3D Reconstruction of Helical Specimens
Many Biological Specimens have helical symmetry

- DNA
- α-Helix
- Viruses (TMV)
- Actin filaments
- Myosin filaments
- Microtubules
- Bacterial Flagella
- Protein-lipid tubes
Reconstruction of Three Dimensional Structures from Electron Micrographs

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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.

Our method starts from the obvious premise that more than one view is generally needed to see an object in three dimensions. We determine the number of views required for reconstructing an object to a given degree of resolution and find a systematic way of obtaining these views. The electron microscope images corresponding to these different views are then combined mathematically, by a procedure which is both quantitative and free from arbitrary assumptions, to give the three dimensional structure in a tangible and permanent form.

Summary of Procedure

Electron micrographs are selected in which the details of the structure are best, as judged for example by the phase and contrast discrimination factor. The optimal density in each image is sampled at regular points on a grid by an automatic microdensitometer linked to a computer (unpublished work of U. W. Arndt, M. A. Overchir and J. F. V. Martin), which converts the image into a set of numbers representing the density at each grid point. These numbers are then translated by computation into a set of Fourier coefficients.
- Helix definition.
- Fourier Transform of a Helix.
- Fourier-Bessel Helical 3D reconstruction
- Real space Helical 3D reconstruction.
- Some examples.
Combining the symmetry operation of translation and rotation (screw) produces a helix.

Possible Symmetry operations:
- Screw.
- n-fold rotation about axis.
- 2-fold rotation perpendicular to axis.
Parameters of a Helix

- **Φ**: Azimuthal rotation angle
- **Pitch** = P
- **Radius** = r
- **Repeat** = C
- **Subunit Axial Translation** = h
Helices give several orientation views of the asymmetric unit from a single view direction.
3D reconstruction approaches

2D crystals

Helical crystals

Single particles/viruses
tomography
No Missing Cone!

2D crystals

Helical crystals

missing cone

point-spread function

Diffraction data to 35 Å used for refinement.
The Helical Latice Radial Projection
Analogy between 2D lattices and Helical Lattices

Analogy between 2D Fourier synthesis and Fourier-Bessel helical synthesis

Summation of 2D waves to produce a 2D density map. (From Jeffery 1972)
The Fourier Transform of a Helix

\[ T(R, \psi, n/P) = J_n(2\pi Rr) \exp \left[ in(\psi + \frac{1}{2}\pi) \right] \]

Cochran, Crick & Vand 1952

Fig. 1. (a) Cartesian \((x, y, z)\) and cylindrical-polar \((r, \phi, z)\) coordinates of a point on a helix. (b) Corresponding coordinates of a point in reciprocal space.

Fig. 2. Illustration of Bessel functions. (Reproduced by kind permission of the publishers from *Tables of Functions* by Jahnke & Emde. New York: Dover Publications.)
The Transform of a discontinuous helix

(b) \( T(A \times B) = T(A) \times T(B) \)
A helix and its corresponding Fourier Transform (Power Spectrum)
The DNA Structure
The Fourier Bessel Transform

\[ T(R, \psi, n/P) = J_n(2\pi R r) \exp \left[ i n(\psi + \frac{1}{2} \pi) \right] \]

Cochran, Crick & Vand 1952

Tranform of group of j atoms at different radii

\[ G_{n,l}(R) = \sum_j f_j J_n(2\pi R r_j) \exp \left[ i \left( -n\varphi_j + \frac{2\pi l z_j}{c} \right) \right]. \]

\[ F(R, \psi, l/c) = \sum_n G_{n,l}(R) \exp \left[ i n(\psi + \frac{1}{2} \pi) \right], \]

Klug, Crick & Wickoff 1958

Fig. 1. (a) Cartesian \((x, y, z)\) and cylindrical-polar \((r, \varphi, z)\) coordinates of a point on a helix. (b) Corresponding coordinates of a point in reciprocal space.
Helical 3D reconstruction Using the Fourier-Bessel Method

DeRosier & Moore J. Mol. Biol. 52:335 1970

Fourier Transform
Reciprocal Space Function

\[ F(R, \Phi, l/c) = \sum_n G_{n,l}(R) \exp \left[ i n (\Phi + \pi/2) \right]. \]  \hspace{1cm} (1)

Selection rule \[ l = tn + um \] \hspace{1cm} (2)

Real space function (structure)

\[ \rho(r, \phi, z) = \sum_l \sum_n g_{n,l}(r) \exp(in\phi) \exp\left(-2\pi ilz/c\right) \] \hspace{1cm} (3)

\[ g_{n,l}(r) = \int G_{n,l}(R)J_n(2\pi Rr) 2\pi RdR \]
Why we can get a 3D structure from a single view?
The real and reciprocal space (Fourier transform) arguments

\[ F(R, \Phi, l/c) = \sum_n G_{n,1}(R) \exp \left[in (\Phi + \pi/2)\right]. \]

\[ F(R, \Phi, l/c) = G_{n,1}(R) \exp \left[in (\Phi + \pi/2)\right]. \]
The Selection Rule

4 units in 1 Turn (RH)

13 units in 6 Turns (LH)


http://www.ccp13.ac.uk/software/program/Helix/INDEX.htm
The Selection Rule

\[ l = tn + um \]

- **l**: Layer line Number.
- **t**: Num. of turns/rep.
- **n**: Num of Helical starts & bessel order.
- **u**: Num. of subunits/rep
- **m**: Integer

If \( k \)-fold rotational symmetry then:
\( n \) must be multiple of \( k \)

\[ Z = n(\Omega/360^0) /h + m /h \]

- **Z**: LL reciprocal spacing
- **\( \Omega \)**: twist angle.
- **h**: rise distance.
Selection Rule Example

\[ l = tn + um \]

\[ l = -6n + 13m \]

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Indexing the diffraction Pattern

Helical 3D reconstruction Using the Fourier-Bessel Method
DeRosier & Moore J. Mol. Biol. 52:335 1970

**Fig. 1.** Schematic diagram of the PHOELIX helical processing package. A detailed description of each step and the programs used is available as part of the PHOELIX distribution.
Boxing & Floating Image

Image

FFT

Non-Floated

Floated
Straightening

Finding Helical Symmetry Selection Rule
Indexing the diffraction Pattern
The Diffraction Pattern of a Helix Has Reflections From Planes in the Front and Back of the Helix

Different from the case of the transform of a 2D lattice where reflections from a set of planes form a spot in Fourier space. In the case of a helix the reflections are continuous Bessel function along “layer” lines.
Drawing the Reciprocal Lattice

Figure 3

Wrong

Right
Clues to trace the Reciprocal Helical Lattice:

- The dimension of the unit vectors should be approximately equal to the inverse of the subunits dimensions. $d = 1.34(m)^{1/3}$ (d in Å, m in daltons) (e.g. actin dimensions ~ 5 nm)

- Approximate value of $|n|$ for each layer line is: $|n| + 2 = 2 \pi Rr$. $r$: Helix radius, $R$: Reciprocal of layer line peak position to the meridian.

- Determine if $n$ is odd or even by looking at mirror symmetric peaks from the meridian. Even if same phase, Odd if phase diff = 180°.

- Determine hand of helical paths (sign of $n$). Shadow or tilt specimens.

- Draw $n,Z$ plot.
$J_n(2\pi Rr)$, $1^{\text{st}}$ max at $R \approx (|n|+2)/(2\pi r)$

Use radial position to determine Bessel order (approximation)
- radius hard to measure with defocus fringes
- different radii of contrast for different helical families
- particle may be flattened
Determining the hand of the helical paths (bessel orders)

Convention:
- Right handed: $n > 0$
- Left handed: $n < 0$
Determining the hand of the helical path by EM metal shadowing

Determining the hand of the helical path by tilting specimen

Left handed

Serrated Pattern on Left
Left Handed
Gathering Amplitude and Phases

\[ \Psi = -\frac{\pi}{2} \]

\[ \Psi = \frac{\pi}{2} \]

\[ F(R, -\pi/2, l/c) = G_{n,l} \]

\[ F(R, +\pi/2, l/c) = G_{n,l} \text{ (for even } n) \]

\[ -G_{n,l} \text{ (for odd } n) \]
Filament image corrections

Plane Shift and out of plane tilt corrections

Phases Differences in Mirror symmetric peaks from the meridian are predicted. $180^0$ for odd $n$ & $0$ for even $n$.

Plane shift and out of plane tilt produce systematic phase differences that can be corrected.

$$F(R, \Phi, l/c) = \sum_n G_n l(R) \exp \left[ i n (\Phi + \pi/2) \right].$$

$$\Delta \alpha_{\text{tilt}} = -2n \times \tan^{-1} \left( \frac{Z \sin (\omega)}{R} \right).$$

$$\Delta \alpha_{\text{shift}} = 4\pi R \Delta x.$$
Lattice distortion Corrections

Alignemt of short filament segments (1/3 of local repeat length) against a reference structure

stretch, bend, helical twist, flattening, tilt, shrinkage.

Beroukhim & Unwin. 1997
Averaging several filament/images:

Case 1: Identical helical symmetry (selection rule).

Case 2: Slightly different symmetry.

Case 3: Different Helical classes
Case 1: Same symmetry (same selection rule)

1) Rotate and Z displace filaments to the same origin. (Minimize phase differences in layer line data sets)

\[ G'_{n, Z}(R, Z) = G_{n, Z}(R, Z) e^{-i n \Delta \phi + 2\pi i \Delta z Z} \]

\( \Delta \phi \): Azimuthal angle difference

\( \Delta z \): Shift along the axis

\[ \sqrt{\langle \Delta \alpha^2_w \rangle} = \sqrt{\frac{1}{\sum_n \sum_j |G_{n, Z}(R_j, Z_n)| |G_{n, Z}(R_j, Z_n)|^2 |\alpha(R_j, Z_n) - \tilde{\alpha}(R_j, Z_n)|^2} \]

Phase residual

2) Average layer line data sets (CTF weighted average if necessary)
Case 2: Slightly different symmetry due to slight winding or unwinding of the helix resulting in changes in axial repeat distance.

13 units in 6 Turns (LH)

54 units in 25 Turns (LH)
Very similar spacings & bessel orders (n) but different layer line number (l).

13 units in 6 Turns (LH)

54 units in 25 Turns (LH)
Case 3: Different Helical Classes

Method 1, Fourier Space Method:

Apply appropriate phase and radial shift to $g_{ni}(r,Z)$ values from different particles and then average them.

Use: If all particles have identical 2D lattices but different circumferential vectors.

**Case 3: Different Helical Classes**

**Method 2, Real Space Method:**

- Calculate separate 3D reconstructions for each filament.
- Carve out the asymmetric unit on each of the reconstructions.
- Generate a new artificial layer line set for each reconstruction by applying helical symmetry (the same for all particles) to the carved out asymmetric unit.
- Align the artificial helices in reciprocal space and average their layer line data (as normally done in Fourier-Bessel helical reconstruction).
- Obtain an averaged map by Fourier Bessel inversion.


*Yonekura & Toyoshima. Ultramicroscopy 84: 15-28 (2000)*
3D Density Map Reconstruction
(Fourier Bessel inversion)

Average $G_{n,l}$ Data set.

$$g_{n,l}(r) = \int G_{n,l}(R)J_n(2\pi R r) 2\pi R dR$$

$$\rho(r, \phi, z) = \sum_l \sum_n g_{n,l}(r) \exp(in \phi) \exp(-2\pi ilz/c)$$
Is it always a single view enough for helical 3D reconstruction?

Yes: If there is no overlap of Bessel functions at the resolution of interest. No if there is overlap.

\[ l = -6n + 13m \]
Resolution Criteria

Phase Residuals Along layer lines

Fourier Shell Correlation

\[
FSC(k, \Delta k) = \frac{\sum_{(k, \Delta k)} F_1(k) F_2^*(k)}{\sqrt{\sum_{(k, \Delta k)} |F_1(k)|^2 \sum_{(k, \Delta k)} |F_2(k)|^2}}^{1/2},
\]
Kinesin13-Microtubule Ring Complex Helical Reconstruction
3D Helical Reconstruction
Examples (All done by Fourier-Bessel method)

Acto-myosin complex

Ca-ATPase
Tubular crystal
$E_2$ state ($VO_4$)

Microtubule-NCD complex

Ach Receptor @ 4A Unwin  PNAS 2005
3D Helical Reconstruction Using Real Space Methods

Individual images are boxed out of the filament at each asymmetric unit axial spacing and a view angle is assigned according to the helical symmetry of the filament.

A 3D volumen is obtained by back-projection of the boxed images.

From Sosa et al. JSB 118: 149-158(1997)
'Single Particle' Helical Reconstruction Methods

Box Filament segments

In-plane Rotational Alignment

Multi-reference alignment with atomic models

8 Å resolution microtubule map

The Iterative Helical Real Space Reconstruction Method (IHRSM)

Some structures solved using the IHRSR method

- Tarantula striated muscle myosin filament (r=2.5 nm). Woodhead et al., 2005
- Dynamin (r=0.2 nm). (Mears et al., 2007)
- Filamentous Bacteriophage (r=0.8 nm). Wang et al., 2009

Fourier-Bessel method vs. IHRSR:
F-Actin @ 6.6 Å resolution
(~120000 asymmetric units)

TMV @ 3.3 Å Resolution
(~1.9 x10^6 asymmetric units)
Software for Helical 3D Reconstruction

Fourier-Bessel

- MRC Package
- Brandeis
- Phoelix & Suprim
- Unwin’s routines
- Toyoshima’s routines
- Ruby-Helix (Kikkawa’s lab)
- EMIP (Stoke’s lab)

Real space or single–particle-like iterative refinement software

- IHRSR (Egelman’s lab)
- SPRING (Sachse’s lab)
- FREALIGN (Grigorieff’s lab)
- EMGlue (Sosa’s lab)
- EMAN, SPARX, SPIDER
3D Helical Reconstruction Workflow

- Pick filaments & mark axis. (EMAN Boxer, Others)
- Preliminary low resolution model (Custom)
- Classify filaments. (Custom, SPIDER, EMAN)
- **Determine helical symmetry** ($\phi$, $h$) (Custom)
- Extract “single particle” boxes (Spider)
- Determine 3D orientation of single particle boxes (projection matching) & make 3D reconstruction (Spider, Custom)
- Refine 3D map (IHRSR).
- Refine 3D map (FREALIGN)
- Refine 3D map (RELION)
- Model Building (UCSF-Chimera, Coot, Phenix, Direx, Modeller)
Docking Atomic Structures into Electron Density Maps

Tan et al., Structure Nov. 2008


