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

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

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:

$$F(R, \Phi) = \sum_n \int_{\text{object}} i^n g_n(r)J_n(2\pi rR)e^{in\Phi}2\pi rd\!r$$

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

$$= \sum_n G_n(R)e^{in(\Phi+\pi/2)}$$

radial variation

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:

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

and \( g_n \) and \( G_n \) are reciprocally related:

\[ g_n(r) = \int_{\text{transform}} G_n(R) J_n(2\pi r R) 2\pi R dR \]
III.D.5 3D Fourier Reconstruction Methods

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

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

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

Interpolation in Polar Coordinates - 2D Case

\[ G_n(R) = \int g_n(r)J_n(2\pi rR)2\pi r dr \]

\[ g_n(r) = \int G_n(R)J_n(2\pi rR)2\pi R dR \]

Overall scheme: Compute from \( F \rightarrow G \rightarrow g \rightarrow \rho \)
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

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

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](image)

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**

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:

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

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

Using shorthand:

$$F_j = \sum_{n} G_n B_{jn}$$

where

$$B_{jn} = i^n e^{in\Phi_j} = e^{in(\Phi_j + \pi/2)}$$
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, $\Phi_j$, 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 $Z = 1/6 \text{ nm}^{-1}$

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

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:

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

(1)

3-D Fourier transform has the form:

\[ F(R, \Phi, Z) = \sum_{n} G_n(R, Z)i^n e^{in\Phi} \]

(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:

\[ F(R, \Phi, Z) = \sum_{n} G_n(R, Z) i^n e^{in\Phi} \]  

(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:

\[ G_n(R,Z) = \int_{\text{object}} g_n(r,Z) J_n(2\pi r R) 2\pi r dr \]  

(2b)

\[ g_n(r,Z) = \int_{\text{transform}} G_n(R,Z) J_n(2\pi r R) 2\pi R dR \]  

(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:

\[ F(R, \Phi, Z) = \sum_{n} G_n(R, Z)i^n e^{in\Phi} \]  

On annulus \( R \) of transform plane \( Z \) (called “annulus \((R,Z)\)”) we have observations \( F(\Phi_j) \) at known \( \Phi_j \), so:

\[ F(\Phi_j) = \sum_{n} G_n i^n e^{in\Phi_j} \]
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

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

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j}$$  \hspace{1cm} (3)

This set of linear equations can be solved for $G_n$

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

$$g_n(r, Z) = \int G_n(R, Z)J_n(2\pi r R)2\pi R dR$$  \hspace{1cm} (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):

$$g_n(r, Z) = \int G_n(R, Z)J_n(2\pi rR)2\pi R dR$$

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

$$\rho(r, \phi, z) = \sum_{n=-\infty}^{\infty} \int_{-\infty}^{\infty} g_n(r, Z)e^{i n \phi} e^{2\pi i z/Z} dZ$$
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

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

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

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

$$F(\Phi_j) = \sum_{n} G_n i^n e^{in\Phi_j}$$  \hspace{1cm} (3)
3-D Reconstruction

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

This set of linear equations is solved by least squares procedures.

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

\[ F = BG \] (4)
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

$$F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j}$$  \hspace{1cm} (3)

$$F = BG$$  \hspace{1cm} (4)

Form the normal equations:

$$B\dagger F = B\dagger BG$$  \hspace{1cm} (5)
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction

\[ F(\Phi_j) = \sum_n G_n i^n e^{in\Phi_j} \]  
\( F = BG \)  
\[ B^\dagger F = B^\dagger BG \]

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

\[ G = \left( B^\dagger B \right)^{-1} B^\dagger F \]
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
III.D.5 3D Fourier Reconstruction Methods

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

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

3-D Reconstruction: Algebra Incorporating Symmetry
III.D.5 3D Fourier Reconstruction Methods

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

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):

\[ F(\Phi_j) = \sum_{all \ n} G_n i^n e^{in\Phi_j} \] (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 \((R,Z)\):

\[
F(\Phi_j) = \sum_{all \ n} G_n i^n e^{in\Phi_j}
\]

Use shorthand:

\[
G'_n = i^n G_n
\]

To get:

\[
F(\Phi_j) = \sum_{all \ n} G'_n e^{in\Phi_j}
\]
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

\[ F(\Phi_j) = \sum_{all \ n} G'_n e^{i n \Phi_j} \] (7)

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

\[
\begin{align*}
\text{At } \Phi_j: \quad A_j + iB_j &= \sum_{\text{positiven}} \left( G'_ne^{i\Phi_j} + G'_{-n}e^{-i\Phi_j} \right) \\
\text{At } -\Phi_j: \quad A_j - iB_j &= \sum_{\text{positiven}} \left( G'_ne^{-i\Phi_j} + G'_{-n}e^{i\Phi_j} \right)
\end{align*}
\] (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

\[ A \phi_j : \quad A_j + iB_j = \sum_{\text{positiven}} \left( G'_n e^{in\phi_j} + G'_{-n} e^{-in\phi_j} \right) \]

\[ A - \phi_j : \quad A_j - iB_j = \sum_{\text{positiven}} \left( G'_n e^{-in\phi_j} + G'_{-n} e^{in\phi_j} \right) \]

\[ 2A_j = \sum_{\text{positiven}} G'_n \left( e^{in\phi_j} + e^{-in\phi_j} \right) + G'_{-n} \left( e^{in\phi_j} + e^{-in\phi_j} \right) \]  \hspace{1cm} \text{(9)}

\[ 2iB_j = \sum_{\text{positiven}} G'_n \left( e^{in\phi_j} - e^{-in\phi_j} \right) - G'_{-n} \left( e^{in\phi_j} - e^{-in\phi_j} \right) \]  \hspace{1cm} \text{(10)}
3-D Reconstruction: Algebra Incorporating Symmetry

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

\[ e^{in\theta} = \cos(n\theta) + i \sin(n\theta) \]
\[ e^{-in\theta} = \cos(n\theta) - i \sin(n\theta) \]
\[ \cos(-n\theta) = \cos(n\theta) \]
\[ \sin(-n\theta) = -\sin(n\theta) \]
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

\[ 2A_j = \sum_{\text{positive } n} G'_n (e^{in\Phi_j} + e^{-in\Phi_j}) + G'_{-n} (e^{in\Phi_j} + e^{-in\Phi_j}) \]  \hspace{1cm} (9)

This can be rearranged to get:

\[ 2A_j = \sum_{\text{positive } n} (G'_n + G'_{-n}) (e^{in\Phi_j} + e^{-in\Phi_j}) \]  \hspace{1cm} (9c)

and

\[ 2A_j = \sum_{\text{positive } n} (G'_n + G'_{-n}) (2 \cos(n\Phi_j)) \]  \hspace{1cm} (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

\[ 2iB_j = \sum_{\text{positive } n} G'_n(e^{in\Phi_j} - e^{-in\Phi_j}) - G'_{-n}(e^{in\Phi_j} - e^{-in\Phi_j}) \]  

(10)

This can be rearranged to get:

\[ 2iB_j = \sum_{\text{positive } n} (G'_n - G'_{-n})(e^{in\Phi_j} - e^{-in\Phi_j}) \]  

(10c)

and

\[ 2iB_j = \sum_{\text{positive } n} (G'_n - G'_{-n})(2i \sin(n\Phi_j)) \]  

(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

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

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

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

$$A_j = \sum (G'_n + G'_{-n}) \cos(n\Phi_j)$$

$$B_j = \sum (G'_n - G'_{-n}) \sin(n\Phi_j)$$

(11)
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

\[
\begin{align*}
A_j &= \sum (G'_n + G'_{-n}) \cos(n\Phi_j) \\
B_j &= \sum (G'_n - G'_{-n}) \sin(n\Phi_j)
\end{align*}
\]

(11)

Since \(A_j, B_j, \Phi_j\) 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 \(G_n\) and \(G_{-n}\) 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

\[
\begin{align*}
A_j &= \sum (G'_n + G'_{-n}) \cos(n\Phi_j) \\
B_j &= \sum (G'_n - G'_{-n}) \sin(n\Phi_j)
\end{align*}
\]

(11)

Since \(A_j, B_j, \Phi_j\) 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 \(n = \text{even}\)

\(G_n\) are imaginary for \(n = \text{odd}\)
III.D.5 3D Fourier Reconstruction Methods

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

3-D Reconstruction: Algebra Incorporating Symmetry

\[
\begin{align*}
A_j &= \sum (G'_n + G'_{-n}) \cos(n\Phi_j) \\
B_j &= \sum (G'_n - G'_{-n}) \sin(n\Phi_j)
\end{align*}
\]  

Since \( A_j, B_j, \Phi_j \) 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 \( n = \text{multiple of 5} \).
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):

$$g_n(r, Z) = \int G_n(R, Z) J_n(2\pi r R) 2\pi R dR$$  \hspace{1cm} (2c)

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

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


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

- Human wart virus
- Tomato bushy stunt
3D Reconstruction of Icosahedral Particles

REFERENCES


General principles of 3DR method

- Fourier-Bessel mathematics
- Common lines
3D Reconstruction of Icosahedral Particles

REFERENCES

- Reference list available as handout

- For die-hards:

3D Reconstruction of Icosahedral Particles

Outline

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3D Reconstruction of Icosahedral Particles

Symmetry

1. Icosahedral (532) point group symmetry

2. Triangulation symmetry
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

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

Asymmetric unit is 1/60^{th} 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
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
3D Reconstruction of Icosahedral Particles

Outline

- Background
  - References; examples; etc.

- Symmetry
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  - Triangulation symmetry

- “Typical” procedure (flow chart)
  - Digitization and boxing
  - Image preprocessing / CTF estimation
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  - 3D reconstruction with CTF corrections
  - Validation (resolution assessment)

- Current and future strategies
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)
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)
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)
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⁻/Å²
Film: SO-163 (12 min, full strength)
Micrographs: 25-100
Particles: 10³-10⁴
Target resolution: 12 - 6 Å