Making the most of symmetry

EMBO Practical Course: Image Processing for cryoEM
What is symmetry?

Perfect rotational symmetry

Nature is not perfect
Learning objectives

1) Describe different types of symmetry common in structural biology

2) Distinguish when it is appropriate to apply symmetry

3) Design computational experiments to solve structures where there is a symmetry break

4) Review and report on the use of symmetry (point group, platonic and helical) and mismatches in cryoEM
Symmetry in structural biology

- Rotational (cyclic) symmetry ($C_n$)
  - Simplest is $C_1$
  - No perpendicular symmetry axes
- Platonic Solids
  - Faces, edges and corners are related by symmetry operations
  - Dihedral ($D_n$)
  - Tetrahedral (T)
  - Octahedral (O)
  - Icosahedral (I)
Cyclic and dihedral symmetries

(Cyclic symmetry)

C2 (2 subunits)

C4 (4 subunits)

(Dihedral symmetry)

D2 (4 subunits)

D4 (8 subunits)

(a) C3 symmetry  C4 symmetry  C22 symmetry

(b) C4 tetramer + C4 tetramer = D4 octamer

(Levy et al., PLoS computational Biology 2006)

(Xu et al., Curr Opin Struct Biol 2019)
Harmonic Analysis of Electron Microscope Images with Rotational Symmetry

R. A. Crowther and Linda A. Amos
Medical Research Council Laboratory of Molecular Biology
Hills Road, Cambridge, England
(Received 9 February 1971)

This paper describes a method of analysing images from electron micrographs of biological specimens believed to possess rotational symmetry. An objective analysis of the symmetry is possible because the method, which is computational, produces a rotational power spectrum of the image. We can then combine just those components which are consistent with the previously determined symmetry to produce a filtered image. The method is applied to the base plate of bacteriophage T4 and to discs of tobacco mosaic virus protein. The advantages of this new approach over the well-known Markham rotation technique are discussed.
Implementation in Xmipp

- Generate rotational power spectra
- K-means classification
- Statistical analysis with eigenvectors
- Limited by noise levels in the images

(Barcena et al., JMB 1998; Scheres et al., Nature Protocols 2008)
Multivariate statistical analysis (MSA) approach

- Finding essential variables
- Representing features as vectors
- Classification based on features

- Eigen images describe particle features
- Can be used as the basis for classification
- Choosing the number of classes

(White et al., Biomedical Research International 2017)
MSA applied to detect symmetry

Main advantage: ability to examine relationships among multiple variables at the same time

(White et al., Biomedical Research International 2017)
Is having symmetry an advantage for cryoEM?

https://b.socrative.com/login/student/

Room FE419C5F
How do I use it to my advantage?

- Smaller asymmetric unit
- Decrease computational resources
- Better averaging improves signal to noise
- Need less particles
$C_n$ symmetry
$D_n$ symmetries

The asymmetric $D_n$ for $d$ symmetries

Location of mirror projections for even $d$ symmetries

Location of mirror projections for odd $d$ symmetries
Platonic symmetries

Interactive asymmetric unit viewing using emimag3dsym.py in EMAN2
## Potential pitfalls

<table>
<thead>
<tr>
<th>Symmetry Group</th>
<th>Notation</th>
<th>Origin</th>
<th>Orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymmetric</td>
<td>C1</td>
<td>User-defined</td>
<td>User-defined</td>
</tr>
<tr>
<td>Cyclic</td>
<td>C&lt;n&gt;</td>
<td>On symm axis, Z user-defined</td>
<td>Symm axis on Z</td>
</tr>
<tr>
<td>Dihedral</td>
<td>D&lt;n&gt;</td>
<td>Intersection of symm axes</td>
<td>principle symm axis on Z, 2-fold on X</td>
</tr>
<tr>
<td>Tetrahedral</td>
<td>T</td>
<td>Intersection of symm axes</td>
<td>3-fold axis on Z (deviating from Heymann et al!)</td>
</tr>
<tr>
<td>Octahedral</td>
<td>O</td>
<td>Intersection of symm axes</td>
<td>4-fold axes on X, Y, Z</td>
</tr>
<tr>
<td>Icosahedral</td>
<td>I&lt;n&gt;</td>
<td>Intersection of symm axes</td>
<td>++</td>
</tr>
</tbody>
</table>

RELION symmetry conventions and axis definitions, but not all programs are the same!
Icosahedral symmetry and viruses

Procedures for three-dimensional reconstruction of spherical viruses by Fourier synthesis from electron micrographs

By R. A. Crowther

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge
Icosahedral symmetry and viruses

- A powerful averaging tool
- Improve your signal to noise
- Need less data
- Smaller asymmetric unit speeds up processing

Zika virus (Sirohi et al., Science 2016)

Herpesvirus

(Yuan et al., Science 2018)
But remember, nature is not perfect!
Symmetry breaks and mismatches

- Symmetry mismatch
- At low resolution seems symmetric
- Flexibility
- Sub-stoichiometric binding

Symmetry breaks and viruses

φ6 phage and its dsRNA genome (Ilca et al., Nature 2019)
Symmetry breaks and viruses

Calicivirus (norovirus)
(Conley et al., Nature 2019)

But infection is an asymmetric process...

Use symmetry to get high resolution....
Symmetry breaks and GroEL

Exploring flexibility of GroEL monomers
How would you do it??
Remove signal from dominant symmetry

Local reconstruction (Scipion)


(Ilca et al., Nature 2019)
Using symmetry to reduce your search range

Symmetry expansion
3D classification no refinement
- Increase the T value (Relion)
- Increase the number of classes
- Don’t make your mask too small

(Conley et al., Nature 2019)
Conformational variability of GroEL
Conformational variability of GroEL
Helical reconstruction
Helical Symmetry

Geometry of a helix: pitch and radius

A single view contains all the necessary info for 3D
2D surface lattice rolled into 3D
Bessel function: cylindrical harmonic
Fourier Bessel analysis

(Diaz et al. Methods in Enzymology 2010)

2D Fourier Transform
Fourier Bessel method for helical reconstruction

Reconstruction of Three Dimensional Structures from Electron Micrographs

by
D. J. DE ROSIER
A. KLUG
MRC Laboratory of Molecular Biology,
Hills Road, Cambridge

General principles are formulated for the objective reconstruction of a three dimensional object from a set of electron microscope images. These principles are applied to the calculation of a three dimensional density map of the tail of bacteriophage T4.

Limitations

- Laborious
- Small inaccuracies in indexing lead to incorrect structures!
- Requires strict helical symmetry
- Requires flat straight helices
Iterative Real-Space Refinement (IHRSR)

Single Particle Approach

Parameters

- Outer and inner radii of cylinder
- Pitch
- Repeat (2x pitch)

Advantages

- Cope with heterogeneous specimens
- Reconstruct filaments that diffract weakly, i.e. when layer lines not visualized.

(Behrmann et al., J Struct Biol 2012)
Iterative Real-Space Refinement (IHRSR)

(Behrmann et al., J Struct Biol 2012)
RELION and helical symmetry

Incorporating prior information about the orientations

(He & Scheres., J Struct Biol 2017)
Potential pitfalls

What to look out for

- Does your map look like a protein?
- Is your FSC smooth?
- What’s happening to your noise?

(He & Scheres., J Struct Biol 2017)
Helical mixed symmetry populations

Approach

- Principal component analysis
- K means classification

(Pothula et al., Ultramicroscopy 2019)
RELION local symmetry applied to helices

Approach

- Define local symmetry operators
- Place PDB into map
- Create masks from PDB
- Global & local search for symmetry operators
- Verification of symmetry operators
- Impose local symmetry in 3D classification/refinement

(Deng et al., PNAS 2017)
MicroTuble Binding Domain of dynein (MTBD) bound to microtubules

(Lacey et al., eLIFE 2019)

- 2D classification: decoration/stoichiometry
- 3D classification: decoration/stoichiometry
- 3D refinement (C1) with 13-fold local symmetry
Think, digest, DRAW!