**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



[R. 164] Sone Materia, Vol. 117, No. 1124, pp. 130 (1), Source 13, 1966

#### Reconstruction of Three Dimensional Structures from Electron Micrographs

#### D. J. DE ROSIER

MLC Laboratory of Hulseular Biology, H.B. Rosel, Cambridge

The standard high resolution shorton moreover has a depth of faces of several theorem 4 department, making the image a two dimensional superposition of different levels is the time dimensional structure. The faces oneset be adjusted to different levels within the object, and so three dimensional structures are different to anotype. Researchering micrographs do not systemate this diffoutly antidenticity, as will be object.

Our method staris from the obvious pressive that more than one view is generally resolute to not an object in three dimensions. We determine first the number of views regulared for resonantenting an object in a given degree of resolution and find a systematic way of obtaining these twices. The determine integration images corresponding is these different views are then multi-out motiomatingly, by a procedure which is both quantitative and free from arbitrary assumptions, to give the three dimensional dimension in tangello and permanent form. The method is most prevential for objects containing symmetricity arranged schematis, for here a single image

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 may of the call of bacteriophage 74.

> effectively contains many different views of the structure. The symmetry of such as object cases be introduced into the process of reconstruction, allowing the three dimensional structure to be reconstructed from a single view, or a small number of views. In principle, however, the method is applicable to may hild of structure, hadaing individual, unsymmetrical particles, or sections of hadapied unsymmetrical particles.

#### Summary of Procedure

Elements interceptspin are indexted in which the details of the structures show up host, as judged for examples in the phage toil described inter, by their optical diffustion patterns<sup>16</sup>. The optical density in real-image, is sampled as regular points in a grid by in entrematic incrediscipationswire links of the asymptotic supplicability work of U, W. Aruch, H. A. Crowther and J. F. W. Malkert, which construct the imagin into a set of numbers representing the density at each grid point. These numbers are nor transformed by computation into a set of Pospise





#### De Rosier & Klug. Nature 217: 130-134 1968

## - Helix definition.

- Fourier Transform of a Helix.
- Fourier-Bessel Helical 3D reconstruction
- Real space Helical 3D reconstruction.
- Some examples.

## **Helical Symmetry**

Combining the 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**



## Helices give several orientation views of the asymmetric unit from a single view direction





## 3D reconstruction approaches



Helical crystals





tomography



Single particles/viruses



## **No Missing Cone !**



0.3

## The Helical Latice Radial Projection



## Analogy between 2D lattices and Helical Lattices





From: Toyoshima (2000) Ultramicroscopy 84: 1-14

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



Summation od 2D waves to produce a 2D density map. (From Jeffery 1972)



A helical wave

## 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  $(r, \varphi, z)$  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



## A helix and its corresponding Fourier Transform (Power Spectrum)



## **The DNA Structure**



#### The Fourier Bessel Transform

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

Cochran, Crick & Vand 1952



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

Tranform of group of j atoms at differnt radii

$$G_{n,l}(R) = \sum_{j} f_j J_n(2\pi R r_j) \exp\left[i\left(-n\varphi_j + rac{2\pi l z_j}{c}
ight)
ight].$$

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

Klug, Crick & Wickoff 1958

## **Helical 3D reconstruction Using the Fourier-Bessel Method**

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

**Fourier Transform** Reciprocal Space Function  $F(R, \Phi, l/c) = \sum G_{n,l}(R) \exp [in (\Phi + \pi/2)].$ 

l = tn + umSelection rule  $\rho(r, \phi, z) = \sum_{l} \sum_{n} g_{n,l}(r) \exp(in \phi) \exp(-2\pi i l z/c)$ Real space function

(structure)

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

(1)

(2)

(3)



Why we can get a 3D structure from a single view? The real and reciprocal space (Fourier transform) arguments

> $F(R, \Phi, l/c) = \sum_{n} G_{n,l}(R) \exp [in (\Phi + \pi/2)].$  $F(R, \Phi, l/c) = G_{n,l}(R) \exp [in (\Phi + \pi/2)].$





## The Selection Rule



Knupp C, Squire JM, HELIX: A helical diffraction simulation program, J Appl Cryst, 2004, Vol: 37, Pages: 832 - 835

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* 

 $Z = n(\Omega/360^{\circ}) / h + m / h$ Z: LL reciprocal spacing  $\Omega$ : twist angle. h: rise distance.



## Selection Rule Example



l = -6n + 13m

## Indexing the diffraction Pattern



From: Diaz et al., Methodsin Enzymol.482: 131-165 (2010)



## Helical 3D reconstruction Using the **Fourier-Bessel Method**

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



FIG. 1. The scheme presented shows the flow of data in the process of three-dimensional reconstruction.

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



#### Non-Floated

Floated

## Straightening

- sIs2.orig		
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- sla2.final	CONTRACTOR DE CARACTERISTICA DE LA CONTRACTOR DE LA CONTRACT	
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sta2.pow.tinal		
		e
*in s_getspacing determining layer line spacing of straightened, intercolated filament		
<pre>*in s finalpeks everching for pesks Intercept 1 : 52.804 Intercept 2 : 64.818 Intercept 3 : 184.846 Intercept 3 : 184.846 Intercept 3 : 184.846 Intercept 5 : 188.88 Intercept 6 : 208.816 Intercept 7 : 231.895 Intercept 8 : 264.119 *in s_mark making dia file of layer line values *in s_csx making files for selection rule 13 6 making files for selection rule 28 13 making files for selection rule 54 25 *in s_checkex checking eelection rule is 54 25 : Chi**2 = 8.986391 The selection rule is 54 25 : Chi**2 = 8.986391 The selection rule of the final scaled filament has not changed. Layer line spacing found to be : 6.2886887 *in s_float</pre>	Intensity x 10 <sup>-3</sup> s1a2.pow.final.colb           140.00         140.00           120.00         100.00           80.00         60.00           40.00         100.00           20.00         100.00	
Displaying final floated image	0.00 0.20 0.40 0.60 0.80	RLU × 10 <sup>3</sup>

From: Carragher et al., JSB 116: 107-112 (1996)

## 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 transfrom 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



#### 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 =  $180^{\circ}$ .

- Determine hand of helical paths (sign of n). Shadow or tilt specimens.
- Draw n,Z plot.



 $J_n(2\pi Rr)$ , 1<sup>st</sup> 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)



### Determining the hand of the helical path by EM metal shadowing



From: Hoenger & Gross J.Struct.Biol. 84: 425 (2008)

## Determining the hand of the helical path by tilting specimen

Left handed







Serrated Pattern on Left



Left Handed

## Gathering Amplitude and Phases



## Filament image corrections

## Plane Shift and out of plane tilt corrections

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

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

$$\Delta \alpha_{tilt} = -2n \times \tan^{-1} \left( \frac{Z \sin (\omega)}{R} \right).$$
$$\Delta \alpha_{shift} = 4\pi R \Delta x.$$

 $F(R, \Phi, l/c) = \sum_{n,l} G_{n,l}(R) \exp [in (\Phi + \pi/2)].$ 



## 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.

Corrected



Uncorrected

REFINE **Overall** procedure Bun SEARCH.COM on Digitise image whole repeats to find ω, x, z, φ and rscale Estimate CTF and axial Run ROTX.COM to repeat distance of tube find 0 by fitting Fourier amplitudes to reference Initial boxing and alignment of whole repeats Bun REPEAT.COM to Iterate until find repeat distance parameters do by maximising not change amplitudes on layer-lines Run REFINE to align each repeat, divide into 1/6-segments, and find w, x, z, φ and rscale for each Run SEARCH.COM again Divide repeats into Use x and z locations of sixths 1/6-segments; run SEARCH.COM on each to find orientations of 1/3-segments SEARCH.COM Divide each repeat into 1/3segments, run SEARCH.COM to Estimate x and w by find (o, x, z, o) and rscale for comparing phases each; plot variations along across meridian Adjust parameters to minimise two-fold phase Find w by residual comparing phases across meridian Extract Fourier terms; find two-fold phase residual Find z,  $\phi$ , and Iterate until x does rscale by comparing not change phases to reference Make complete distortioncorrected dataset Refine x by comparing phases to reference

Beroukhim & Unwin. 1997

## **Averaging several filament/images:**

Case 1: Identical helical symmetry (selection rule).

Case2: Slightly different symmetry.

Case 3: Different Helical classes

## **Case 1: Same symmetry (same selection rule)**

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

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

 $\Delta \Phi$ : Azimuthal angle difference  $\Delta z$ : Shift along the axis

$$\sqrt{\langle \Delta \alpha_W^2 \rangle} = \sqrt{\frac{1}{\sum_{n} \sum_{j} |G_{n,Z}(R_j, Z_n)|} \sum_{n} \sum_{j} |G_{n,Z}(R_j, Z_n)| |\alpha(R_j, Z_n) - \overline{\alpha}(R_j, Z_n)|^2}}$$
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.



## Very similar spacings & bessel orders (n) but different layer line number (l).





54 units in 25 Turns (LH)

13 units in 6 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.

DeRosier, Stokes & Darst. J. Mol. Biol. 289: 159 (1999)

$$F(R, \Phi, l/c) = \sum_{n} G_{n,l}(R) \exp \left[in \left(\Phi + \pi/2\right)\right].$$
$$g_{n,l}(r) = \int G_{n,l}(R) J_n(2\pi Rr) 2\pi R dR$$
$$\rho(r, \phi, z) = \sum_{l} \sum_{n} g_{n,l}(r) \exp(in \phi) \exp\left(-2\pi i l z/c\right)$$



## 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.

Zhang et al., Nature 392: 835-840 (1998). Yonekura & Toyoshima. Ultramicroscopy 84: 15-28 (2000)

## **3D Density Map Reconstruction** (Fourier Bessel inversion)

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

$$\rho(r,\phi,z) = \sum_{l} \sum_{n} g_{n,l}(r) \exp(in\phi) \exp(-2\pi i l z/c)$$



# 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



$$\operatorname{FSC}(k, \Delta k) = \frac{\sum_{(k,\Delta k)} F_1(\mathbf{k}) F_2^{\dagger}(\mathbf{k})}{\left[\sum_{(k,\Delta k)} |F_1(\mathbf{k})|^2 \sum_{(k,\Delta k)} |F_2(\mathbf{k})|^2\right]^{1/2}},$$

## Kinesin13-Microtubule Ring Complex Helical Reconstruction





0.023	0.047	0.070	0.034 N,L -6, 45	<ul> <li>Include</li> <li>↓ Exclude</li> </ul>
0.023	0.047	0.070	0.094 N,L 11, 31	<ul> <li>Include</li> <li>⇒ Exclude</li> </ul>
0.023	0.047	0.070	N,L -4, 30	<ul> <li>Include</li> <li>⇒ Exclude</li> </ul>
0.023	0.047	0.070	N,L 13, 16	<ul> <li>Include</li> <li>⇒ Exclude</li> </ul>
0.023	0.047	0.070	N,L -2, 15	◆ Include ↓ Exclude
0.023	0.047	0.070	N,L 30, 2	◆ Include ◇ Exclude
0.023	0.047	0.070	N,L 15, 1	<ul> <li>Include</li> <li>Exclude</li> </ul>
	0.047	0.070	N,L 0,0	<ul> <li>♦ Include</li> <li>↓ Exclude</li> </ul>
N, L, Radius, Amp, Phase:	-2,15: 0	0.045222 5.158E+01	1.660E+02	



## 3D Helical Reconstruction Examples (All done by Fourier-Bessel method)





Ca-ATPase Tubular crystal  $E_2$  state (VO<sub>4</sub>)



#### Microtubule-NCD complex



Ach Receptor @ 4A Unwin PNAS 2005

Acto-myosin complex

## 3D Helical Reconstruction Using Real Space Methods



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



A 3D volumen is obtained by backprojection of the boxed images.



#### From Sosa et al. JSB 118: 149-158(1997)

## 'Single Particle' Helical Reconstruction Methods



Multi-reference alignment with atomic models



8 Å resolution microtubule map

Box Filament segments

In-plane Rotational Alignment

From: Li et al., (2002) Structure <u>10</u>: 1317-1328.

## The Iterative Helical Real Space Reconstruction Method (IHRSR)



From: Egelman E.H. (2000) Ultramicroscopy 85: 225-234.

## Some structures solved using the IHRSR method





Dynamin (r=0.2nm). (Mears et al., 2007)





Filamentous Bacteriophage (r=0.8 nm) Wang et al., 2009

Tarantula striated muscle myosin filament (r=2.5 nm). Woodhead etal., 2005

Bacteria Adhesion Pili (r=1.25 nm)). Mu et al., (2008)

Fourier-Bessel method vs. IHRSR: Pomfret et al., (2007) J. Struct Biol 157: 106







**TMV @ 3.3 A Resolution** Ge & Zhou. PNAS 108: 9637 (2011) (~1.9 x10<sup>6</sup> asymmetric units)

**F-Actin @ 6.6 A resolution** Fujii et al. Nature 467: 724 (2010) (~120000 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' 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)



## Firefox

UCSF-Chimer







media









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Save CTF curve





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## Docking Atomic Structrures into Electron Density Maps



Tan et al., Structure Nov. 2008



Ge & Zhou. PNAS 108: 9637 (2011)

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