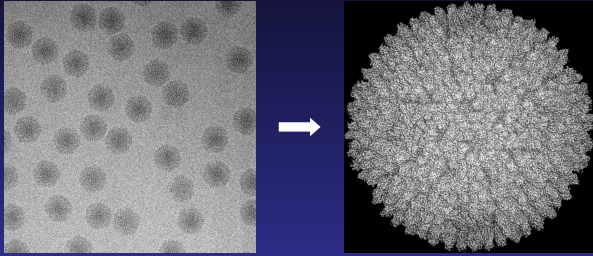


3D Reconstruction of Icosahedral Particles

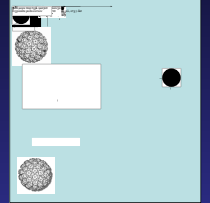


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3D Reconstruction of Icosahedral Particles

Outline

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



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3D Reconstruction of Icosahedral Particles

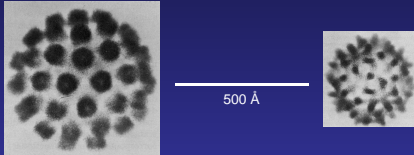
REFERENCES

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



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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

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3D Reconstruction of Icosahedral Particles

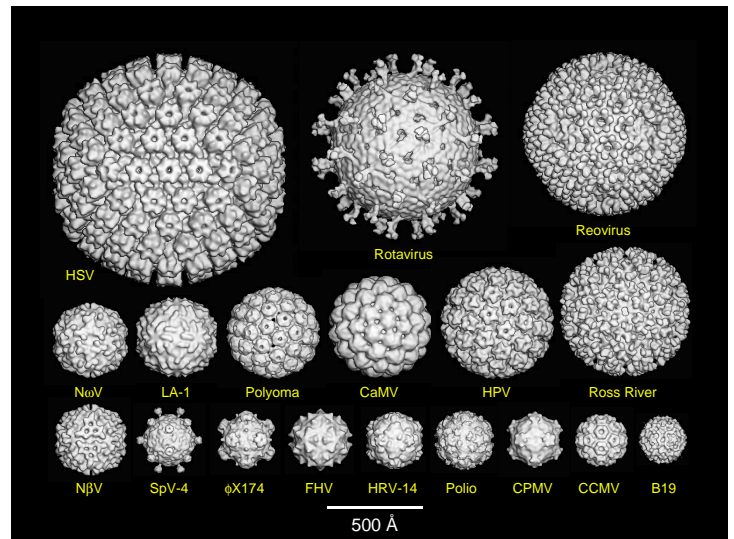
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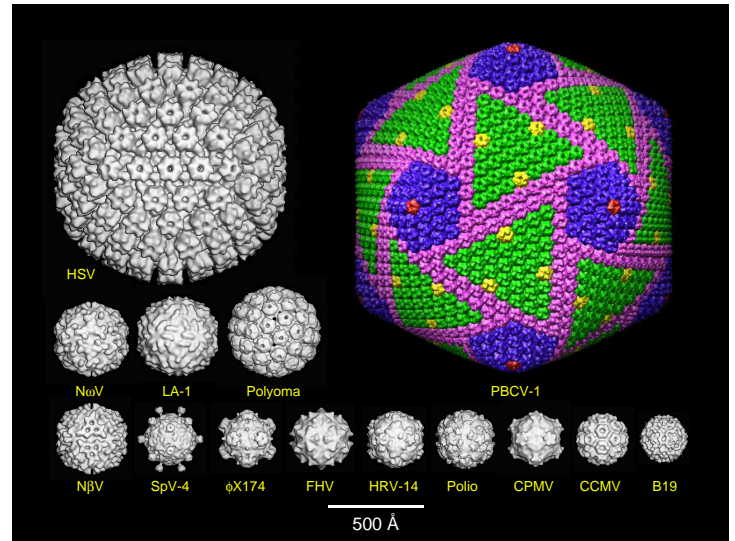
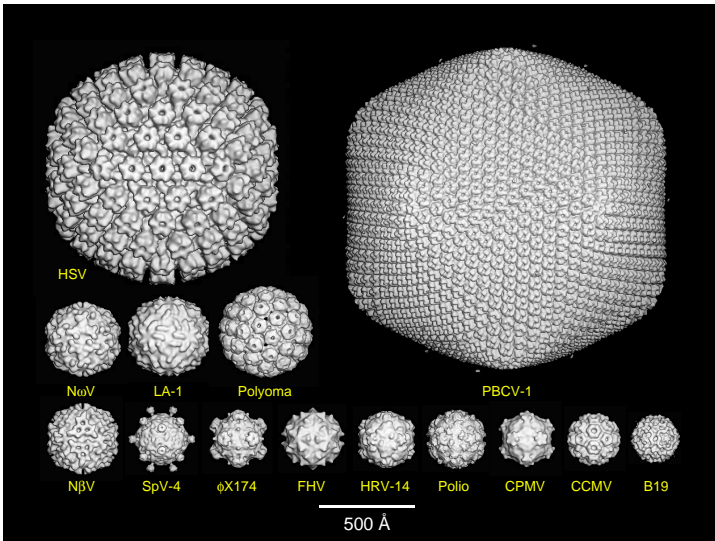
- Reference list available as handout

- 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**

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3D Reconstruction of Icosahedral Particles

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3D Reconstruction of Icosahedral Particles

Symmetry

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

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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**

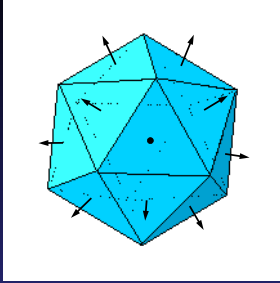
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Icosahedral (532) Point Group Symmetry

12 vertices (5-fold)

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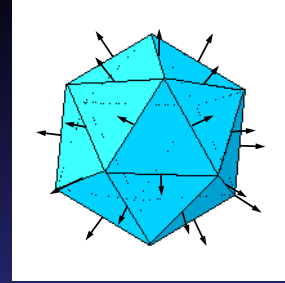
Icosahedral (532) Point Group Symmetry



12 vertices (5-
fold)
20 faces (3-
fold)

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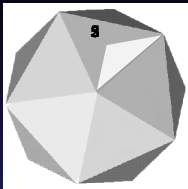
Icosahedral (532) Point Group Symmetry



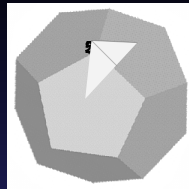
12 vertices (5-
fold)
20 faces (3-
fold)
30 edges (2-
fold)

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Icosahedron



Dodecahedron



Different shapes, but both have 532 symmetry

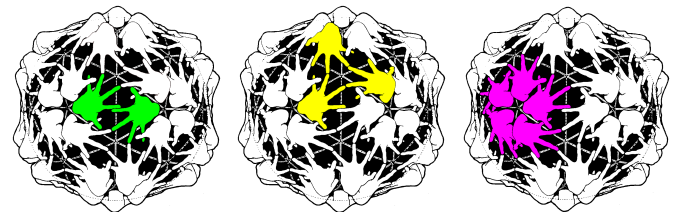
12 vertices, 20 faces, 30 edges (6 5-folds, 10 3-folds, 15 2-folds) 20 vertices, 12 faces, 30 edges (10 3-folds, 6 5-folds, 15 2-folds)

Asymmetric unit is 1/60th of whole object

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

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Icosahedral (532) Point Group Symmetry



30 dimers

20 trimers

12 pentamers

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

3D Reconstruction of Icosahedral Particles Symmetry

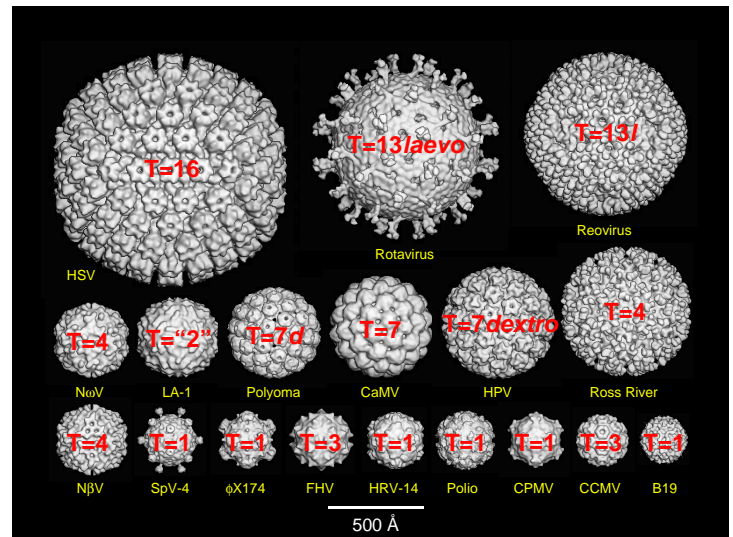
- ➡ 1. Icosahedral (532) point group symmetry
- ➡ 2. Triangulation symmetry

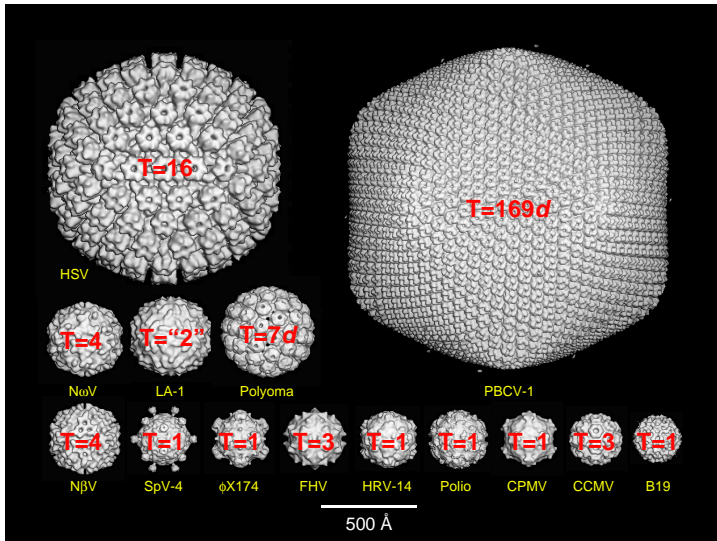
Purely mathematical concept (concerns lattices)

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

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

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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

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3D Reconstruction of Icosahedral Particles

Triangulation Number

Key Concept:

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

In other words: What you determine is the structure of one asymmetric unit of the object

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3D Reconstruction of Icosahedral Particles

Two Basic Assumptions:

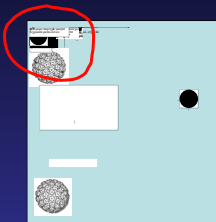
- Specimen consists of stable particles with 'identical' structures (else averaging is invalid)
- Programs test for and assume presence of icosahedral (532) symmetry

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3D Reconstruction of Icosahedral Particles

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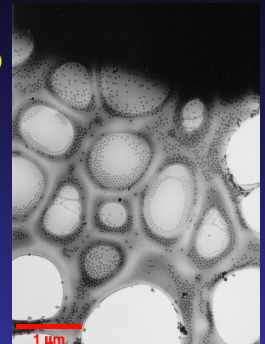
3D Reconstruction of Icosahedral Particles

Protocol

Electron Cryo-Microscopy

Sample : ~2-3 μ l at 1-5 mg/ml

Specimen support: holey carbon film (1-2 μ m)



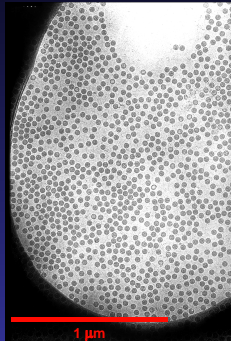
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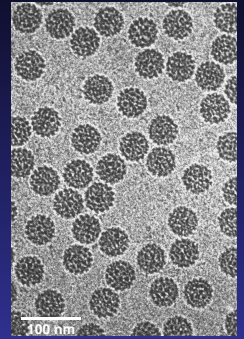
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3D Reconstruction of Icosahedral Particles

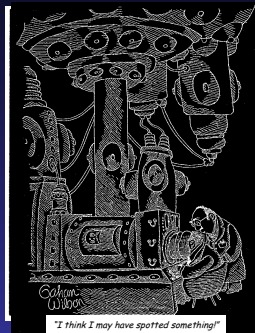
Protocol

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

Microscope: 200-300 keV with FEG



3D Reconstruction of Icosahedral Particles

Protocol

Electron Cryo-Microscopy

Sample : ~2-3 μl at 1-5 mg/ml

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

Microscope: 200-300 keV with FEG

Defocus range: 1-3 μm underfocus

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

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

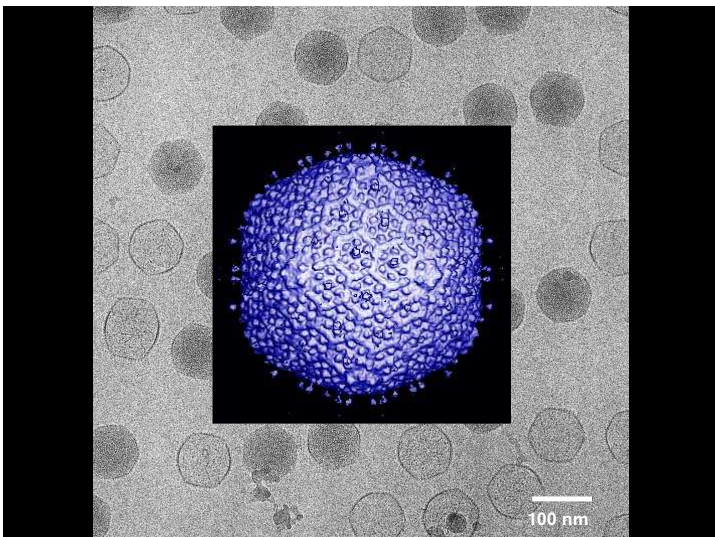
Micrographs: 50-100-->1000s(?)

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

Target resolution: 10 - 6 \AA --> 4 \AA (?)



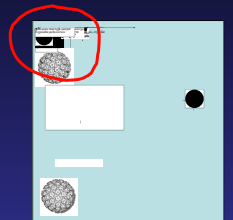
FEI Tecnai F30 Polara



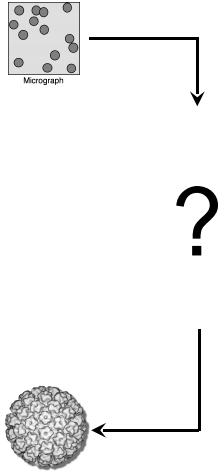
3D Reconstruction of Icosahedral Particles

Outline

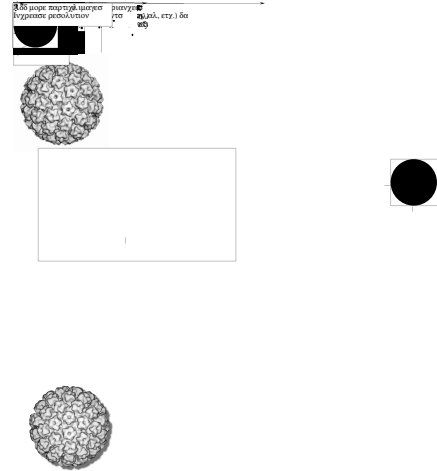
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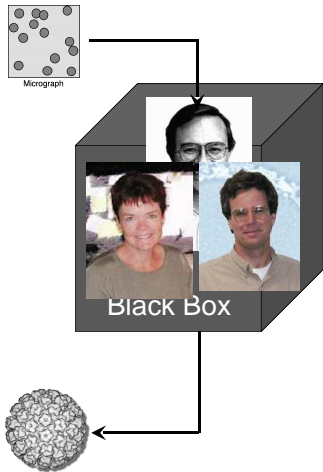
Icosahedral Particle Image Reconstruction Scheme



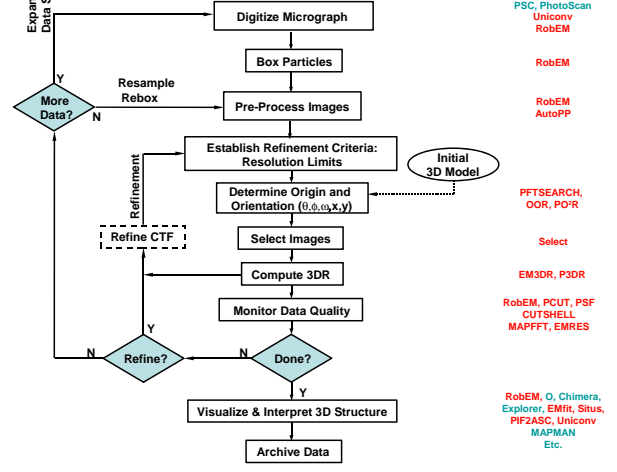
Icosahedral Particle Image Reconstruction Scheme



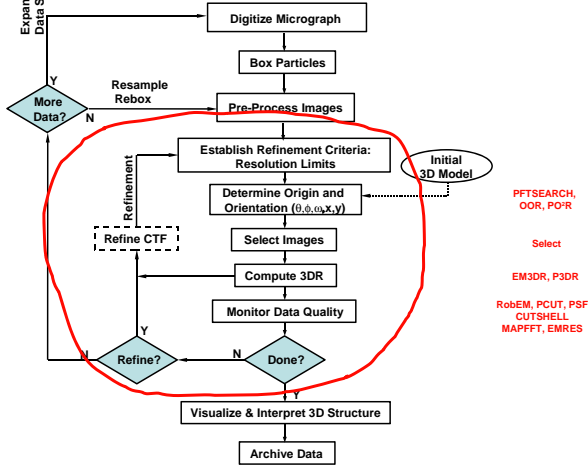
Icosahedral Particle Image Reconstruction Scheme



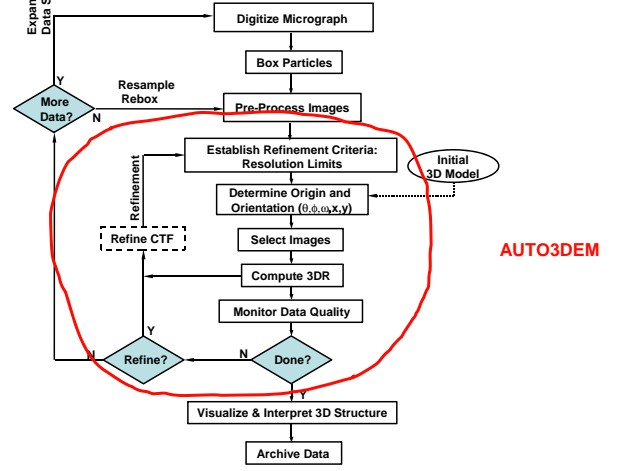
Icosahedral Virus 3D Reconstruction Scheme



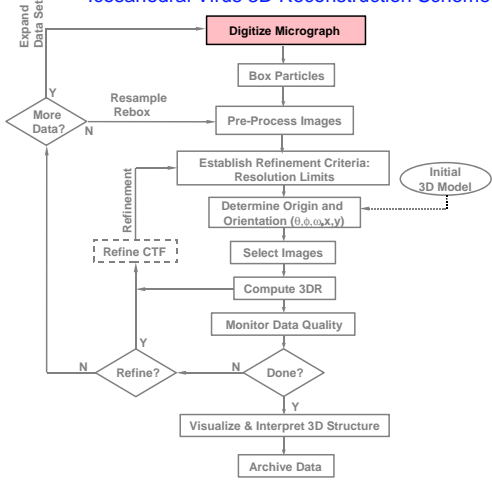
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



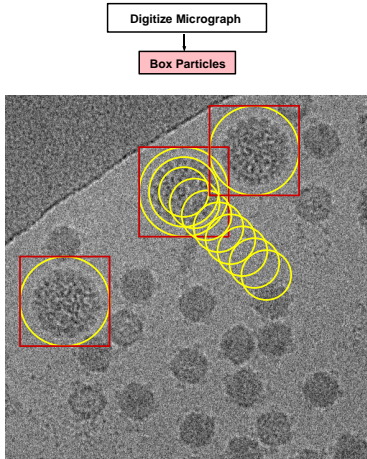
Icosahedral Virus 3D Reconstruction Scheme



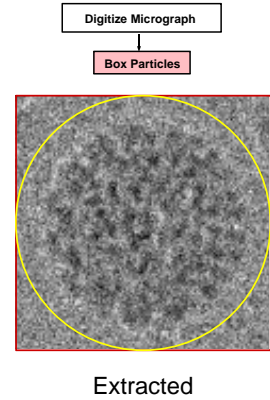
Icosahedral Virus 3D Reconstruction Scheme



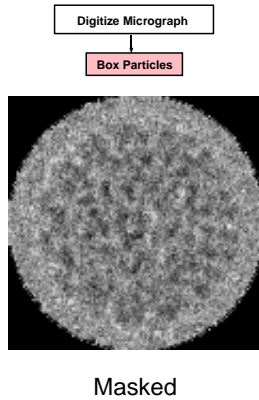
Icosahedral Virus 3D Reconstruction Scheme



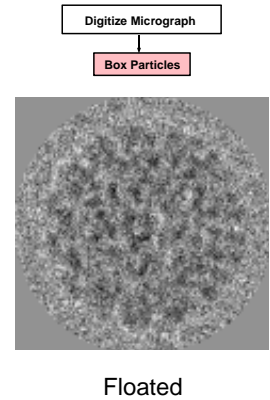
Icosahedral Virus 3D Reconstruction Scheme



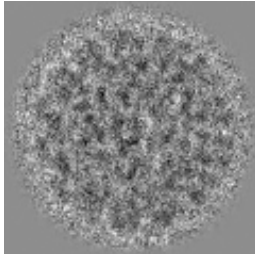
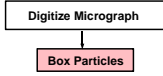
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

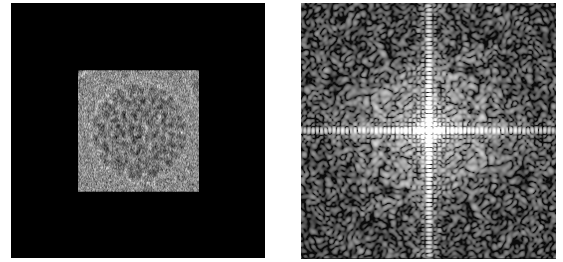
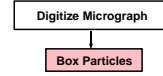


Icosahedral Virus 3D Reconstruction Scheme



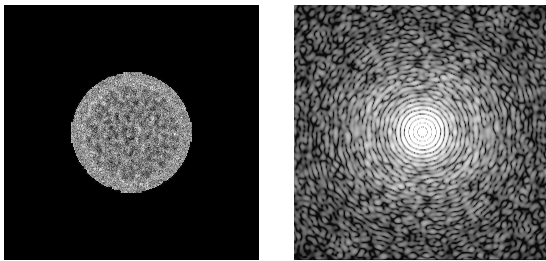
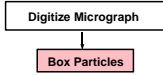
Apodized

Icosahedral Virus 3D Reconstruction Scheme



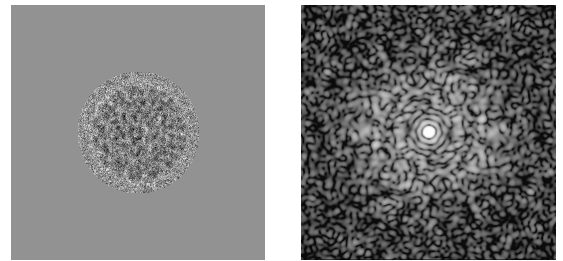
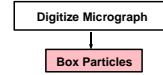
Square mask; unfloated

Icosahedral Virus 3D Reconstruction Scheme



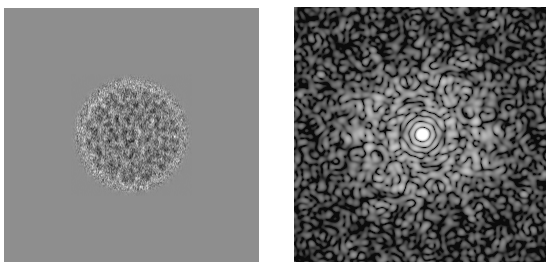
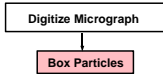
Circular mask; unfloated

Icosahedral Virus 3D Reconstruction Scheme



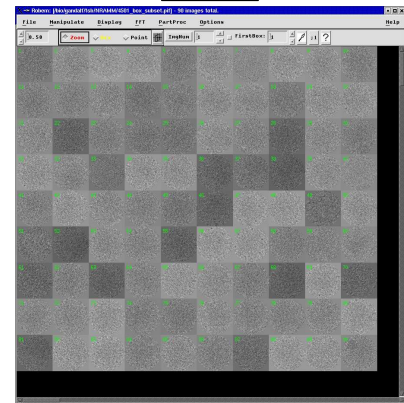
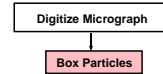
Circular mask; floated

Icosahedral Virus 3D Reconstruction Scheme

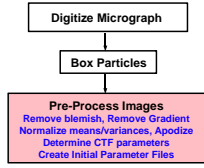


Circular mask; floated & apodized

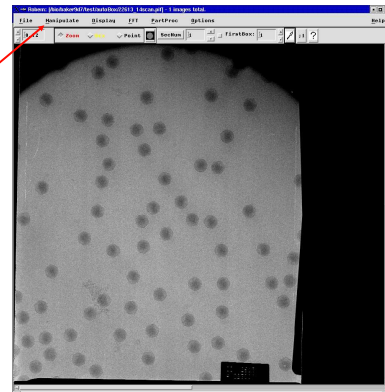
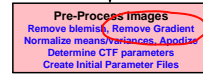
Icosahedral Virus 3D Reconstruction Scheme



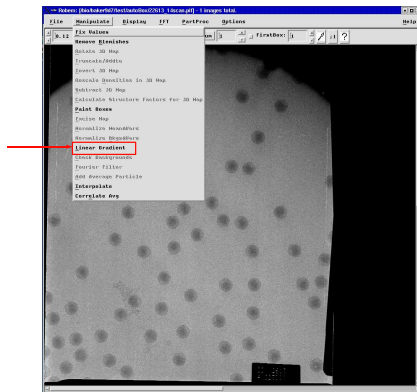
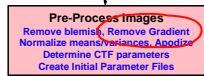
Icosahedral Virus 3D Reconstruction Scheme



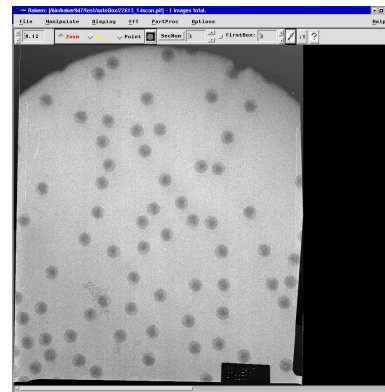
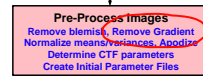
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

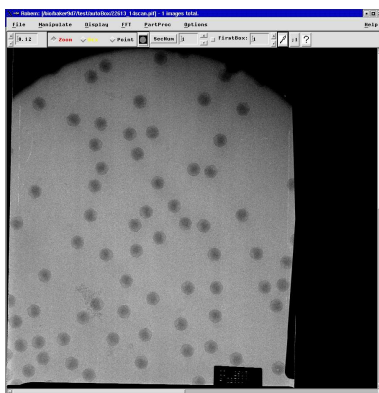
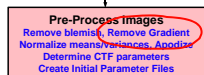


Icosahedral Virus 3D Reconstruction Scheme



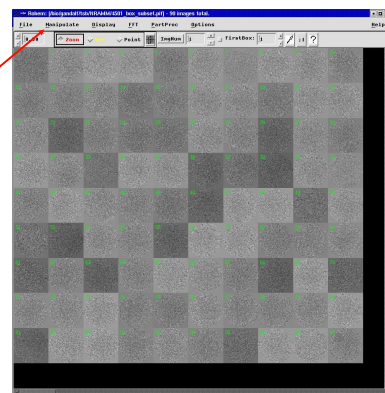
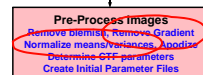
Gradient removed

Icosahedral Virus 3D Reconstruction Scheme



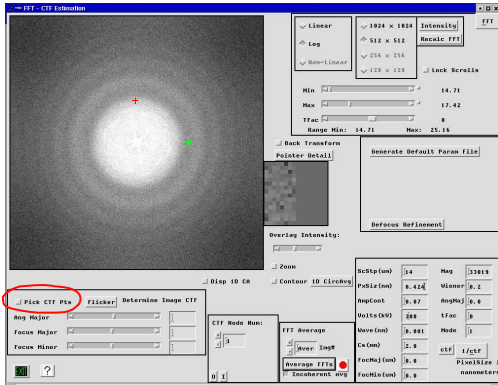
Gradient not removed

Icosahedral Virus 3D Reconstruction Scheme



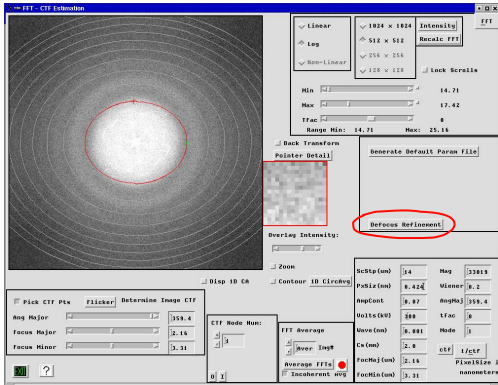
Icosahedral Virus 3D Reconstruction Scheme

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



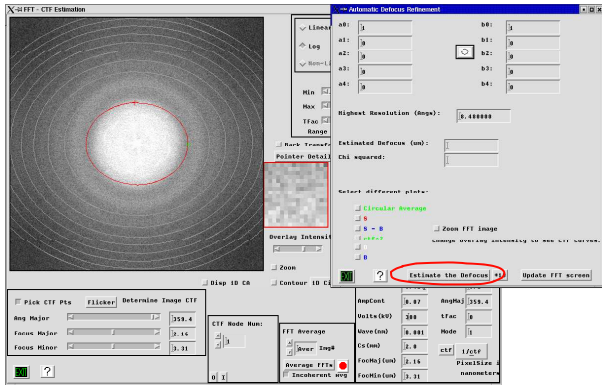
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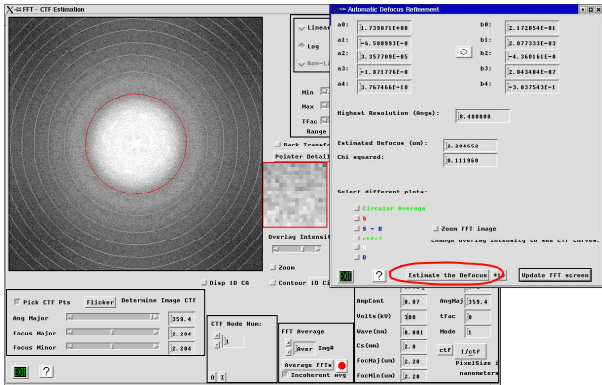
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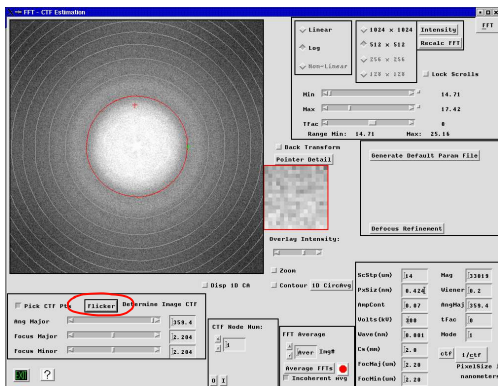
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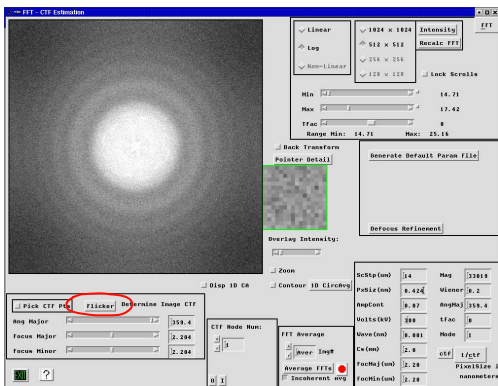
Icosahedral Virus 3D Reconstruction Scheme

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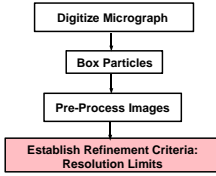


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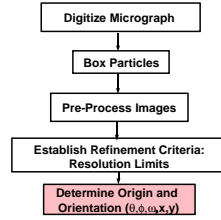
Pre-Process Images
 Remove blemish, Remove Gradient
 Normalize means/variances, Apodize
 Determine CTF parameters
 Create Initial Parameter Files



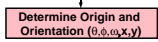
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

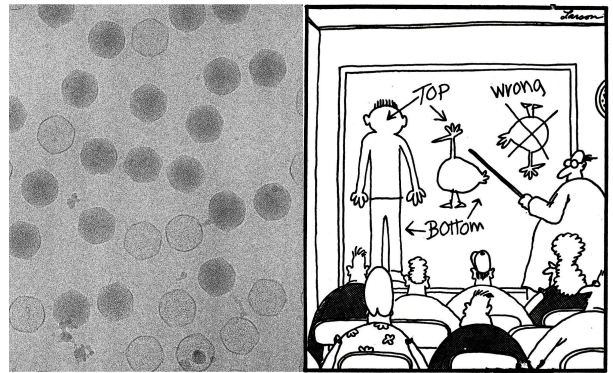
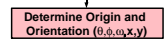


Goal: determine phase origin and view orientation for each boxed particle

MOST IMPORTANT STEP?

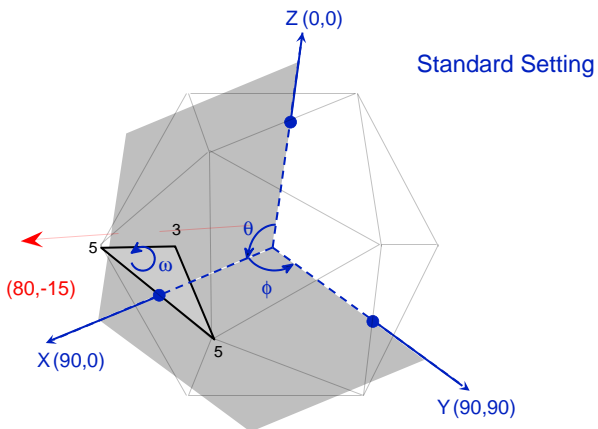
Garbage in -----> garbage out

Icosahedral Virus 3D Reconstruction Scheme

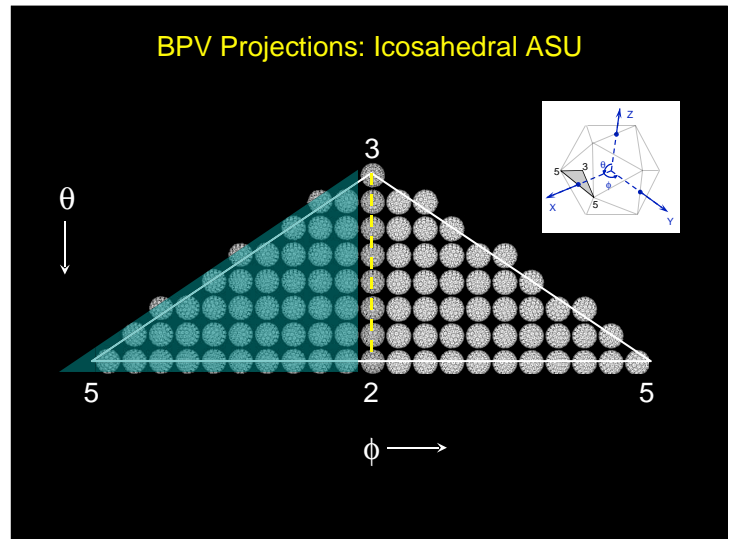


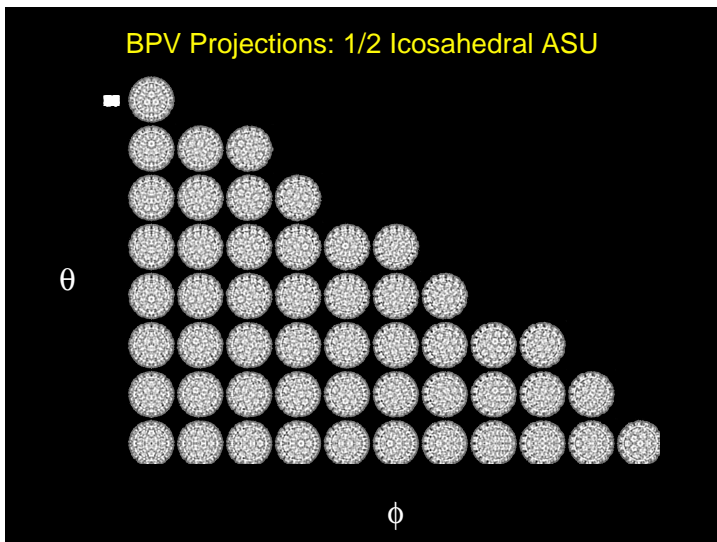
People who don't know which end is up

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

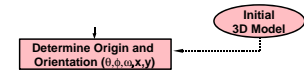


BPV Projections: Icosahedral ASU





Icosahedral Virus 3D Reconstruction Scheme



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

Two methods:

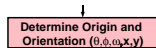
1. Common lines

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



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

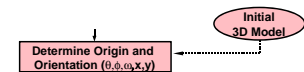
Two methods:

1. *Ab initio* (e.g. Common lines)

New or unknown structure

2. By guess and by golly

Icosahedral Virus 3D Reconstruction Scheme



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

Two methods:

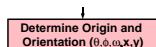
1. *Ab initio* (e.g. Common lines)

New or unknown structure

2. Model-based (template) matching

General features of structure are known or a crude model can be generated (...or, sometimes, even a lousy model will work)

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

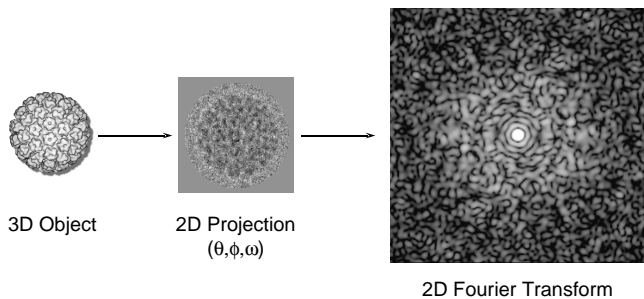
The 'gospel' continued:

"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"

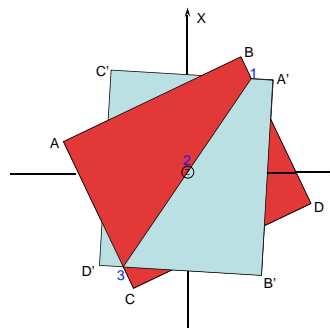
"The angular positions of these lines are dependent on the orientation of the particle."

Orientation Determination by Common Lines



Orientation Determination by Common Lines

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



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

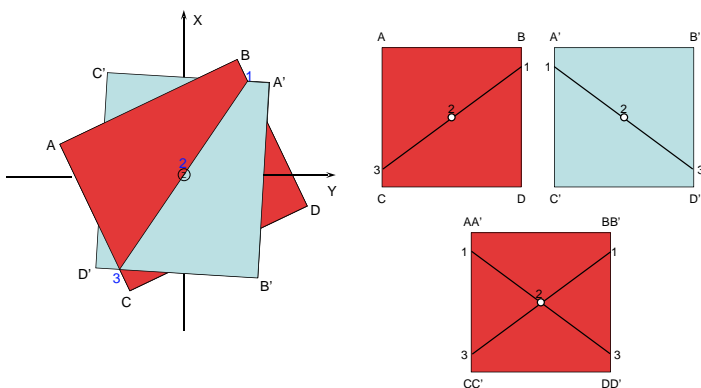
Let Z-direction coincide with **3-fold** axis of symmetry

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

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

Adapted from Moody (1990) Fig. 7.68, p.246

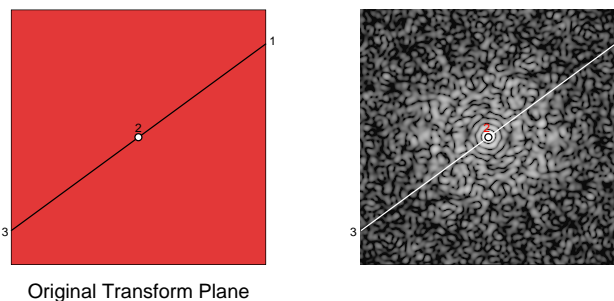
Orientation Determination by Common Lines



Adapted from Moody (1990) Fig. 7.68, p.246

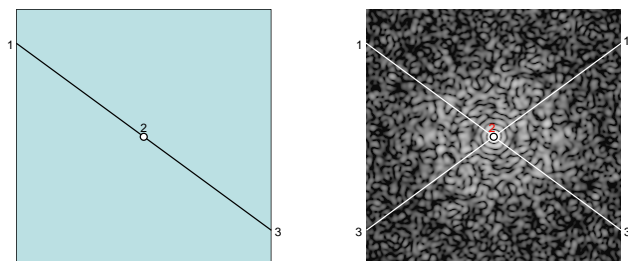
Adapted from Moody (1990) Fig. 7.69, p.246

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:

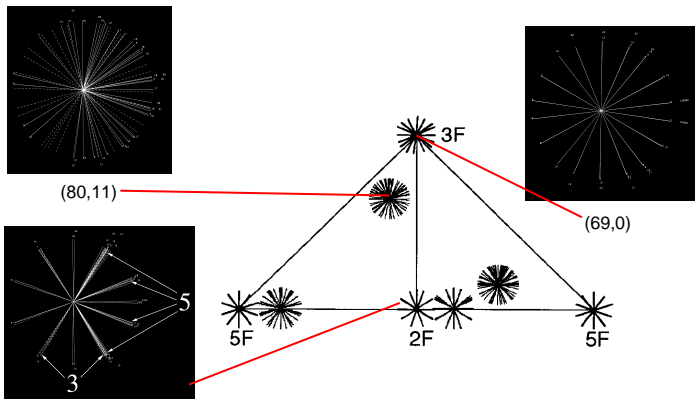
5-folds: $\frac{12}{2} \times 2 = 12$ pairs

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

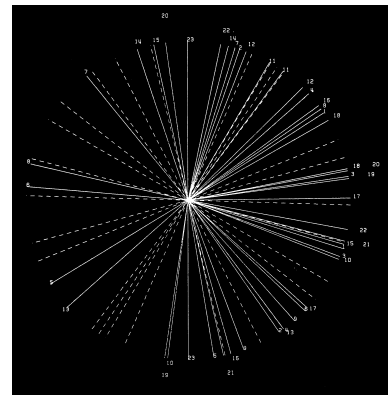
2-folds: $\frac{30}{2} \times 1 = 15$ real lines

37 common lines

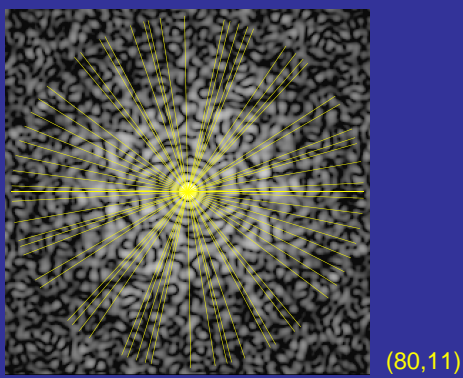
Orientation Determination by Common Lines



Orientation Determination by Common Lines

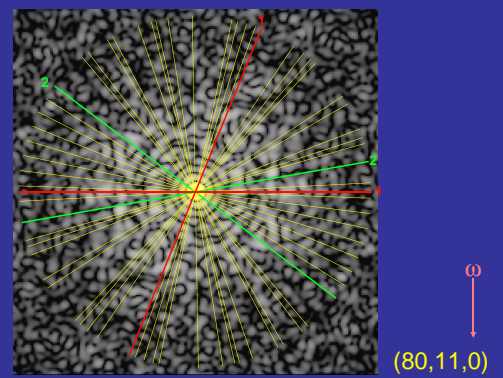


Orientation Determination by Common Lines

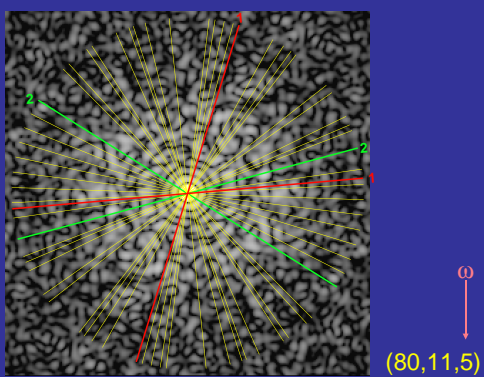


What is (θ, ϕ, ω) for this particle?

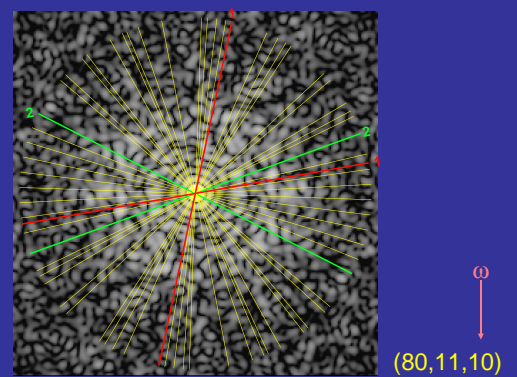
Orientation Determination by Common Lines



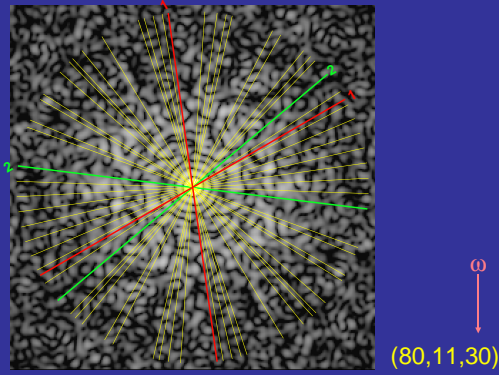
Orientation Determination by Common Lines



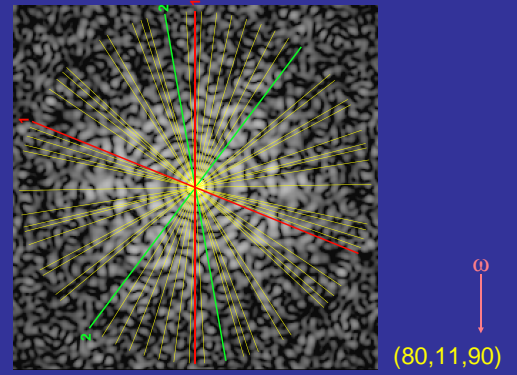
Orientation Determination by Common Lines



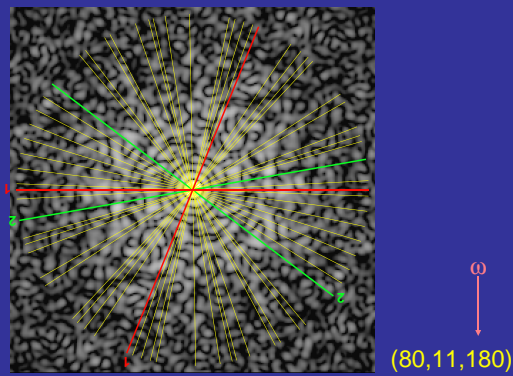
Orientation Determination by Common Lines



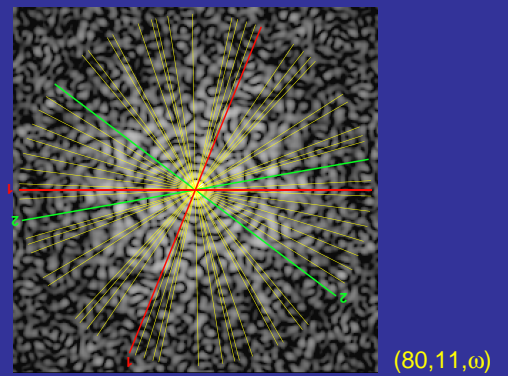
Orientation Determination by Common Lines



Orientation Determination by Common Lines

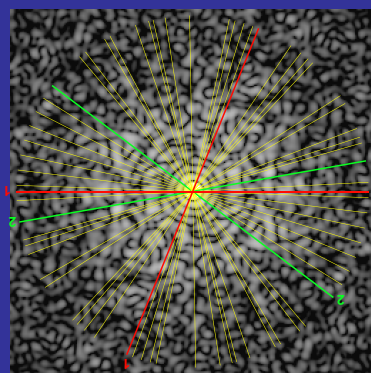


Orientation Determination by Common Lines



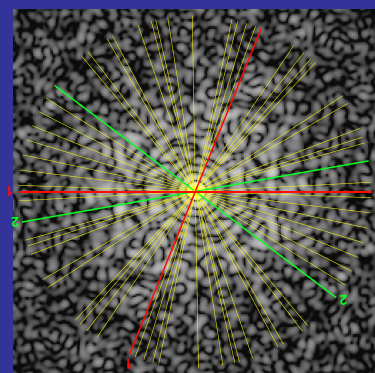
Metric: Identify ω that gives lowest phase residual

Orientation Determination by Common Lines



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

Orientation Determination by Common Lines



> 250,000 combinations for 1° angular search intervals

Icosahedral Virus 3D Reconstruction Scheme

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

Common Lines

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

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

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

Icosahedral Virus 3D Reconstruction Scheme

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

Initial 3D Model

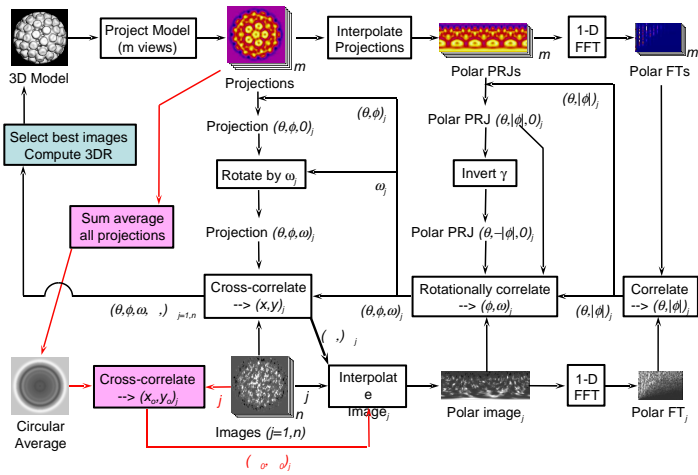
Recall: two methods to determine $(\theta, \phi, \omega, x, y)$:

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

Bulk of structures now solved this way

Details discussed in practical session

PFTSEARCH Program Flowchart



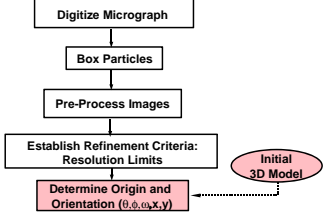
Icosahedral Virus 3D Reconstruction Scheme

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

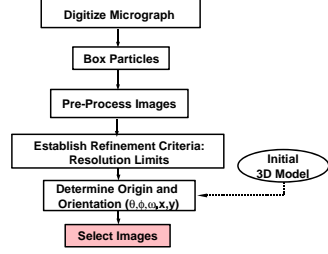
Initial 3D Model

ID	θ	ϕ	ω	x	y
1	80.000	-13.201	313.594	48.751	48.849
2	85.000	23.089	49.922	49.267	48.429
3	77.000	-5.152	334.688	49.051	48.364
4	81.000	5.062	15.469	49.215	49.139
5	81.000	0.000	149.399	49.225	48.819
6	79.000	-2.037	338.203	49.320	48.202
7	82.000	1.000	175.781	49.147	48.801
8	83.000	-3.023	32.344	49.147	48.801
9	80.000	1.001	15.469	49.845	49.142
10	75.000	-7.247	159.203	47.863	49.139
11	87.000	-7.010	222.891	48.247	48.675
12	86.000	-1.052	19.689	49.127	48.761
13	80.000	-5.077	68.906	49.397	48.623
14	84.000	5.029	379.141	49.489	48.600
15	86.000	3.022	175.781	49.628	49.787
16	87.000	11.015	2.813	49.397	49.182
17	89.000	30.006	89.297	49.754	48.739
18	85.000	1.004	170.156	49.710	49.074
19	86.000	0.000	136.563	49.944	48.914
20	89.000	5.001	190.391	49.925	48.704
21	78.000	7.156	108.281	49.310	49.342
22	81.000	2.025	399.078	49.317	48.173
23	80.000	-3.046	108.000	49.873	48.515
24	86.000	-1.022	291.863	49.849	48.789
25	84.000	10.022	148.247	49.332	48.281
26	84.000	15.023	5.629	49.824	48.378
27	83.000	-4.030	149.703	48.892	48.974

Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme

Select Images

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

Icosahedral Virus 3D Reconstruction Scheme

Select Images

ID	θ	ϕ	ω	x	y				
1	80.000	-13.201	313.594	48.751	48.849	1.015	0.492	0.713	0.557
2	85.000	23.088	49.922	49.287	48.429	1.010	0.998	0.725	0.609
3	77.000	-5.132	334.698	49.061	48.364	1.005	0.726	0.749	0.616
4	81.000	5.062	15.469	49.215	49.139	1.000	0.718	0.778	0.656
5	81.000	0.000	148.399	49.681	48.119	1.005	0.725	0.712	0.646
6	79.000	-2.037	338.203	49.220	48.202	1.005	0.746	0.738	0.647
7	83.000	-3.022	175.781	49.223	48.802	1.010	0.687	0.703	0.620
8	83.000	1.004	15.469	49.147	48.911	1.005	0.656	0.762	0.574
9	88.000	1.001	15.469	49.845	49.042	1.005	0.732	0.736	0.662
10	75.000	-7.247	158.203	47.863	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	1.004	170.156	49.127	48.761	1.005	0.784	0.753	0.657
13	80.000	-5.077	68.946	48.997	48.623	1.000	0.720	0.780	0.640
21	78.000	1.156	108.281	49.310	49.242	1.000	0.732	0.792	0.657
15	86.000	3.022	175.781	49.535	49.787	1.000	0.625	0.760	0.589
16	87.000	11.015	2.813	49.797	49.852	1.000	0.707	0.761	0.609
17	89.000	30.005	89.297	49.754	48.838	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
22	86.000	0.000	126.563	49.944	48.914	1.000	0.775	0.809	0.669
20	89.000	5.001	290.391	49.325	48.704	1.000	0.699	0.765	0.642
23	84.000	-3.025	279.141	49.445	48.600	1.000	0.707	0.761	0.652
25	81.000	2.025	295.078	49.317	48.172	1.000	0.729	0.766	0.632
24	80.000	-3.046	180.000	49.673	48.513	1.005	0.756	0.730	0.578
26	86.000	-9.022	291.563	49.649	48.789	1.005	0.609	0.760	0.573
28	84.000	15.053	5.625	49.824	48.378	1.000	0.548	0.737	0.621
32	80.000	4.030	180.703	48.852	48.376	0.995	0.726	0.770	0.654

Icosahedral Virus 3D Reconstruction Scheme

Select Images

PRJ CC
PFT CC
CMP CC

ID	θ	ϕ	ω	x	y	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	49.287	48.429	1.010	0.998	0.725	0.609
3	77.000	-5.132	334.698	49.061	48.364	1.005	0.726	0.749	0.616
4	81.000	5.062	15.469	49.215	49.139	1.000	0.718	0.778	0.656
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6	79.000	-2.037	338.203	49.220	48.202	1.005	0.746	0.738	0.647
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13	80.000	-5.077	68.946	48.997	48.623	1.000	0.720	0.780	0.640
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15	86.000	3.022	175.781	49.535	49.787	1.000	0.625	0.760	0.589
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28	84.000	15.053	5.625	49.824	48.378	1.000	0.548	0.737	0.621
32	80.000	4.030	180.703	48.852	48.376	0.995	0.726	0.770	0.654

0.784

0.567 ?

0.775

0.548 ?

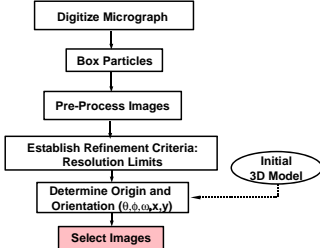
Icosahedral Virus 3D Reconstruction Scheme

Select Images

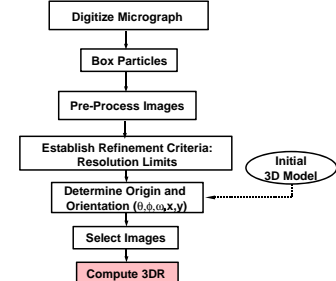
ID	θ	ϕ	ω	x	y	PRJ CC	PFT CC	CMP CC	
1	80.000	-13.201	313.594	48.751	48.849	1.015	0.492	0.713	0.557
2	85.000	23.088	49.922	49.287	48.429	1.010	0.998	0.725	0.609
3	77.000	-5.132	334.698	49.061	48.364	1.005	0.726	0.749	0.616
4	81.000	5.062	15.469	49.215	49.139	1.000	0.718	0.778	0.656
5	81.000	0.000	148.399	49.681	48.119	1.005	0.725	0.712	0.646
6	79.000	-2.037	338.203	49.220	48.202	1.005	0.746	0.738	0.647
7	83.000	-3.022	175.781	49.223	48.802	1.010	0.687	0.703	0.620
8	83.000	1.004	15.469	49.147	48.911	1.005	0.656	0.762	0.574
9	88.000	1.001	15.469	49.845	49.042	1.005	0.732	0.736	0.662
10	75.000	-7.247	158.203	47.863	49.199	1.000	0.744	0.784	0.622
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12	86.000	1.004	170.156	49.127	48.761	1.005	0.784	0.753	0.657
13	80.000	-5.077	68.946	48.997	48.623	1.000	0.720	0.780	0.640
21	78.000	1.156	108.281	49.310	49.242	1.000	0.732	0.792	0.657
15	86.000	3.022	175.781	49.535	49.787	1.000	0.625	0.760	0.589
16	87.000	11.015	2.813	49.797	49.852	1.000	0.707	0.761	0.609
17	89.000	30.005	89.297	49.754	48.838	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
22	86.000	0.000	126.563	49.944	48.914	1.000	0.775	0.809	0.669
20	89.000	5.001	290.391	49.325	48.704	1.000	0.699	0.765	0.642
23	84.000	-3.025	279.141	49.445	48.600	1.000	0.707	0.761	0.652
25	81.000	2.025	295.078	49.317	48.172	1.000	0.729	0.766	0.632
24	80.000	-3.046	180.000	49.673	48.513	1.005	0.756	0.730	0.578
26	86.000	-9.022	291.563	49.649	48.789	1.005	0.609	0.760	0.573
28	84.000	15.053	5.625	49.824	48.378	1.000	0.548	0.737	0.621
32	80.000	4.030	180.703	48.852	48.376	0.995	0.726	0.770	0.654

PFT Coefficient 0.679 ± 0.075

Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



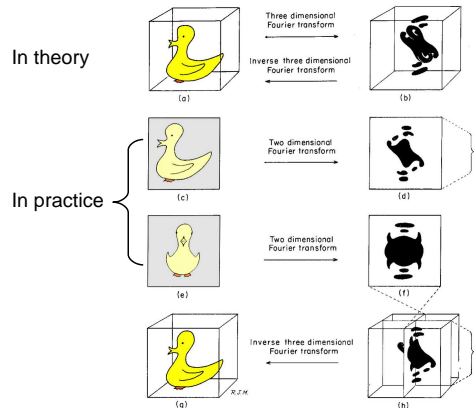
Icosahedral Virus 3D Reconstruction Scheme

Compute 3DR

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

Icosahedral Virus 3D Reconstruction Scheme

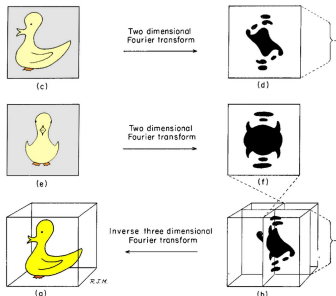
Compute 3DR



From Lake (1972), p.174

Icosahedral Virus 3D Reconstruction Scheme

Compute 3DR



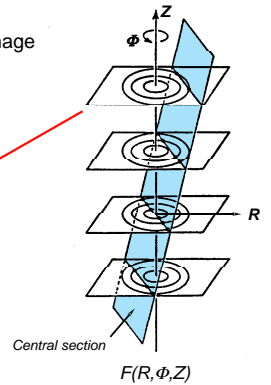
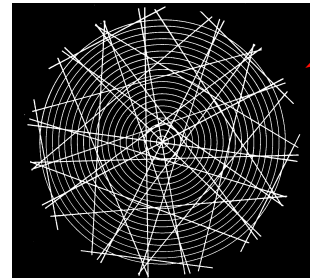
Overall scheme: $\rho \leftarrow g \leftarrow G \leftarrow F$

Icosahedral Virus 3D Reconstruction Scheme

Compute 3DR

Steps:

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



Crowther, DeRosier and Klug, 1970, p.329

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

Icosahedral Virus 3D Reconstruction Scheme

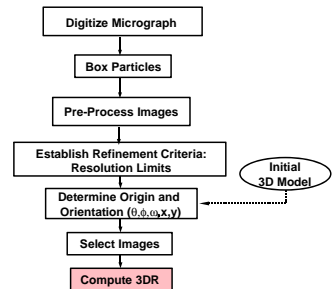
Compute 3DR

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

Steps:

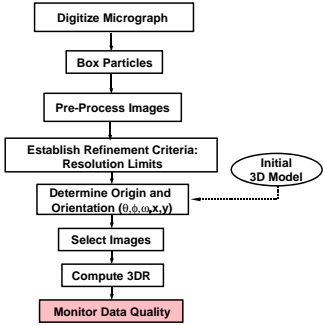
1. Compute 2D FFT of each particle image
2. Combine all 2D FFTs to build up 3D Fourier-Bessel transform
3. Compute G_n 's on each annulus $G = (B B)^{-1} B F$
4. Compute g_n 's from G_n 's (Fourier-Bessel transform)
5. Compute polar density map ($\rho(r, \phi, z)$) from g_n 's
6. Convert from polar to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

Icosahedral Virus 3D Reconstruction Scheme

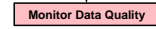


Option: correct for CTF effects in particle FFTs before FFTs are merged to form the 3D FFT

Icosahedral Virus 3D Reconstruction Scheme

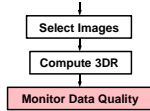


Icosahedral Virus 3D Reconstruction Scheme

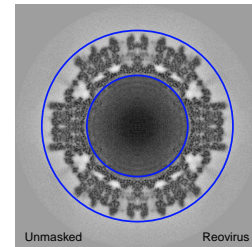
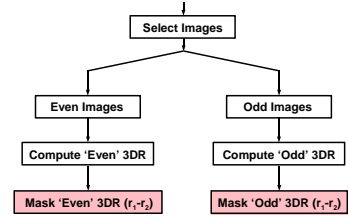


Goal: assess resolution of 3D density map to determine what to do next

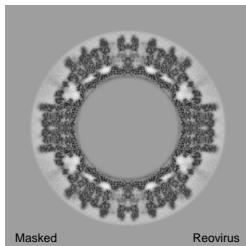
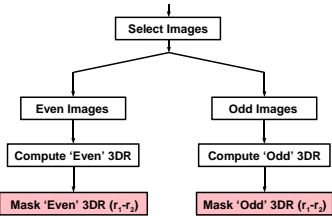
Icosahedral Virus 3D Reconstruction Scheme



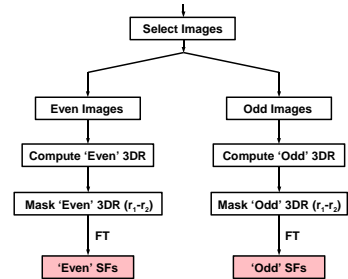
Icosahedral Virus 3D Reconstruction Scheme



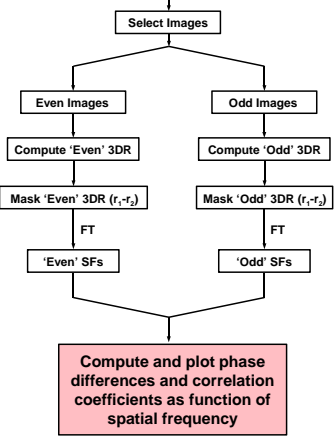
Icosahedral Virus 3D Reconstruction Scheme



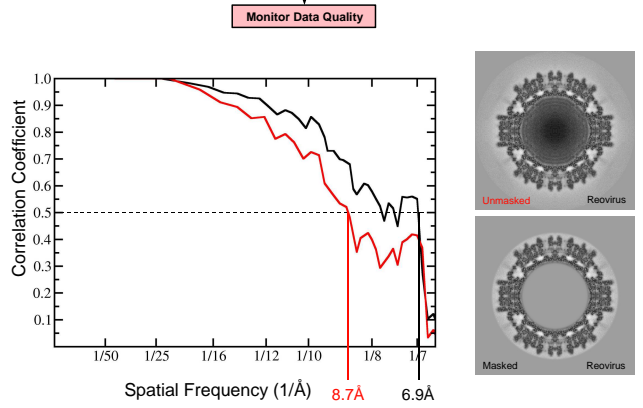
Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



Icosahedral Virus 3D Reconstruction Scheme



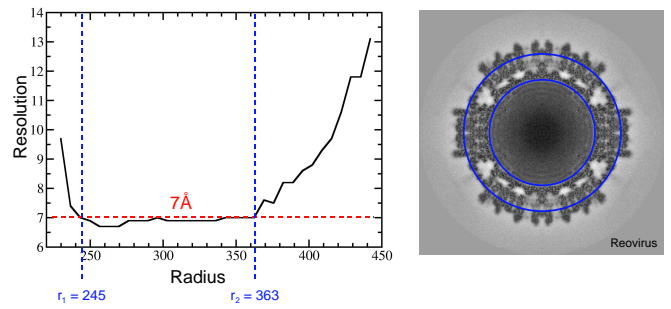
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality

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

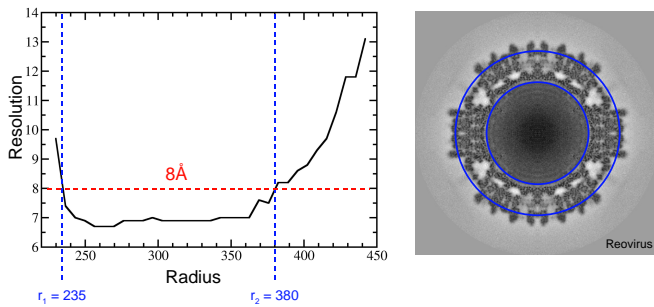
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality



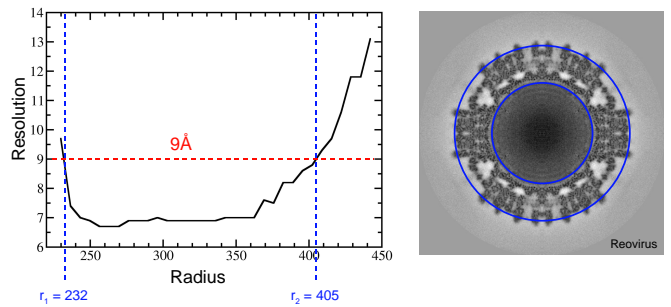
Icosahedral Virus 3D Reconstruction Scheme

Monitor Data Quality

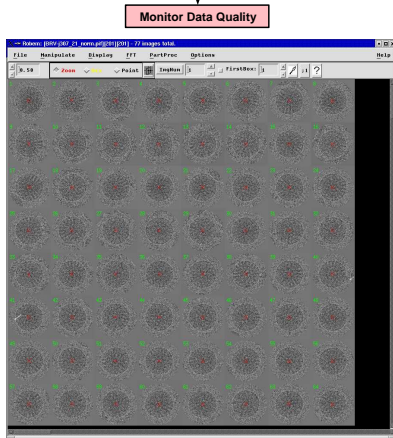


Icosahedral Virus 3D Reconstruction Scheme

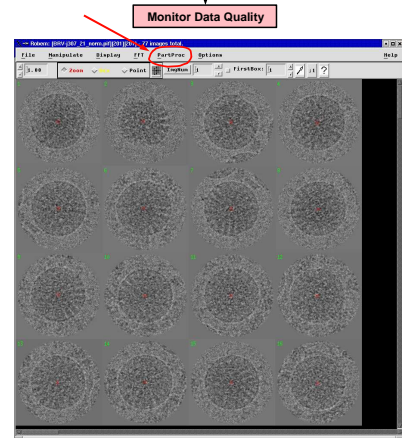
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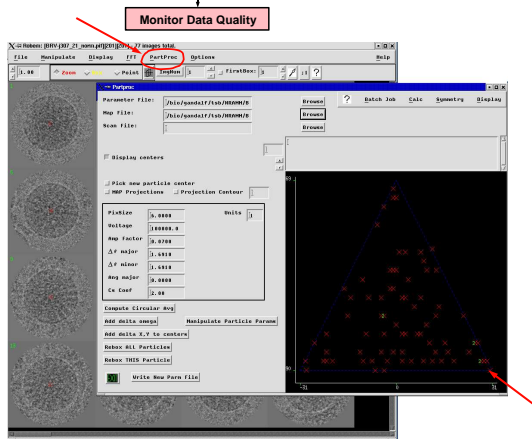
Icosahedral Virus 3D Reconstruction Scheme



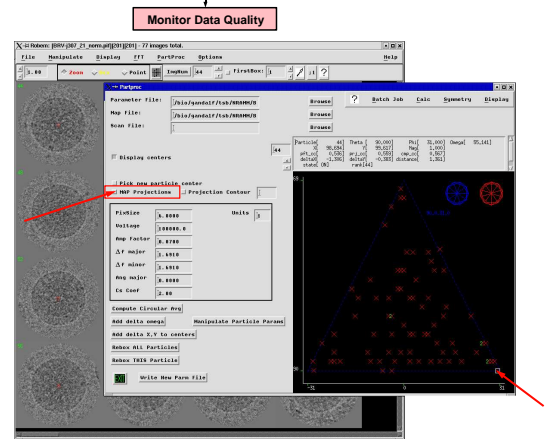
Icosahedral Virus 3D Reconstruction Scheme



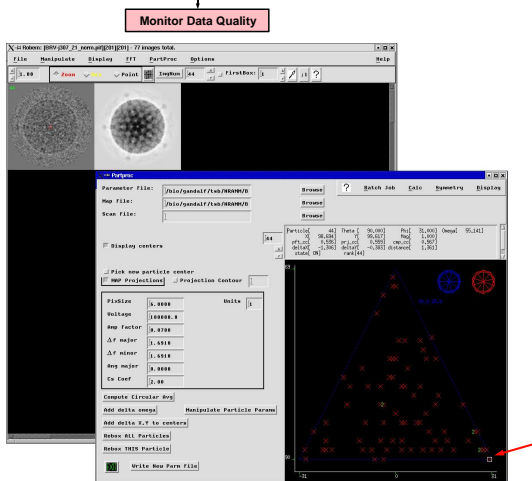
Icosahedral Virus 3D Reconstruction Scheme



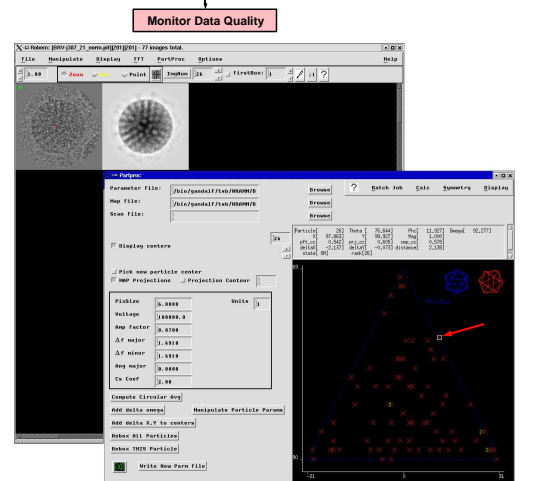
Icosahedral Virus 3D Reconstruction Scheme



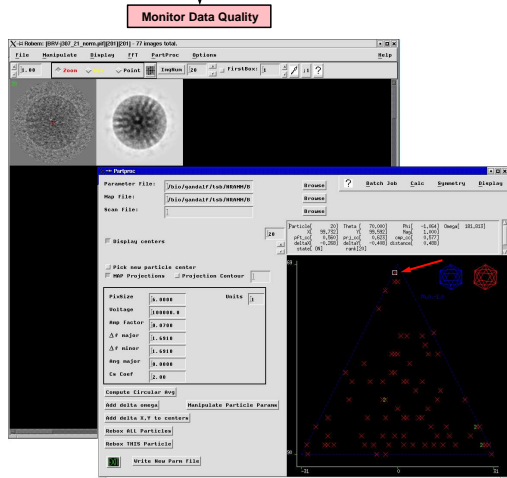
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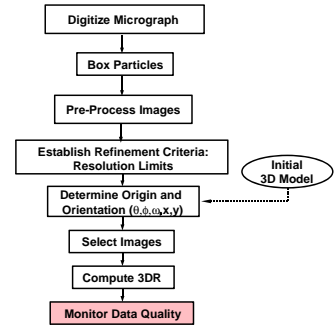
Icosahedral Virus 3D Reconstruction Scheme



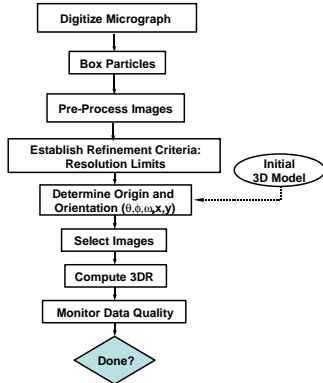
Icosahedral Virus 3D Reconstruction Scheme



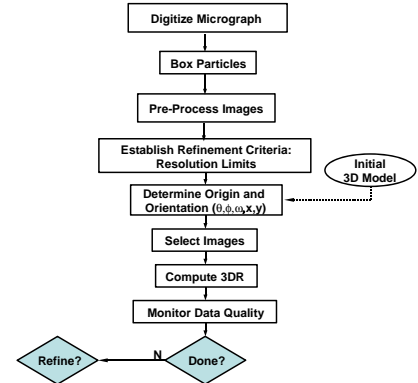
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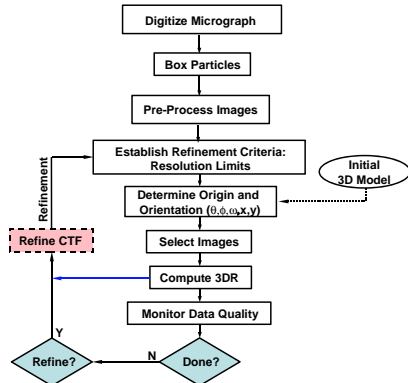
Icosahedral Virus 3D Reconstruction Scheme



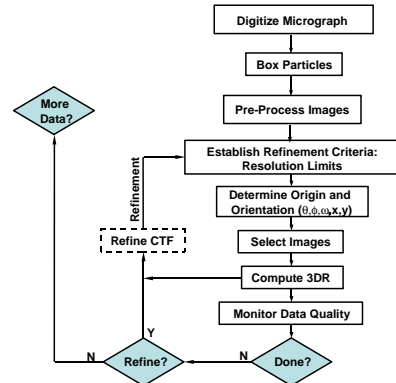
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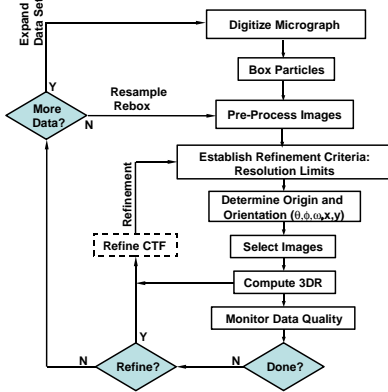
Icosahedral Virus 3D Reconstruction Scheme



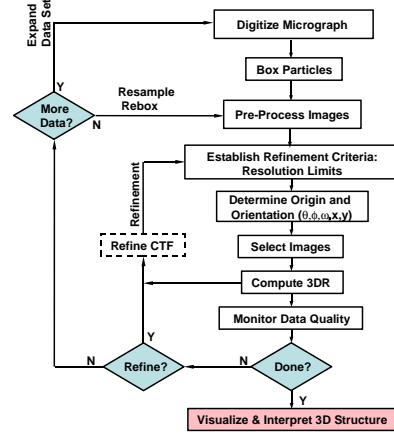
Icosahedral Virus 3D Reconstruction Scheme



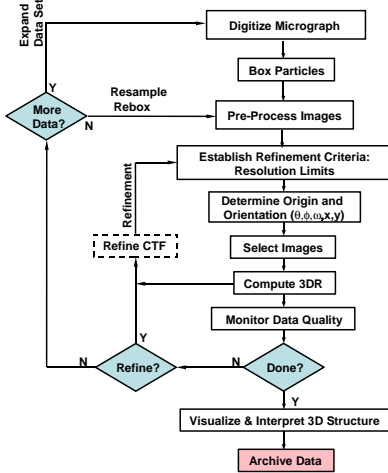
Icosahedral Virus 3D Reconstruction Scheme



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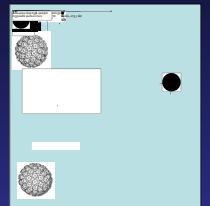
Icosahedral Virus 3D Reconstruction Scheme



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



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

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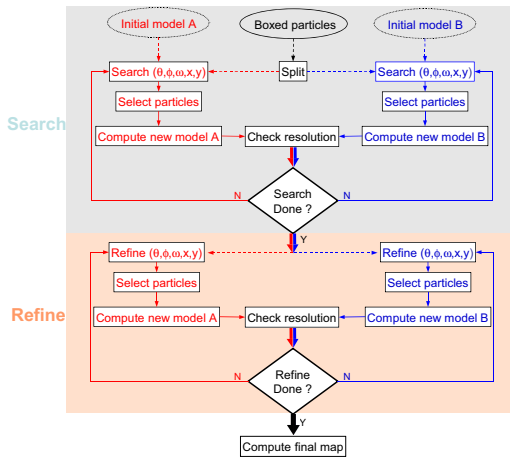
3D Reconstruction of Icosahedral Particles

Current and Future Strategies

- Parallelization and new algorithms
 - "Parallel" versions of EM3DR, PFTSEARCH, OOR
 - EM3DR ---> P3DR
 - OOR -----> PO²R
- Automation
 - Semi-auto boxing (RobEM)
 - Automated origin/orientation refinement (AUTO3DEM)
- Split data set processing
 - Divide image data at very beginning and refine 'even' and 'odd' data independently.
 - Minimizes (eliminates ?) bias in resolution assessment
 - Combine independent reconstructions to obtain 'final' 3DR with highest S/N

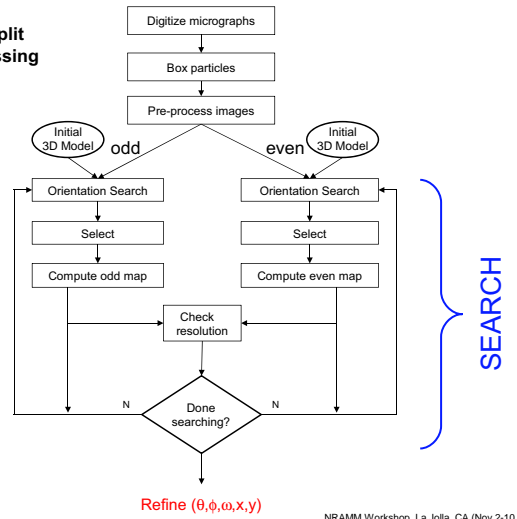
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Structure Determination Flow Chart



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Data Flow for Split Data Set Processing



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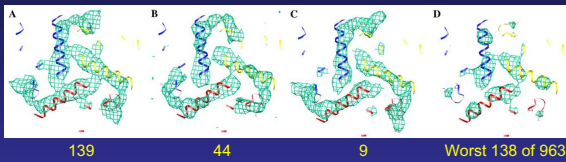
3D Reconstruction of Icosahedral Particles

Current and Future Strategies

- Data selection

Trying to improve resolution by substantially increasing the number of images averaged *ad infinitum* may prove less beneficial than simply applying more rigorous quality control measures to weed out 'bad' data.

Borgnia, M. J., D. Shi, P. Zhang and J. L. Milne (2004) Visualization of α -helical features in a density map constructed using 9 molecular images of the 1.8 MDa icosahedral core of pyruvate dehydrogenase. *J. Struct. Biol.* **147**:136-145.



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