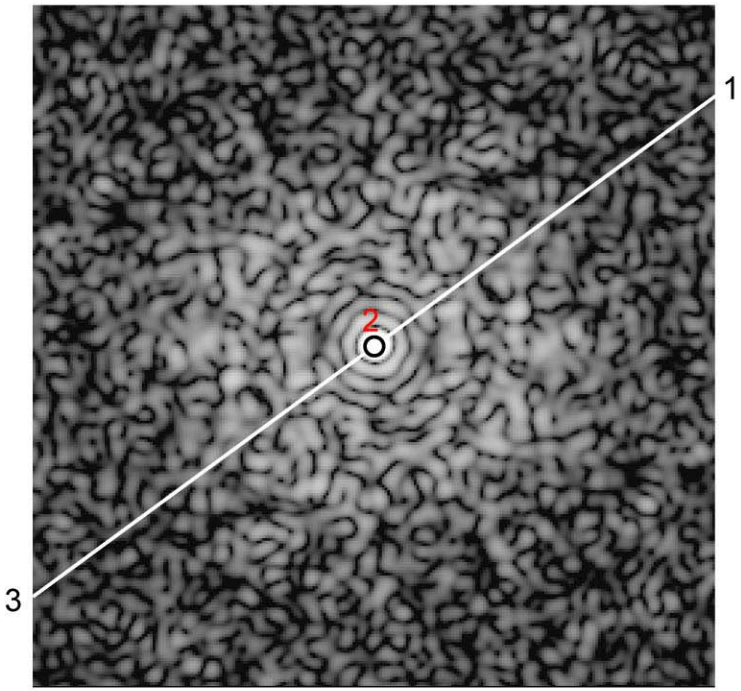


Adapted from Moody (1990) Fig. 7.69, p.246

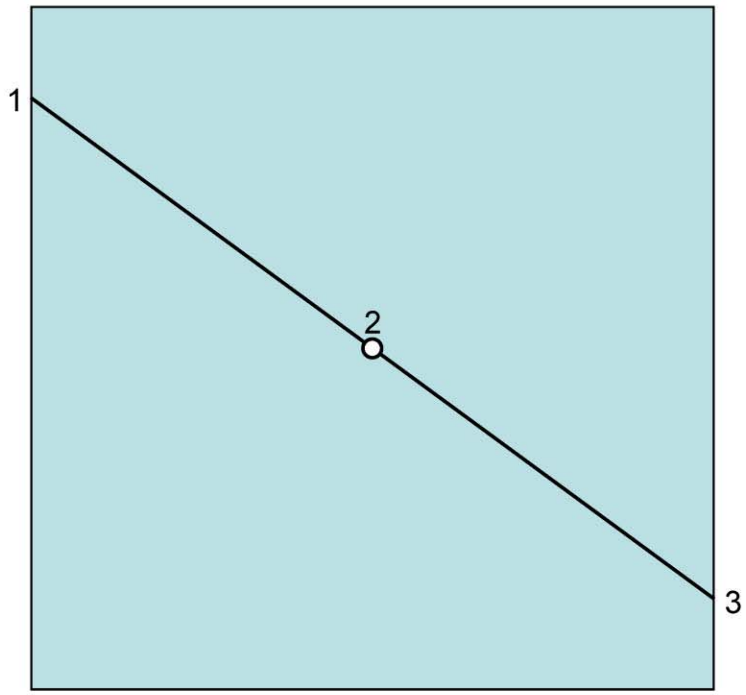
Orientation Determination by Common Lines



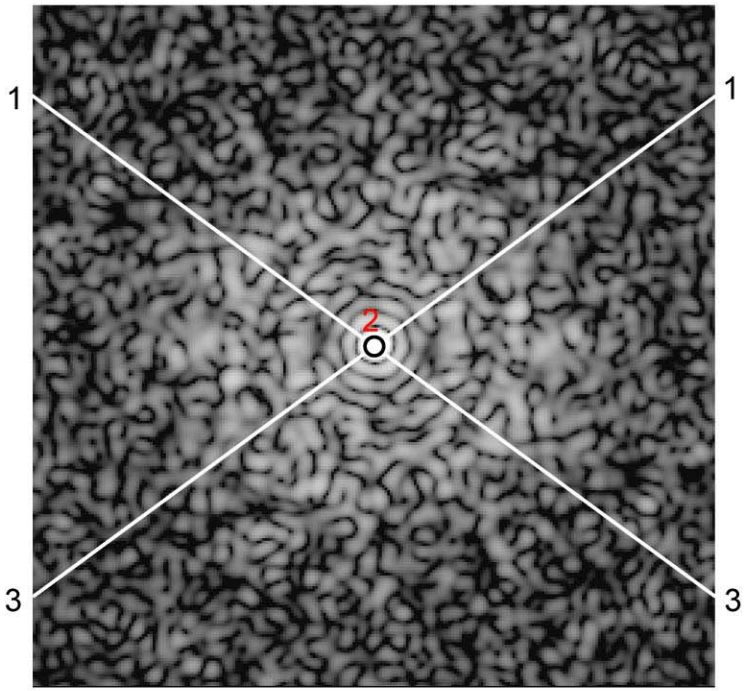
Original Transform Plane



Orientation Determination by Common Lines



Symmetry-Related
Transform Plane



Orientation Determination by Common Lines

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

What about an object with 532 symmetry?

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

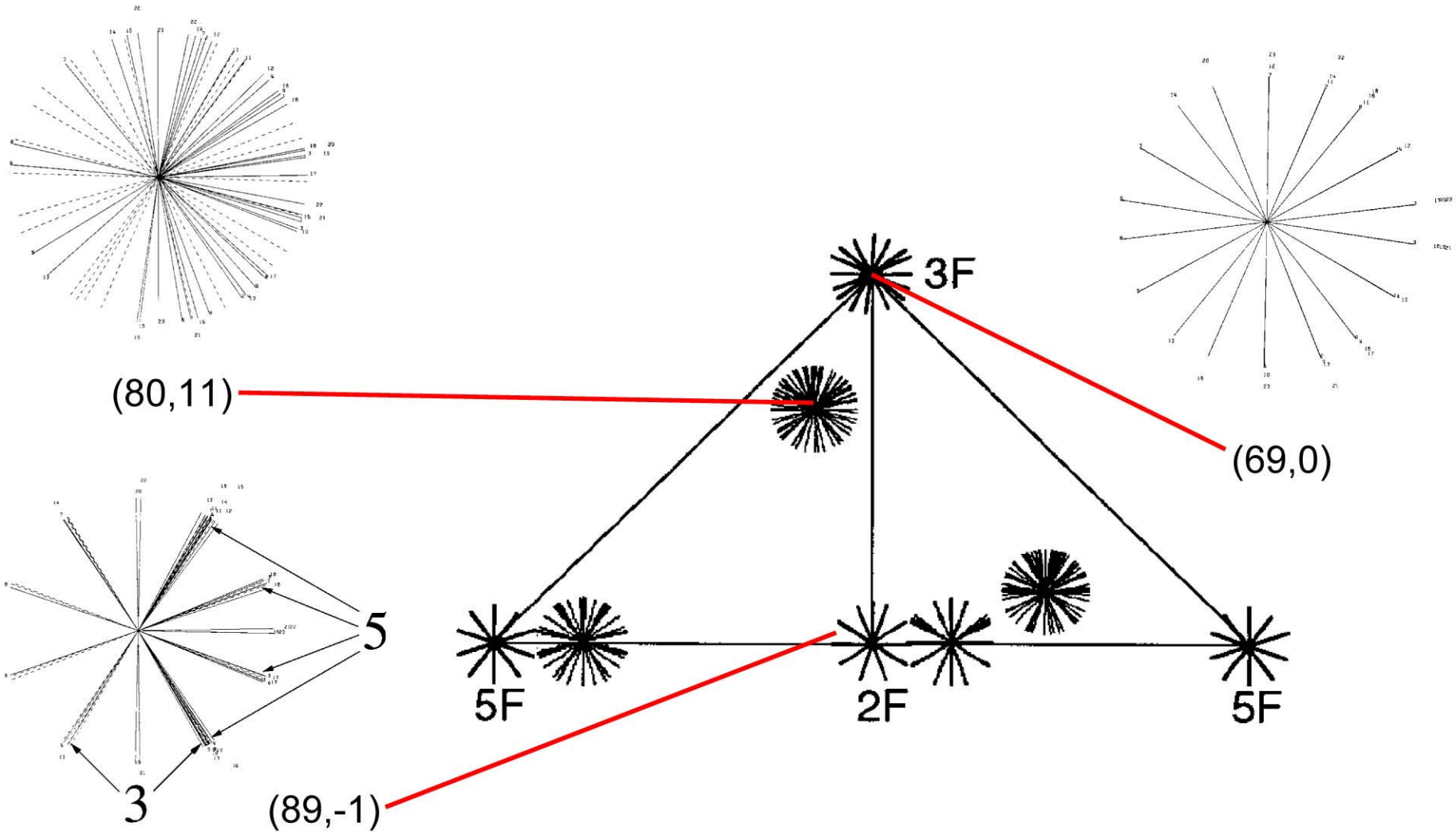
5-folds: $\frac{12}{2}$ x 2 = 12 pairs

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

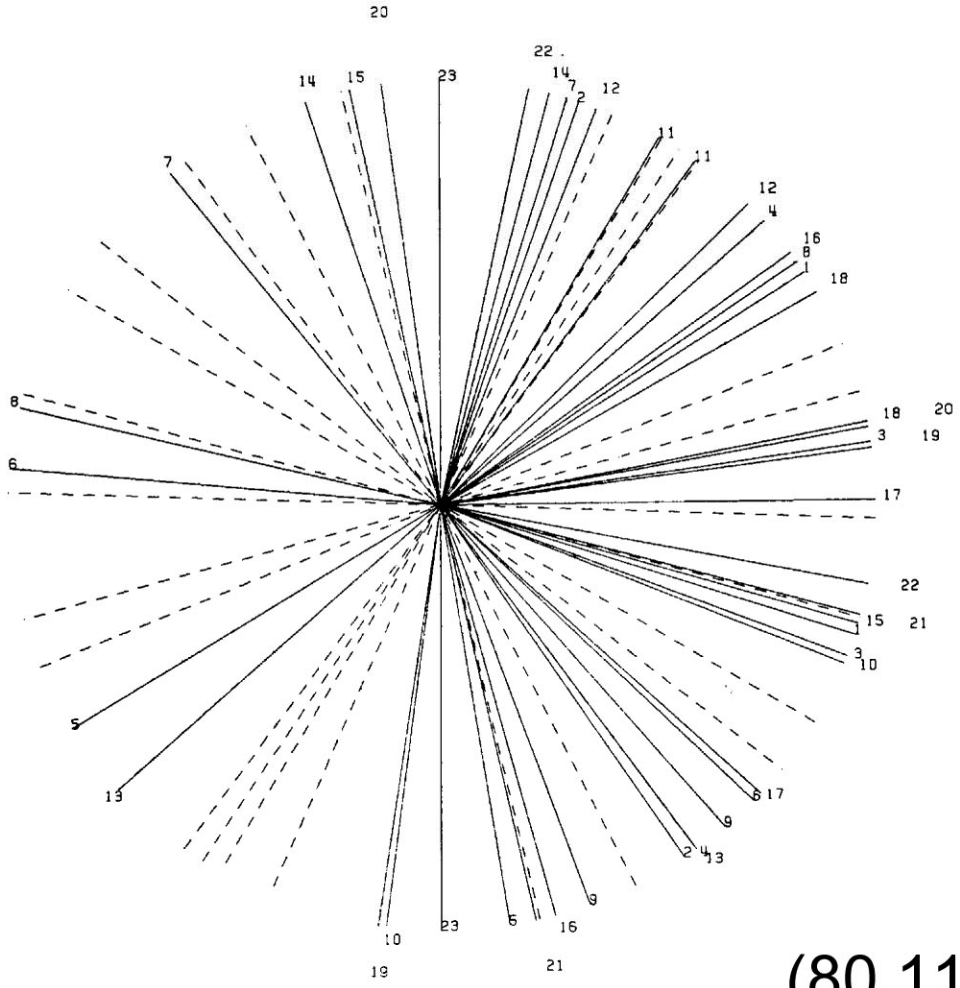
2-folds: $\frac{30}{2}$ x 1 = 15 real lines

37 common lines

Orientation Determination by Common Lines

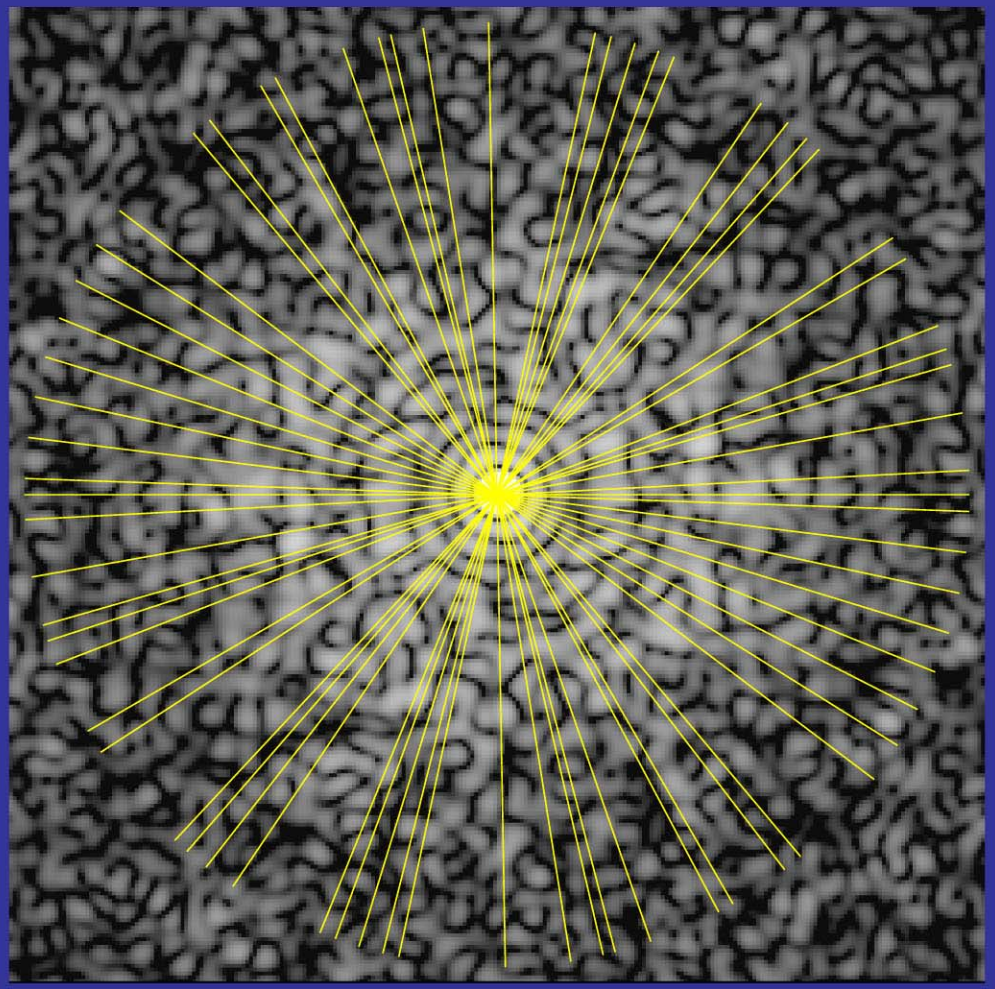


Orientation Determination by Common Lines



(80,11)

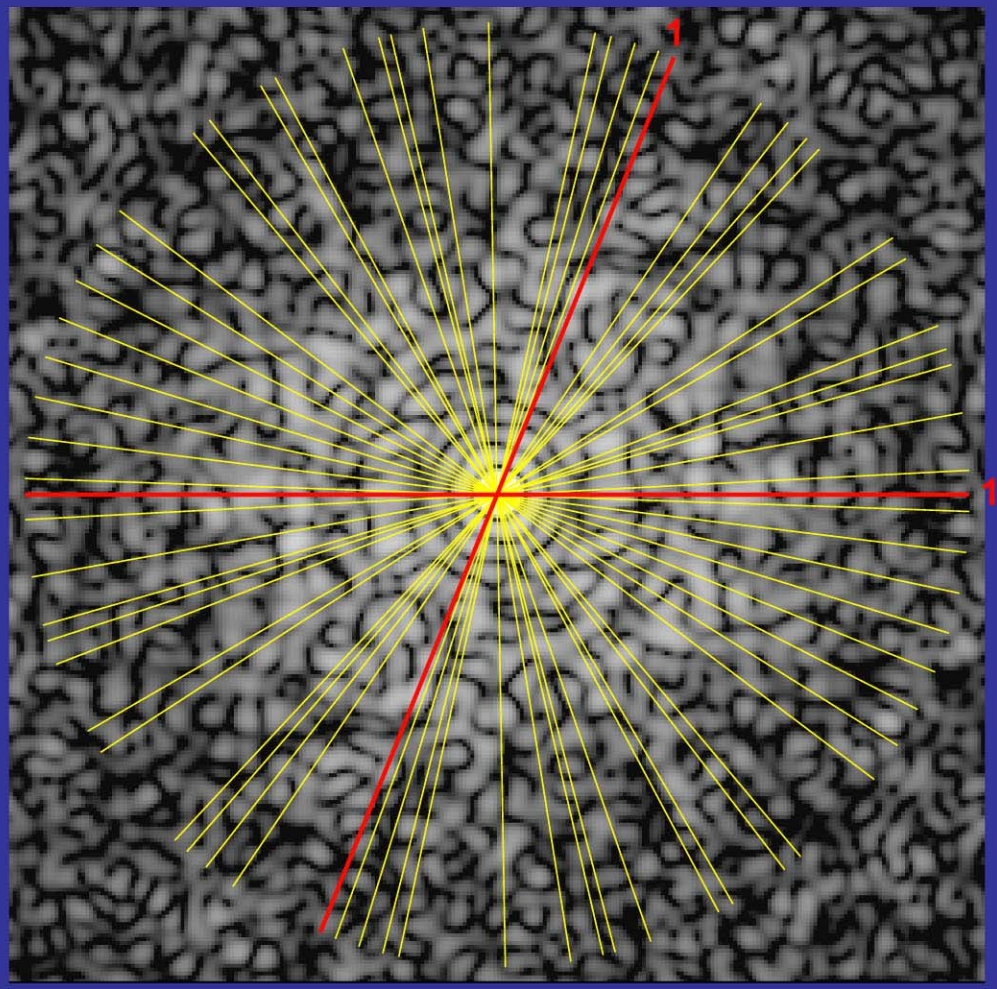
Orientation Determination by Common Lines



(80,11)

What is (θ,ϕ,ω) for this particle?

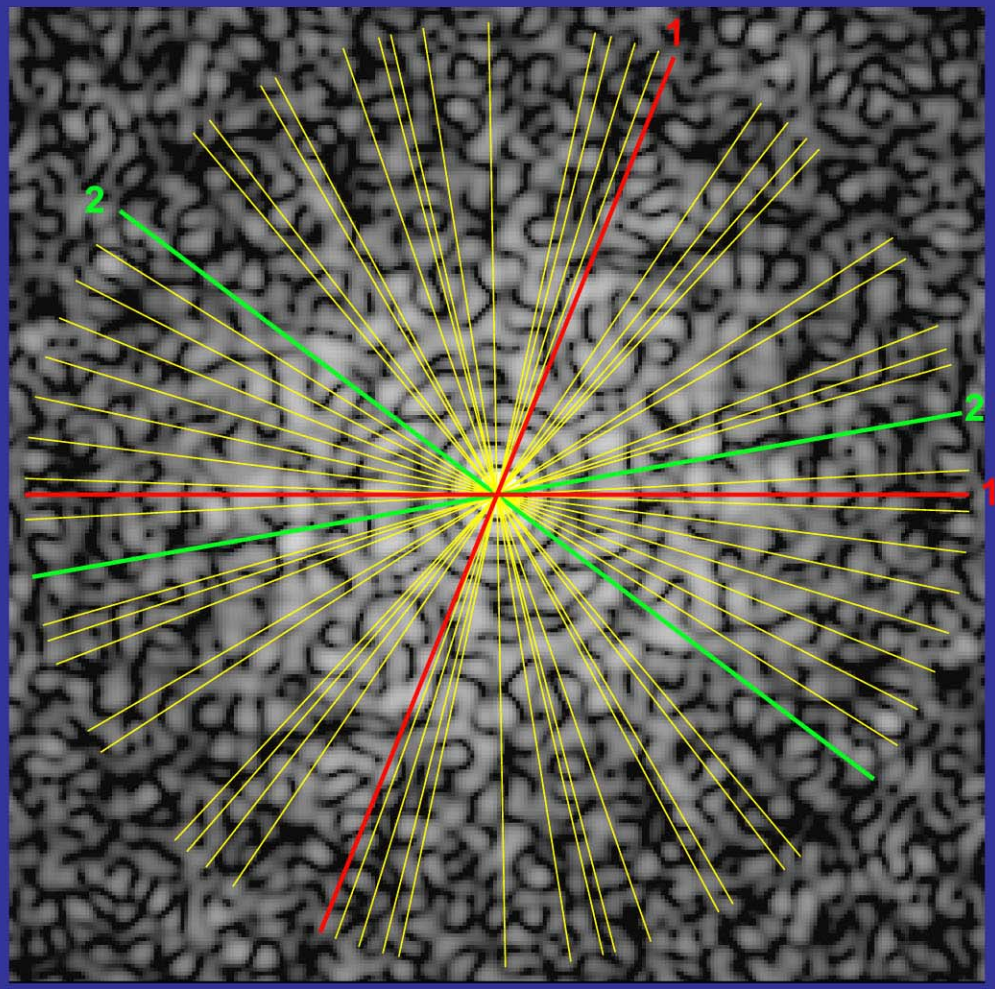
Orientation Determination by Common Lines



ω

(80,11,0)

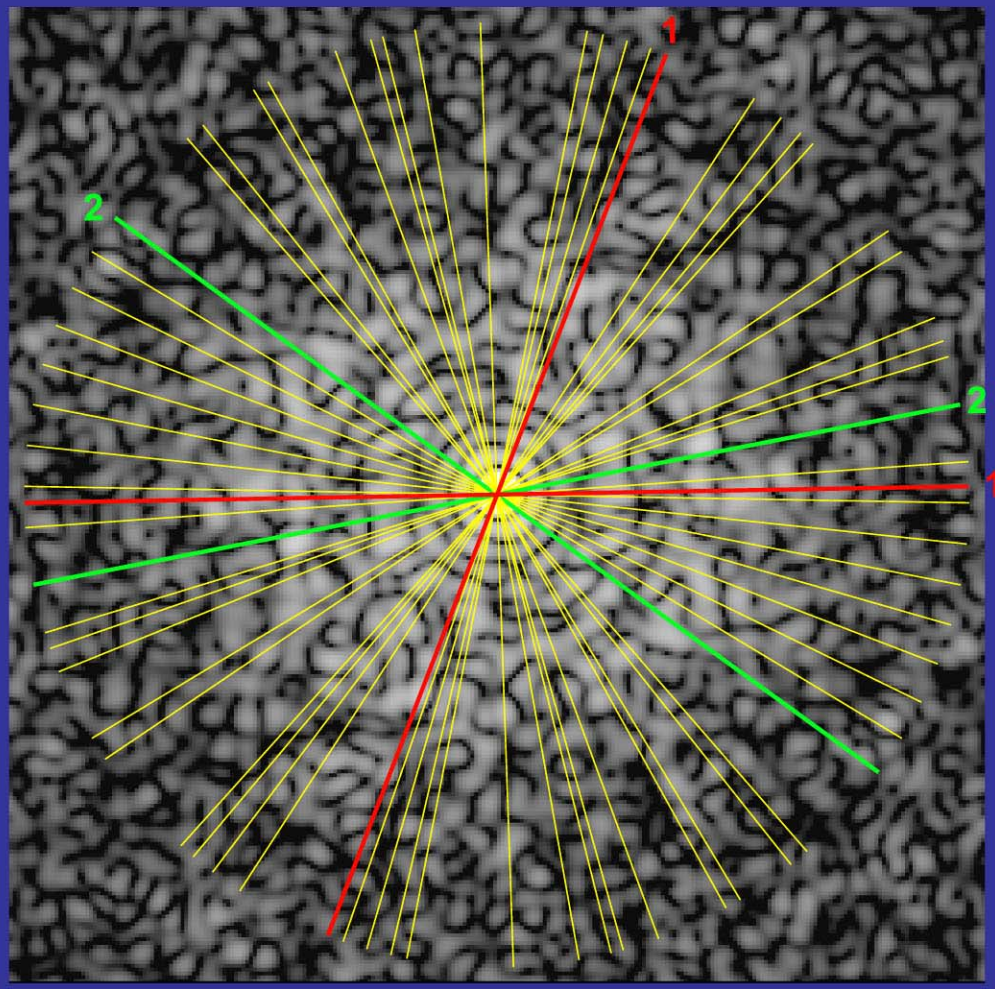
Orientation Determination by Common Lines



ω

(80,11,0)

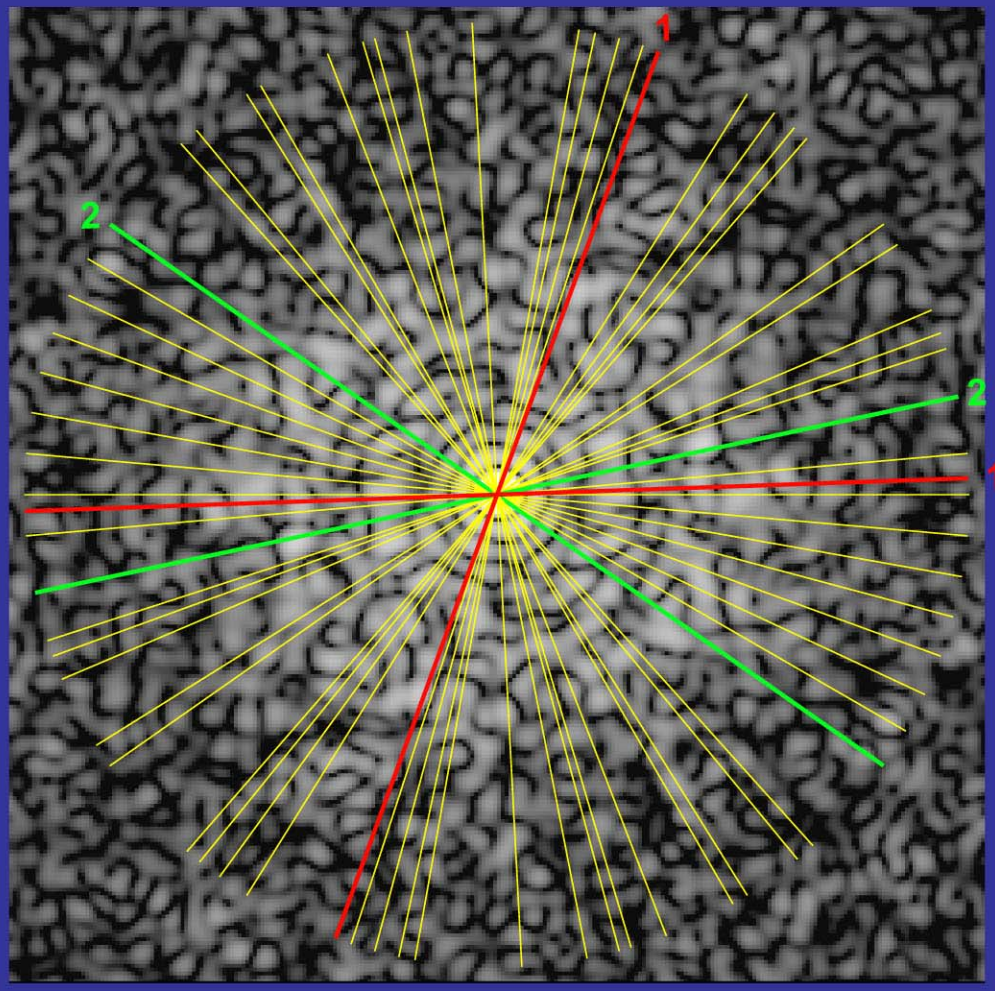
Orientation Determination by Common Lines



ω

(80,11,1)

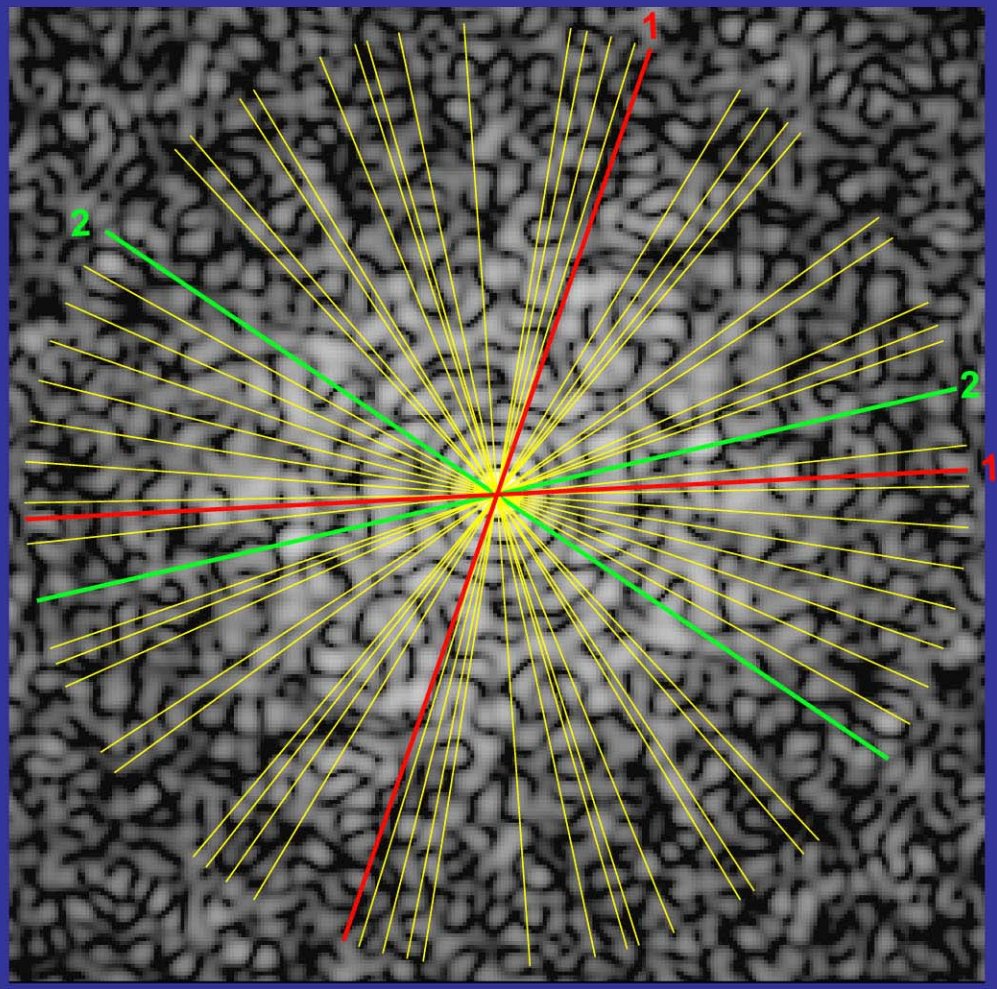
Orientation Determination by Common Lines



ω

(80,11,2)

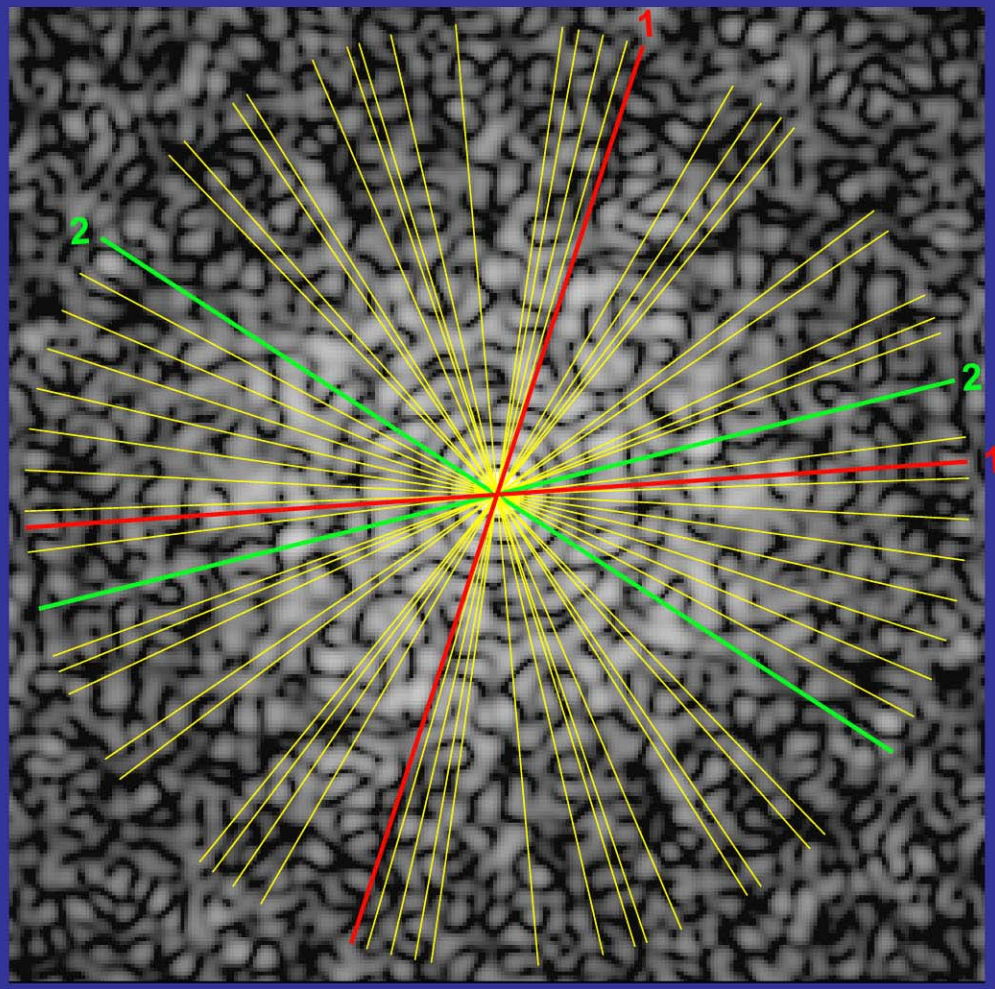
Orientation Determination by Common Lines



ω

(80,11,3)

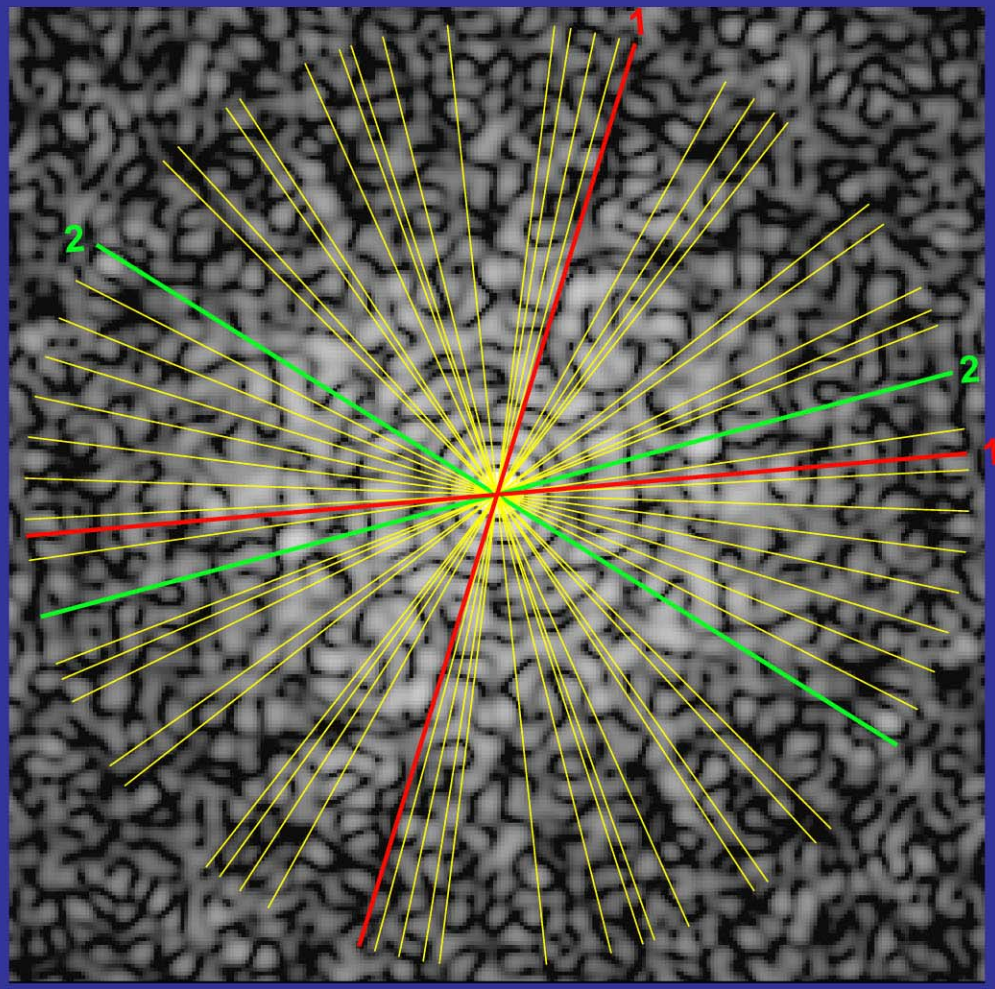
Orientation Determination by Common Lines



ω

(80,11,4)

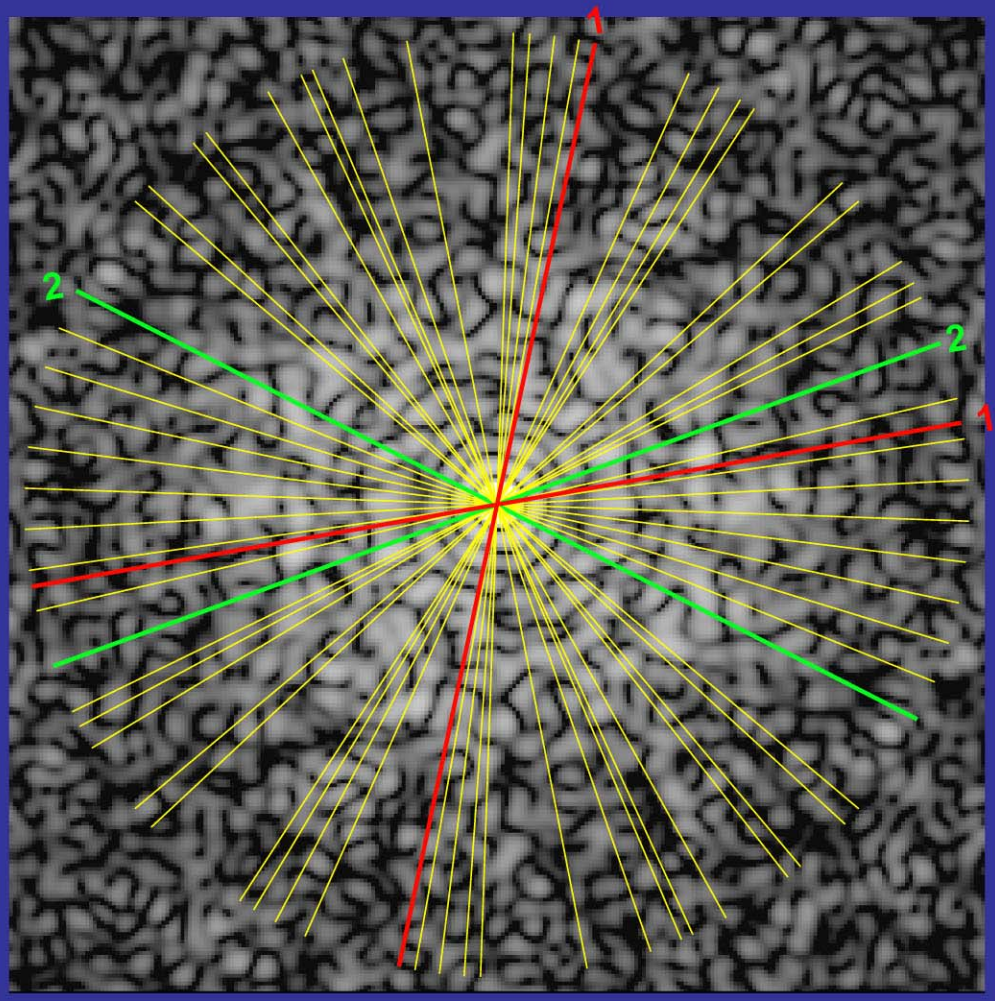
Orientation Determination by Common Lines



ω

(80,11,5)

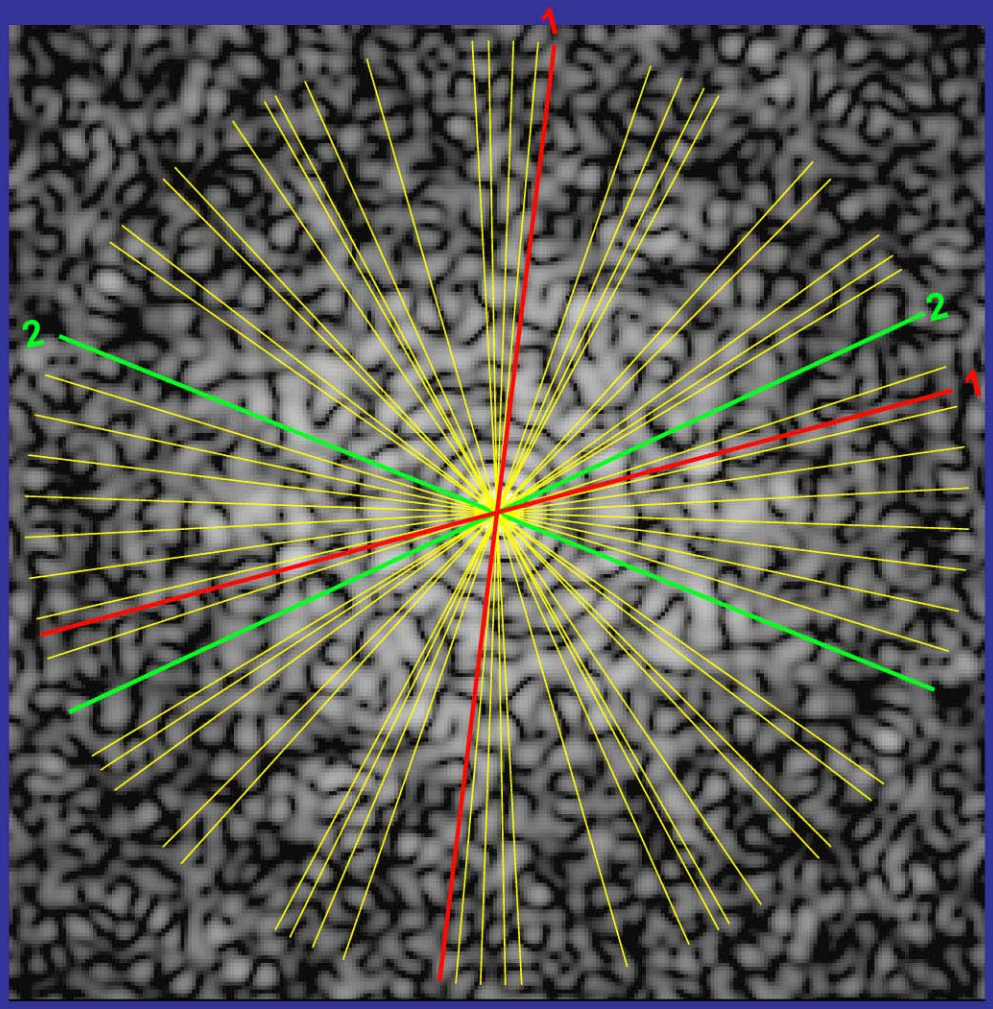
Orientation Determination by Common Lines



ω

(80,11,10)

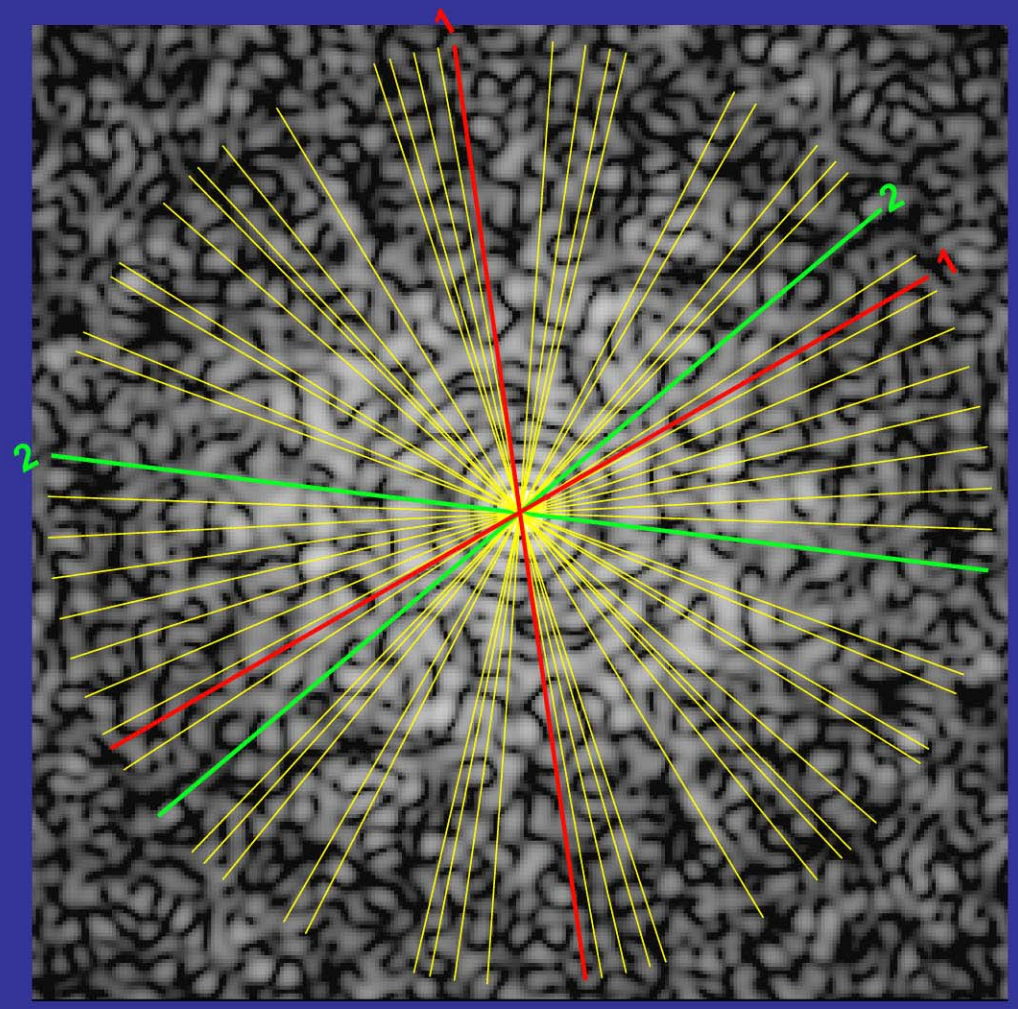
Orientation Determination by Common Lines



ω

(80,11,15)

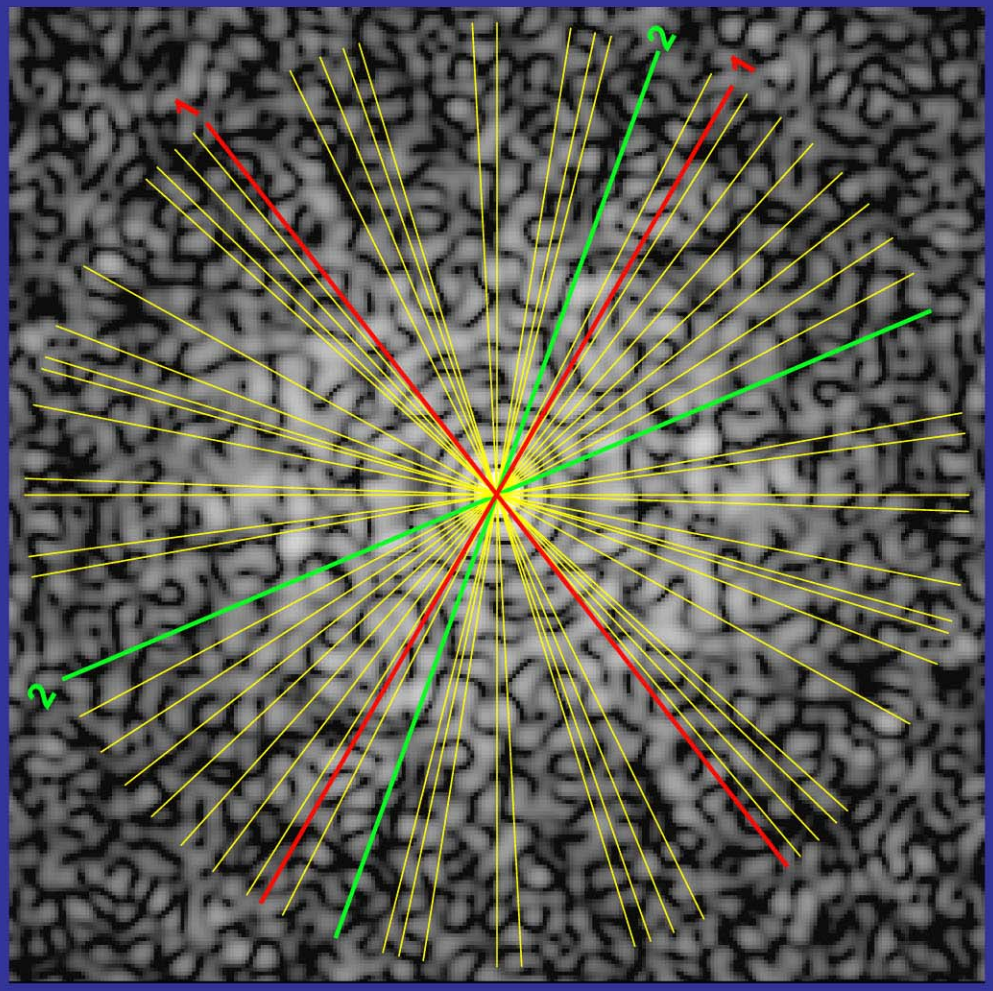
Orientation Determination by Common Lines



ω

(80,11,30)

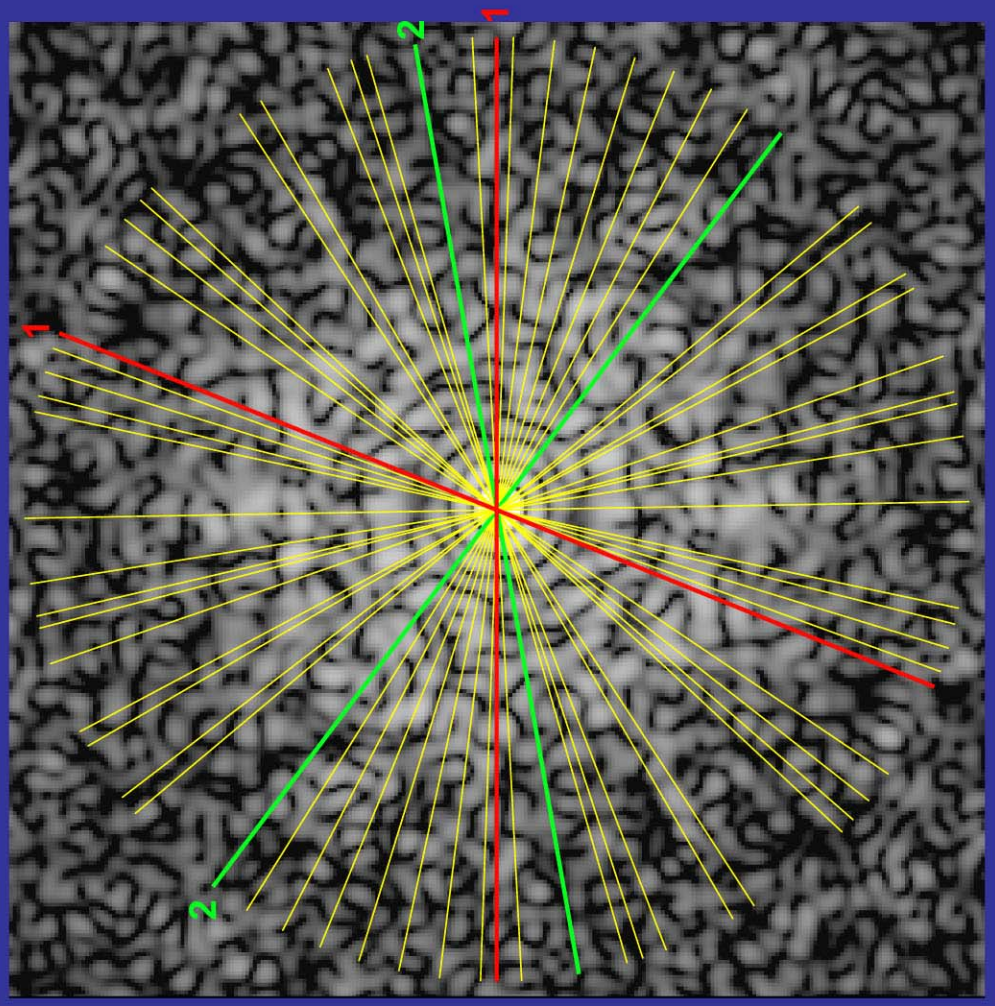
Orientation Determination by Common Lines



ω

(80,11,60)

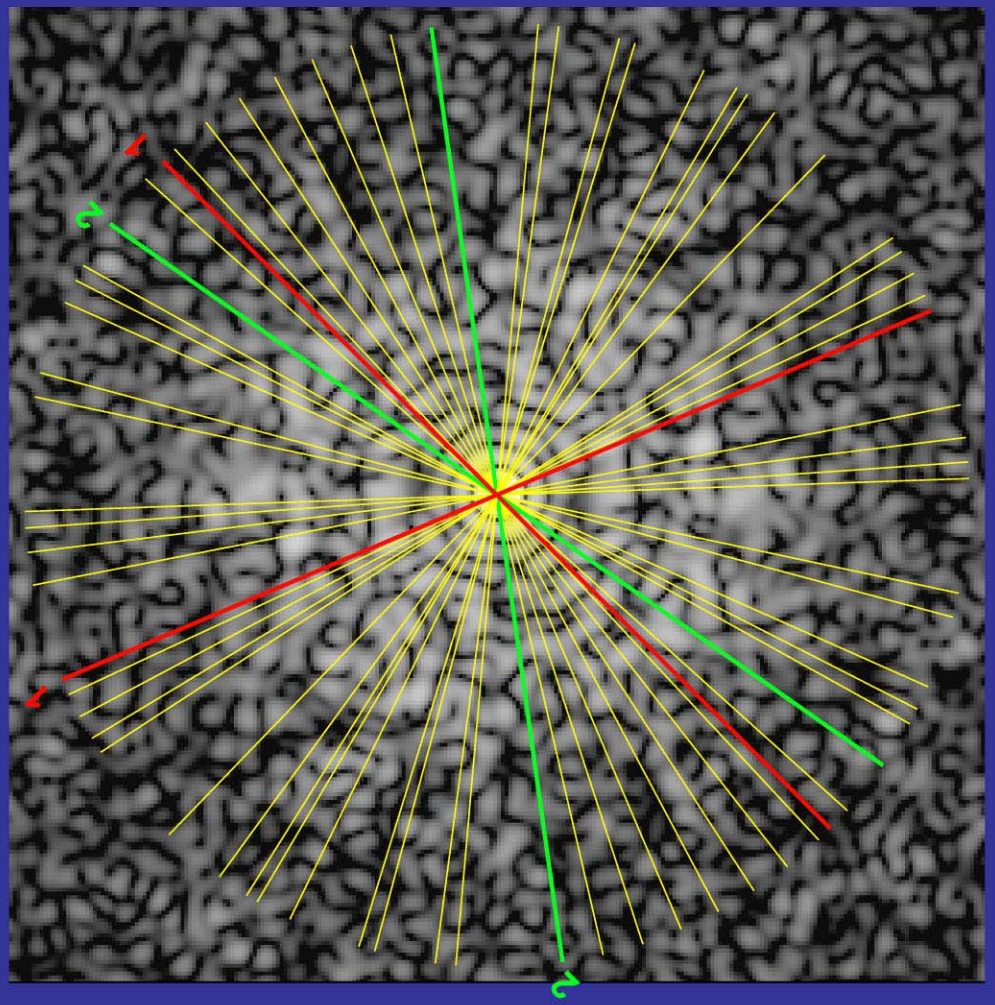
Orientation Determination by Common Lines



ω

(80,11,90)

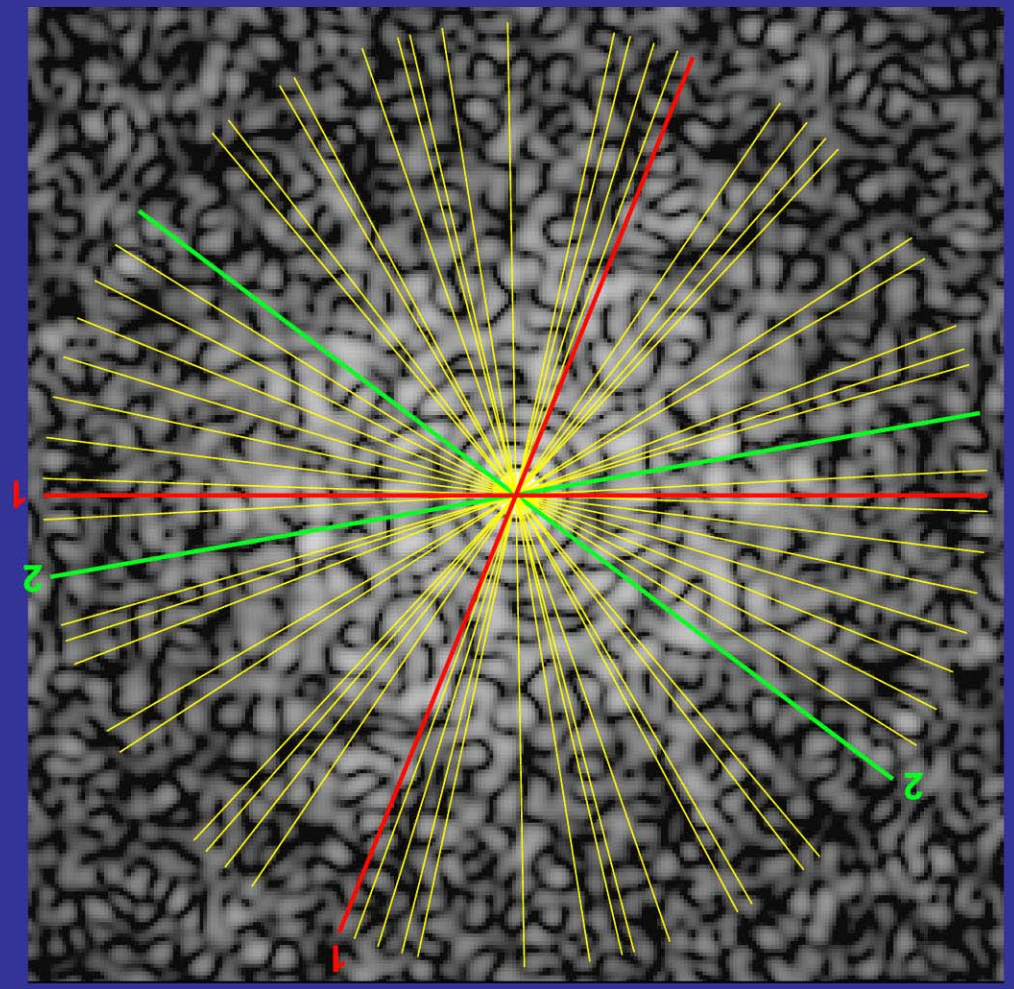
Orientation Determination by Common Lines



ω

(80,11,135)

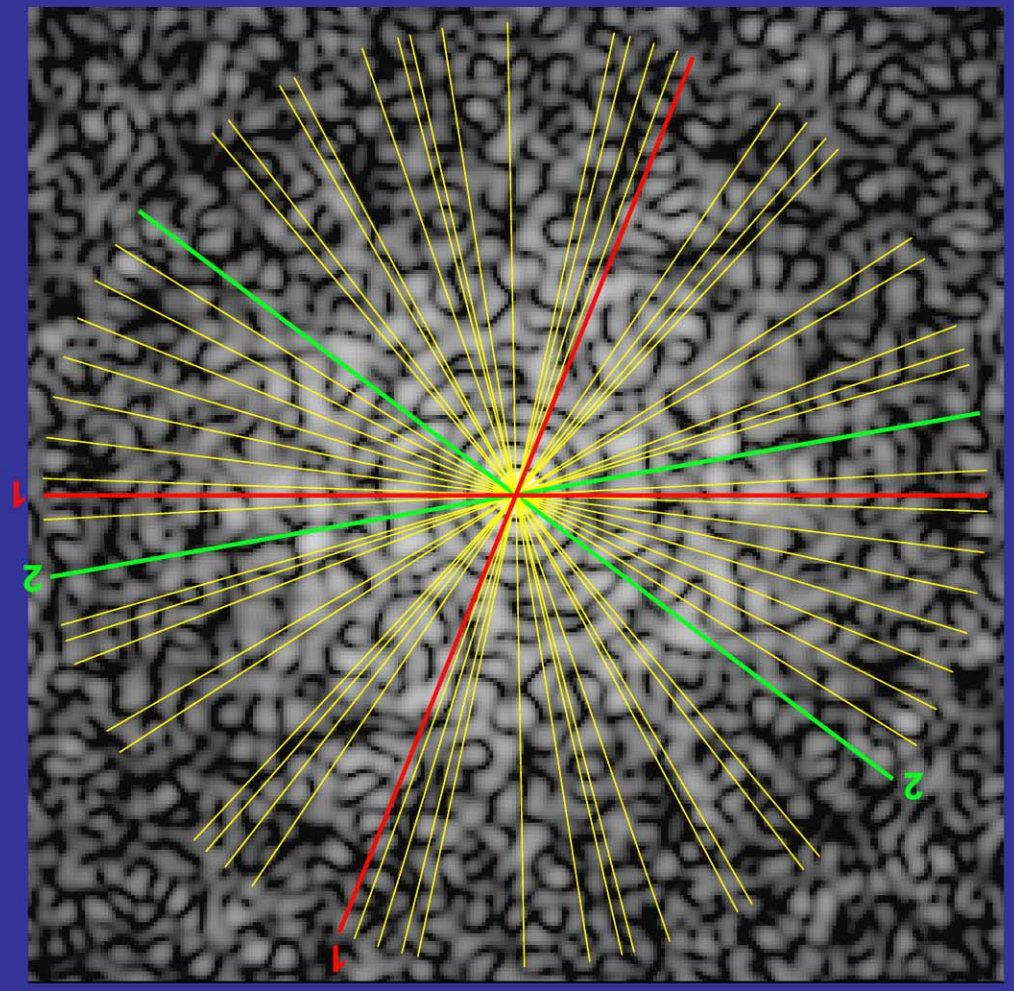
Orientation Determination by Common Lines



ω

(80,11,180)

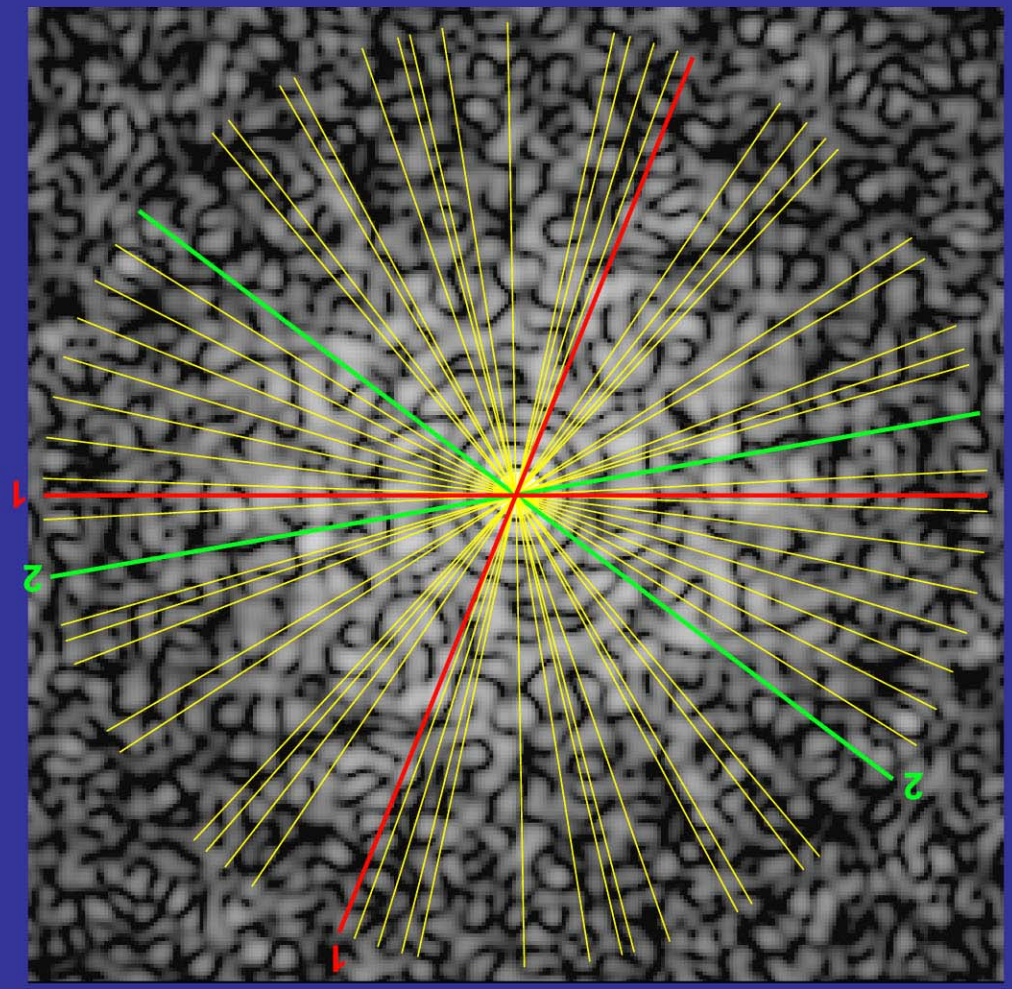
Orientation Determination by Common Lines



(80,11,ω)

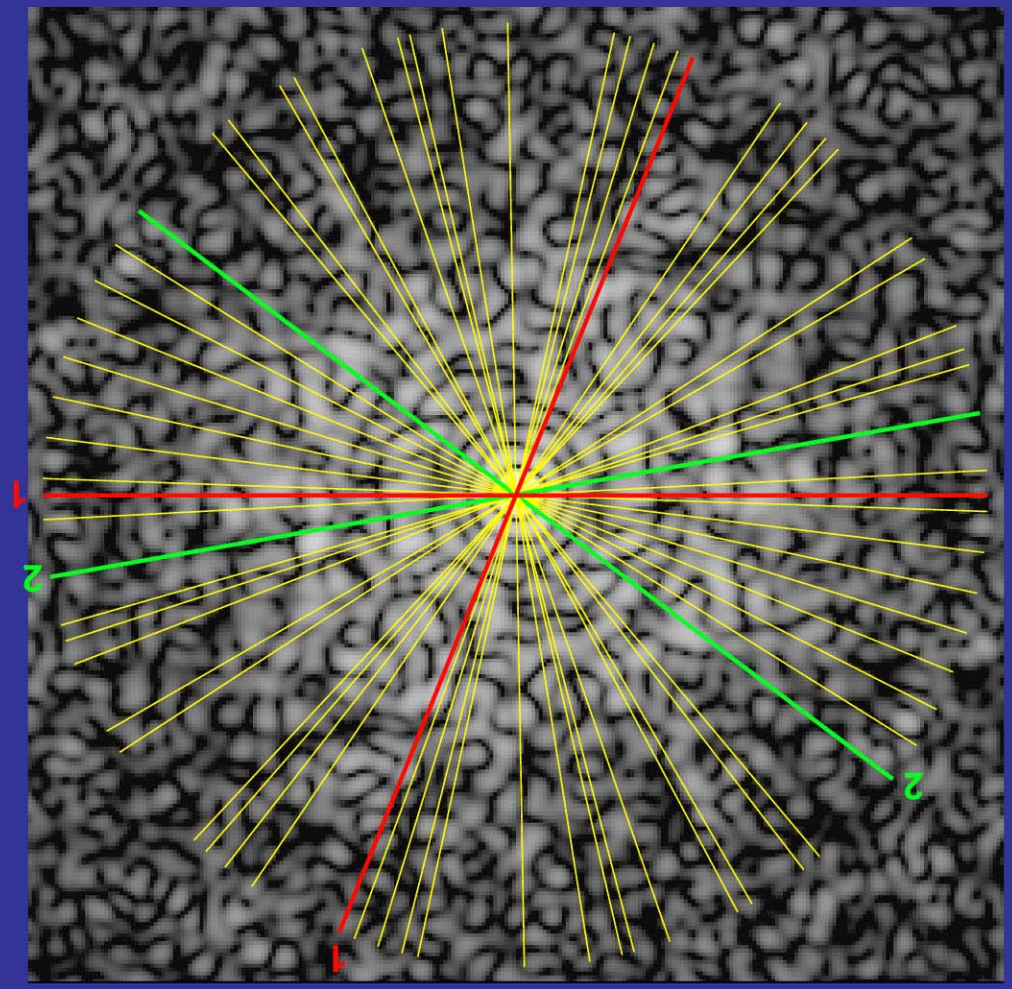
Metric: Identify ω that gives lowest phase residual

Orientation Determination by Common Lines



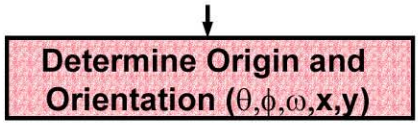
Repeat process for all possible (θ,ϕ,ω) combinations

Orientation Determination by Common Lines



> 250,000 combinations for 1° angular search intervals

Orientation Determination by Common Lines



Common Lines

The (θ, ϕ, ω) that results in the lowest phase residual is selected as the best estimate for the particle view orientation

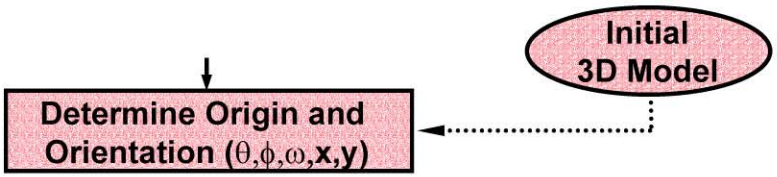
The 'common lines' procedure is similarly used to determine the particle phase origin (x, y)

Minimizing Phase Differences Among Common Lines

$$P_i(\phi,\theta,\omega,x,y) = \frac{\sum_{j=1}^{N}\sum_{k=1}^{k_{\max}(j)}\sum_{R=R_{\min}}^{R_{\max}}\left|\psi_i(R,x_i,y_i,\alpha_{i,j,k}) - \psi_j(R,x_j,y_j,\alpha_{j,i,k})\right| \times w(R,\alpha_{i,j,k},\alpha_{j,i,k})}{(R_{\max}-R_{\min})\sum_{j=1}^{N}k_{\max}(j)}$$

P is the phase residual
Ψ is the phase value in Fourier space
i and j refer to particles or symmetry-related sections
x and y define the phase origin
k refers to the common line
αijk are the angles of the *k*-th common-lines
Rmin and Rmax define the frequency range within which the phase residuals are evaluated
kmax is total number of common lines (here: 37)
N is the number of particles
w defines a weighting function for the Fourier elements at different frequency and orientations

Icosahedral Particle Reconstruction Scheme



Recall: two methods to determine (θ, ϕ, ω, x, y):

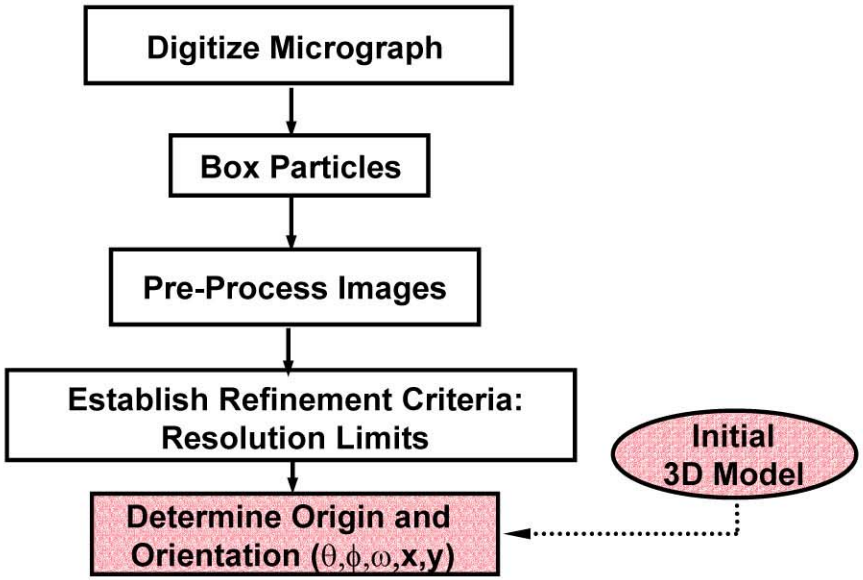
1. Common lines

2. Model-based (template) matching

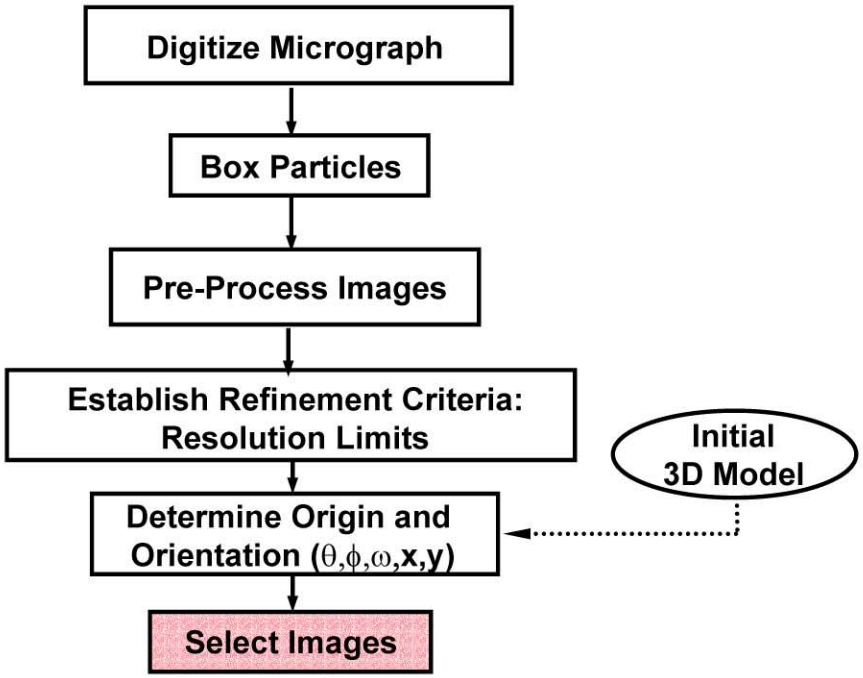
- Many structures now solved this way
- Same as reference based alignment in single-particle processing in earlier session...

3D Reconstruction

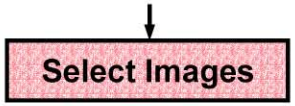
Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme

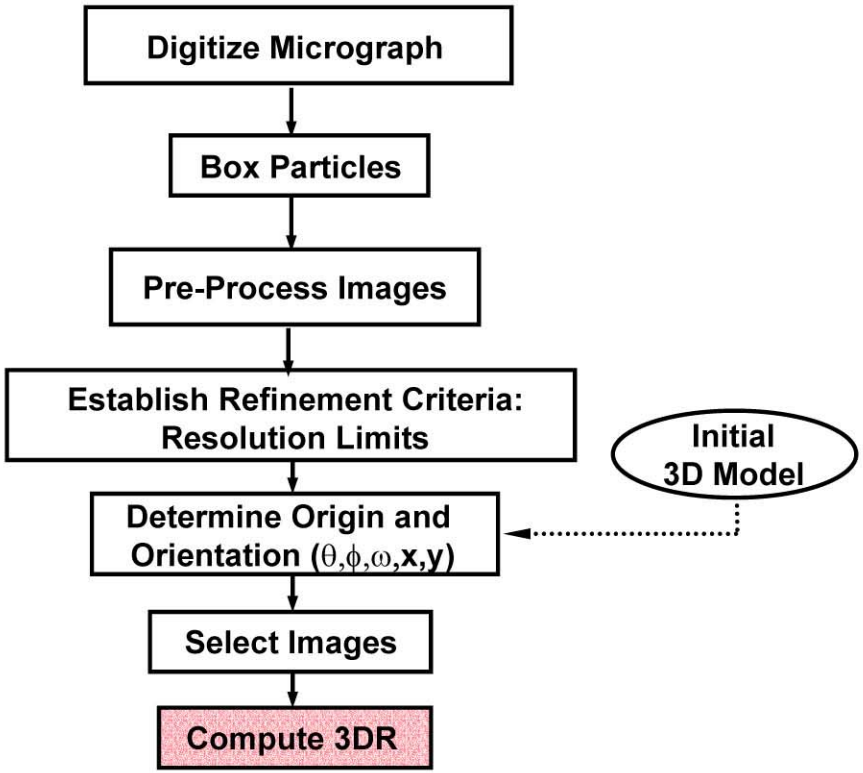


Icosahedral Particle Reconstruction Scheme

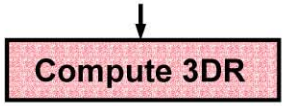


Goal: weed out ‘bad’ particle images before computing 3D reconstruction

Icosahedral Particle Reconstruction Scheme

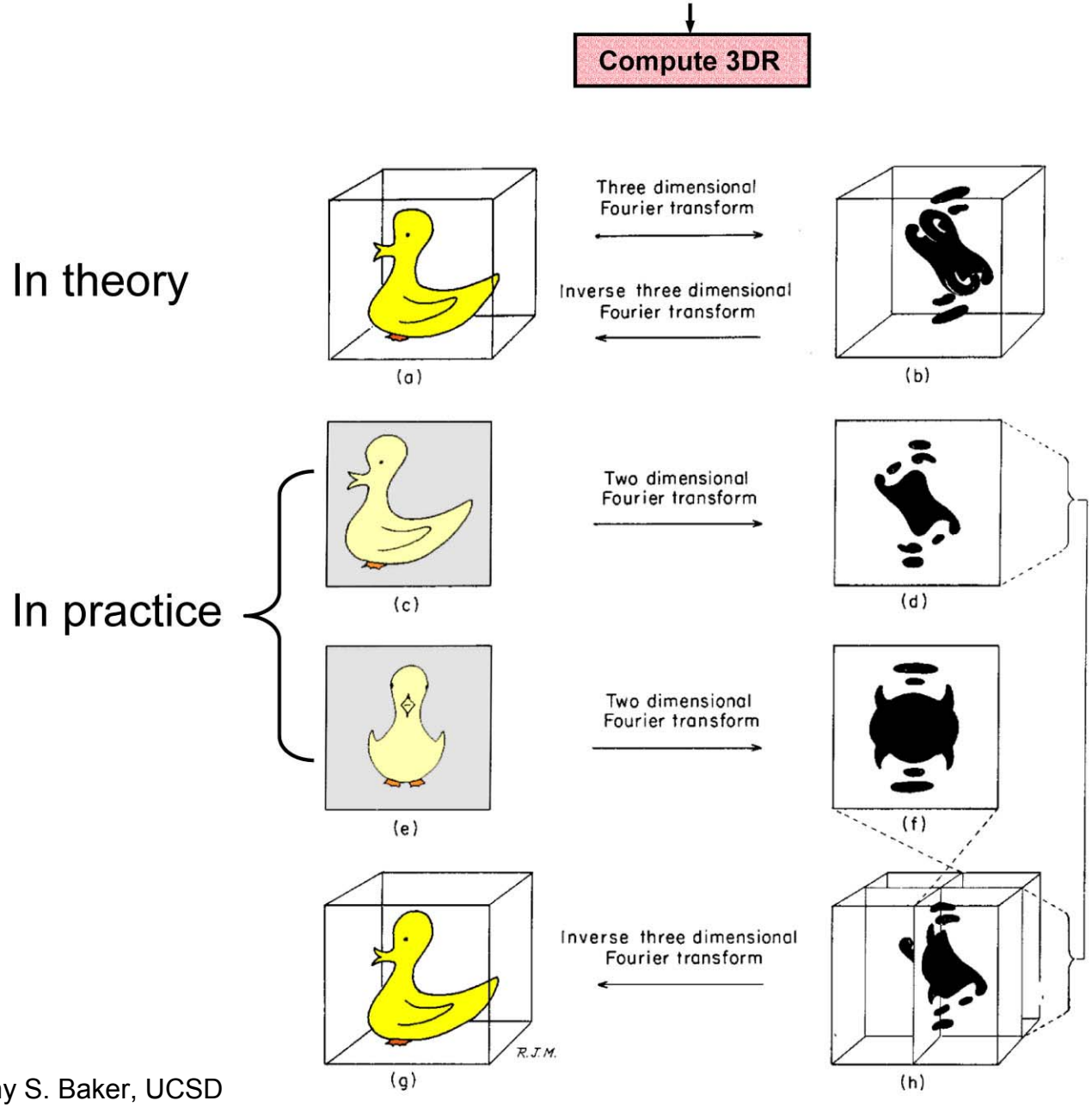


Icosahedral Particle Reconstruction Scheme



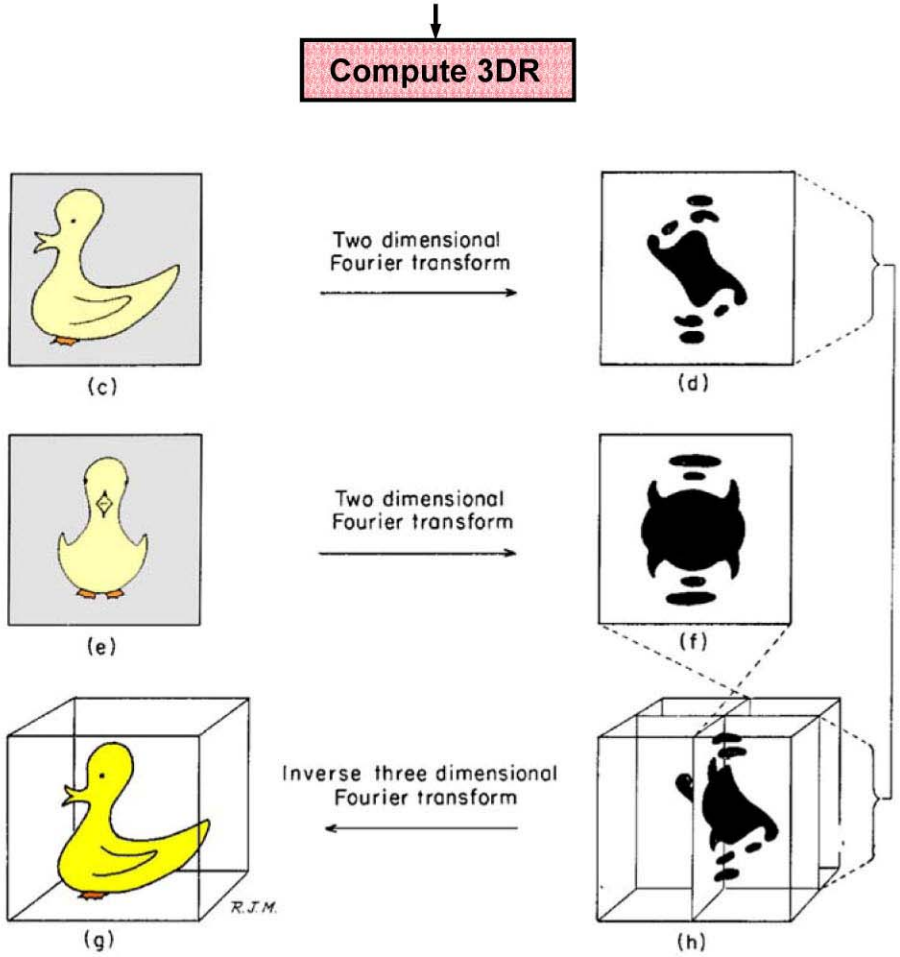
Goal: combine “good” particle images to compute a 3D density map

Icosahedral Particle Reconstruction Scheme



From Lake (1972), p.174

Icosahedral Particle Reconstruction Scheme



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

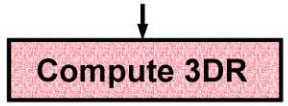
Remember? Fourier-Bessel Formalism

$$\rho(r,\varphi,z) = \sum_{n=-\infty}^{\infty} \int_{-\infty}^{\infty} g_n(r,Z) e^{in\varphi} e^{2\pi i z Z} dZ$$

$$g_n(r,Z) = \int_0^{\infty} G_n(R,Z) J_n(2\pi R r) 2\pi R dR$$

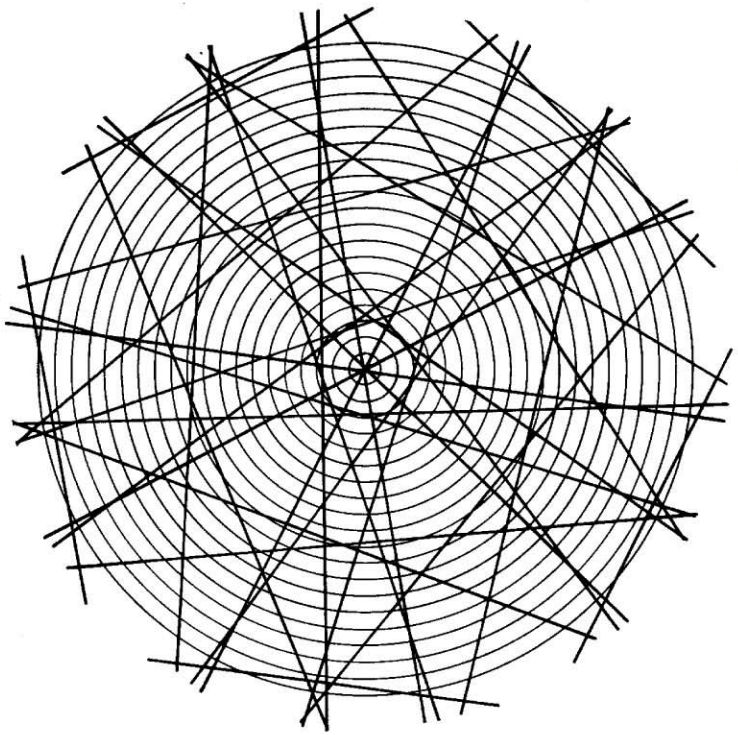
$$F(R,\Phi,Z) = \sum_{n=-\infty}^{\infty} G_n(R,Z) i^n e^{in\Phi}$$

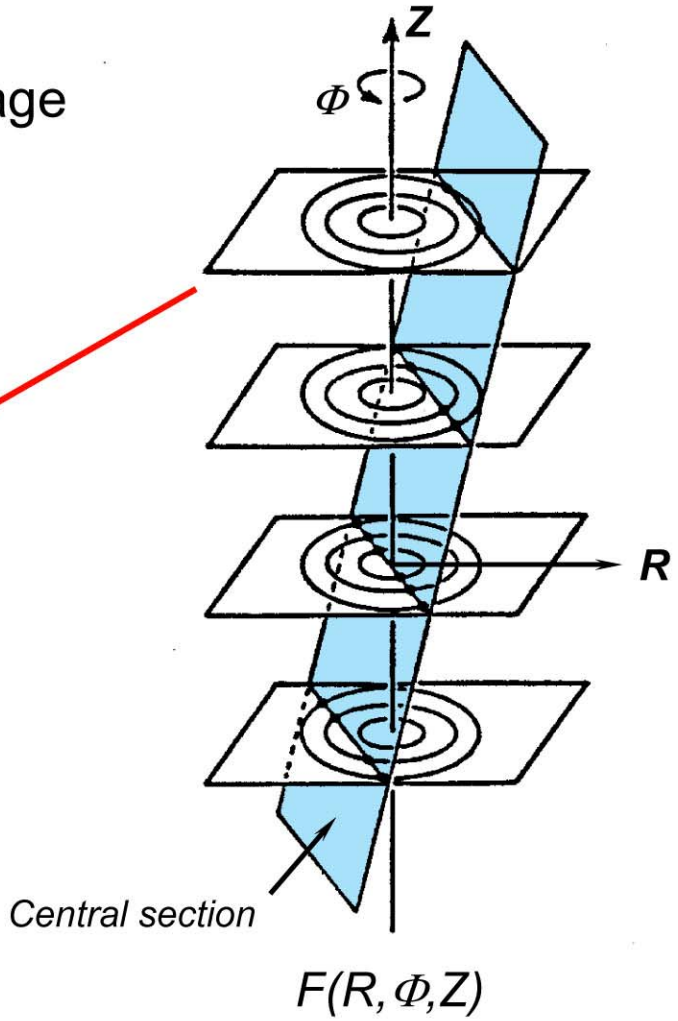
Icosahedral Particle Reconstruction Scheme



Steps:

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

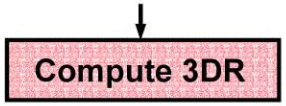




Crowther, DeRosier and Klug, 1970, p.329

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

Icosahedral Particle Reconstruction Scheme

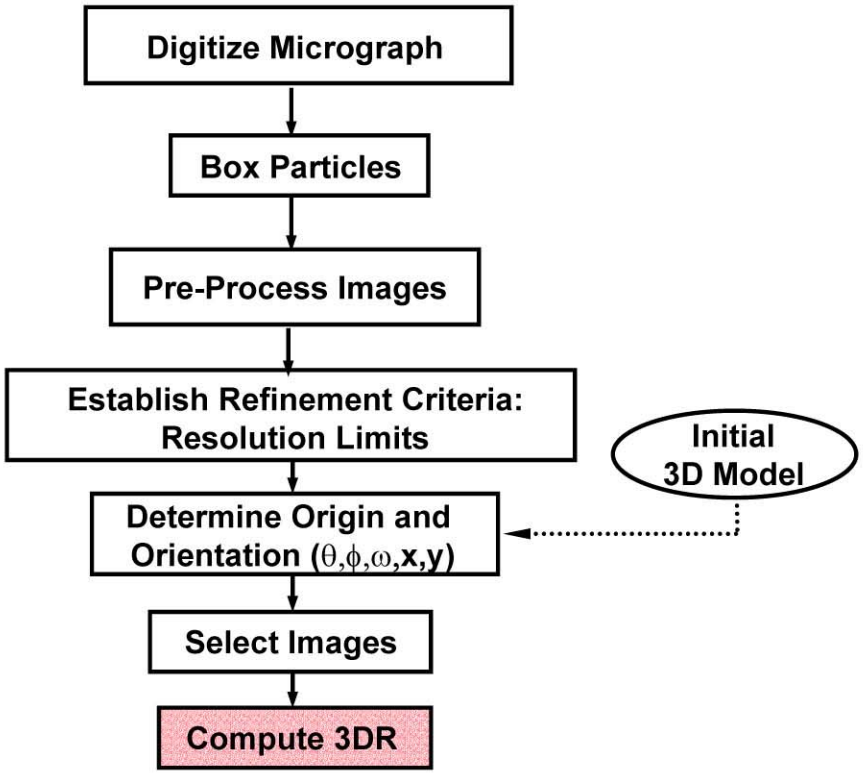


$\rho \leftarrow g \leftarrow G \leftarrow F$

Steps:

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

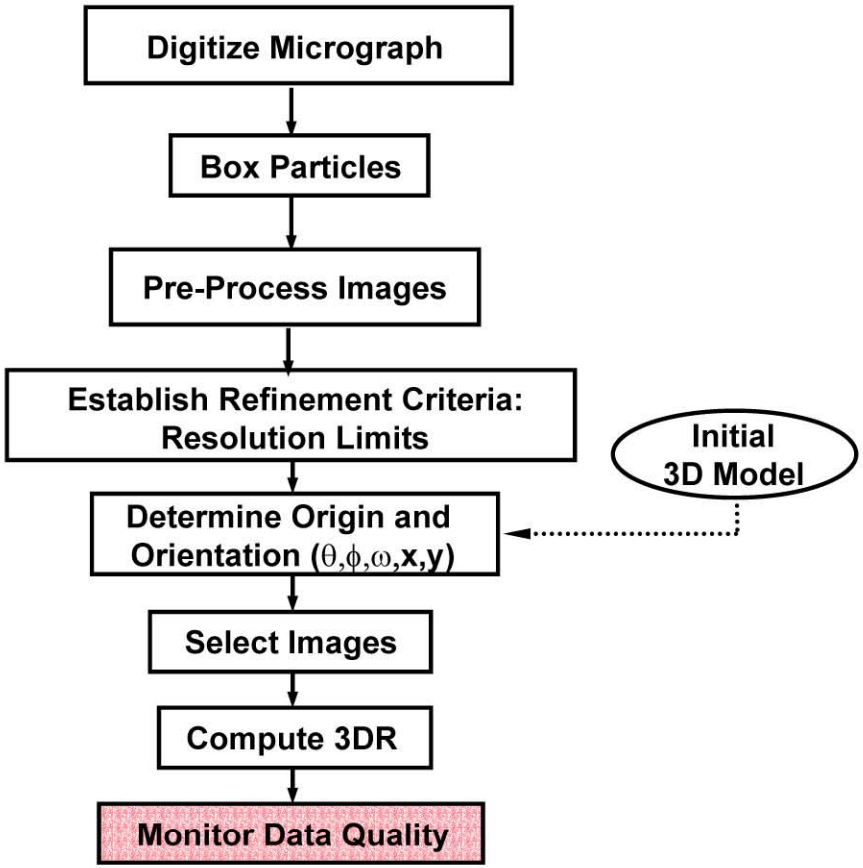
Icosahedral Particle Reconstruction Scheme



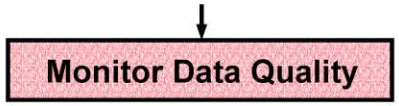
Option: correct for CTF effects in particle FFTs before FFTs are merged to form the 3D FFT

Resolution Estimation and Quality Control

Icosahedral Particle Reconstruction Scheme

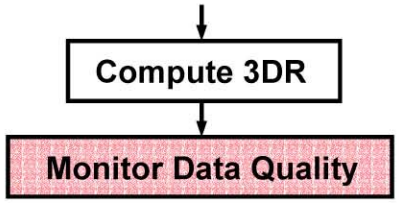


Icosahedral Particle Reconstruction Scheme

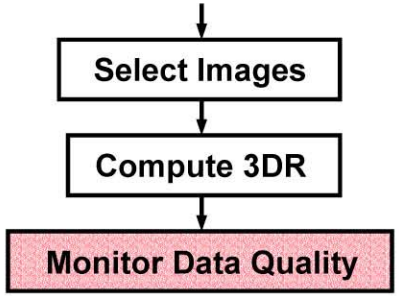


Goal: assess resolution of 3D density map
to determine what to do next

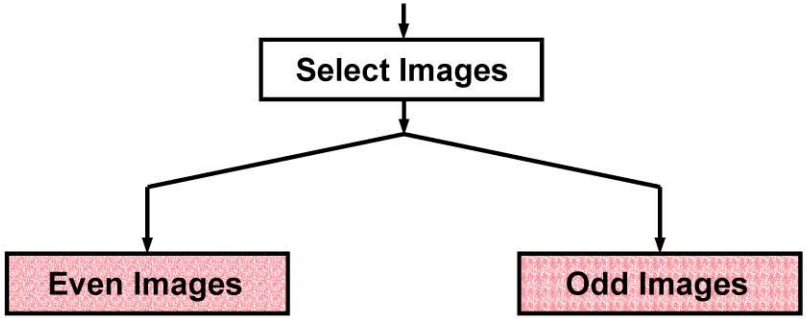
Icosahedral Particle Reconstruction Scheme



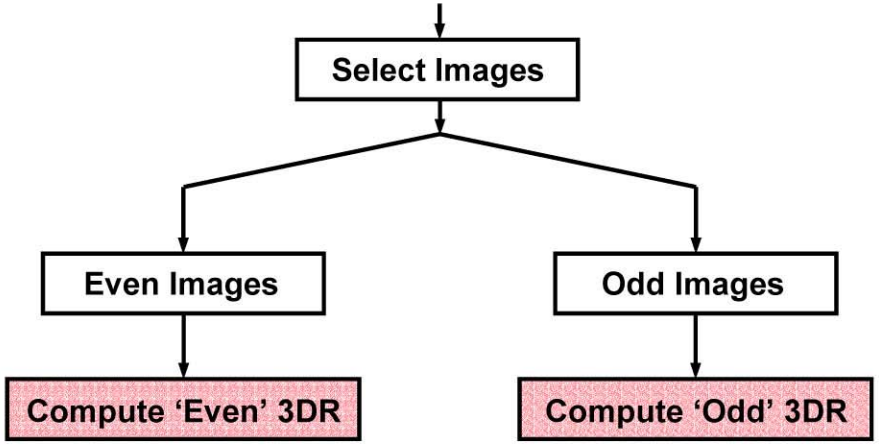
Icosahedral Particle Reconstruction Scheme



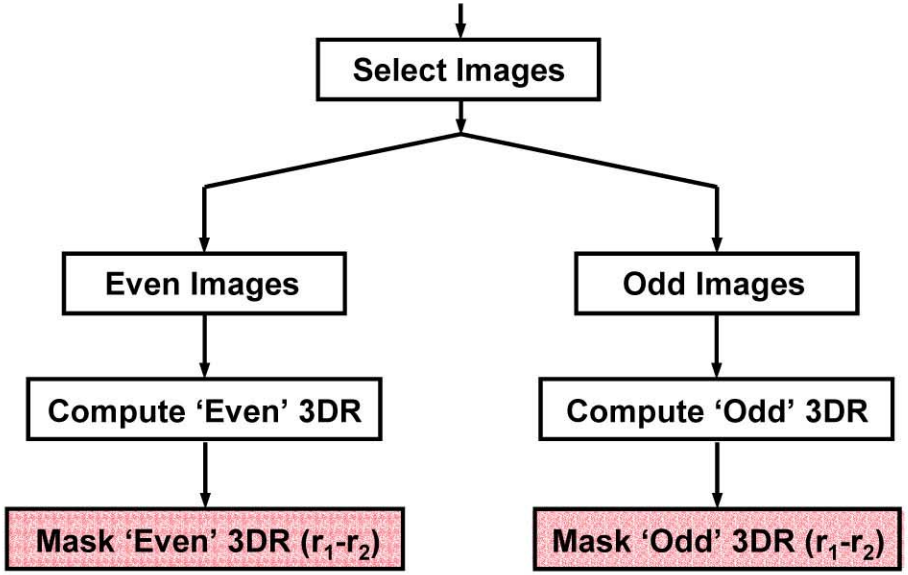
Icosahedral Particle Reconstruction Scheme

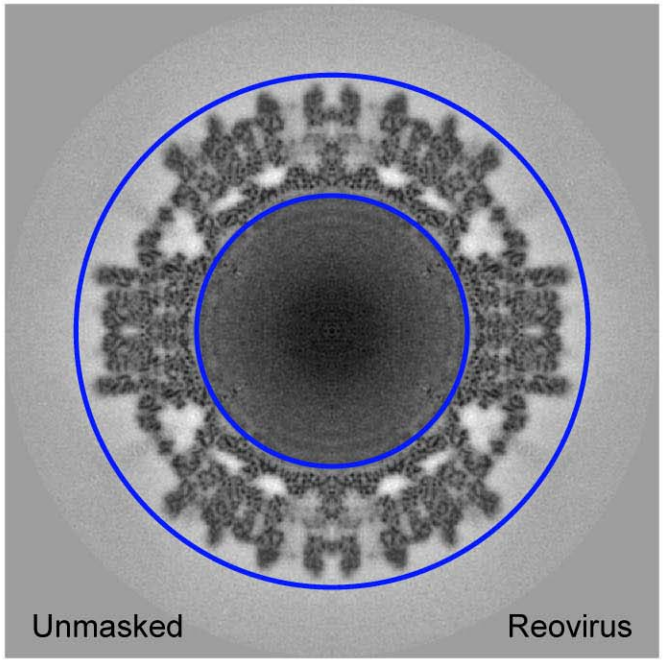


Icosahedral Particle Reconstruction Scheme

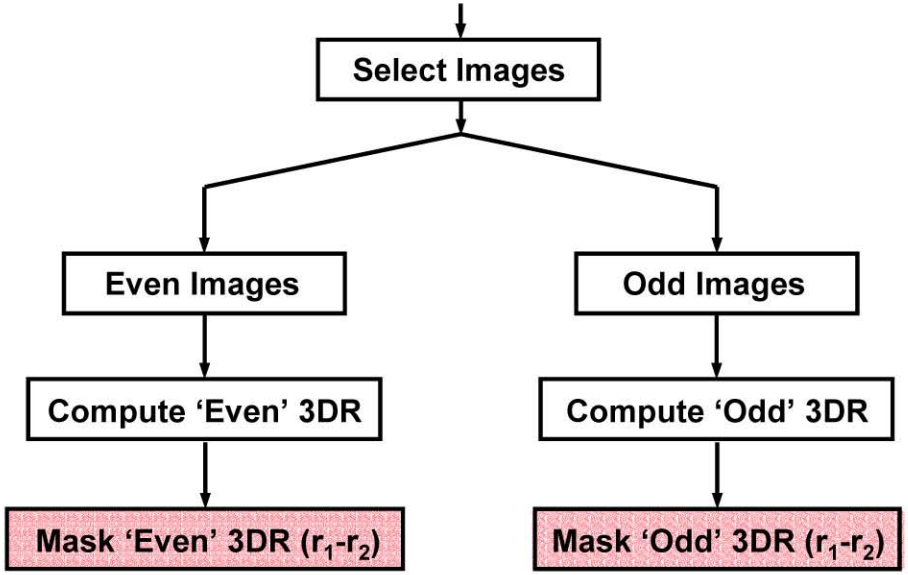


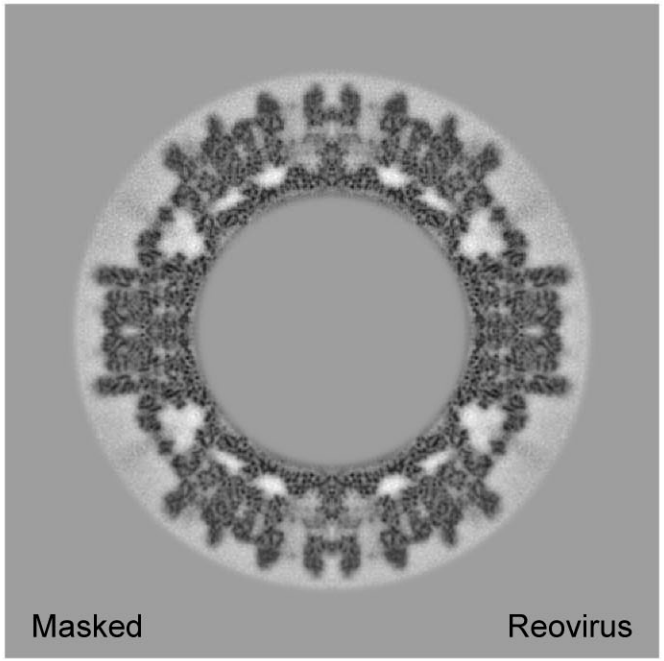
Icosahedral Particle Reconstruction Scheme



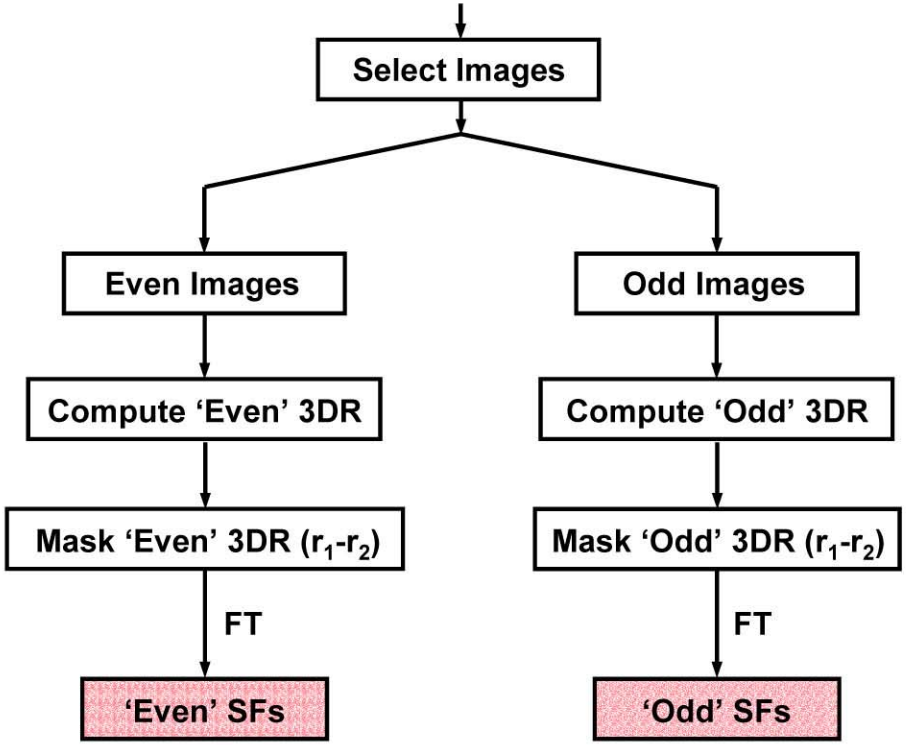


Icosahedral Particle Reconstruction Scheme

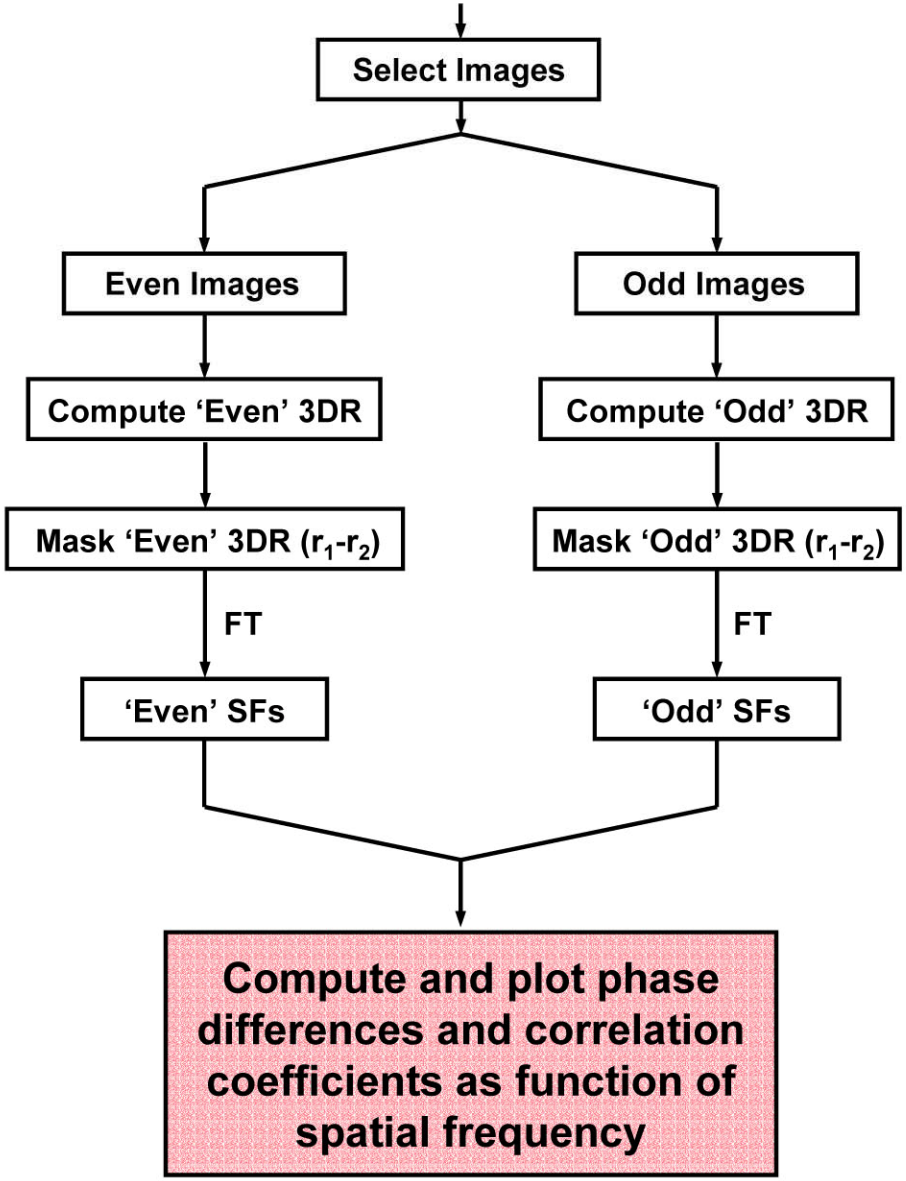




Icosahedral Particle Reconstruction Scheme

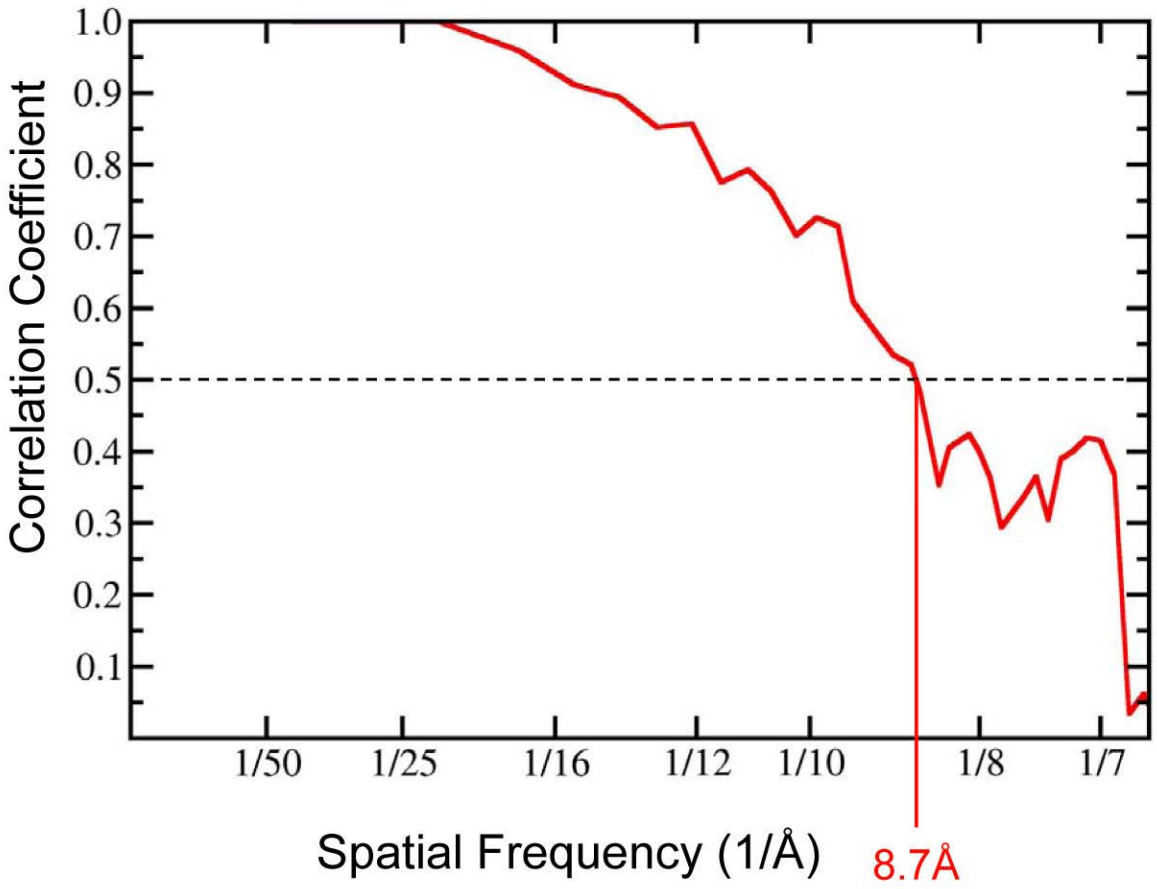


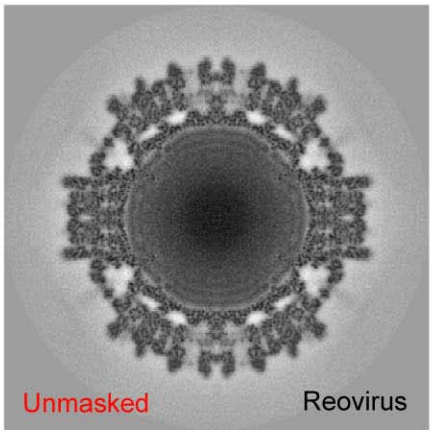
Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme

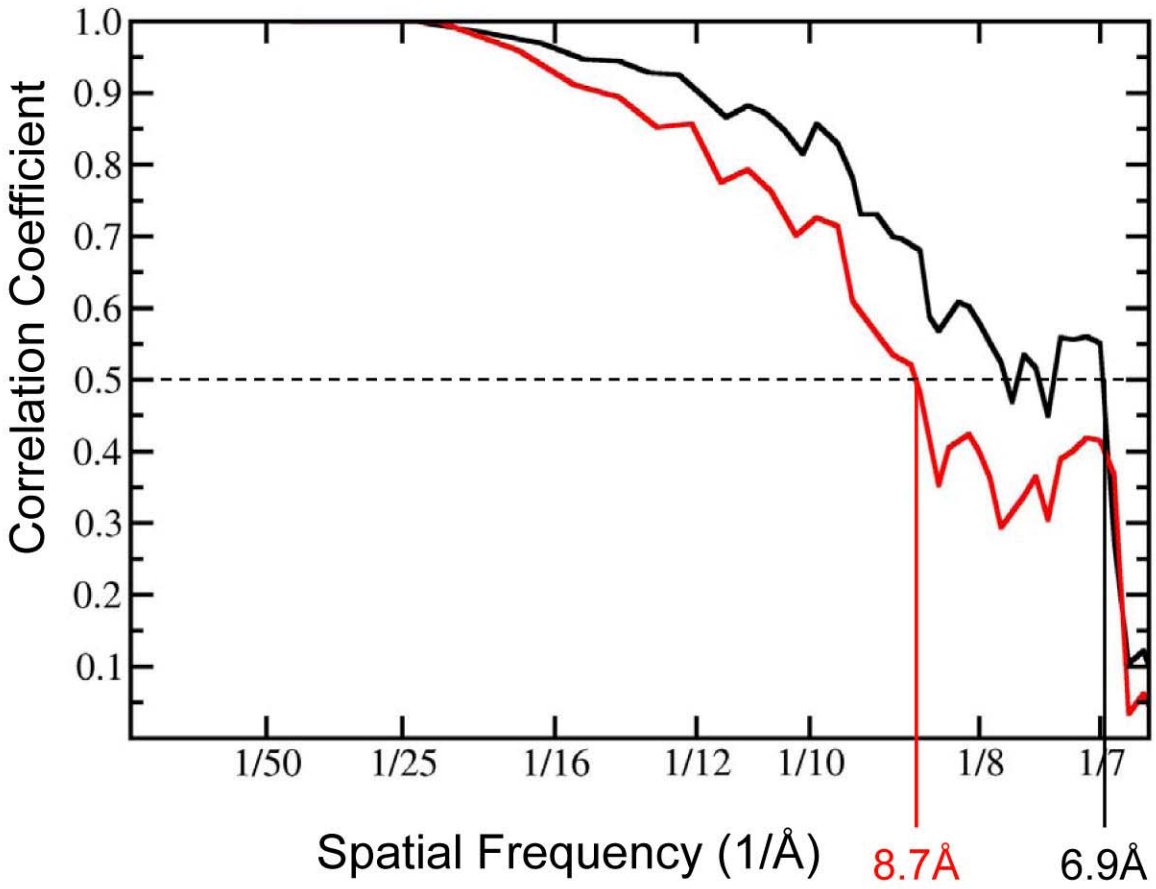
Monitor Data Quality

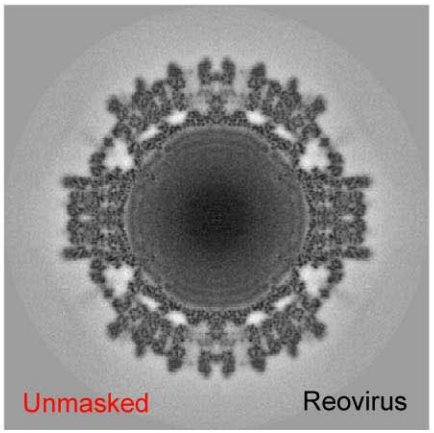


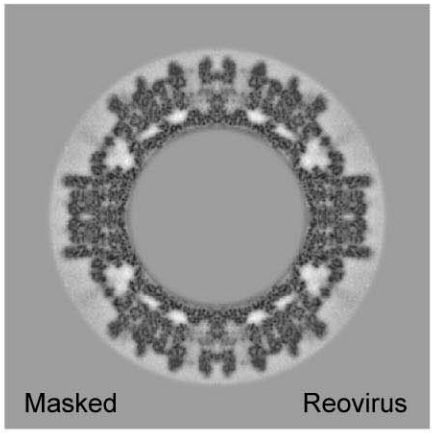


Icosahedral Particle Reconstruction Scheme

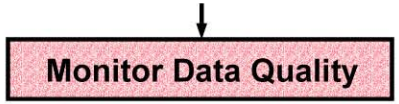
Monitor Data Quality







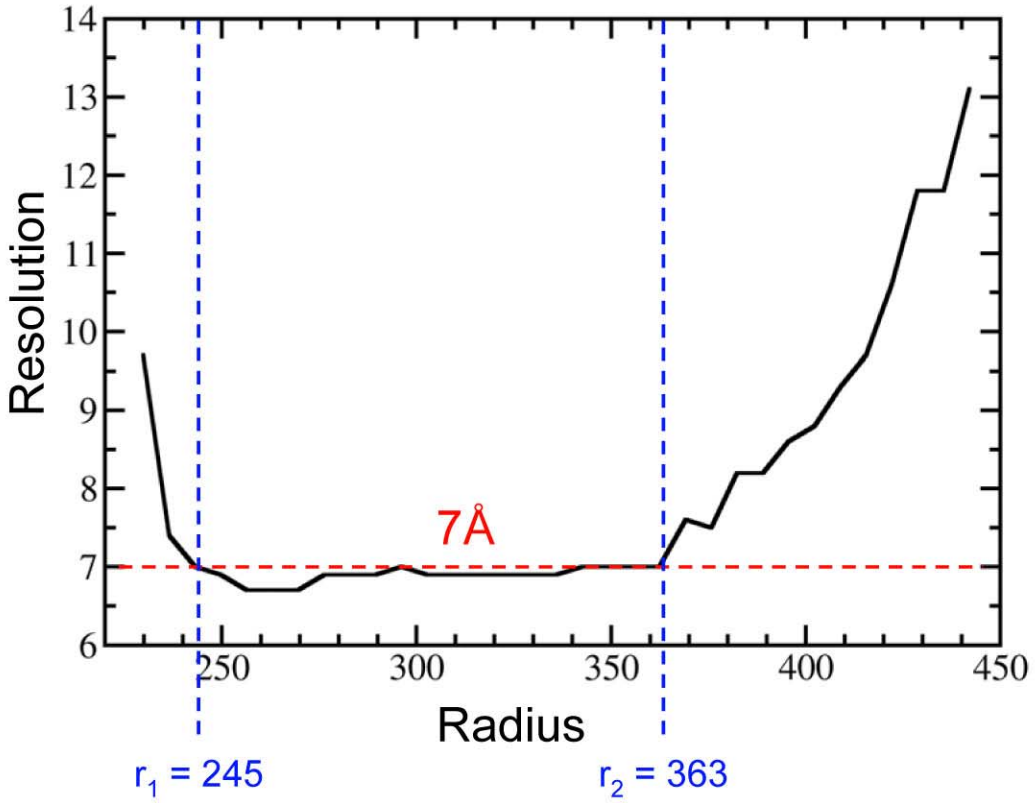
Icosahedral Particle Reconstruction Scheme

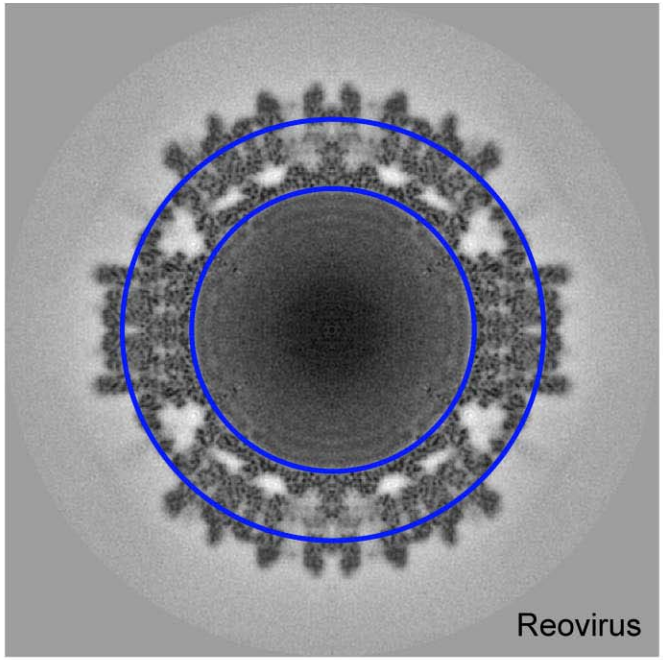


Note: quality of 3D density map is not the identical throughout the map

Icosahedral Particle Reconstruction Scheme

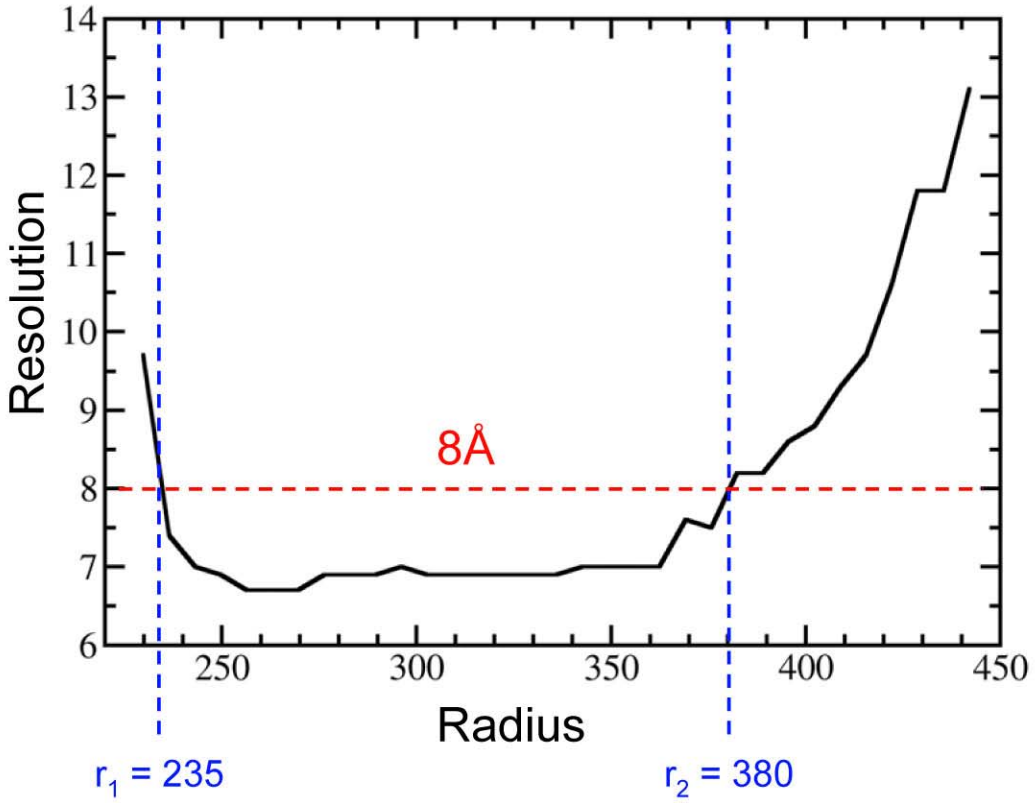
Monitor Data Quality

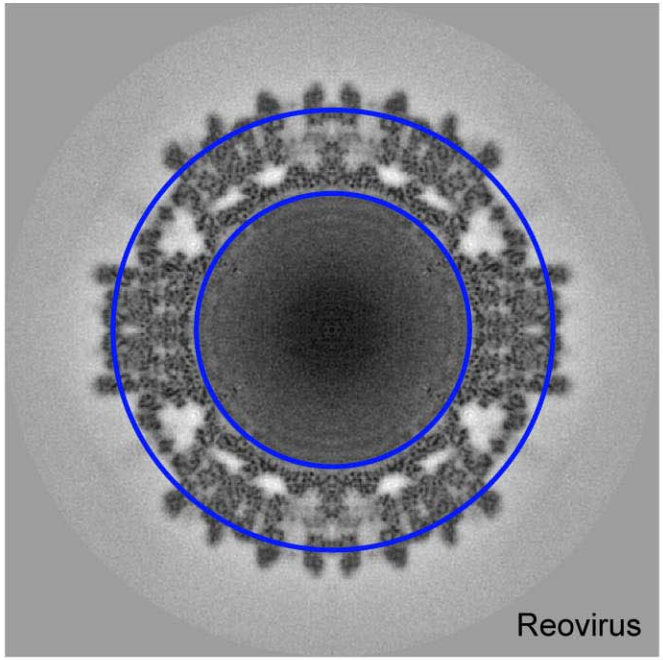




Icosahedral Particle Reconstruction Scheme

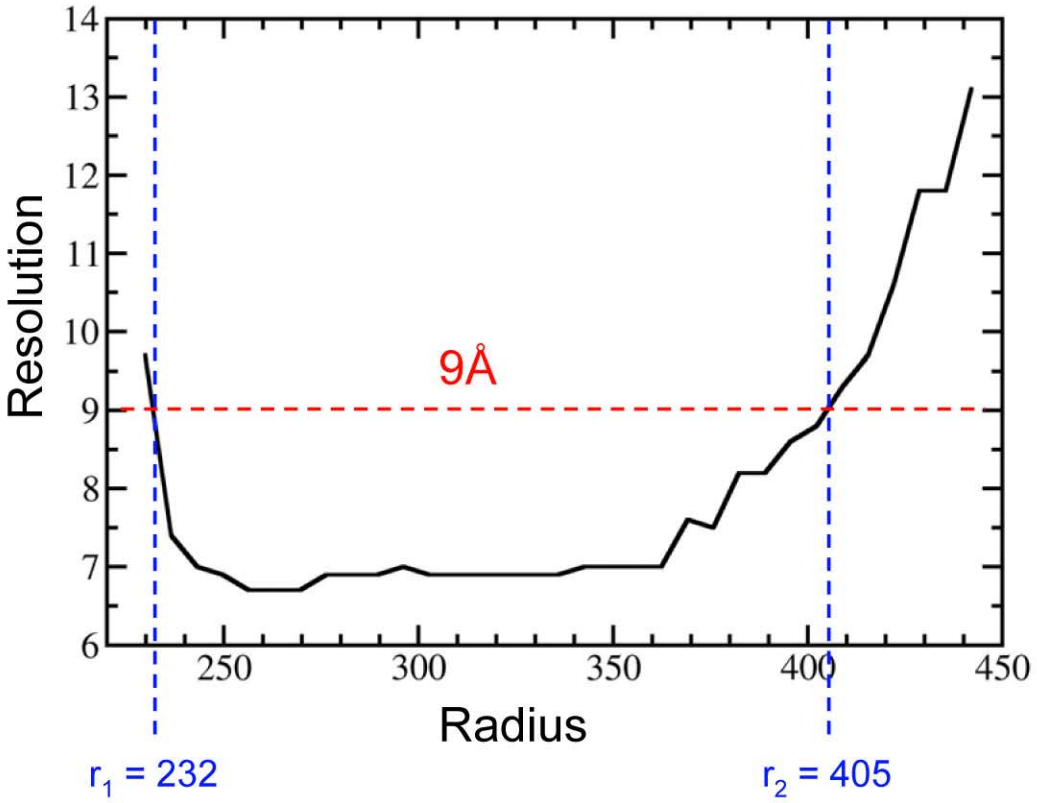
Monitor Data Quality

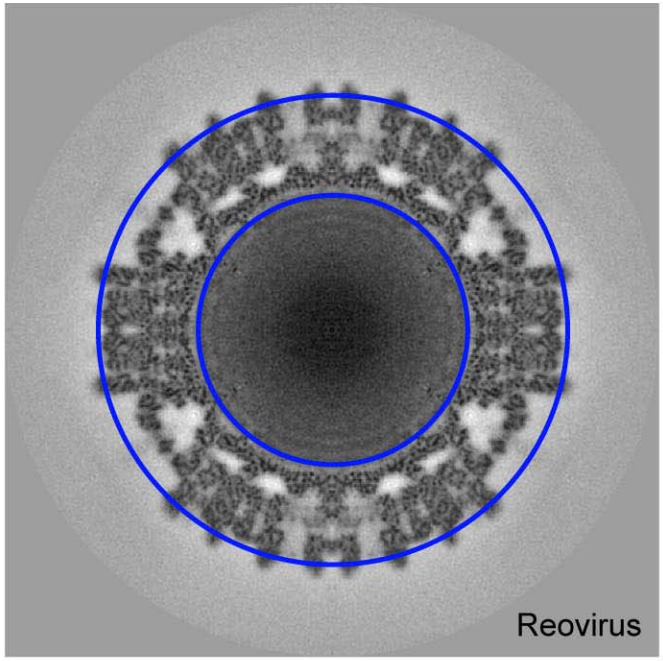




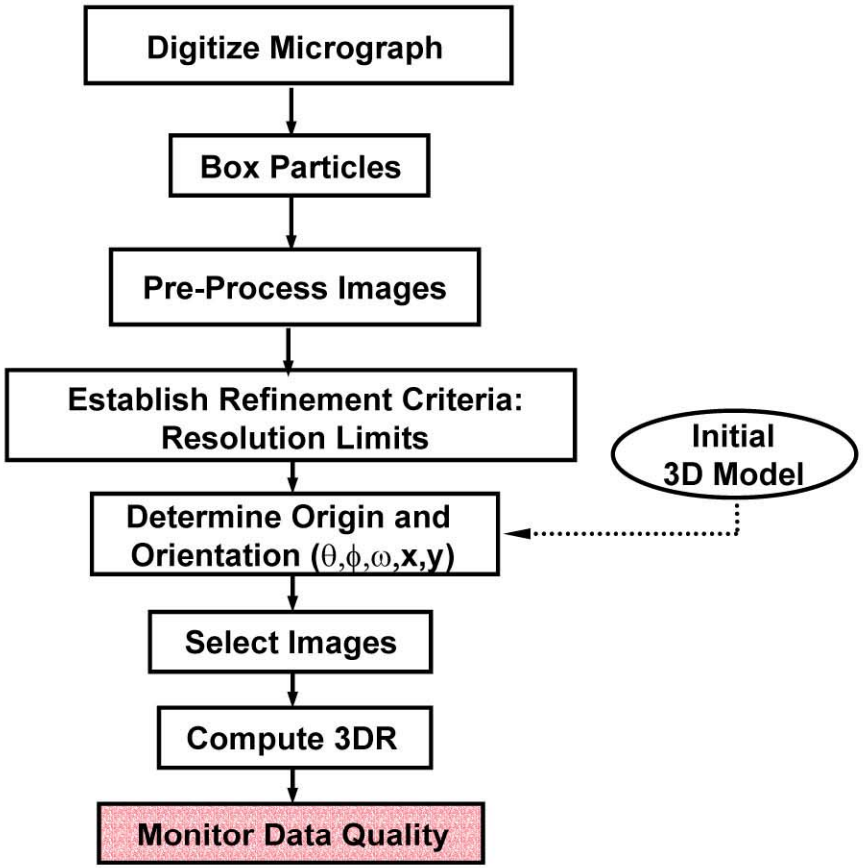
Icosahedral Particle Reconstruction Scheme

Monitor Data Quality

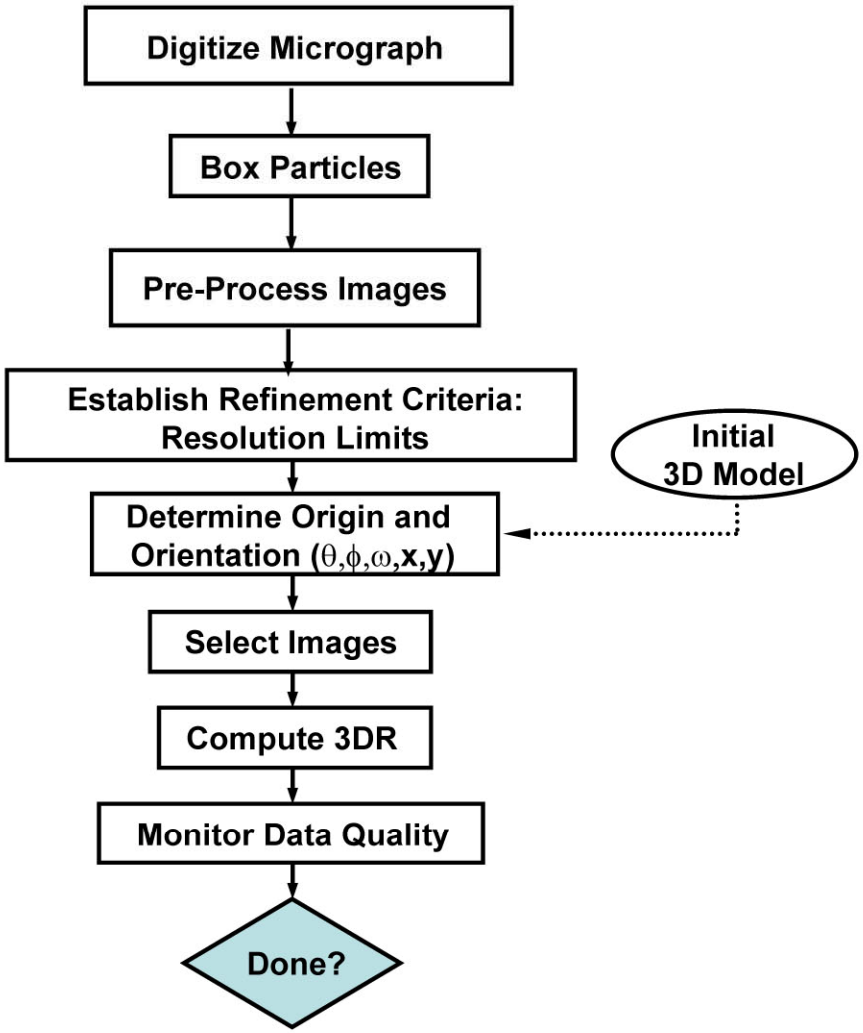




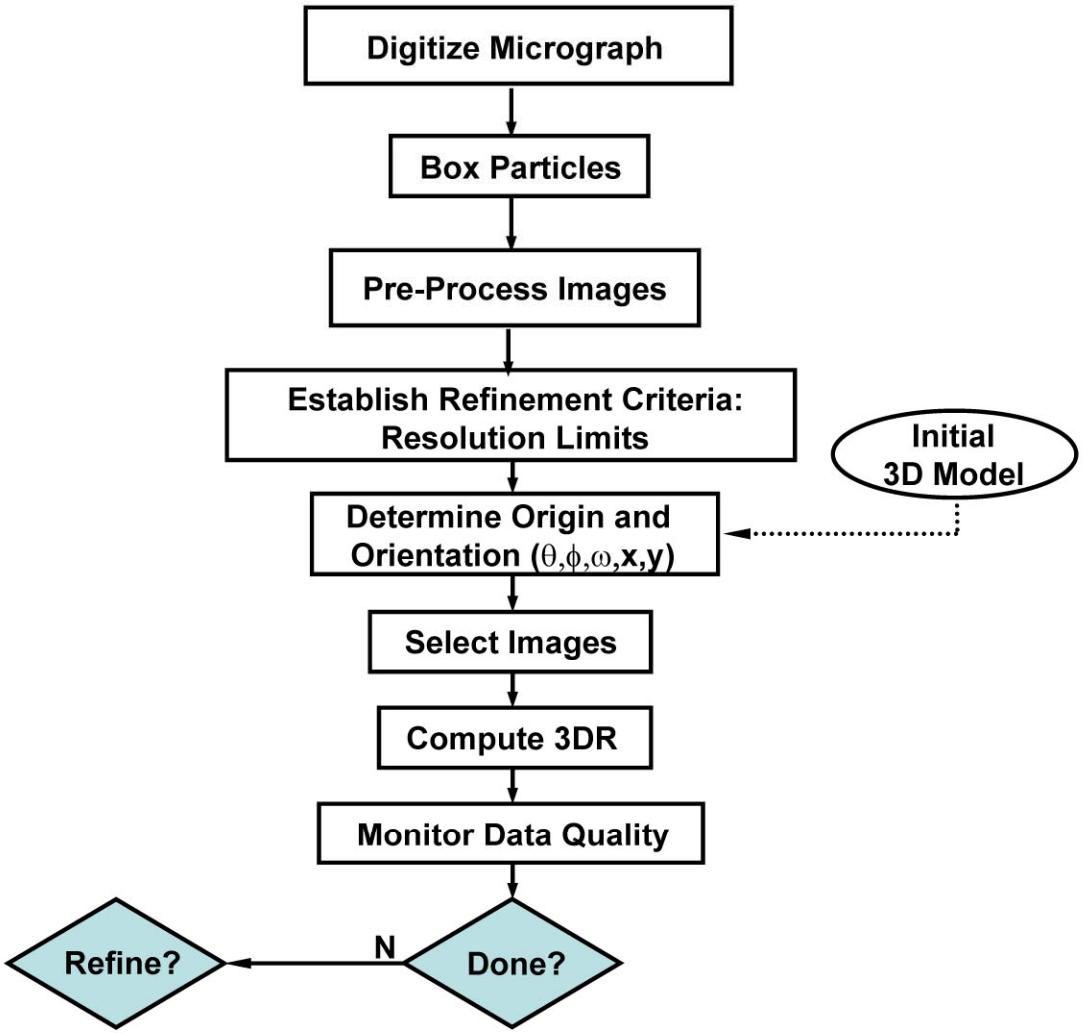
Icosahedral Particle Reconstruction Scheme



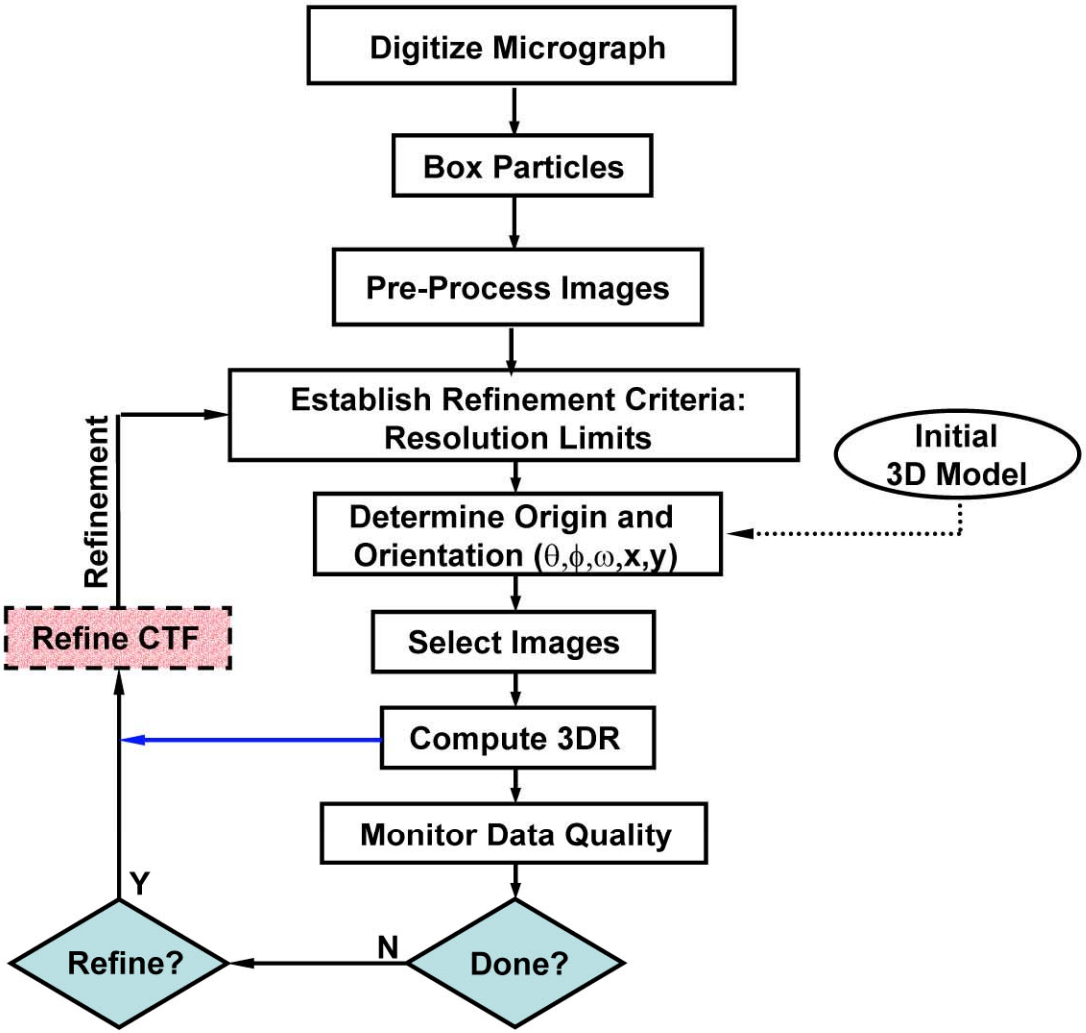
Icosahedral Particle Reconstruction Scheme



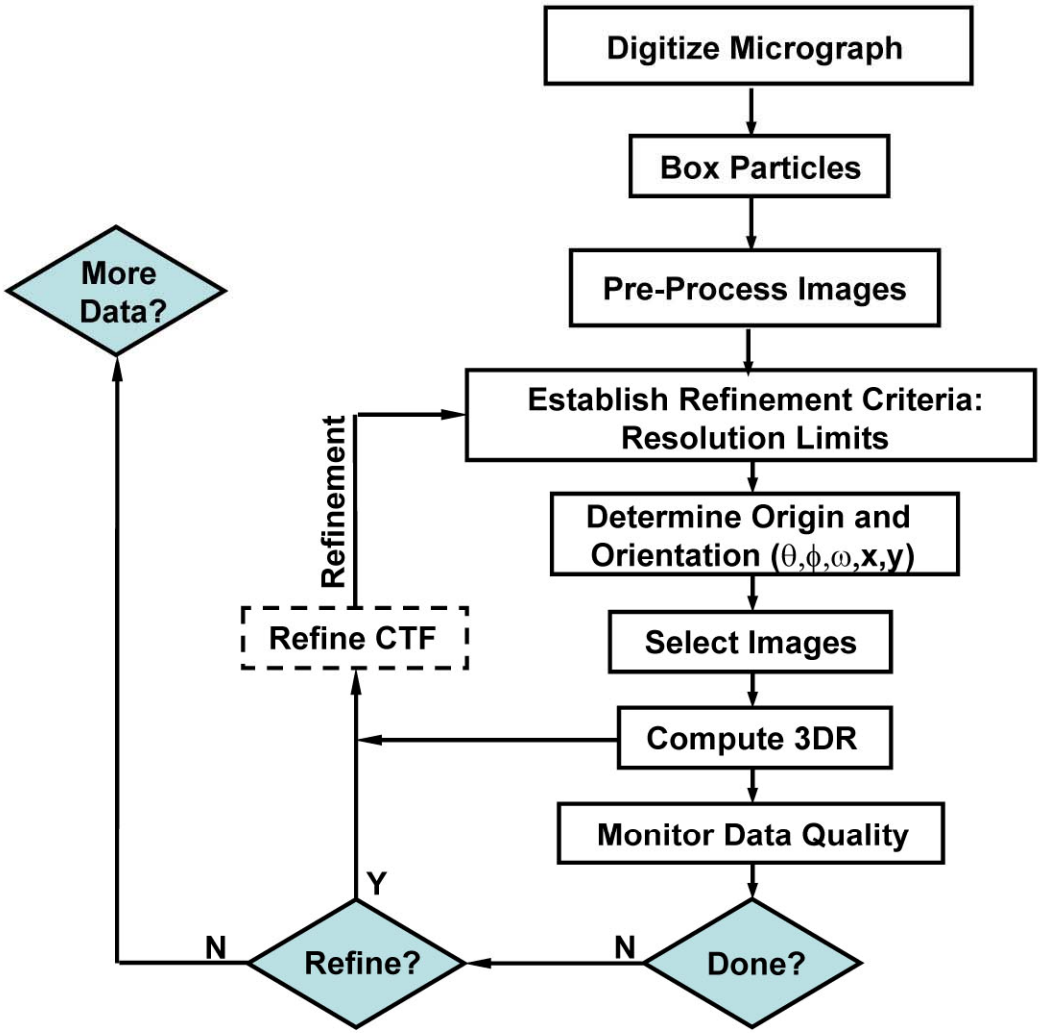
Icosahedral Particle Reconstruction Scheme



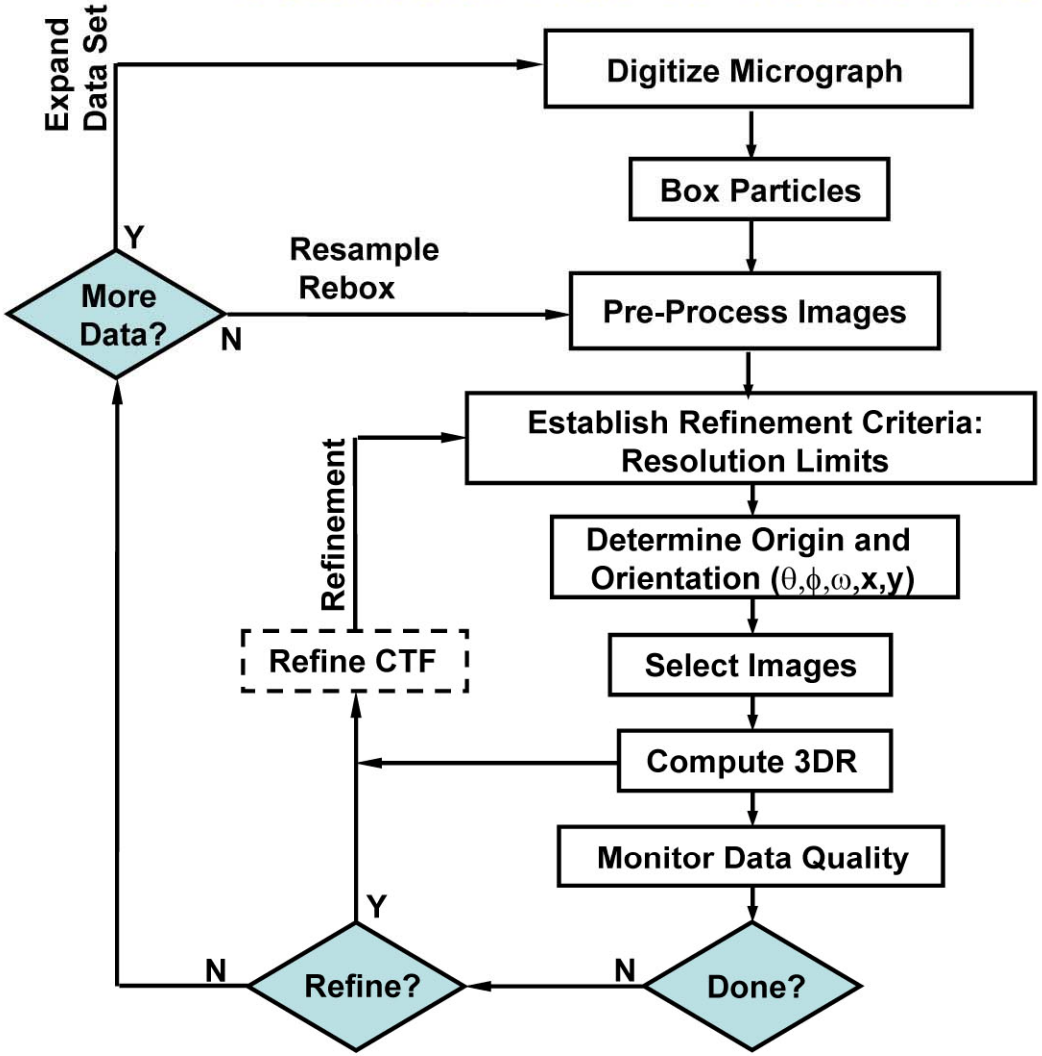
Icosahedral Particle Reconstruction Scheme



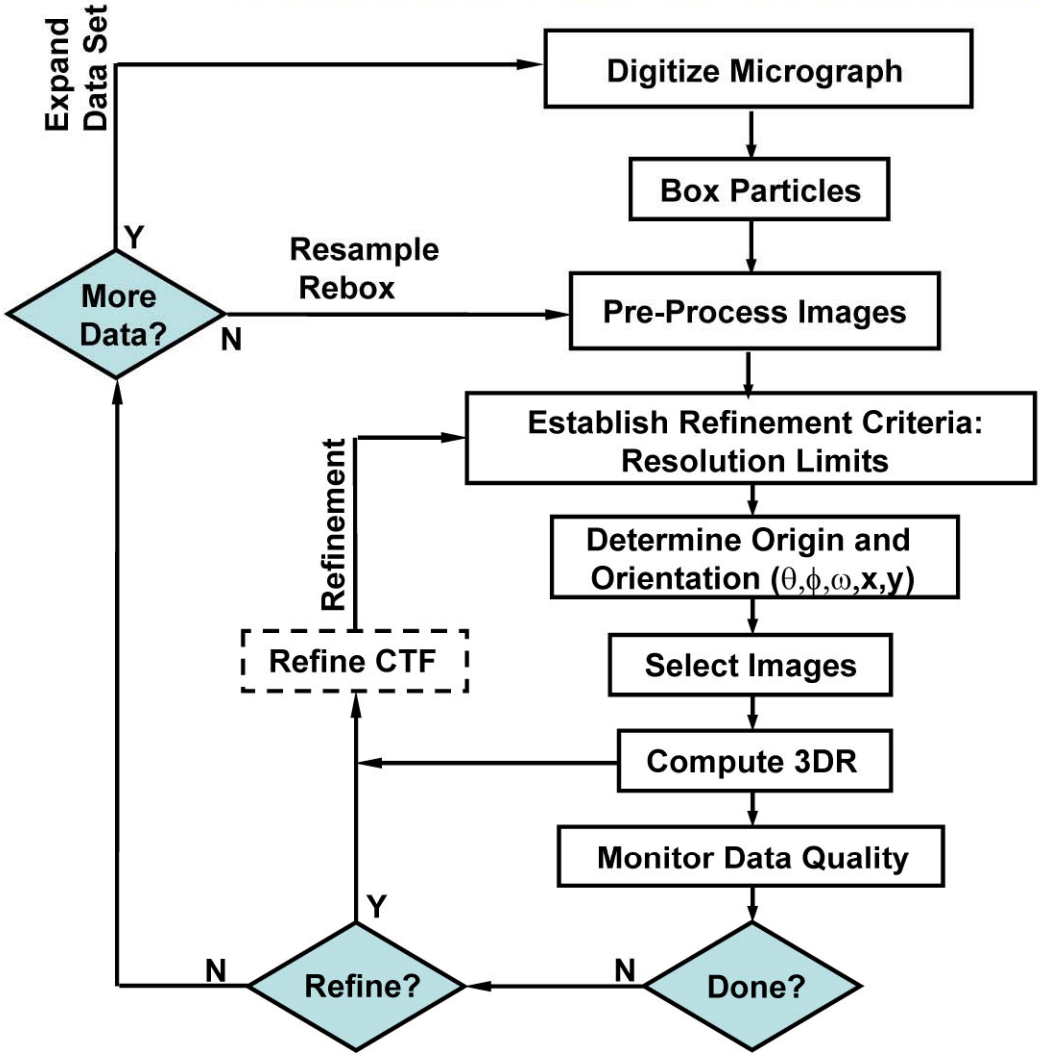
Icosahedral Particle Reconstruction Scheme



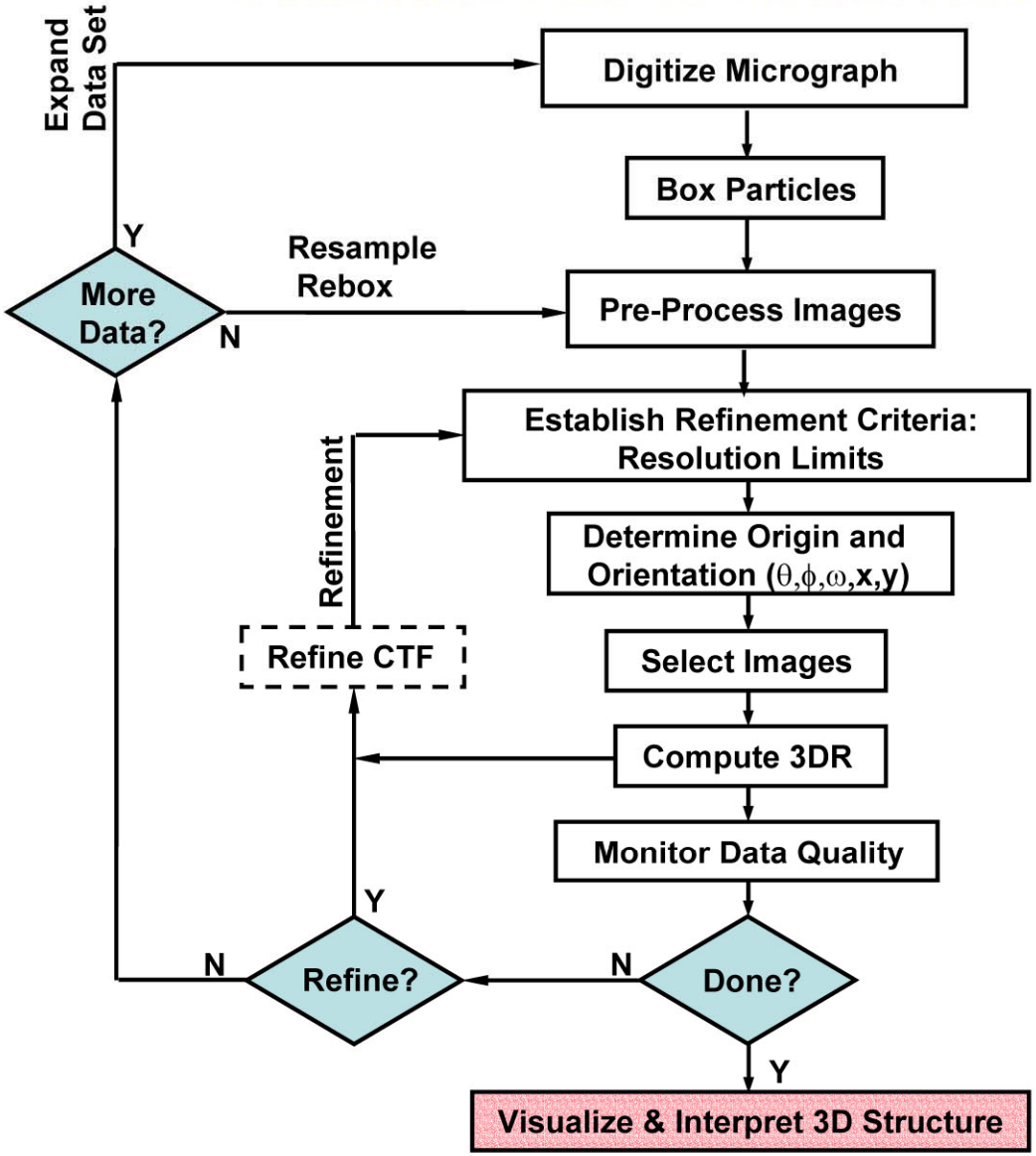
Icosahedral Particle Reconstruction Scheme



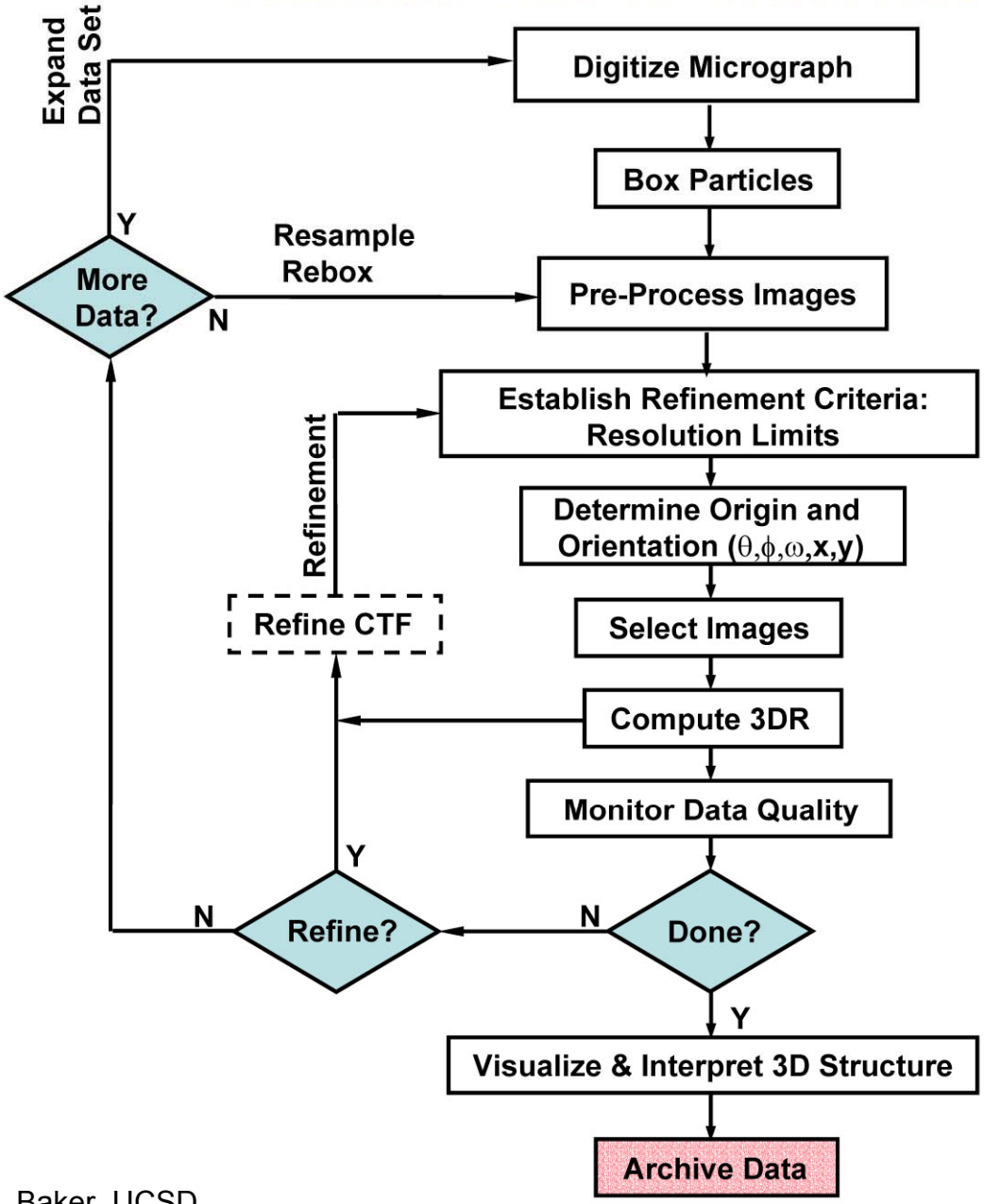
Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme



Icosahedral Particle Reconstruction Scheme



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