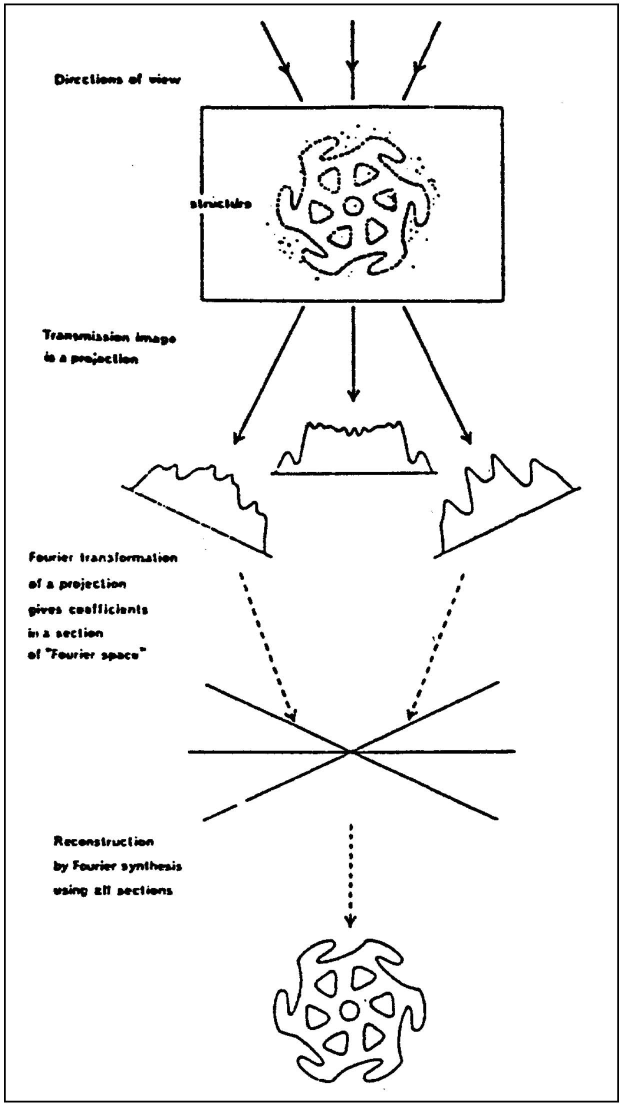
III.D.5 3D Fourier Reconstruction Methods

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

Overall Scheme for 3-D Reconstruction



Generally the available views of the specimen do not give an even sampling of data in the Fourier transform

III.D.5 3D Fourier Reconstruction Methods

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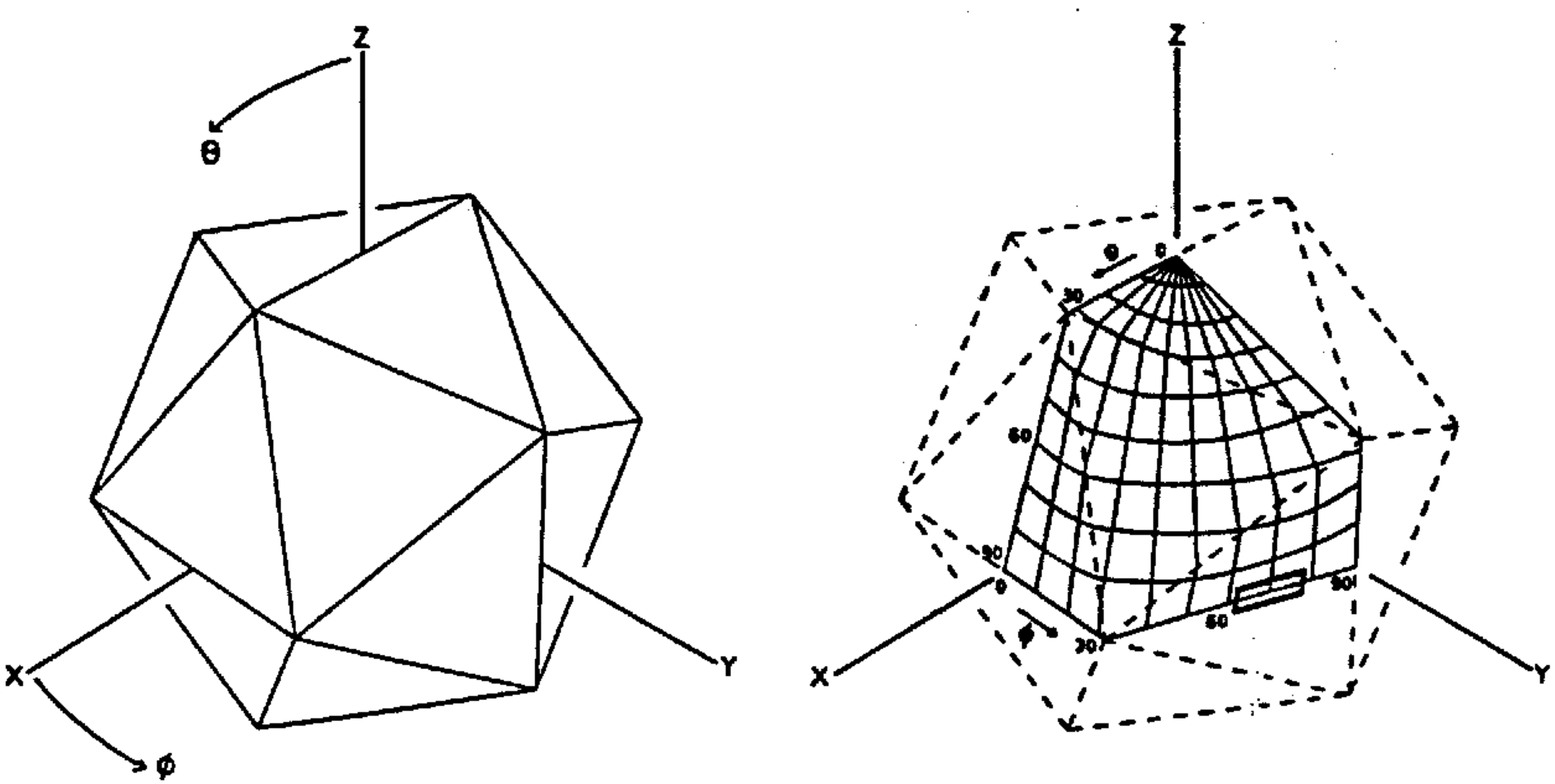
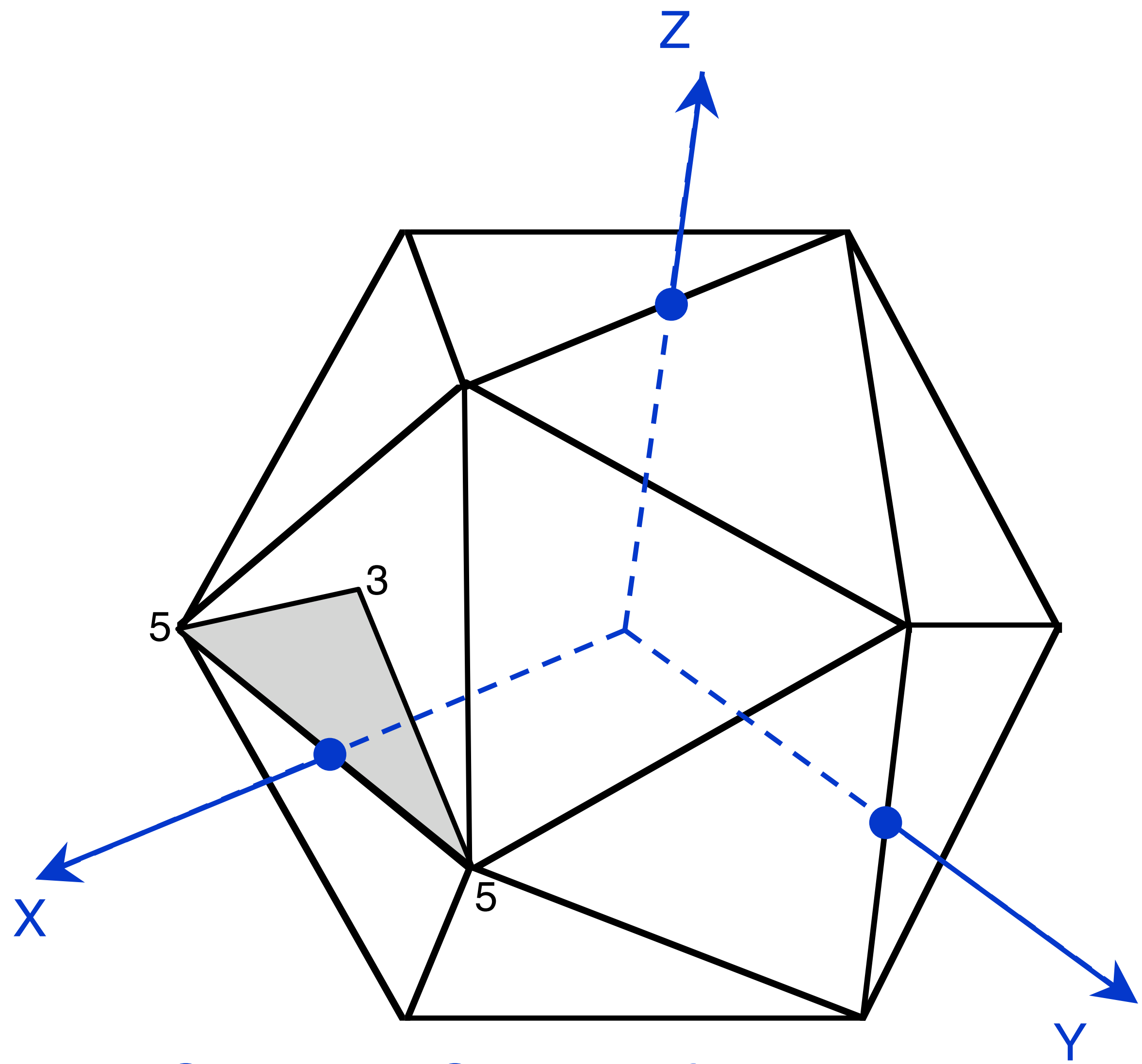


2-fold setting used for specifying direction of view

III.D.5 3D Fourier Reconstruction Methods

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



Standard Setting of Icosahedron

III.D.5 3D Fourier Reconstruction Methods

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

Interpolation in Polar Coordinates - 2D Case

Expanded object density: $$\rho(r,\phi) = \sum_n g_n(r) e^{in\phi}$$

Fourier transform: $$F(R,\Phi) = \sum_n \int_{object} i^n g_n(r) J_n(2\pi rR) e^{in\Phi} 2\pi r dr$$

$$= \sum_n \int_{object} g_n(r) J_n(2\pi rR) e^{in(\Phi+\pi/2)} 2\pi r dr$$

$$= \sum_n \underbrace{G_n(R)}_{\text{radial variation}} \underbrace{e^{in(\Phi+\pi/2)}}_{\text{angular variation}}$$

III.D.5 3D Fourier Reconstruction Methods

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

Interpolation in Polar Coordinates - 2D Case

Recall from the discussion of rotationally symmetric objects that the Fourier-Bessel (or Hankel) transform relations are:

$$G_n(R) = \int_{object} g_n(r) J_n(2\pi rR) 2\pi r dr$$

and g_n and G_n are reciprocally related:

$$g_n(r) = \int_{transform} G_n(R) J_n(2\pi rR) 2\pi R dR$$

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Interpolation in Polar Coordinates - 2D Case

$$G_n(R) = \int_{object} g_n(r) J_n(2\pi rR) 2\pi r dr$$

$$g_n(r) = \int_{transform} G_n(R) J_n(2\pi rR) 2\pi R dR$$

Each g_n is a **real wave** and each G_n represents a particular J_n **weighted** by the strength of that angular component at a particular radius, *R*, in the transform

III.D.5 3D Fourier Reconstruction Methods

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Interpolation in Polar Coordinates - 2D Case

$$G_n(R) = \int_{object} g_n(r) J_n(2\pi r R) 2\pi r dr$$

$$g_n(r) = \int_{transform} G_n(R) J_n(2\pi r R) 2\pi R dR$$

Overall scheme: Compute from F → G → g → ρ

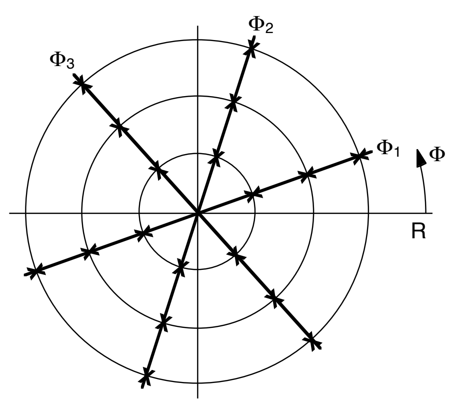
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

To obtain sufficient image data to compute a 3-D reconstruction, projected views of the object **may be** collected by tilts about a single axis

As a consequence of the projection theorem, such strategy would produce a **sampling** (a star of lines) in each **Z-plane** of the 3-D Fourier transform:



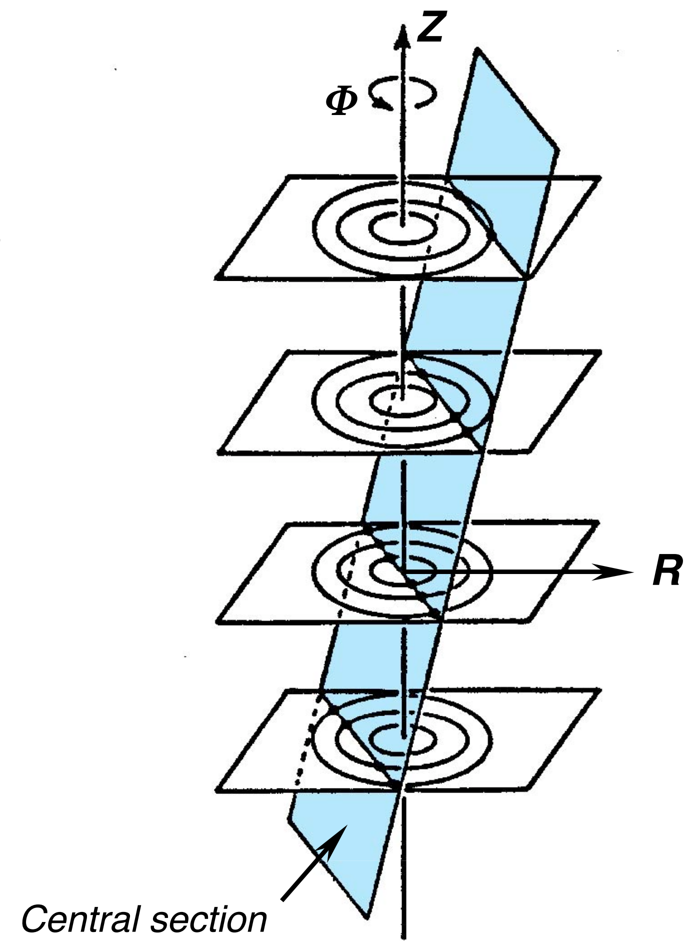
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

Cylindrical polar coordinate system used to describe the 3-D transform, which is **sampled on planes of constant Z** and on **annuli of constant *R*** within each plane

Example of how one **central plane**, corresponding to a particular view of the object, cuts the various annuli is depicted



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

III.D.5 3D Fourier Reconstruction Methods

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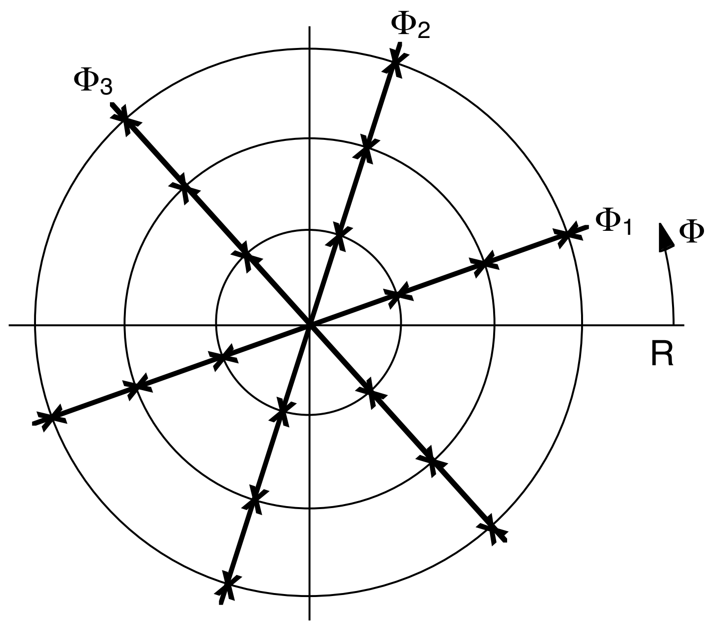
3-D Reconstruction

As a consequence of the projection theorem, such strategy would produce a **sampling** (a star of lines) in each **Z-plane** of the 3-D Fourier transform:



2D illustration of interpolation in polar coordinates

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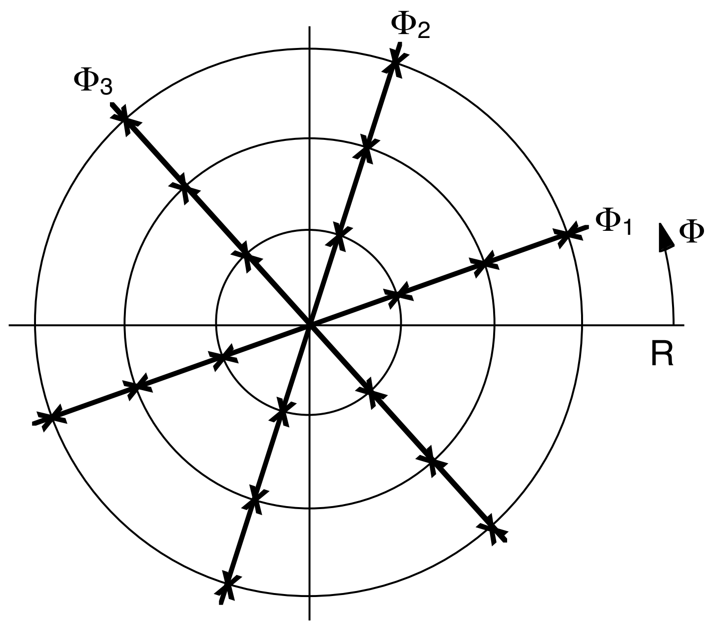
3-D Reconstruction

A **cylindrical expansion** is made on **each annulus**, using transform values at points where the available data planes cut the annulus (indicated by crosses)



2D illustration of interpolation in polar coordinates

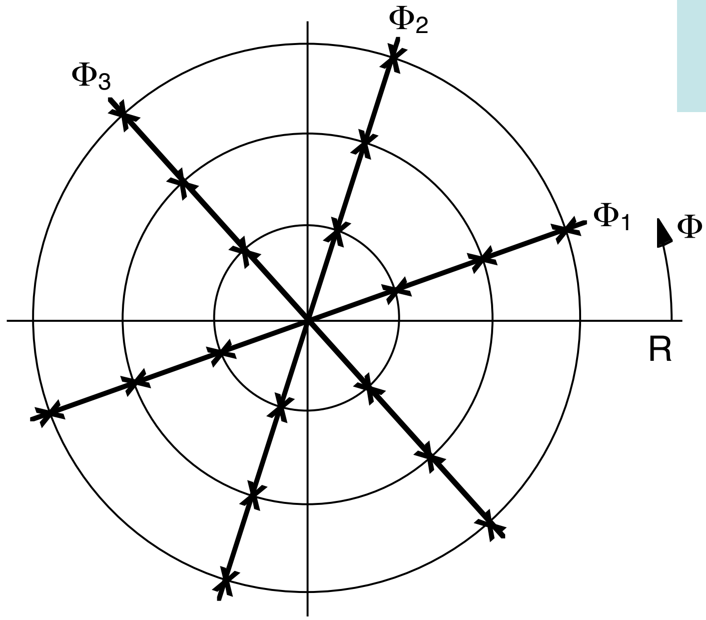
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

On annulus R the Fourier transform is given by:

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j}$$



2D illustration of interpolation in polar coordinates

III.D.5 3D Fourier Reconstruction Methods

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3-D Reconstruction

On annulus R the Fourier transform is given by:

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j}$$

Using shorthand:

$$F_j = \sum_n G_n B_{jn}$$

where

$$B_{jn} = i^n e^{in\Phi_j} = e^{in(\Phi_j + \pi/2)}$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

Transform on annulus R:

$$F_j = \sum_n G_n B_{jn}$$

and

$$B_{jn} = e^{in(\Phi_j + \pi/2)}$$

For given angular positions in the Fourier transform, Φ_j, and observed F_j, these are **linear equations** that can be **solved for G_n**, provided a sufficient number of views can be included

III.D.5 3D Fourier Reconstruction Methods

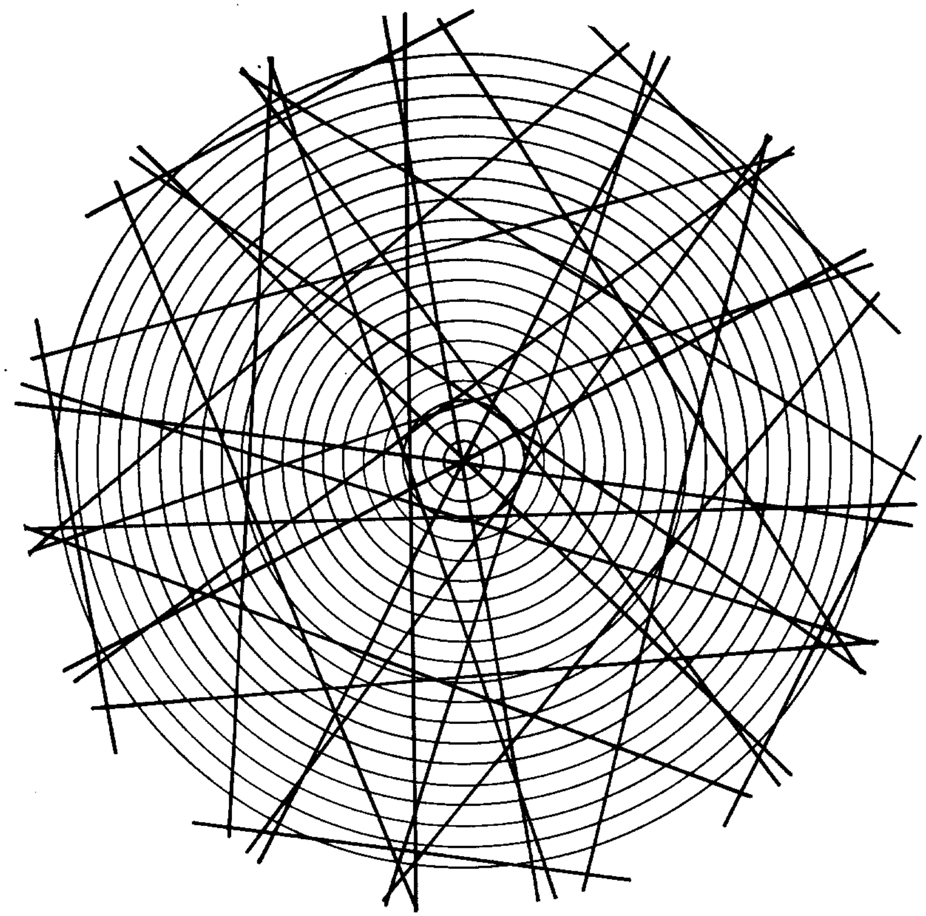
III.D.5.a 3D Reconstruction of Objects with Icosahedral Symmetry

3-D Reconstruction

Arrangement of lines where a primary plane of data, normal to a two-fold axis of an icosahedral particle, and the planes related to it by symmetry intersect the transform plane Z = 1/6 nm^{-1}

Spacing of annuli = 1/60 nm^{-1}

Note the uneven nature of the sampling around each annulus



Crowther, DeRosier and Klug, 1970, p.329

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

3-D object density given by following expansion equation:

$$\rho(r,\phi,z) = \sum_{n=-\infty}^{\infty} \int_{-\infty}^{\infty} g_n(r,Z)\, e^{in\phi} e^{2\pi i z Z} dZ \qquad (1)$$

3-D Fourier transform has the form:

$$F(R,\Phi,Z) = \sum_{n} G_n(R,Z)\, i^n e^{in\Phi} \qquad (2)$$

Recall - in 2-D the FT is: $F(R,\Phi) = \sum_{n} G_n(R)\, i^n e^{in\Phi}$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

3-D Fourier transform:

$$F(R,\Phi,Z) = \sum_{n} G_n(R,Z) i^n e^{in\Phi} \quad (2)$$

Recall: Just as was true in 2-D, in 3-D $G_n(R,Z)$ and $g_n(r,Z)$ are related by the Fourier Bessel transformations:

$$G_n(R,Z) = \int_{object} g_n(r,Z) J_n(2\pi rR) 2\pi r dr \quad (2b)$$

$$g_n(r,Z) = \int_{transform} G_n(R,Z) J_n(2\pi rR) 2\pi R dR \quad (2c)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

3-D Fourier transform:

$$F(R,\Phi,Z) = \sum_n G_n(R,Z) i^n e^{in\Phi} \tag{2}$$

On annulus *R* of transform plane *Z* (called "annulus *(R,Z)*") we have observations $F(\Phi_j)$ at known Φ_j , so:

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j} \tag{3}$$

III.D.5 3D Fourier Reconstruction Methods

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3-D Reconstruction

On annulus *(R,Z)* we have observations $F(\Phi_j)$ at known Φ_j, so:

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j} \qquad (3)$$

This set of linear equations can be solved for G_n

The g_n are then computed using eqn. (2c):

$$g_n(r,Z) = \int_{transform} G_n(R,Z) J_n(2\pi rR) 2\pi R dR \qquad (2c)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

Compute g_n from (2c):

$$g_n(r,Z) = \int_{transform} G_n(R,Z) J_n(2\pi rR) 2\pi R dR \quad \text{(2c)}$$

The 3-D density $\rho(r,\phi,z)$ is computed from (1):

$$\rho(r,\phi,z) = \sum_{n=-\infty}^{\infty} \int_{-\infty}^{\infty} g_n(r,Z) e^{in\phi} e^{2\pi izZ} dZ \quad \text{(1)}$$

III.D.5 3D Fourier Reconstruction Methods

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3-D Reconstruction

The 3-D density $\rho(r,\phi,z)$ is computed from (1):

$$\rho(r,\phi,z) = \sum_{n=-\infty}^{\infty} \int_{-\infty}^{\infty} g_n(r,Z)\, e^{in\phi} e^{2\pi i z Z} dZ \quad (1)$$

In general: It is necessary to include a sufficient number of views so that the set of linear equations (3) will contain **many <u>MORE</u> observations than unknowns**

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j} \quad (3)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j} \qquad (3)$$

This set of linear equations is solved by least squares procedures

Equation (3) is rewritten in matrix form as observational equations:

$$F = BG \qquad (4)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j} \quad (3)$$

$$F = BG \quad (4)$$

Form the normal equations:

$$B^{\dagger} F = B^{\dagger} B G \quad (5)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j} \quad (3)$$

$$F = BG \quad (4)$$

$$B^\dagger F = B^\dagger B G \quad (5)$$

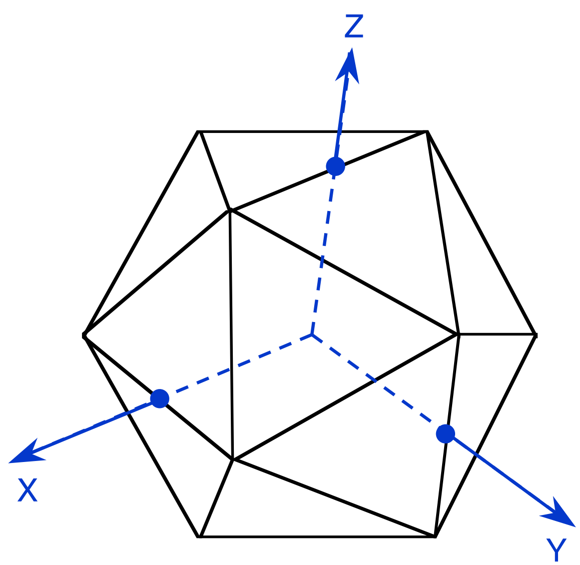
This gives a least squares solution of (4) as:

$$G = \left(B^\dagger B\right)^{-1} B^\dagger F \quad (6)$$

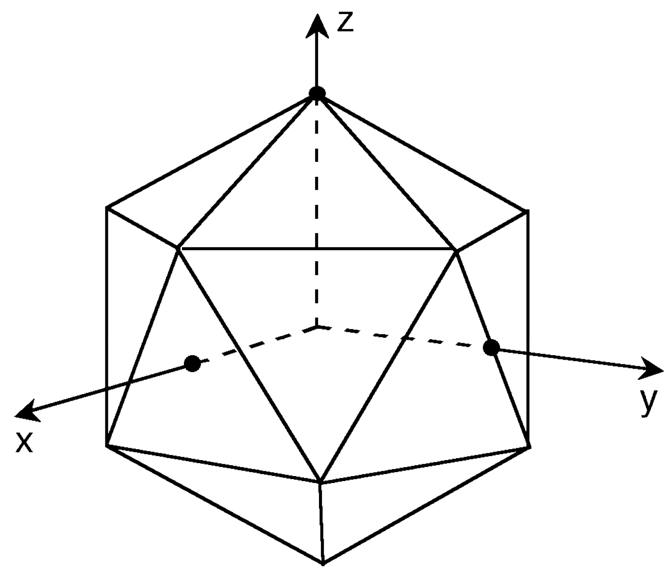
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry



Standard Setting



5-fold setting used for computing reconstruction

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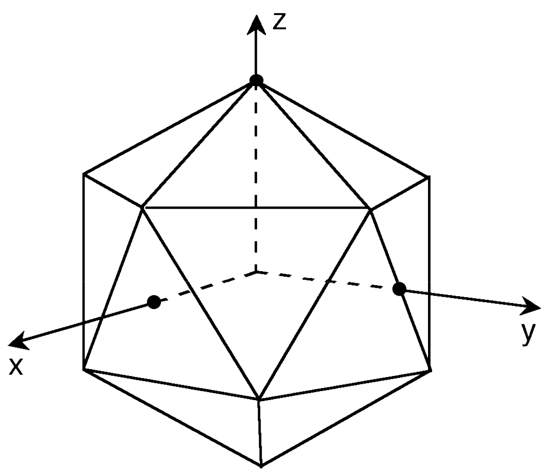
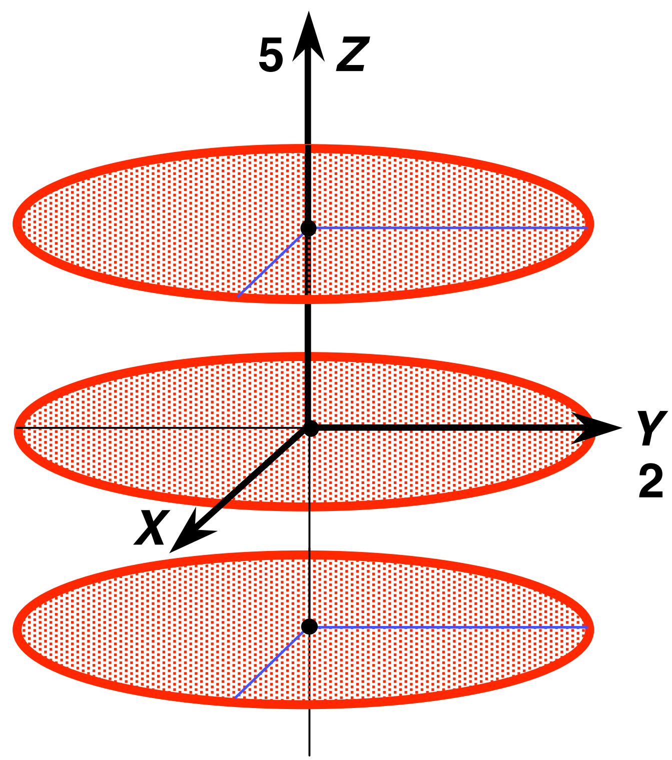
III.D.5.a 3D Reconstruction of Objects with Icosahedral Symmetry

3-D Reconstruction: Algebra Incorporating Symmetry



5-fold setting used for computing reconstruction



Fourier Space

III.D.5 3D Fourier Reconstruction Methods

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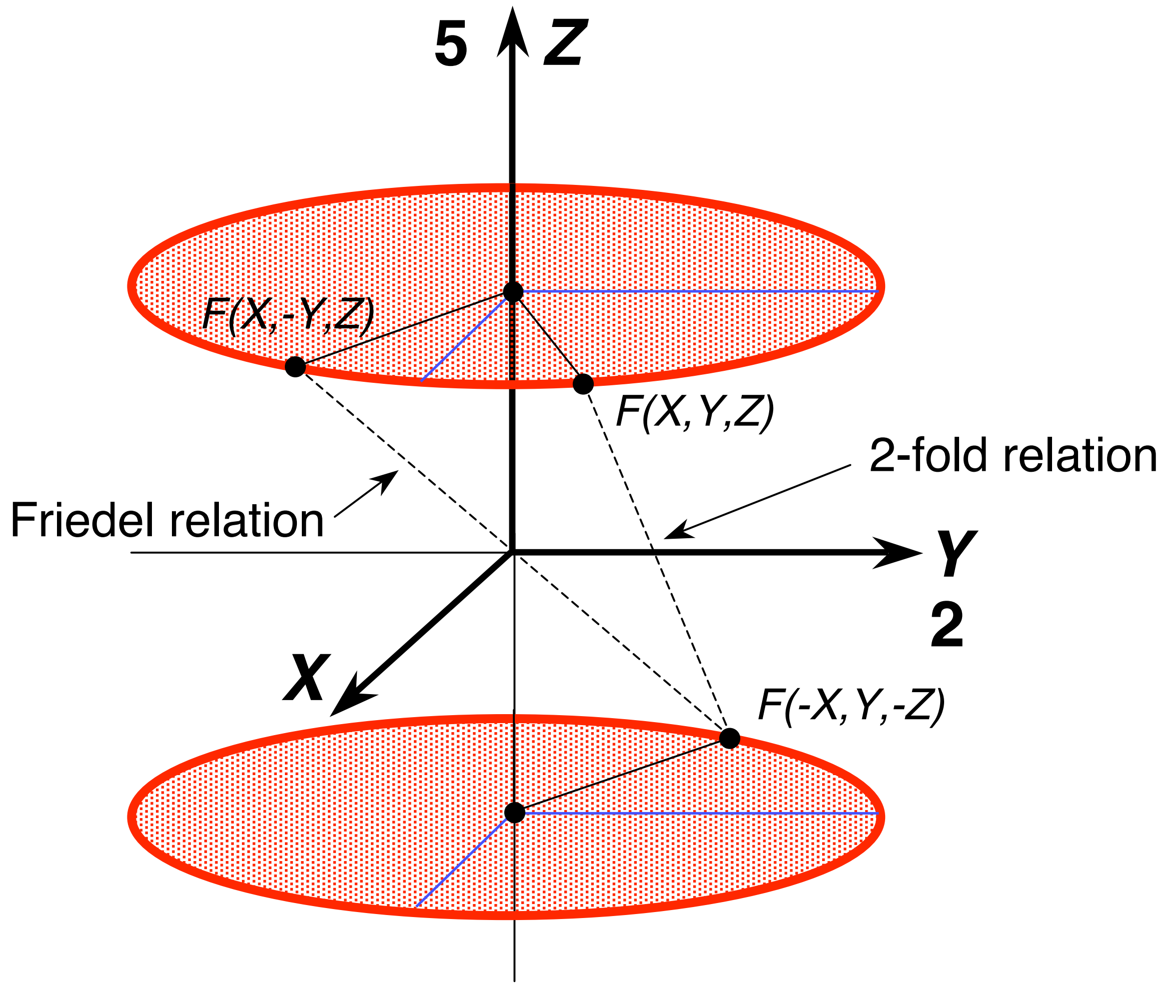
3-D Reconstruction: Algebra Incorporating Symmetry



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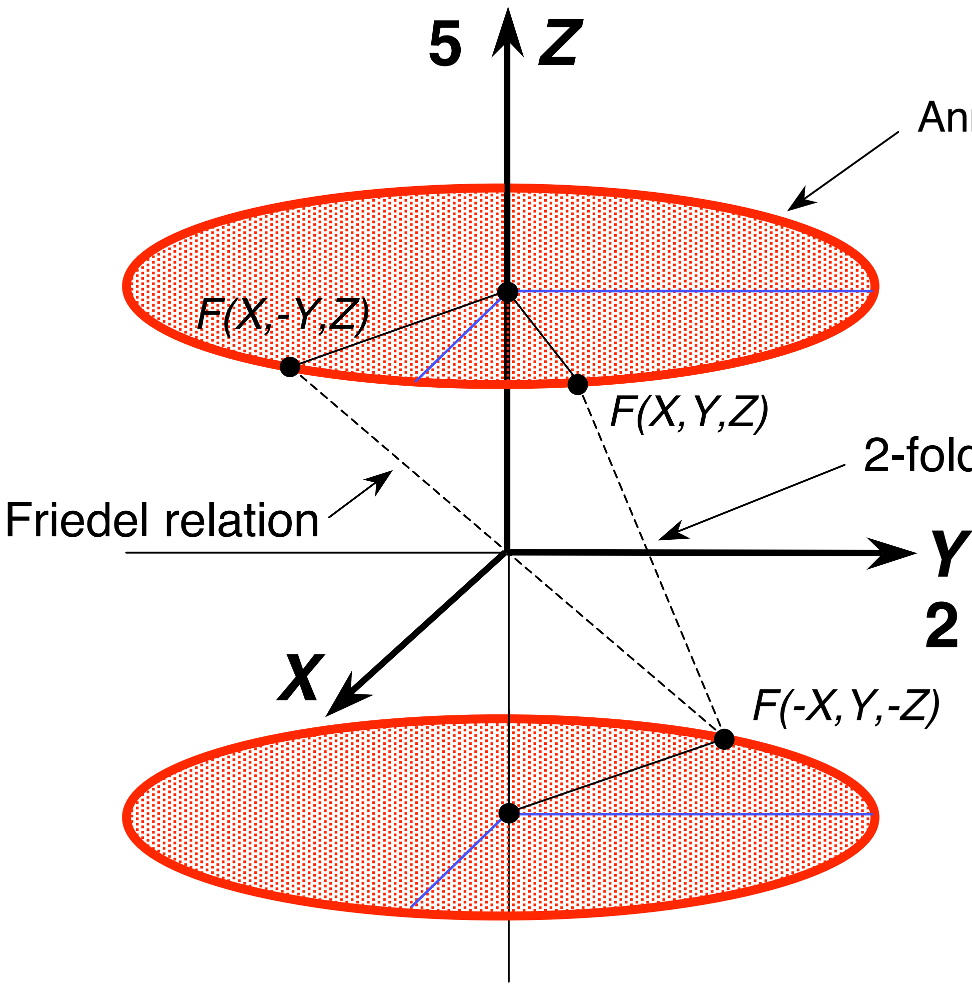
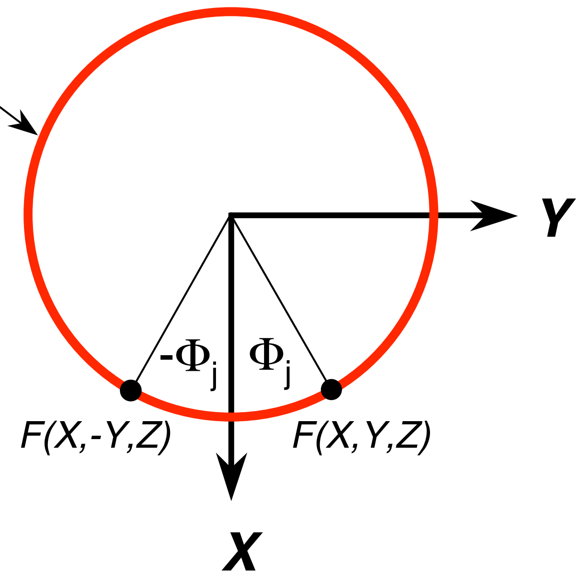
3-D Reconstruction: Algebra Incorporating Symmetry





Let $F(-\Phi_j) = F(X,-Y,Z)$

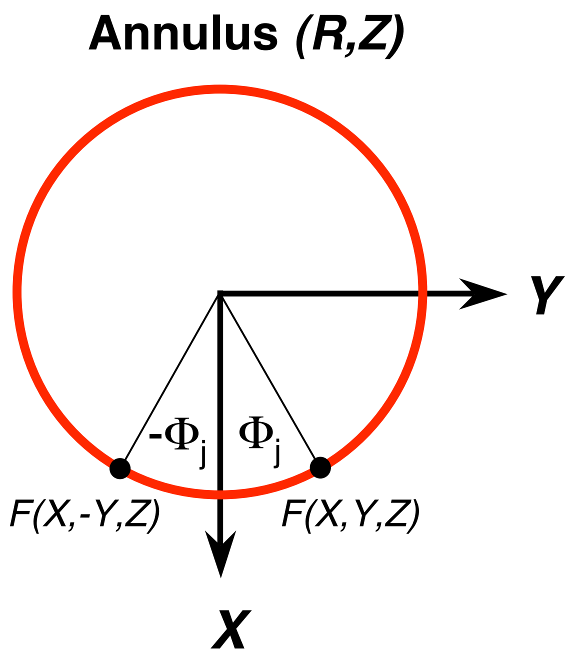
$F(\Phi_j) = F(X,Y,Z)$

Though $F(-\Phi_j)$ and $F(\Phi_j)$ are **not** a Friedel pair, because of 2-fold along Y axis, they obey the Friedel relationship

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry



Recall:

On annulus (R,Z):

$$F(\Phi_j) = \sum_{all\ n} G_n i^n e^{in\Phi_j} \quad (3)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

On annulus (R,Z):

$$F(\Phi_j) = \sum_{all\ n} G_n i^n e^{in\Phi_j} \qquad (3)$$

Use shorthand:

$$G_n' = i^n G_n$$

To get:

$$F(\Phi_j) = \sum_{all\ n} G_n' e^{in\Phi_j} \qquad (7)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

$$F(\Phi_j) = \sum_{all\ n} G_n{}' e^{in\Phi_j} \tag{7}$$

Express each structure factor, *F*, in its component **real *(A)* and imaginary *(B)* parts** to get:

$$\left.\begin{aligned} At\ \Phi_j: \quad A_j + iB_j &= \sum_{\underline{positive n}} \left(G'_n e^{in\Phi_j} + G'_{-n} e^{-in\Phi_j} \right) \\ At\ -\Phi_j: A_j - iB_j &= \sum_{\underline{positive n}} \left(G'_n e^{-in\Phi_j} + G'_{-n} e^{in\Phi_j} \right) \end{aligned}\right\} \tag{8}$$

Remember, $F(-\Phi_j)$ obeys Friedel relation with $F(\Phi_j)$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

$$\left.\begin{aligned} &At\ \Phi_j: \quad A_j + iB_j = \sum_{positive\,n}\left(G'_n e^{in\Phi_j} + G'_{-n} e^{-in\Phi_j}\right) \\ &At\ -\Phi_j: A_j - iB_j = \sum_{positive\,n}\left(G'_n e^{-in\Phi_j} + G'_{-n} e^{in\Phi_j}\right) \end{aligned}\right\} \quad (8)$$

Add

$$2A_j = \sum_{positive\,n} G'_n\left(e^{in\Phi_j} + e^{-in\Phi_j}\right) + G'_{-n}\left(e^{in\Phi_j} + e^{-in\Phi_j}\right) \quad (9)$$

Subtract

$$2iB_j = \sum_{positive\,n} G'_n\left(e^{in\Phi_j} - e^{-in\Phi_j}\right) - G'_{-n}\left(e^{in\Phi_j} - e^{-in\Phi_j}\right) \quad (10)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

Equations (9) and (10) can be reduced if we rearrange the equations and recall the following relations:

$$
\begin{aligned}
e^{in\theta} &= \cos(n\theta) + i\sin(n\theta) \\
e^{-in\theta} &= \cos(n\theta) - i\sin(n\theta) \\
\cos(-n\theta) &= \cos(n\theta) \\
\sin(-n\theta) &= -\sin(n\theta)
\end{aligned}
$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

$$2A_j = \sum_{positive n} G'_n\left(e^{in\Phi_j} + e^{-in\Phi_j}\right) + G'_{-n}\left(e^{in\Phi_j} + e^{-in\Phi_j}\right) \quad (9)$$

This can be rearranged to get:

$$2A_j = \sum_{positive\ n} \left(G'_n + G'_{-n}\right)\left(e^{in\Phi_j} + e^{-in\Phi_j}\right) \quad (9c)$$

and

$$2A_j = \sum_{positive n} \left(G'_n + G'_{-n}\right)\left(2\cos\left(n\Phi_j\right)\right) \quad (9d)$$

because:

$$e^{in\theta} = \cos(n\theta) + i\sin(n\theta)$$
$$e^{-in\theta} = \cos(n\theta) - i\sin(n\theta)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

$$2iB_j = \sum_{positive n} G'_n\left(e^{in\Phi_j} - e^{-in\Phi_j}\right) - G'_{-n}\left(e^{in\Phi_j} - e^{-in\Phi_j}\right) \quad (10)$$

This can be rearranged to get:

$$2iB_j = \sum_{positive\ n} \left(G'_n - G'_{-n}\right)\left(e^{in\Phi_j} - e^{-in\Phi_j}\right) \quad (10c)$$

and

$$2iB_j = \sum_{positive\ n} \left(G'_n - G'_{-n}\right)\left(2i\sin\left(n\Phi_j\right)\right) \quad (10d)$$

because:

$$e^{in\theta} = \cos(n\theta) + i\sin(n\theta)$$
$$e^{-in\theta} = \cos(n\theta) - i\sin(n\theta)$$

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

$$\cancel{2}A_j = \sum_{positive\, n} \left(G'_n + G'_{-n}\right)\left(\cancel{2}\cos\left(n\Phi_j\right)\right) \qquad (9d)$$

$$\cancel{2i}B_j = \sum_{positive\ n} \left(G'_n - G'_{-n}\right)\left(\cancel{2i}\sin\left(n\Phi_j\right)\right) \qquad (10d)$$

Divide equation (9d) by 2 and equation (10d) by 2i to get:

$$\left.\begin{aligned} A_j &= \sum (G'_n + G'_{-n})\cos\left(n\Phi_j\right) \\ B_j &= \sum (G'_n - G'_{-n})\sin\left(n\Phi_j\right) \end{aligned}\right\} \qquad (11)$$

3-D Reconstruction: Algebra Incorporating Symmetry

$$\left.\begin{aligned} A_j &= \sum (G'_n + G'_{-n}) \cos\left(n\Phi_j\right) \\ B_j &= \sum (G'_n - G'_{-n}) \sin\left(n\Phi_j\right) \end{aligned}\right\} \qquad (11)$$

Since A_j, B_j, Φ_j, are known (recall, we have experimental $F(\Phi)$ values), it is necessary to solve for $(G'_n + G'_{-n})$ and $(G'_n - G'_{-n})$

Hence, values of G'_n and G'_{-n} can be found, and, because the relation between G'_n and G_n is known (*i.e.* $G'_n = i^n G_n$), the values of $\boldsymbol{G_n}$ and $\boldsymbol{G_{-n}}$ can be determined

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

$$\left.\begin{aligned} A_j &= \sum (G'_n + G'_{-n}) \cos\left(n\Phi_j\right) \\ B_j &= \sum (G'_n - G'_{-n}) \sin\left(n\Phi_j\right) \end{aligned}\right\} \qquad (11)$$

Since A_j, B_j, Φ_j, are known (recall, we have experimental $F(\Phi)$ values), it is necessary to solve for $(G'_n + G'_{-n})$ and $(G'_n - G'_{-n})$

Note: G_n are **real** for ***n = even***

G_n are **imaginary** for ***n = odd***

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

$$\left.\begin{aligned} A_j &= \sum (G'_n + G'_{-n}) \cos\left(n\Phi_j\right) \\ B_j &= \sum (G'_n - G'_{-n}) \sin\left(n\Phi_j\right) \end{aligned}\right\} \qquad (11)$$

Since A_j, B_j, Φ_j, are known (recall, we have experimental $F(\Phi)$ values), it is necessary to solve for $(G'_n + G'_{-n})$ and $(G'_n - G'_{-n})$

Note: For the 3D reconstruction of icosahedral particles, the above summations are **ONLY** evaluated for terms for which ***n* = multiple of 5**

III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

Compute g_n from (2c):

$$g_n(r,Z) = \int_{transform} G_n(R,Z) J_n(2\pi rR) 2\pi R dR \quad \text{(2c)}$$

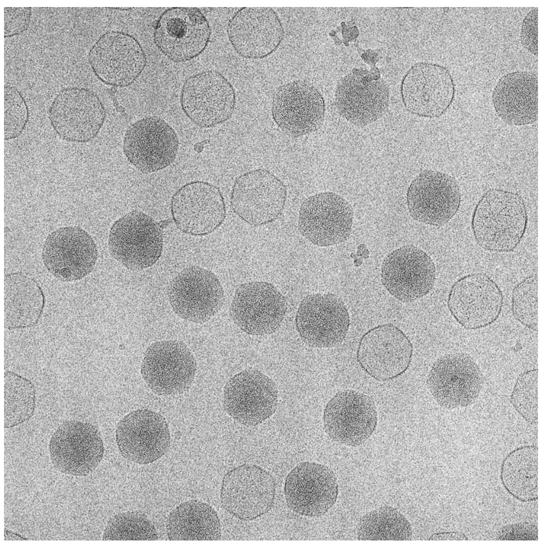
The 3-D density $\rho(r,\phi,z)$ is computed from (1):

$$\rho(r,\phi,z) = \sum_{n=-\infty}^{\infty} \int_{-\infty}^{\infty} g_n(r,Z) e^{in\phi} e^{2\pi izZ} dZ \quad \text{(1)}$$

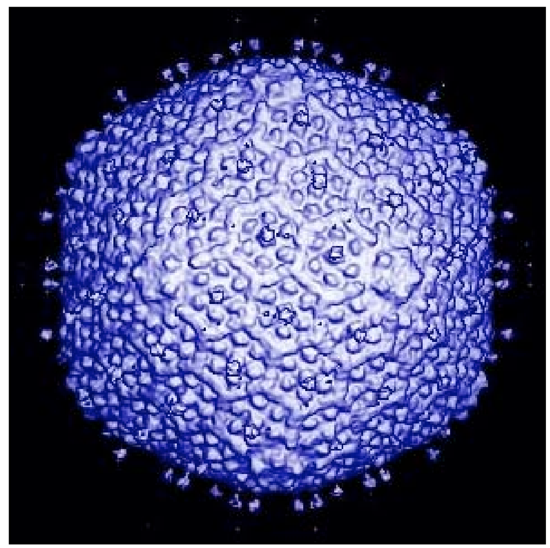
III.D.5 3D Fourier Reconstruction Methods

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

OK, let's get practical about icosahedral particle processing



2D

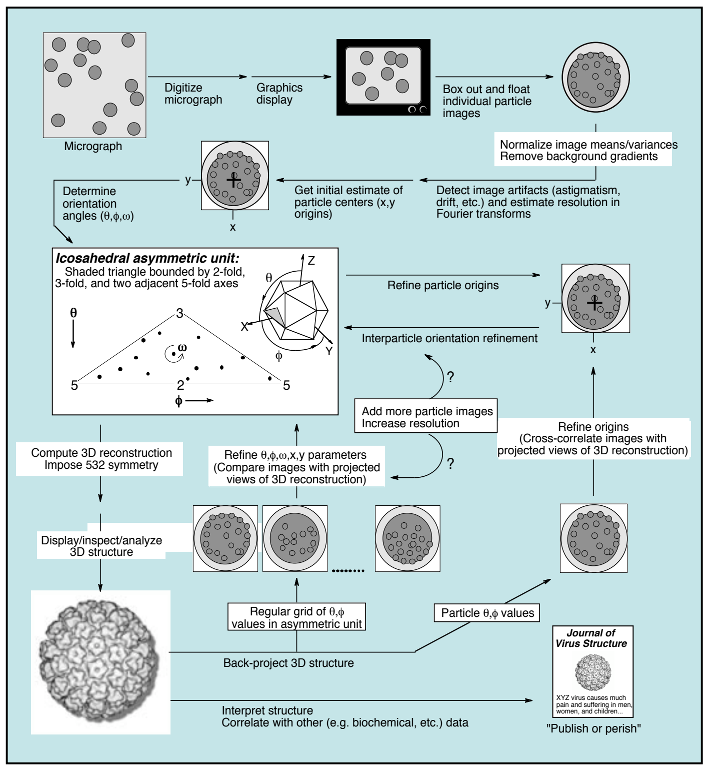


3D

III.D.5 3D Fourier Reconstruction Methods

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

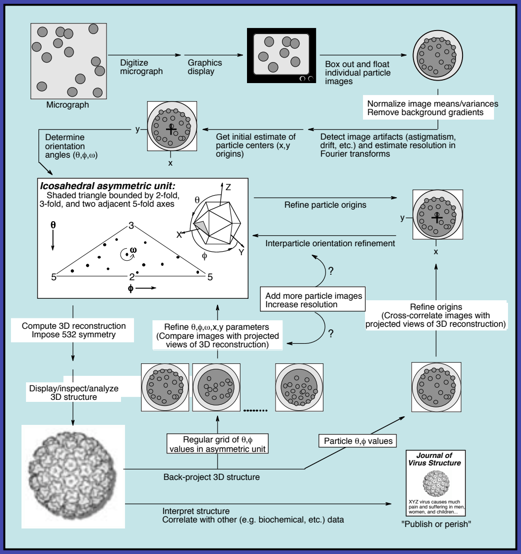
OK, let's get practical about icosahedral particle processing



3D Reconstruction of Icosahedral Particles

Outline

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



3D Reconstruction of Icosahedral Particles

REFERENCES

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

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

- Human wart virus
- Tomato bushy stunt

3D Reconstruction of Icosahedral Particles

REFERENCES

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

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

General principles of 3DR method

- Fourier-Bessel mathematics
- Common lines

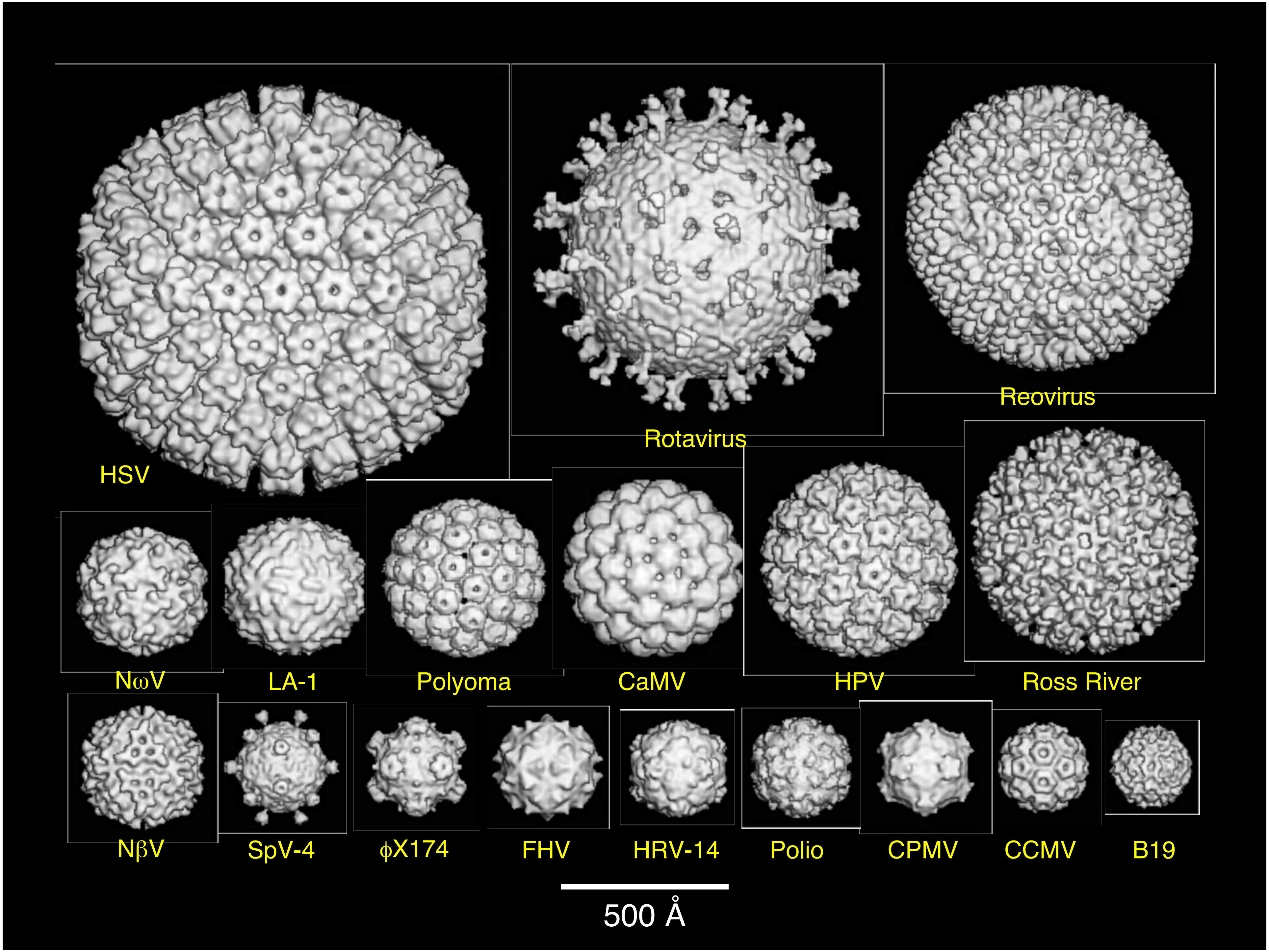
3D Reconstruction of Icosahedral Particles

REFERENCES

- Reference list available as handout

- For die-hards:

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


HSV
Rotavirus
Reovirus
NωV
LA-1
Polyoma
CaMV
HPV
Ross River
NβV
SpV-4
φX174
FHV
HRV-14
Polio
CPMV
CCMV
B19
500 Å

3D Reconstruction of Icosahedral Particles

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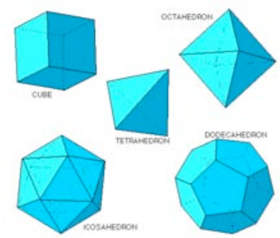
3D Reconstruction of Icosahedral Particles

Symmetry

1. Icosahedral (532) point group symmetry

2. Triangulation symmetry

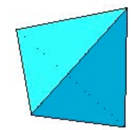


Regular Polyhedra

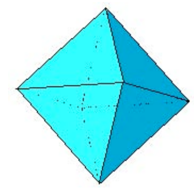
(Platonic Solids)

There are just five platonic solids:

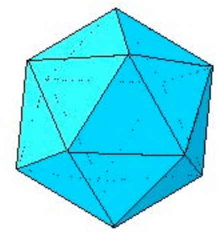
From **equilateral triangles** you can make:
with 3 faces at each vertex, a **tetrahedron**



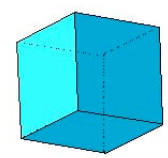
with 4 faces at each vertex, an **octahedron**



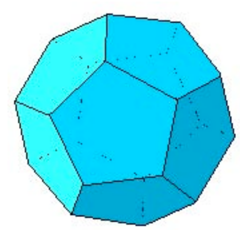
with 5 faces at each vertex, an **icosahedron**



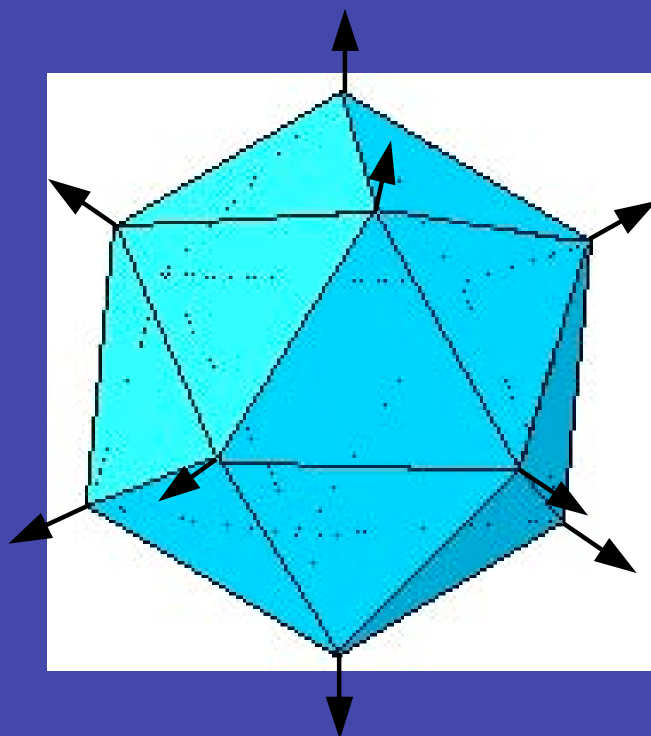
From **squares** you can make:
with 3 faces at each vertex, a **cube**



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

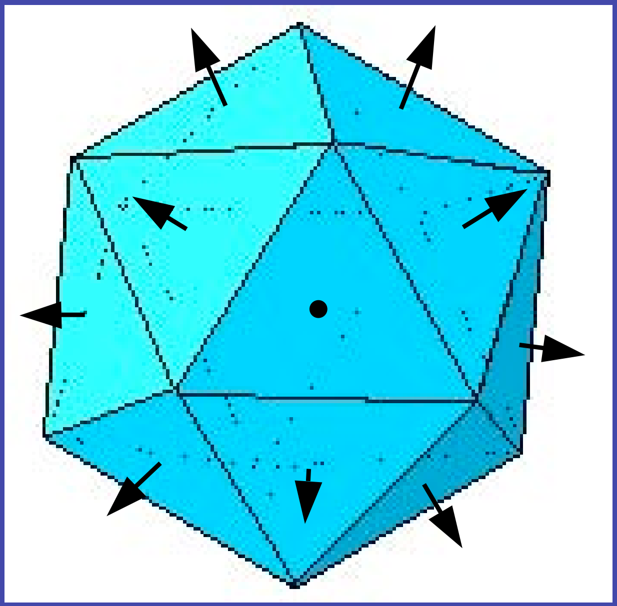


Icosahedral (532) Point Group Symmetry



12 vertices (5-fold)

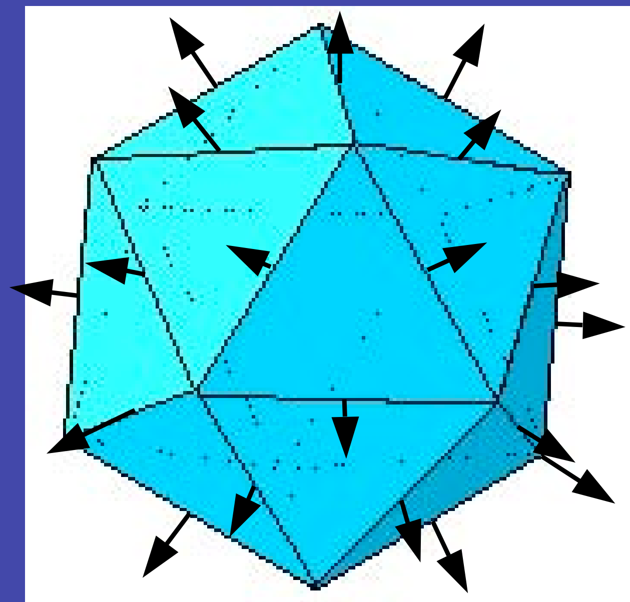
Icosahedral (532) Point Group Symmetry



12 vertices (5-fold)

20 faces (3-fold)

Icosahedral (532) Point Group Symmetry



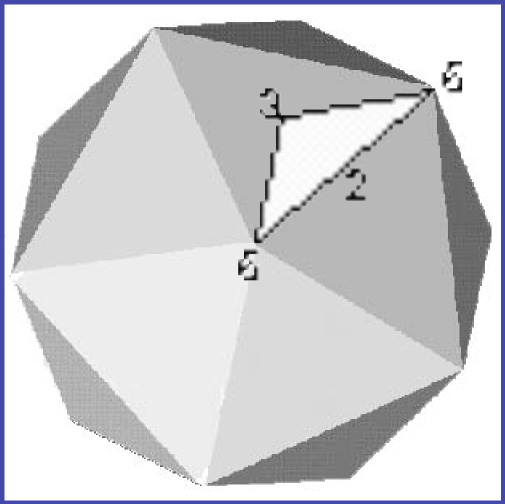
12 vertices (5-fold)

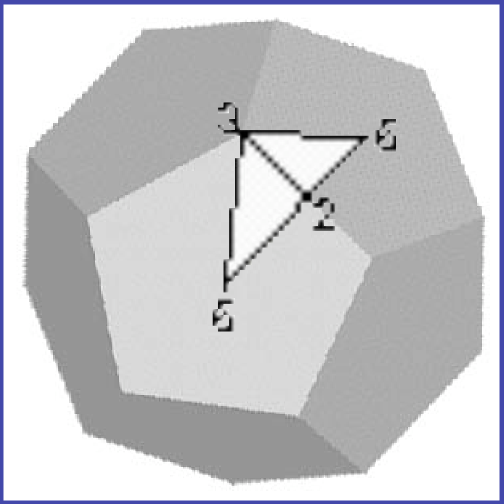
20 faces (3-fold)

30 edges (2-fold)

Icosahedron

Dodecahedron





Different shapes, but **both** have 532 symmetry

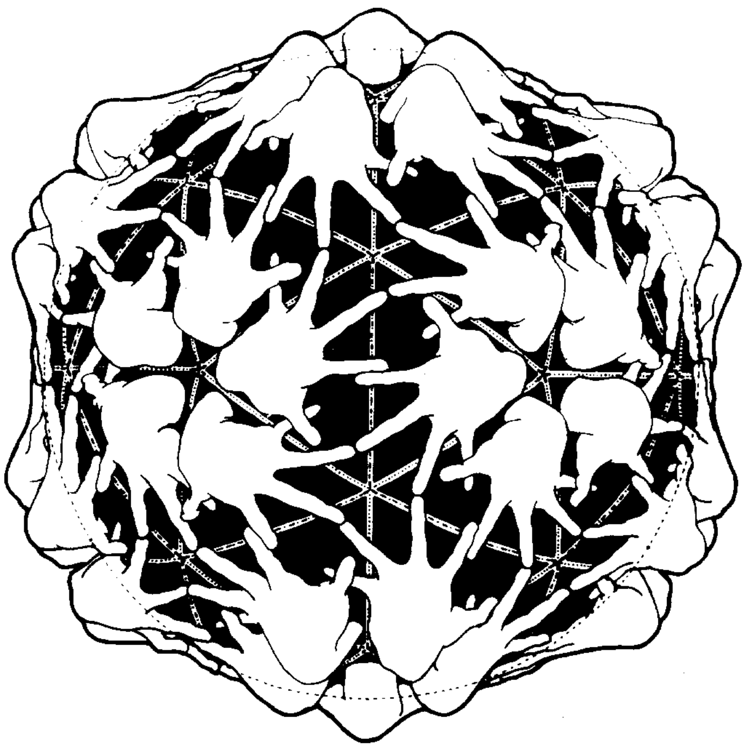
12 vertices, 20 faces, 30 edges
(6 5-folds, 10 3-folds, 15 2-folds)

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

Asymmetric unit is 1/60th of whole object

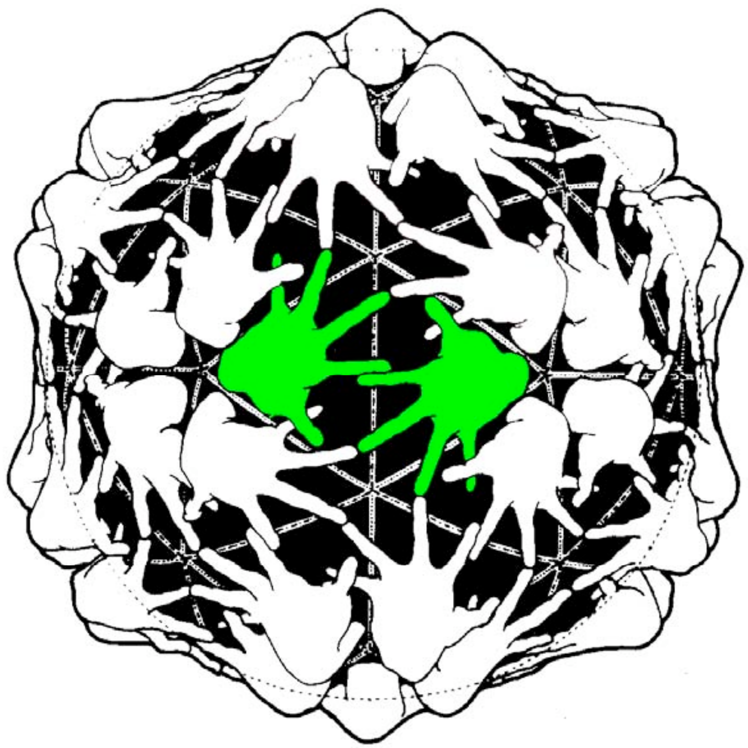
Object consists of 60 identical 'subunits' arranged with icosahedral symmetry

Icosahedral (532) Point Group Symmetry



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

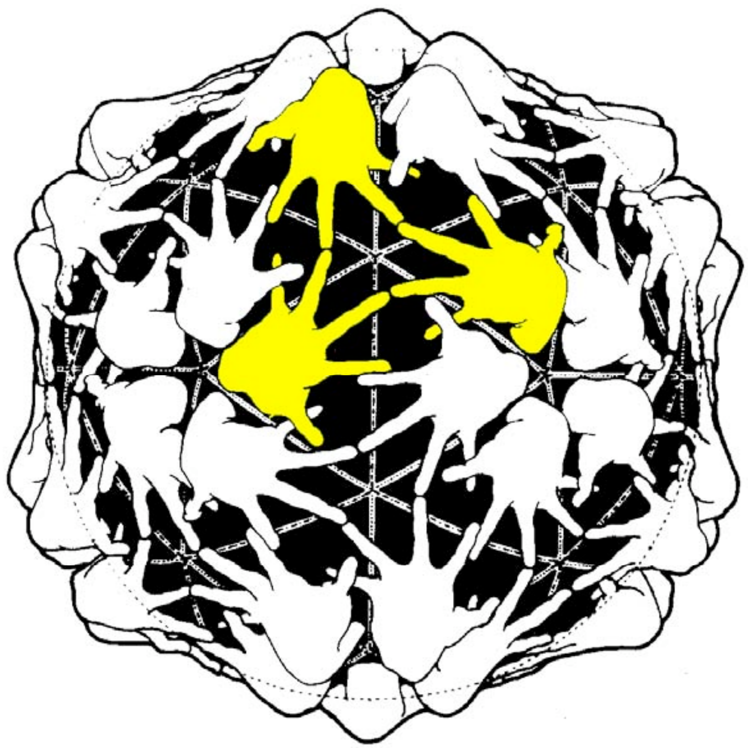
Icosahedral (532) Point Group Symmetry



30 dimers

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

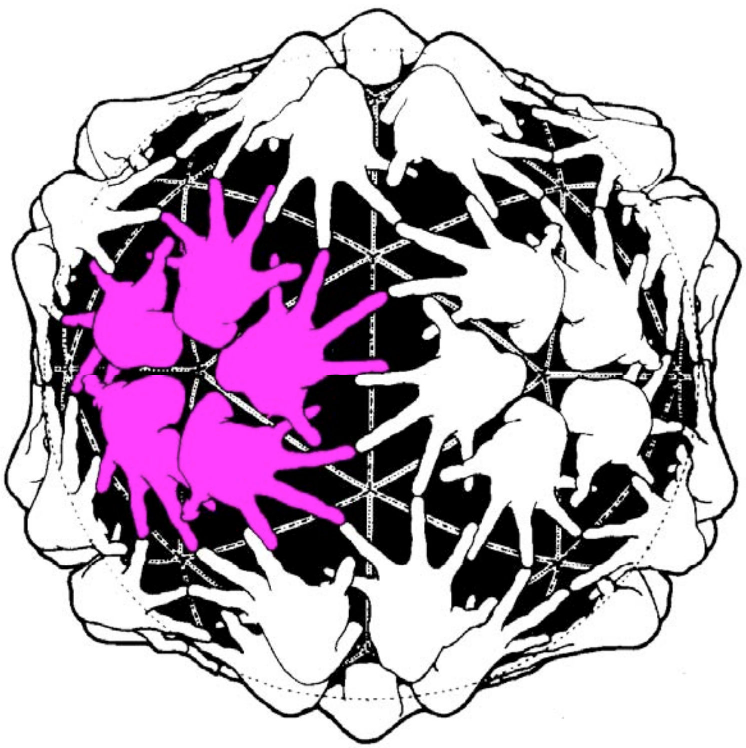
Icosahedral (532) Point Group Symmetry



20 trimers

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

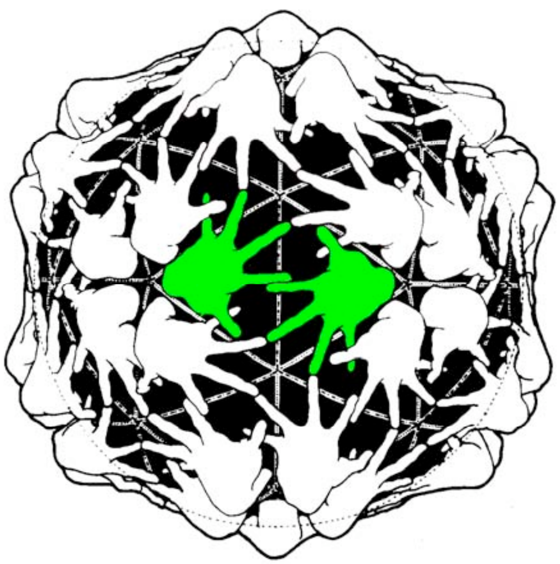
Icosahedral (532) Point Group Symmetry



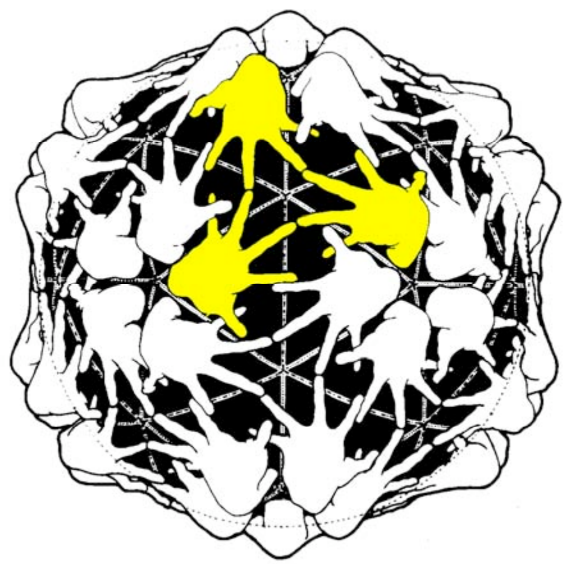
12 pentamers

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

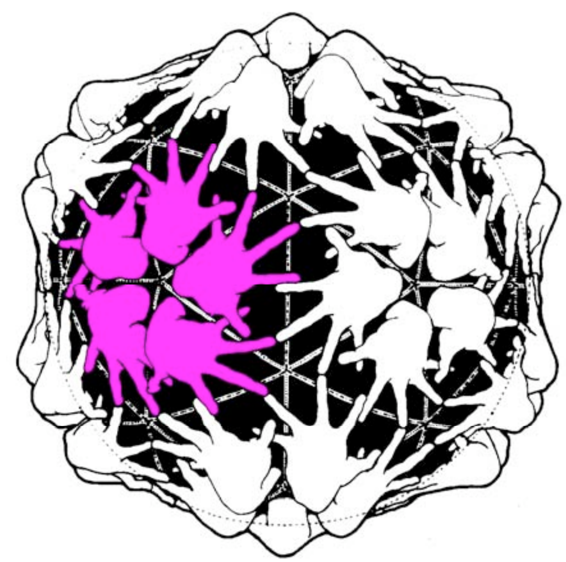
Icosahedral (532) Point Group Symmetry



30 dimers



20 trimers



12 pentamers

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

3D Reconstruction of Icosahedral Particles

Symmetry

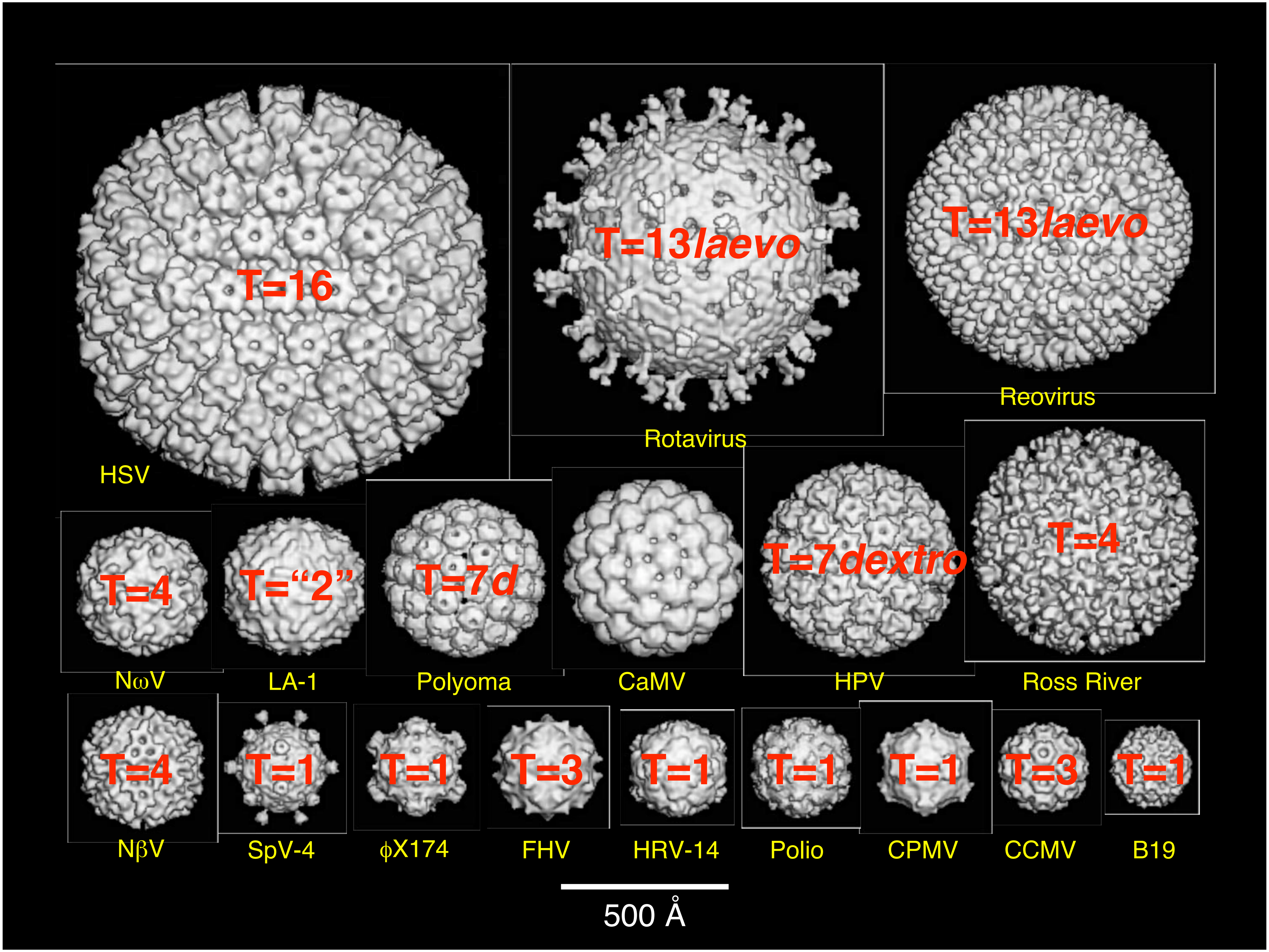
1. Icosahedral (532) point group symmetry

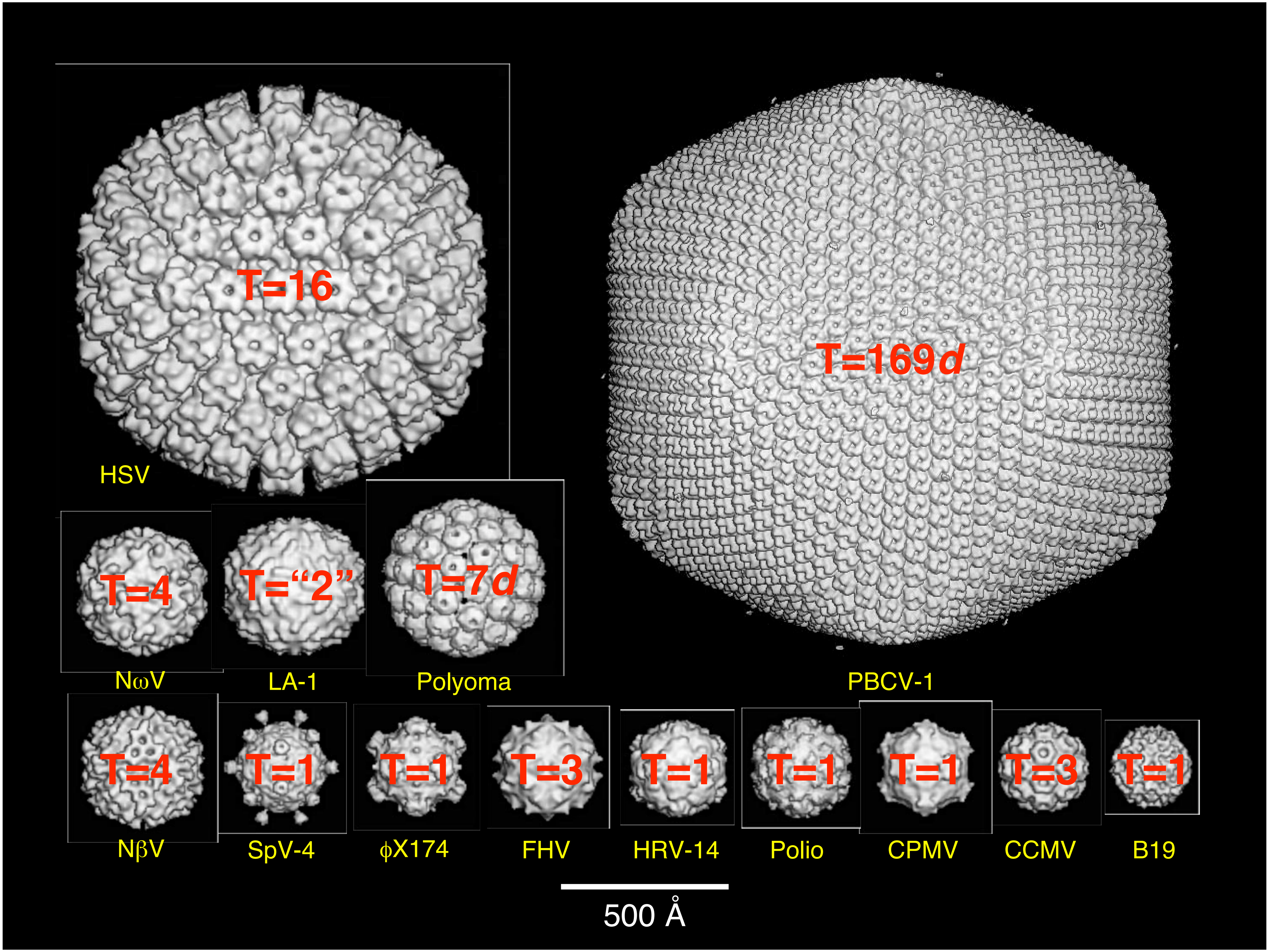
2. Triangulation symmetry

Purely mathematical concept (concerns lattices)

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

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


T=16
HSV
T=13laevo
Rotavirus
T=13laevo
Reovirus
T=4
NωV
T="2"
LA-1
T=7d
Polyoma
CaMV
T=7dextro
HPV
T=4
Ross River
T=4
NβV
T=1
SpV-4
T=1
φX174
T=3
FHV
T=1
HRV-14
T=1
Polio
T=1
CPMV
T=3
CCMV
T=1
B19
500 Å


T=16
HSV
T=169d
PBCV-1
T=4
NωV
T="2"
LA-1
T=7d
Polyoma
T=4
NβV
T=1
SpV-4
T=1
φX174
T=3
FHV
T=1
HRV-14
T=1
Polio
T=1
CPMV
T=3
CCMV
T=1
B19
500 Å

3D Reconstruction of Icosahedral Particles

Triangulation Number

Key Concept:

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

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

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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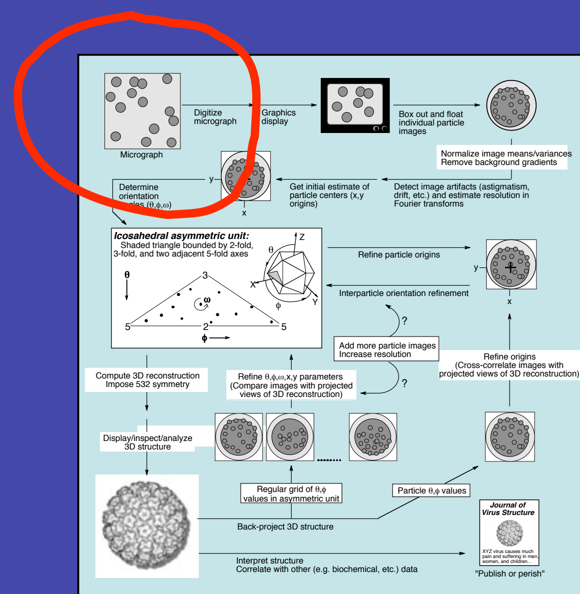


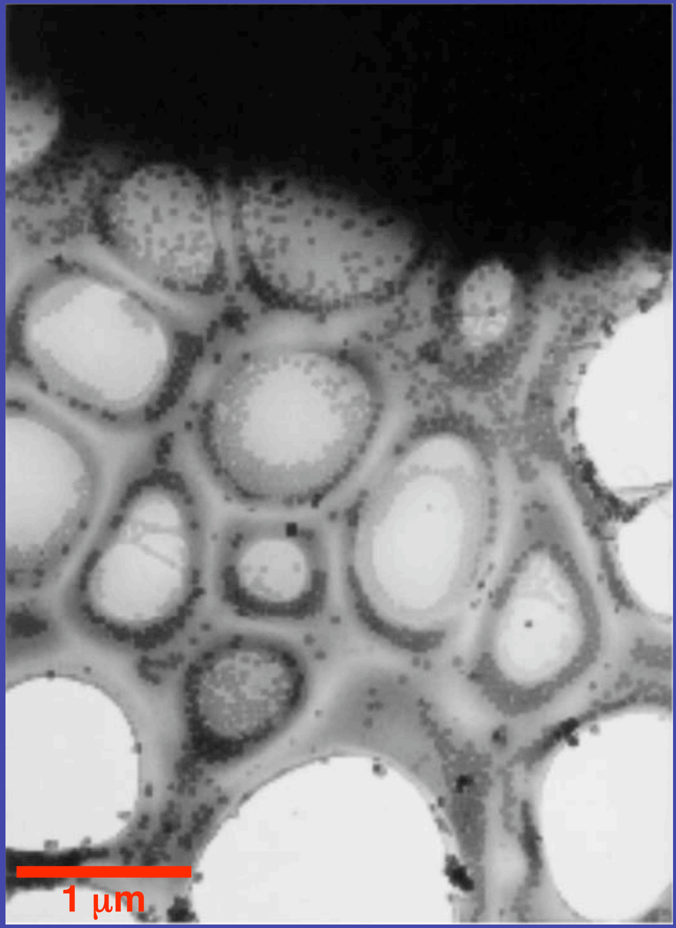
3D Reconstruction of Icosahedral Particles

Protocol

Electron Cryo-Microscopy

Sample : ~2-3 μl at 1-5 mg/ml

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



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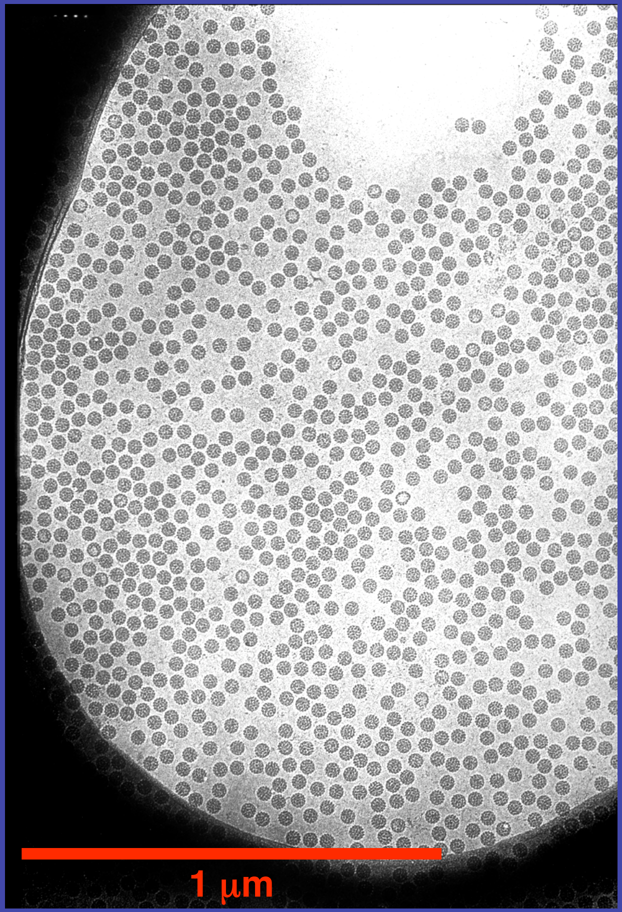

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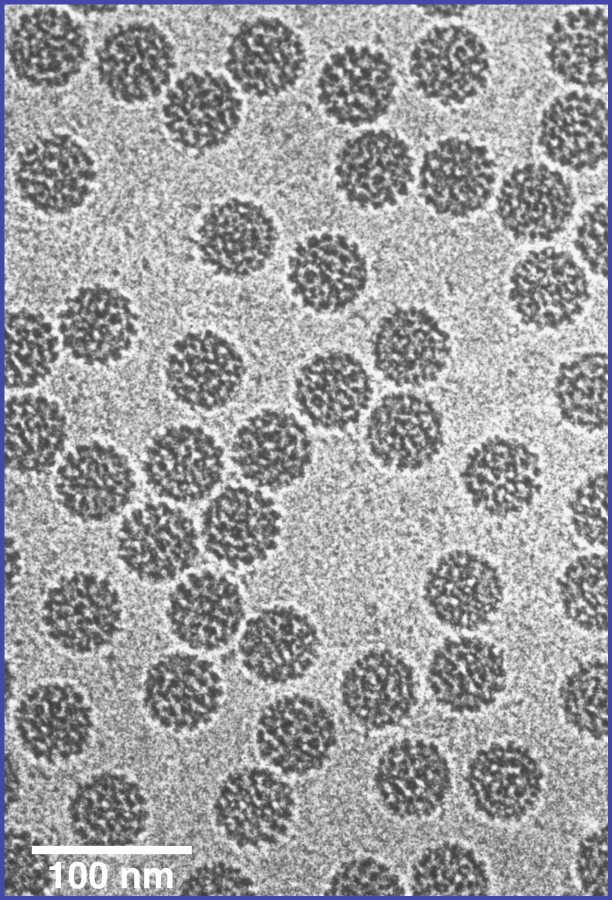


3D Reconstruction of Icosahedral Particles

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

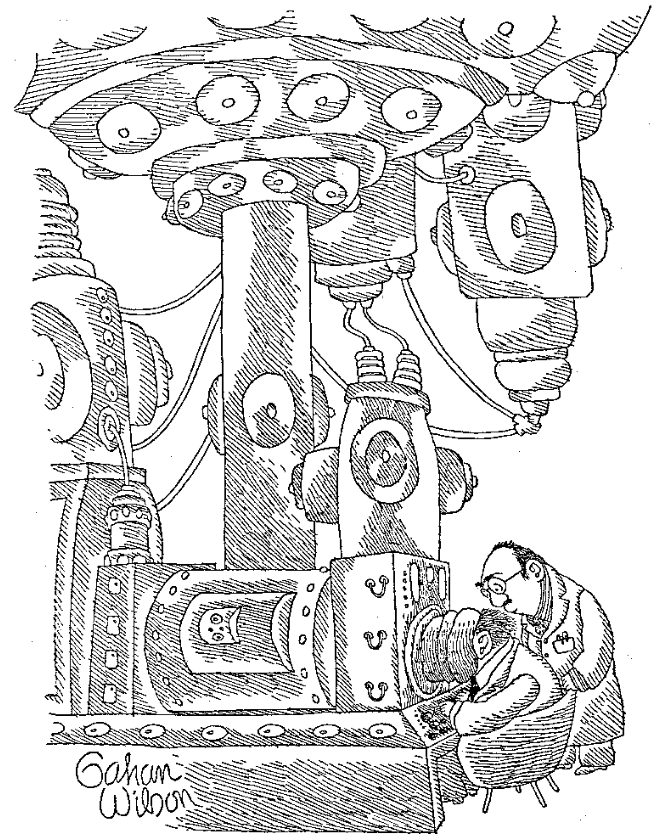
Dose: 10-20 e^-/Å^2

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

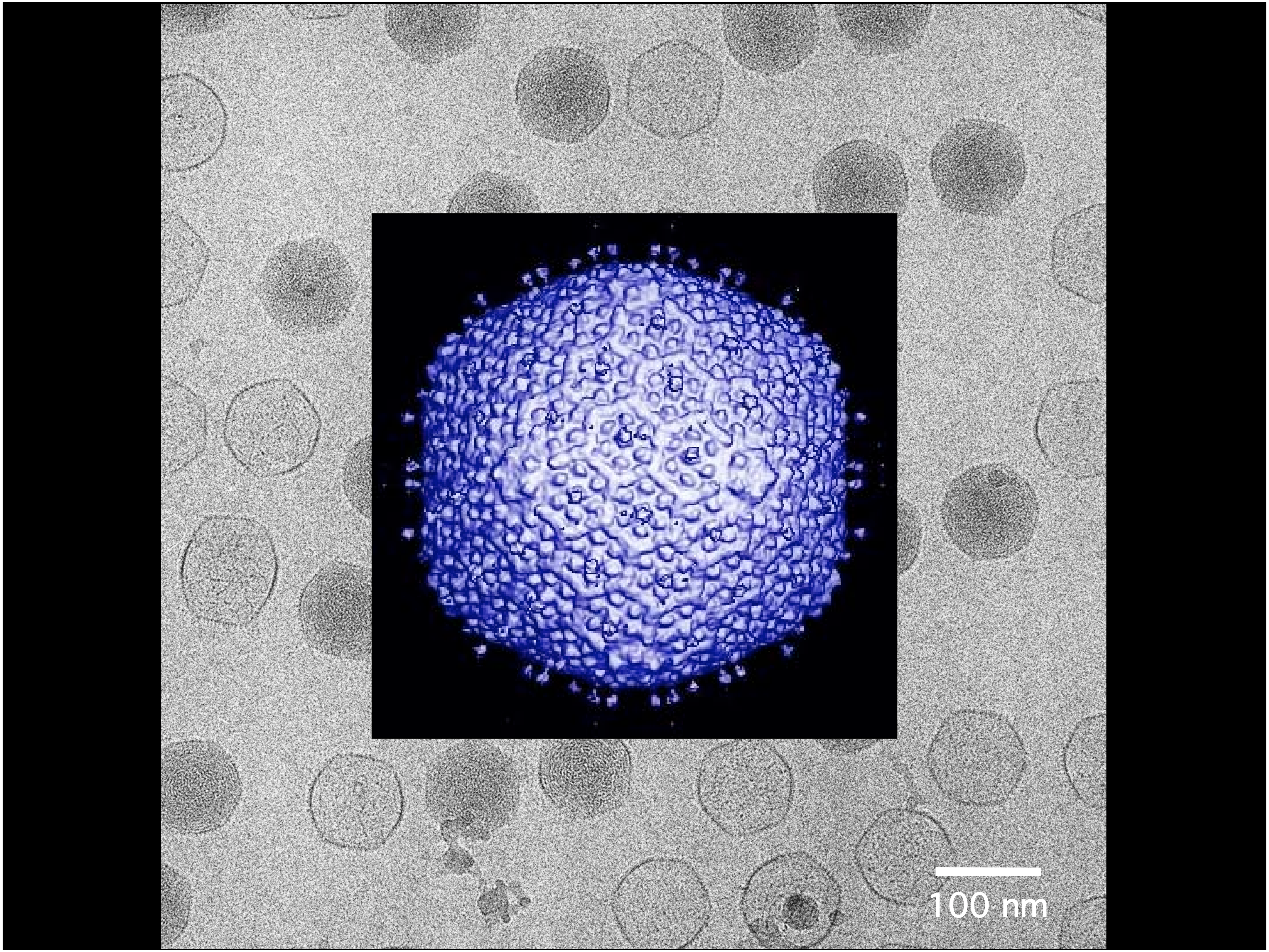
Micrographs: 25-100

Particles: 10^3-10^4

Target resolution: 12 - 6 Å



"I think I may have spotted something!"


100 nm