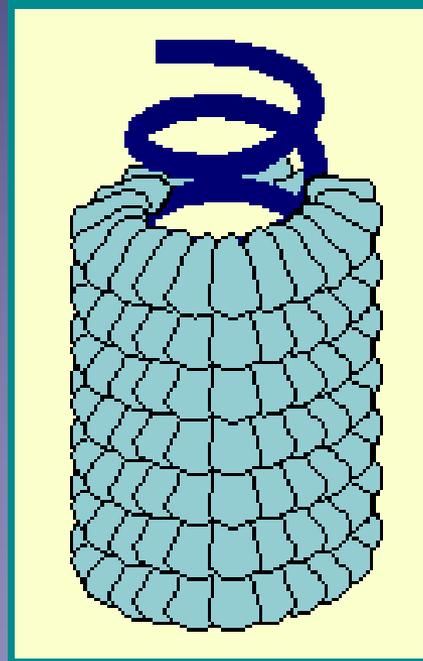


3D Reconstruction of Helical Specimens



Topics

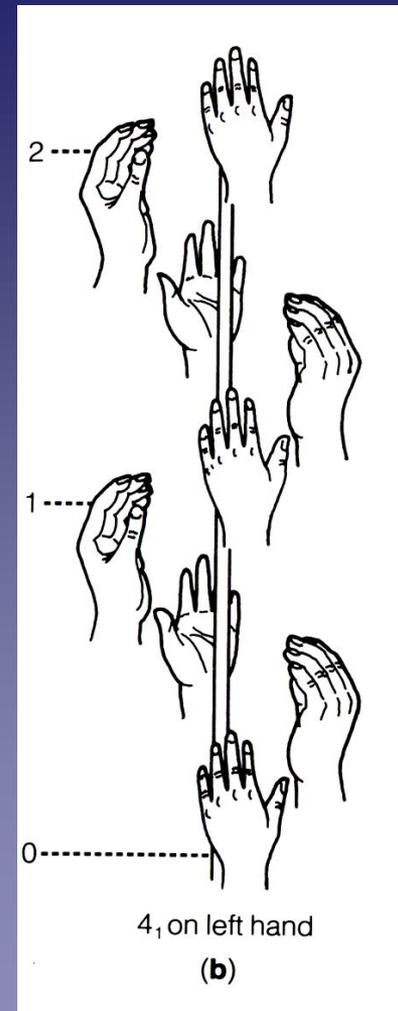
- History
- Helix definition
- Fourier Transform of a helix
- Fourier-Bessel helical 3D reconstruction
- Determining sample helical symmetry (twist & rise, selection rule)
- Real space/single particle helical 3D reconstruction
- Resolving asymmetric features in helical assemblies
- Examples.

Helical Symmetry

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.



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

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.

The standard high resolution electron microscope has a depth of focus of several thousand Ångströms, making the image a two dimensional superposition of different levels in the three dimensional structure. The focus cannot be adjusted to different levels within the object, and so three dimensional structures are difficult to analyse. Stereo-electron micrographs do not overcome this difficulty satisfactorily, as will be shown.

Our method starts from the obvious premise that more than one view is generally needed to see an object in three dimensions. We determine first 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. The method is most powerful for objects containing symmetrically arranged subunits, for here a single image

effectively contains many different views of the structure. The symmetry of such an object can 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 any kind of structure, including individual, unsymmetrical particles, or sections of biological specimens.

Summary of Procedure

Electron micrographs are selected in which the details of the structure show up best, as judged for example in the phage tail described later, by their optical diffraction patterns^{1,2}. The optical 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, R. A. Crowther and J. F. W. Mallett), which converts the image into a set of numbers representing the density at each grid point. These numbers are now transformed by computation into a set of Fourier

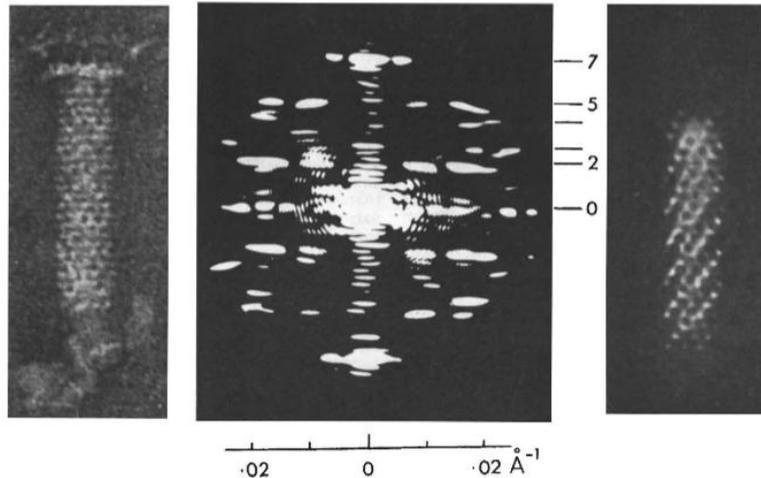


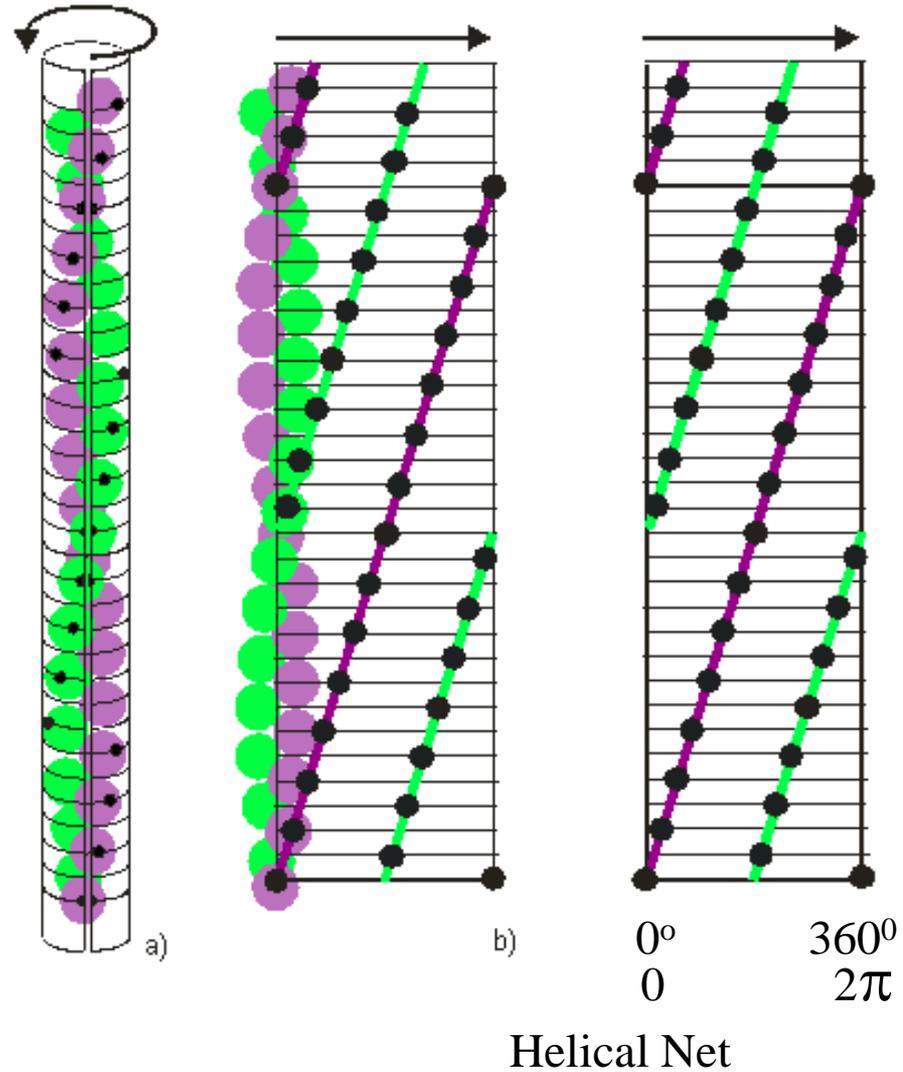
Fig. 1

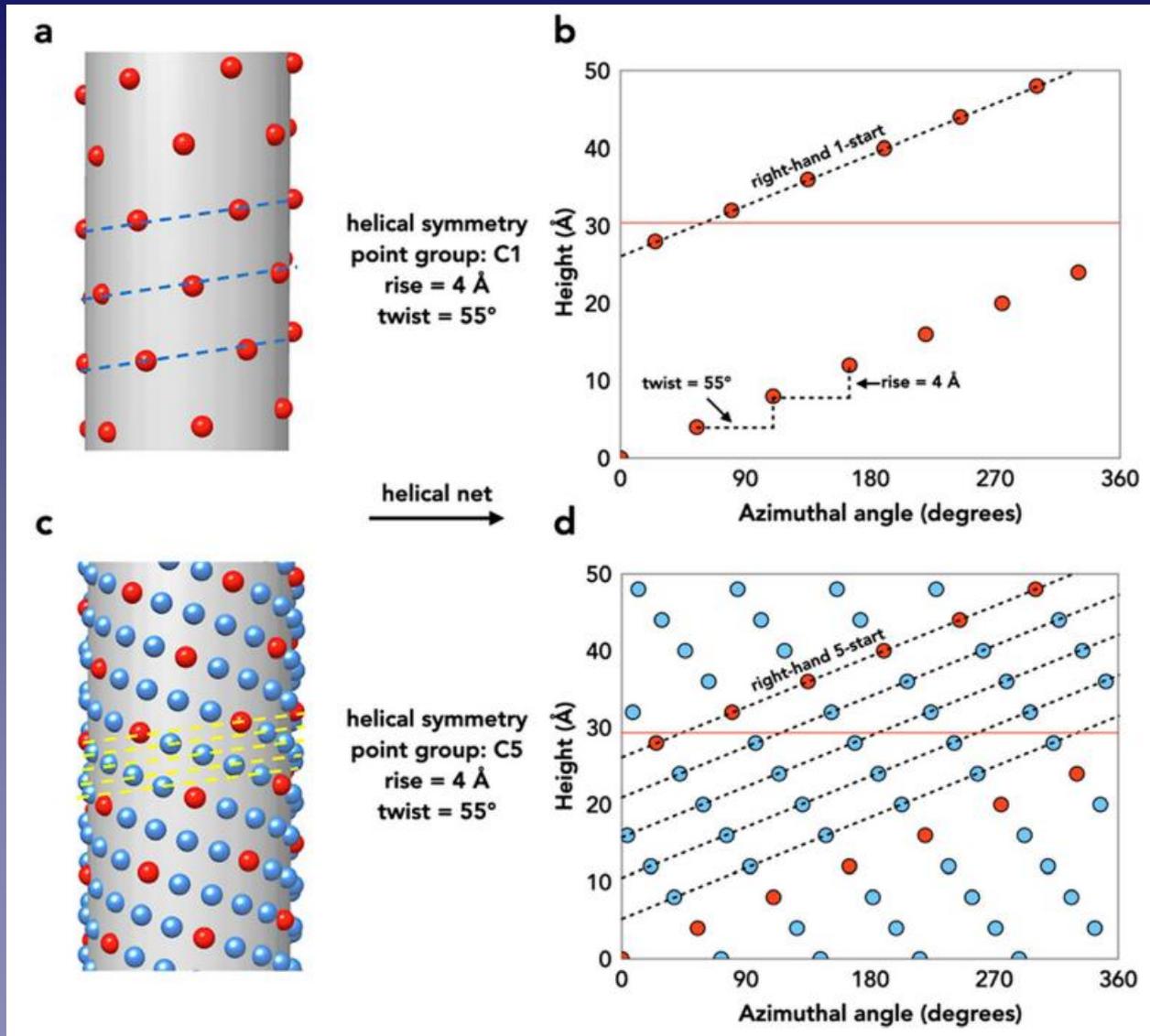
Fig. 2

Fig. 3

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

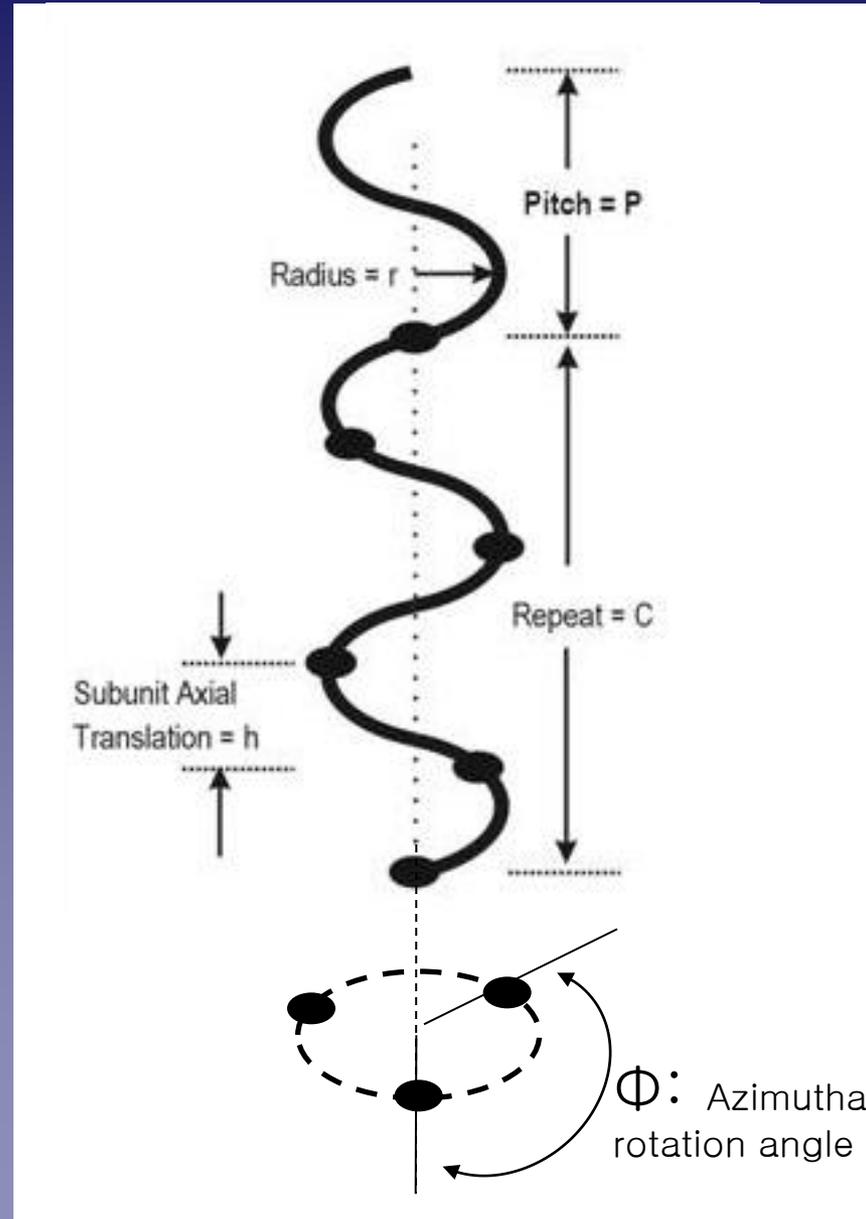
The Helical Lattice Radial Projection



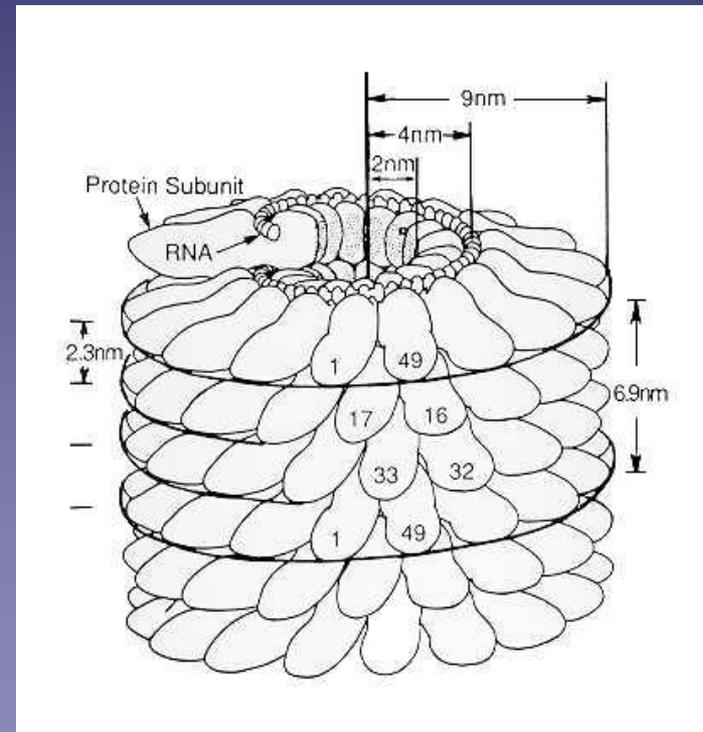
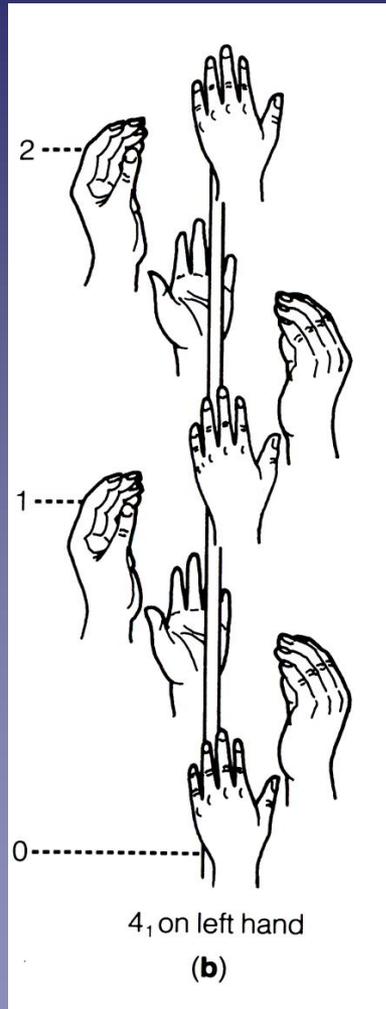


From: Wang et al., 2022

Parameters of a Helix

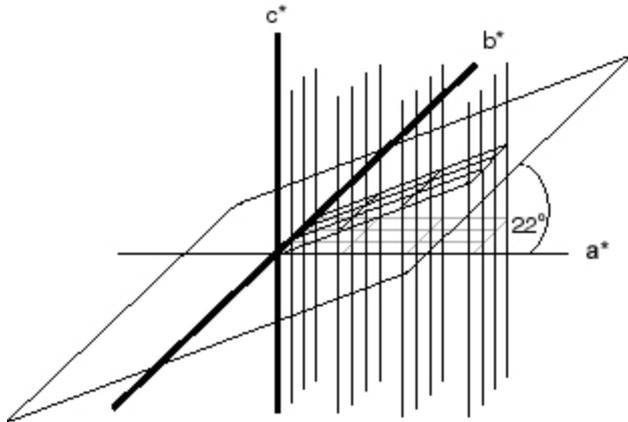


Helices give several orientation views of the asymmetric unit from a single view direction

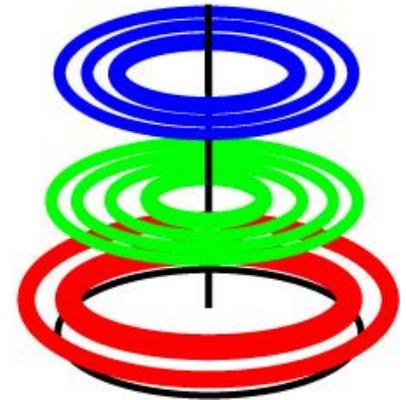
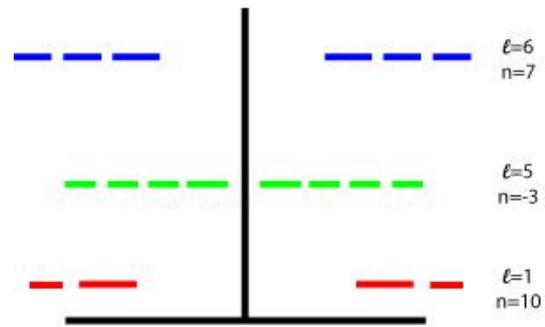


No Missing Cone !

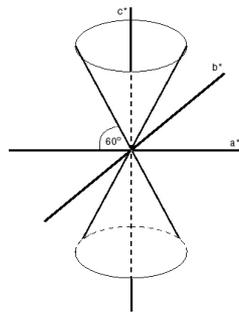
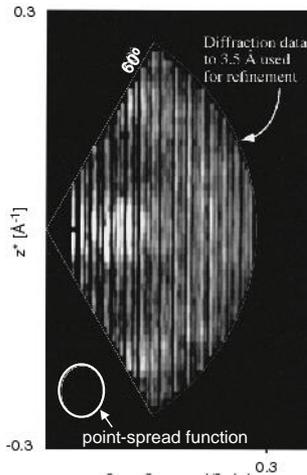
2D crystals



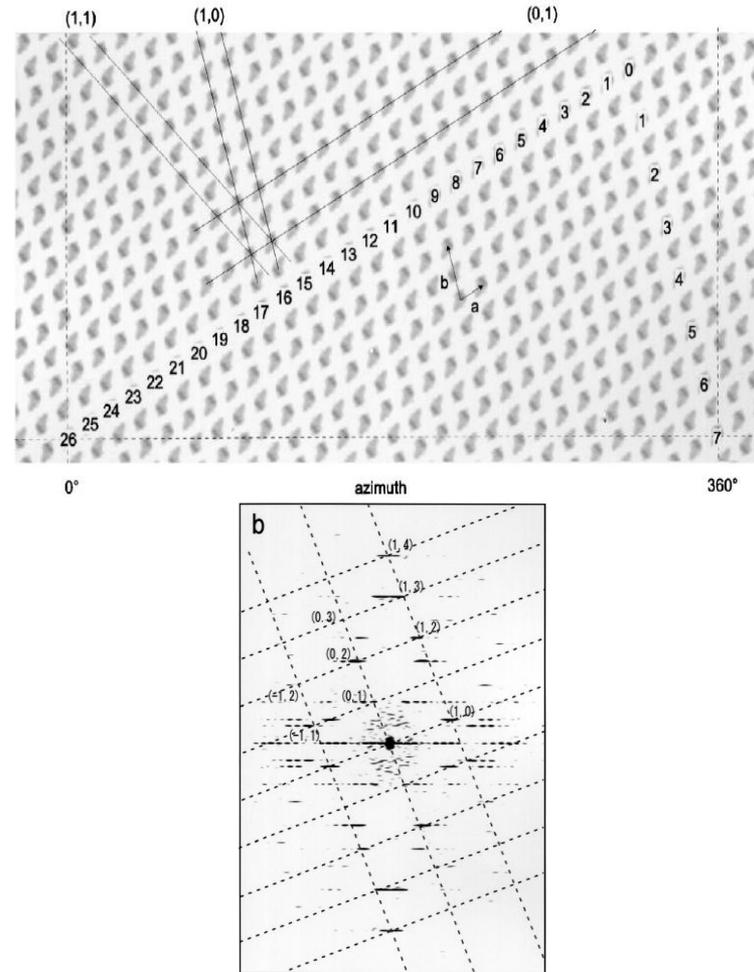
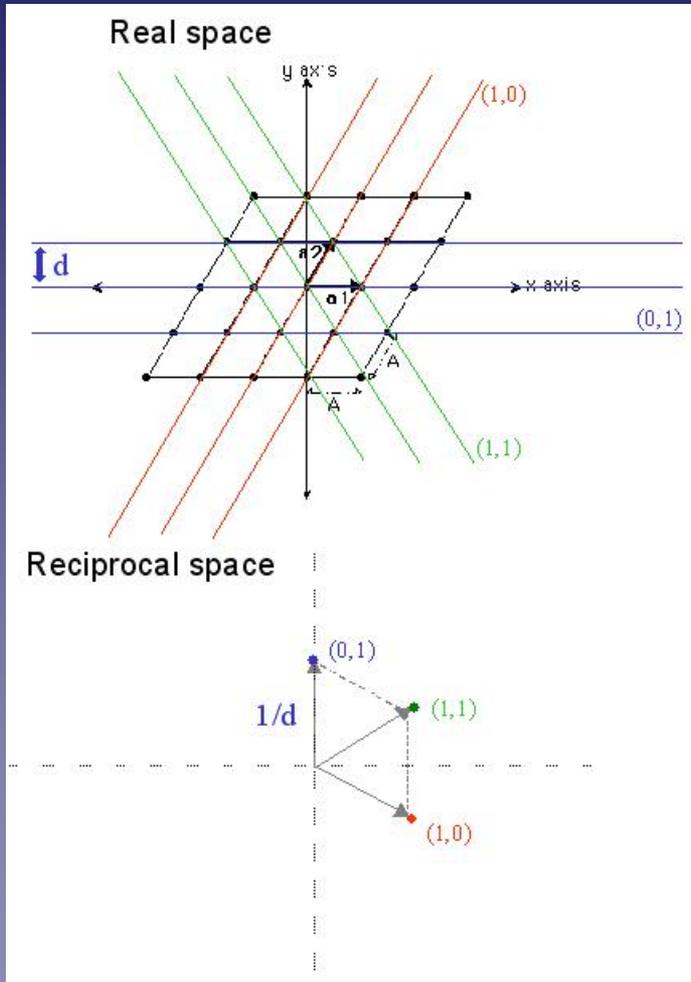
Helical crystals



missing cone

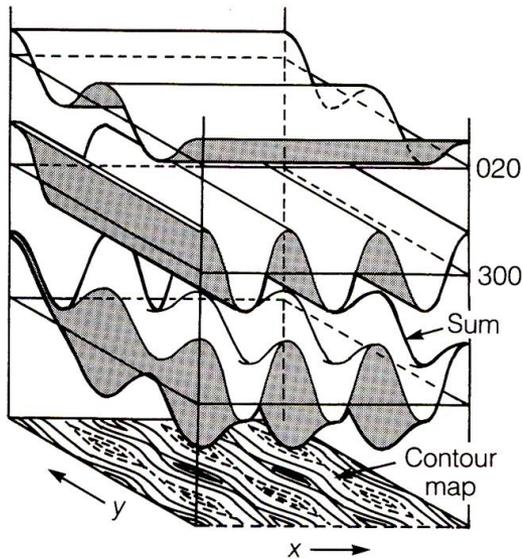
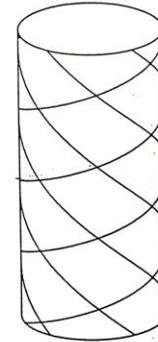
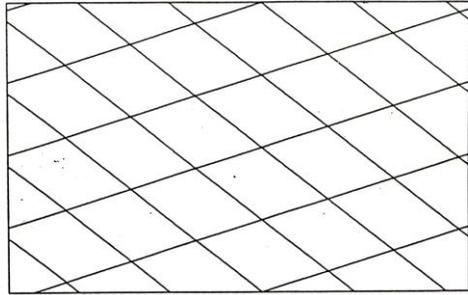


Analogy between 2D lattices and Helical Lattices



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

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



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

A helical wave

The Fourier Transform of a Helix

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

Cochran, Crick & Vand 1952

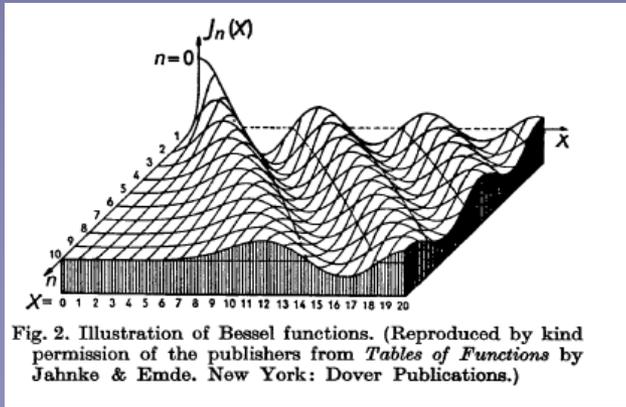
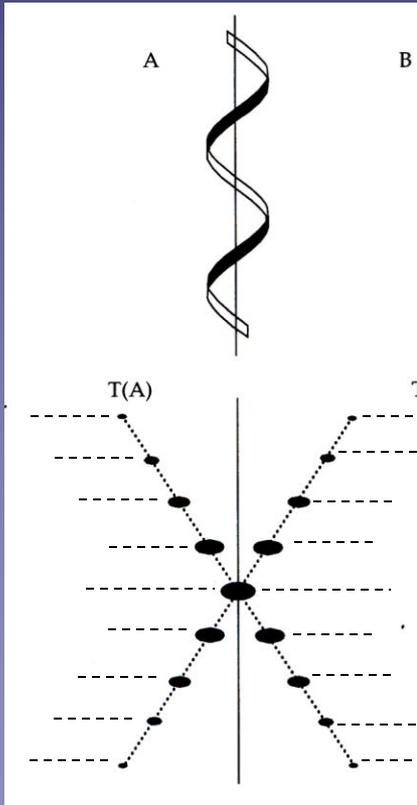
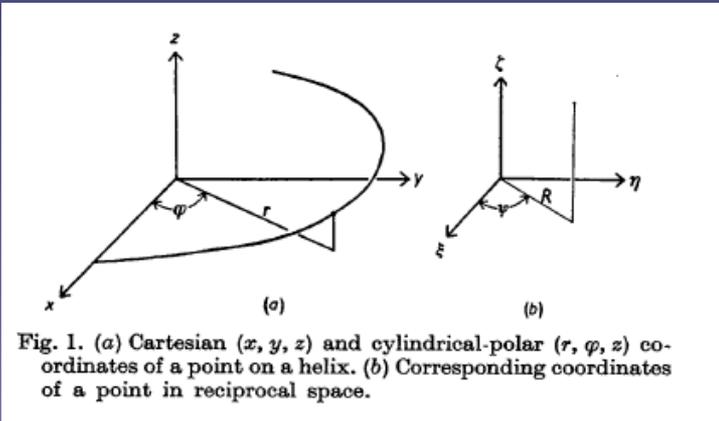
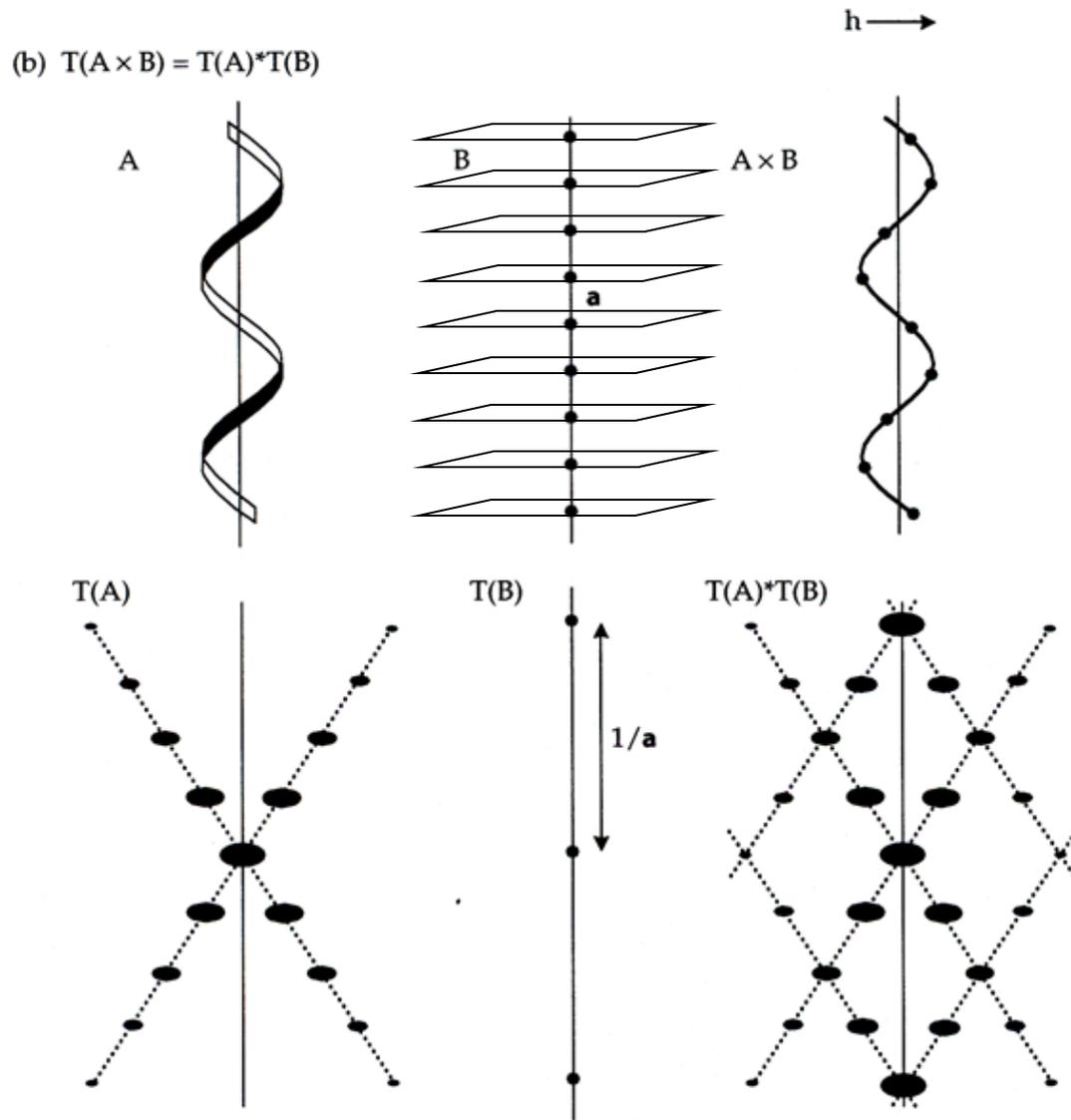
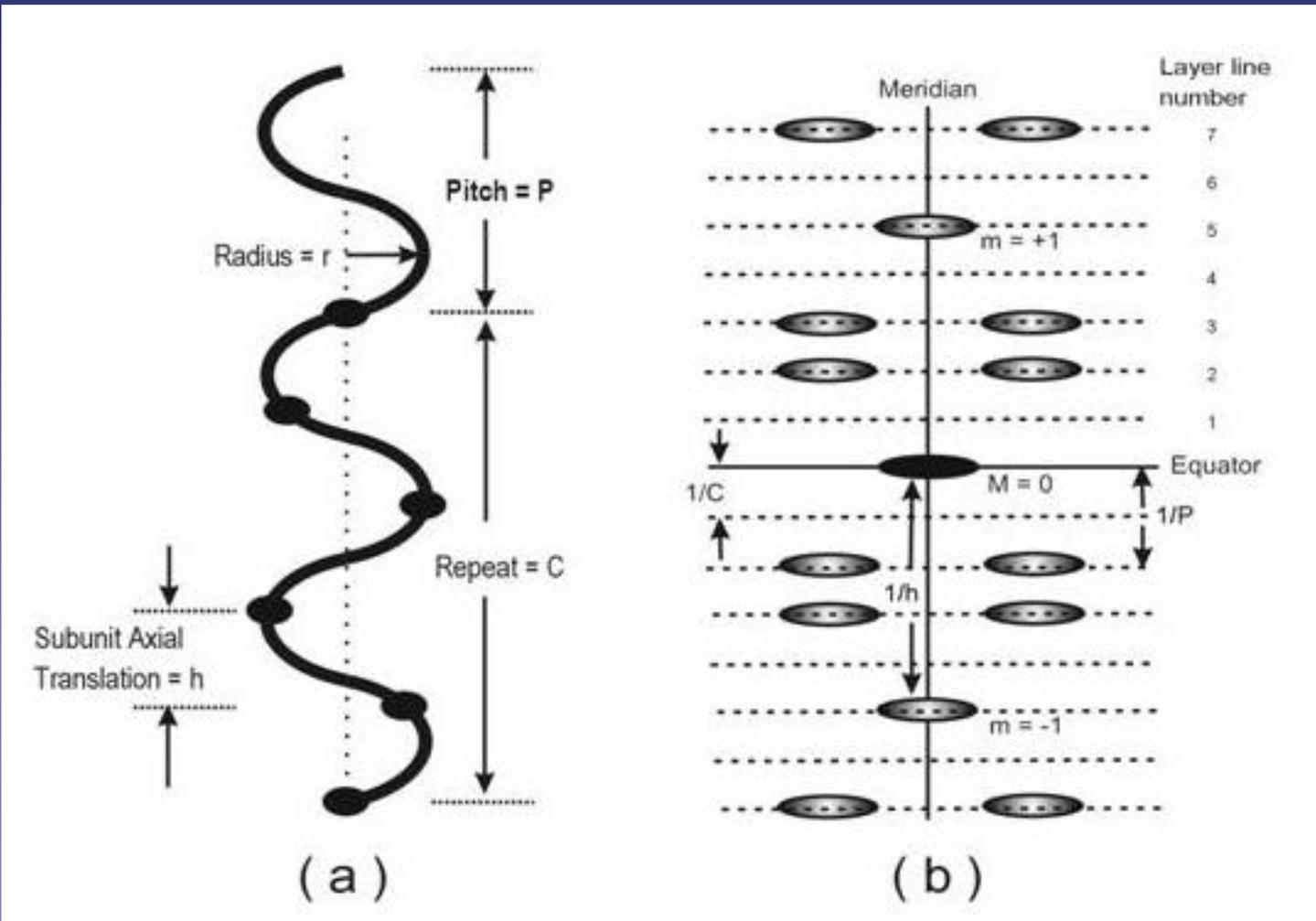


Fig. 2. Illustration of Bessel functions. (Reproduced by kind permission of the publishers from *Tables of Functions* by Jahnke & Emde. New York: Dover Publications.)

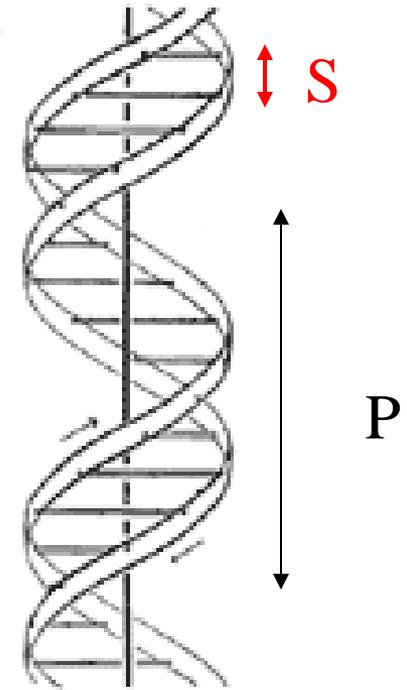
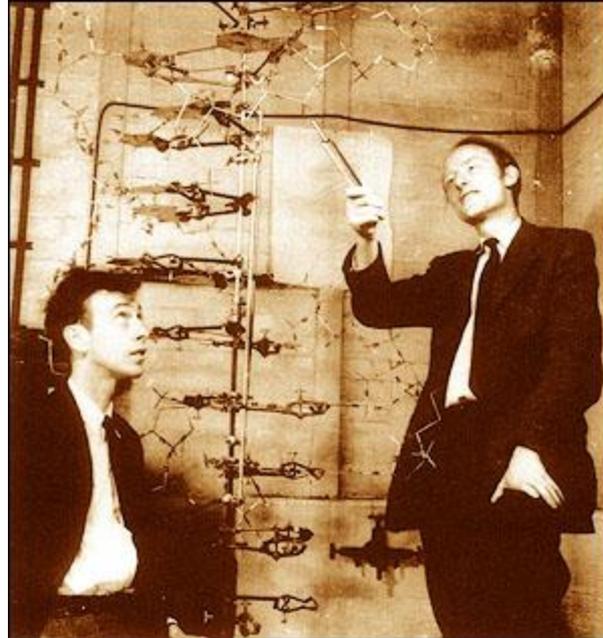
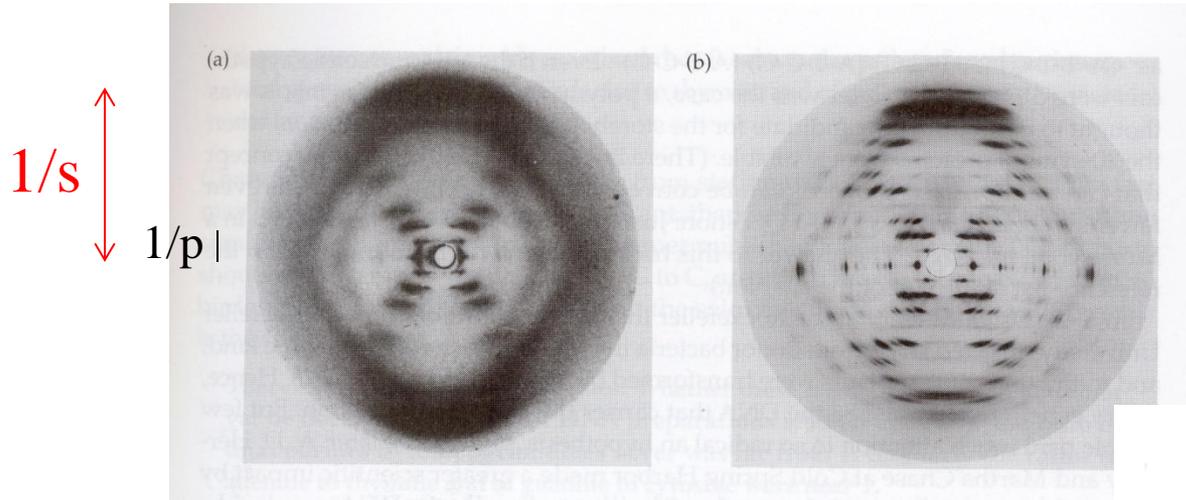
The Transform of a discontinuous helix



A helix and its corresponding Fourier Transform (Power Spectrum)



The DNA Structure



Helical 3D reconstruction Using the Fourier-Bessel Method

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

Fourier Transform

Reciprocal Space Function

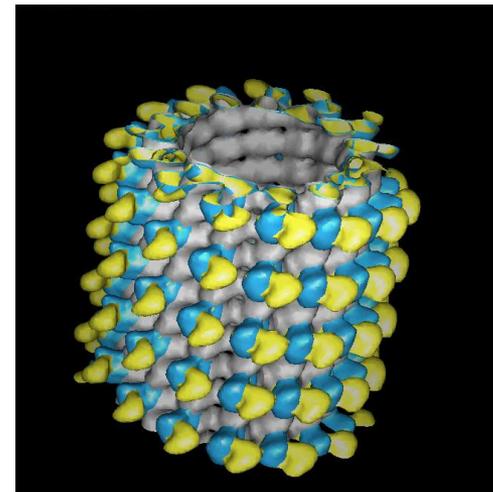
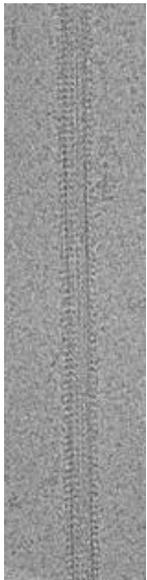
$$F(R, \Phi, l/c) = \sum_n G_{n,l}(R) \exp[in(\Phi + \pi/2)]. \quad (1)$$

Selection rule $l = tn + um$ (2)

Real space function
(structure)

$$\rho(r, \phi, z) = \sum_l \sum_n g_{n,l}(r) \exp(in\phi) \exp(-2\pi ilz/c) \quad (3)$$

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



Helical 3D reconstruction Using the Fourier-Bessel Method

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

358

D. J. DEROSIER AND P. B. MOORE

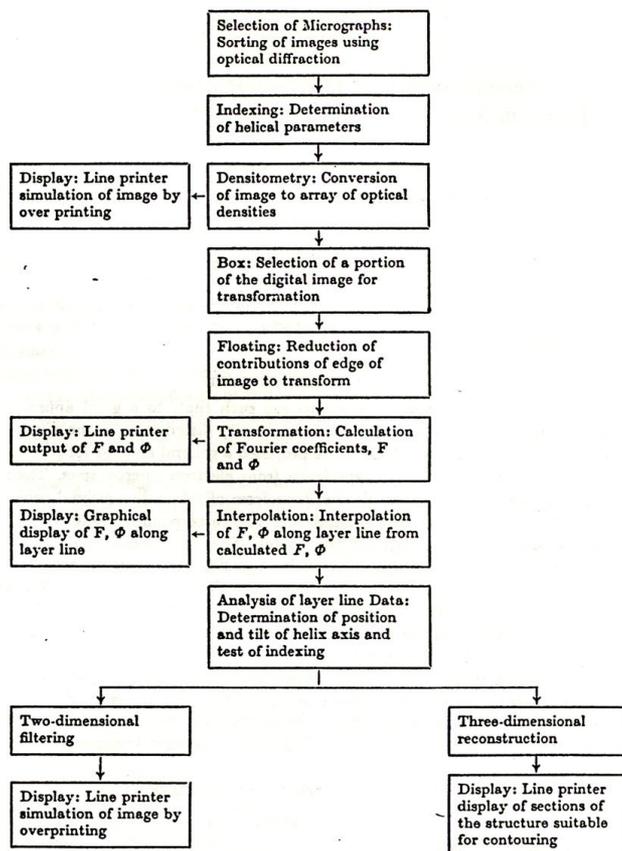


FIG. 1. The scheme presented shows the flow of data in the process of three-dimensional reconstruction.

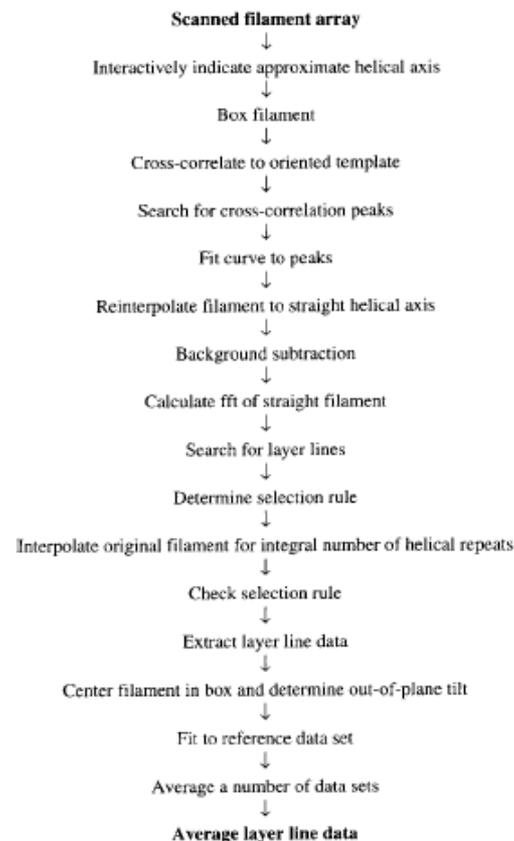
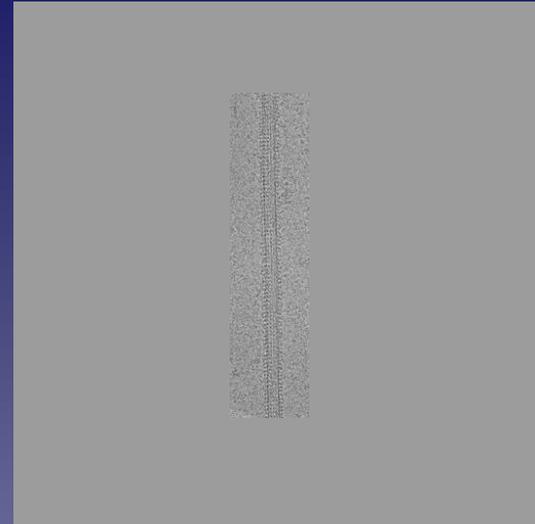
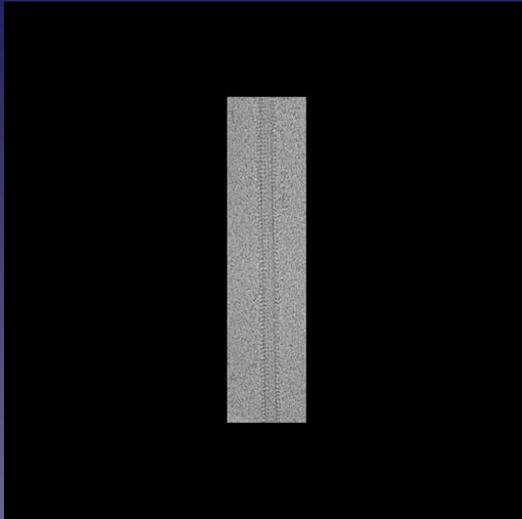


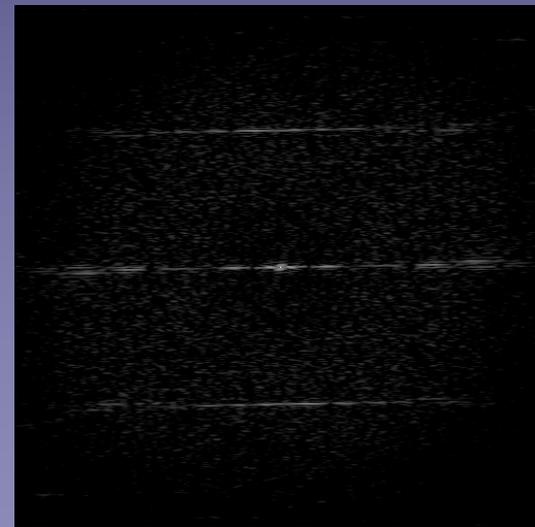
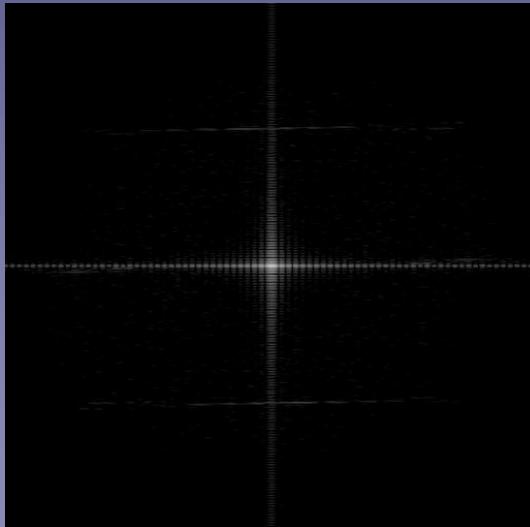
FIG. 1. Schematic diagram of the PHOELIX helical processing package. A detailed description of each step and the programs used is available as part of the PHOELIX distribution.

Boxing & Floating Image

Image



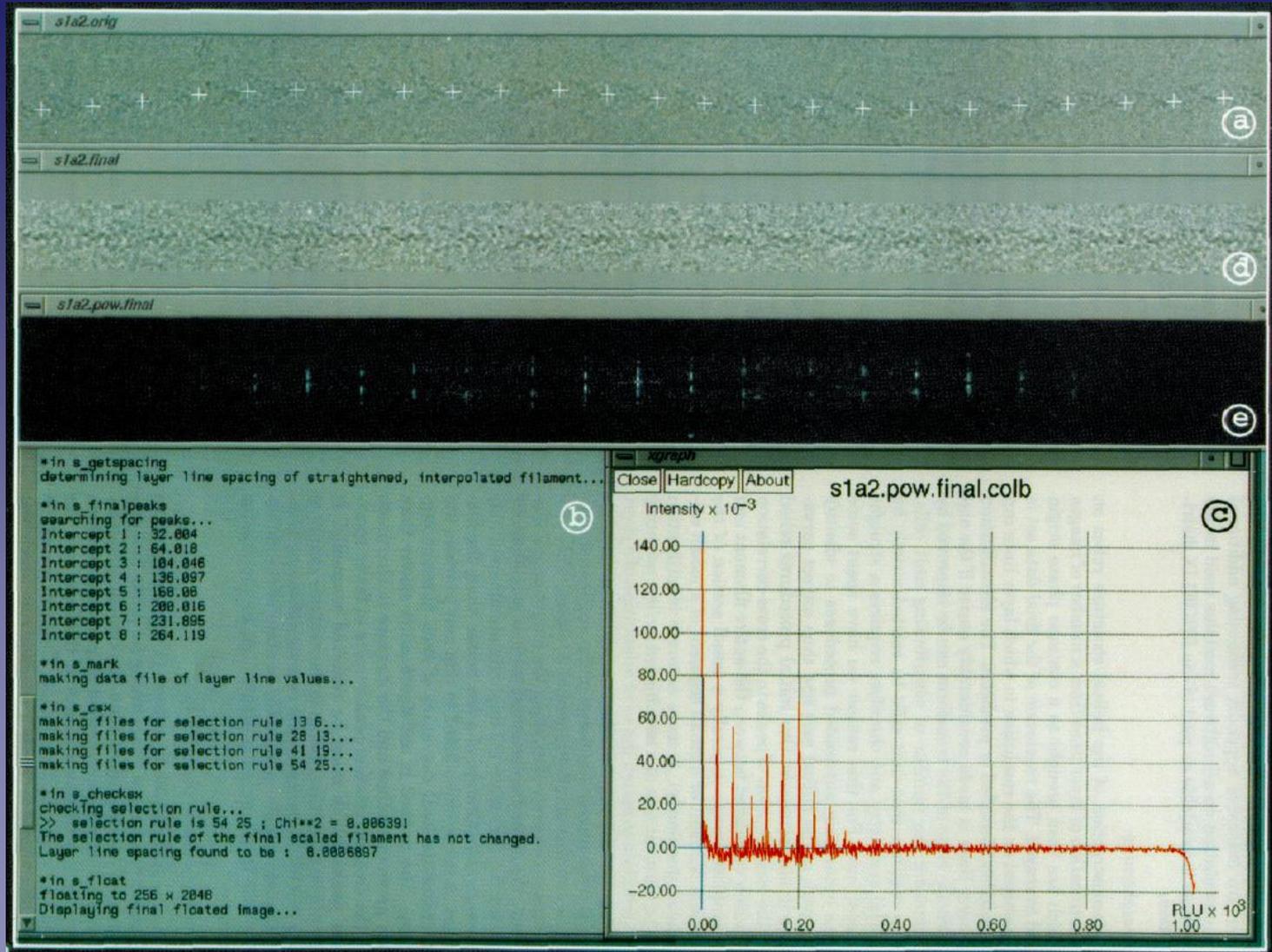
FFT



Non-Floated

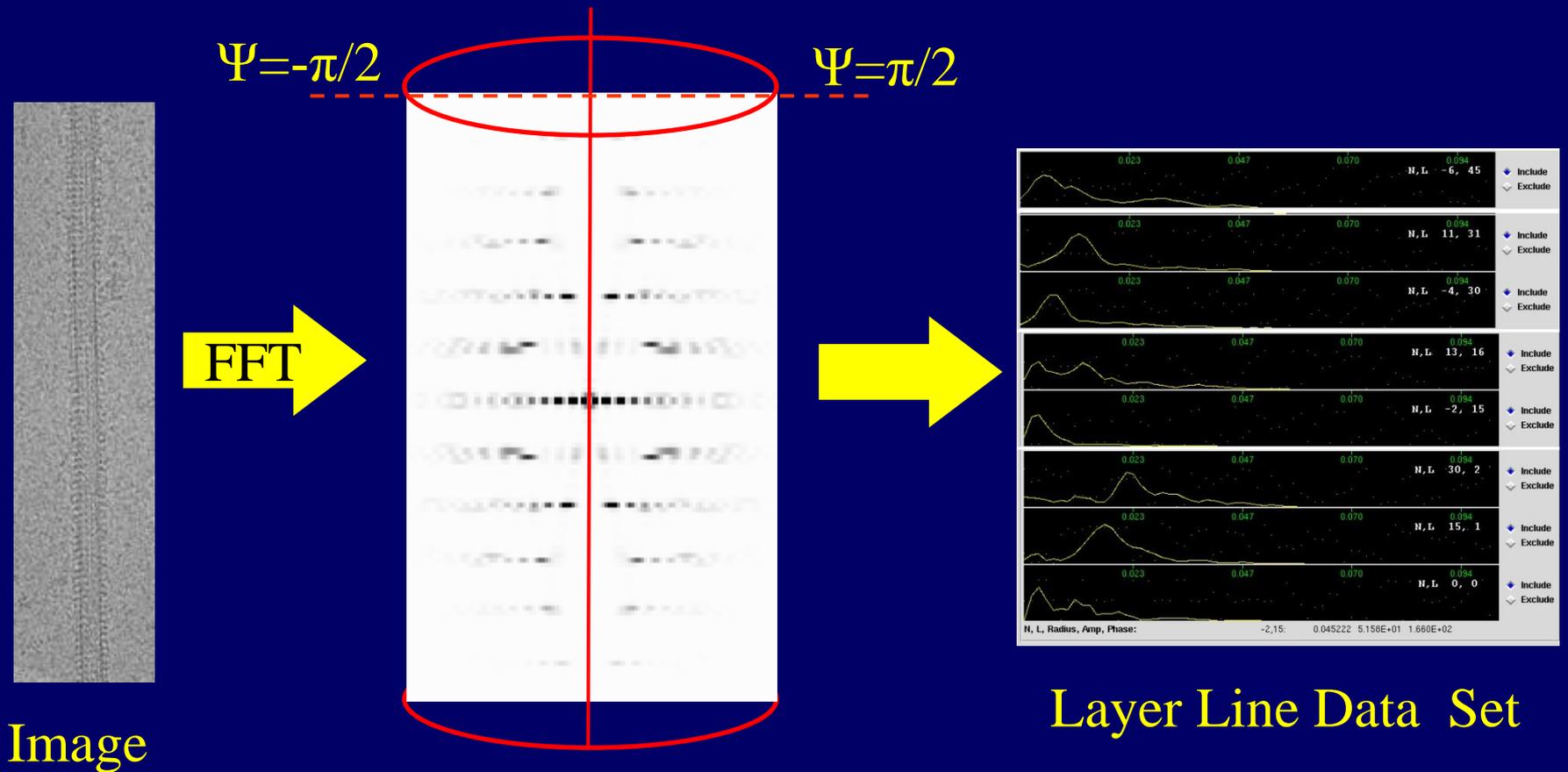
Floated

Straightening



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

Gathering Amplitude and Phases



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

$F(R, -\pi/2, l/c) = G_{n,l}$
 $F(R, +\pi/2, l/c) = \begin{matrix} G_{n,l} & (\text{for even } n) \\ -G_{n,l} & (\text{for odd } n) \end{matrix}$



hlx_run2.py H.S. v Nov 13 2009

Help

TIF to mrc/suprim file format

Define pixel size etc

----- Single Filament procedures -----

Power spectrum average (CTF ring inspection)

Estimate CTF parameters

Do CTF Phase flipping correction

Eliminate density gradients

Straightening filament

Normalize, apodize & pad image

Find selection rule & LL positions

Cut to integral number of repeats and reinterpolate

Extract layer lines

Create lline ranges file

Fix xshift & out of plane tilt

Make avlist file for averaging

----- Several Filament procedures -----

Edit list with files to average

Shift ll phase origin to a template & make average

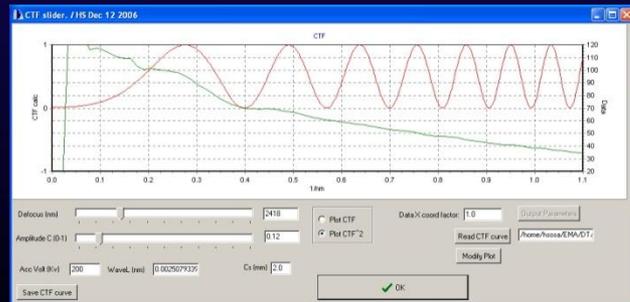
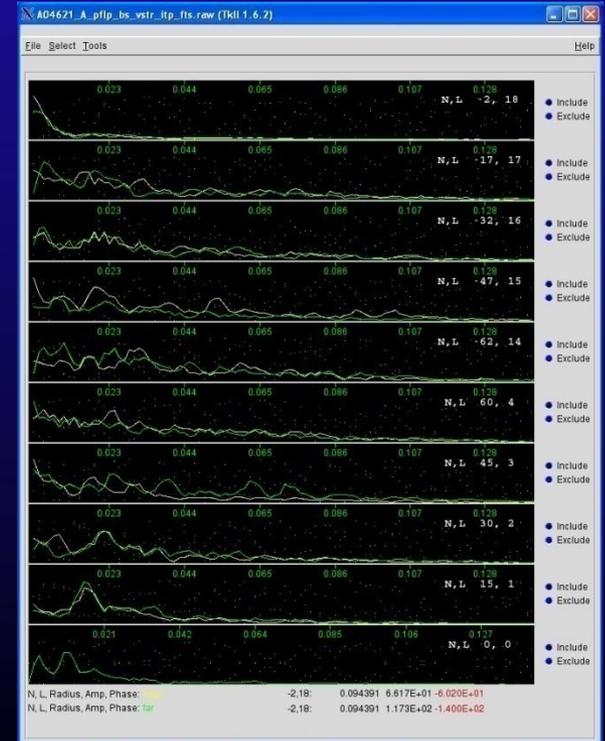
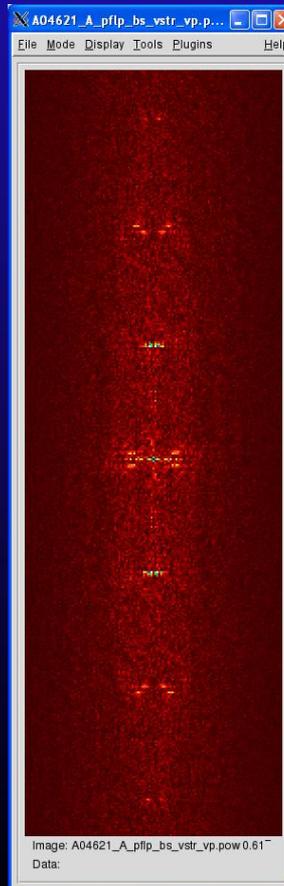
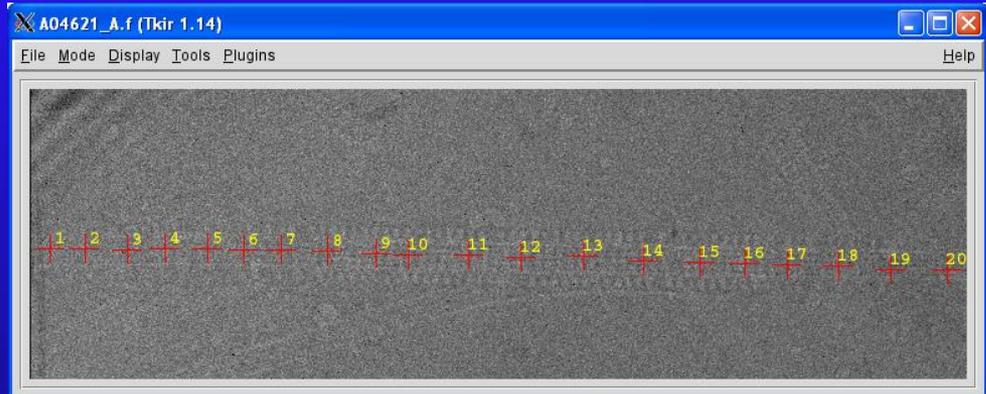
OverPlot make overplot of selected LL

----- General procedures -----

Calculate 3D map

Calculate Fourier Correlation Shell

Exit

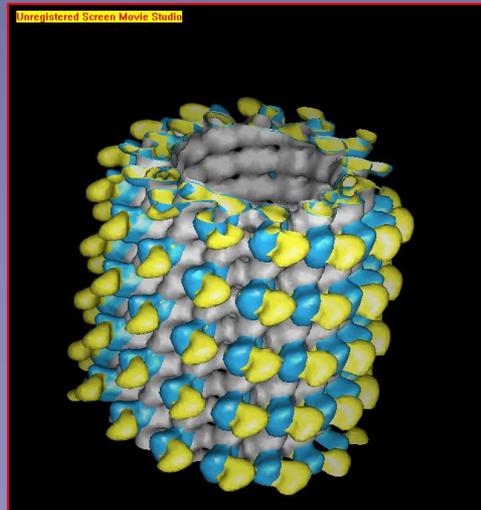


3D Density Map Reconstruction (Fourier Bessel inversion)

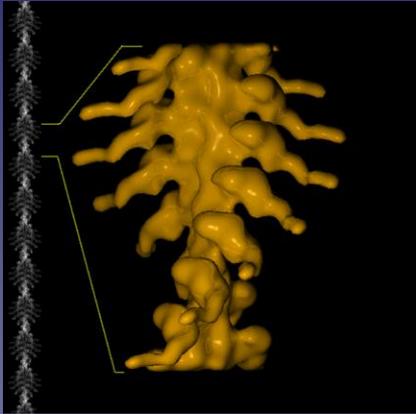
Average $G_{n,l}$ Data set.

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

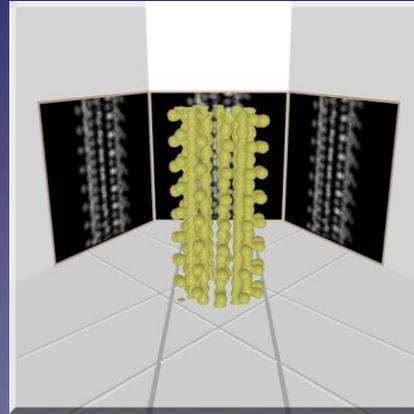
$$\rho(r, \phi, z) = \sum_l \sum_n g_{n,l}(r) \exp(in \phi) \exp(-2\pi ilz/c)$$



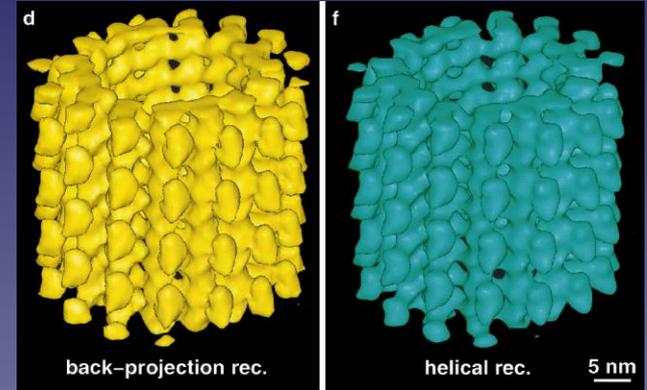
3D Helical Reconstruction Using Real Space Methods



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.

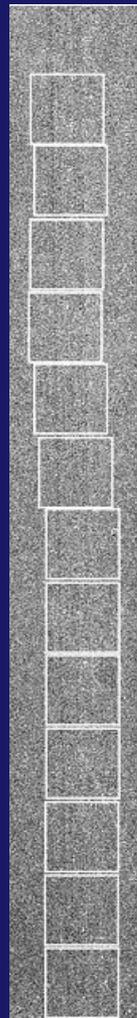


A 3D volume is obtained by back-projection of the boxed images.

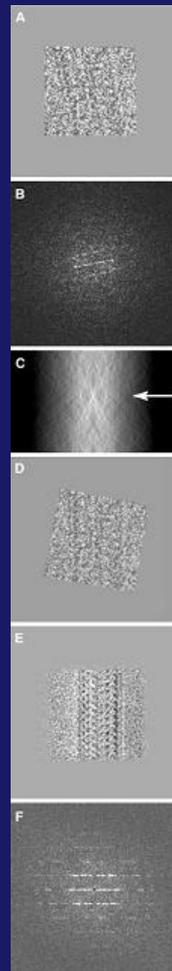


From Sosa et al. JSB 118: 149-158(1997)

'Single Particle' Helical Reconstruction Methods



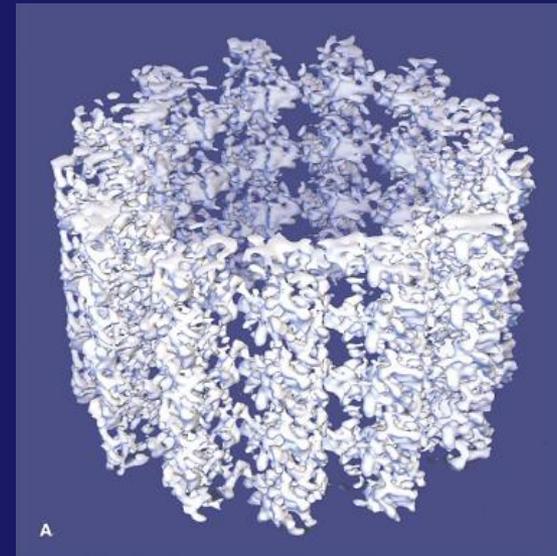
Box Filament segments



In-plane Rotational Alignment



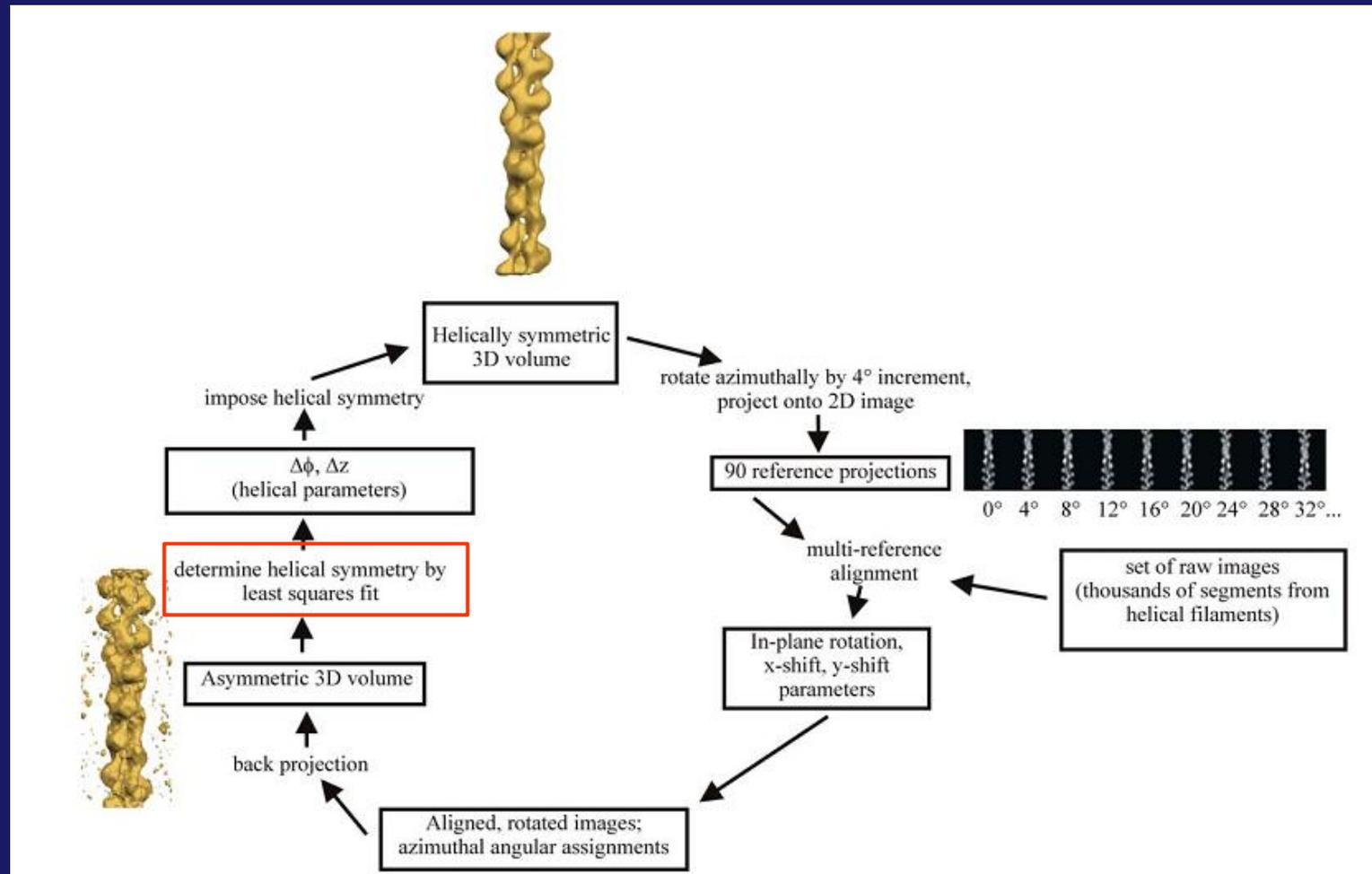
Multi-reference alignment with atomic models



8 Å resolution microtubule map

From: Li et al., (2002) Structure 10: 1317-1328.

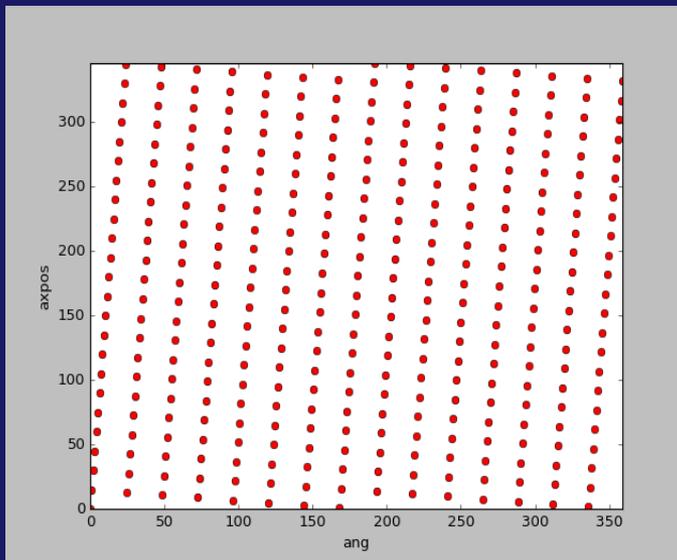
The Iterative Helical Real Space Reconstruction Method (IHRSR)



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

A first requirement for 3D reconstruction of a helical specimen, regardless of the method to be used (Fourier/Bessel or real space/single particle) is a good estimate of the helical parameters of the specimen:

- Rise per repeating unit (h).
- Azimuthal rotation per repeating unit (Φ).



l	n
0	0
1	15
2	30
22	-17
23	-2
24	13
25	28
45	-19
46	-4
47	11
48	26
68	-21
69	-6
70	9
71	24
91	-23
92	-8
93	7
94	22

$$l = tn + um$$

l : Layer line Number.

t : Num. of turns/rep.

n : Num of Helical starts
& bessel order.

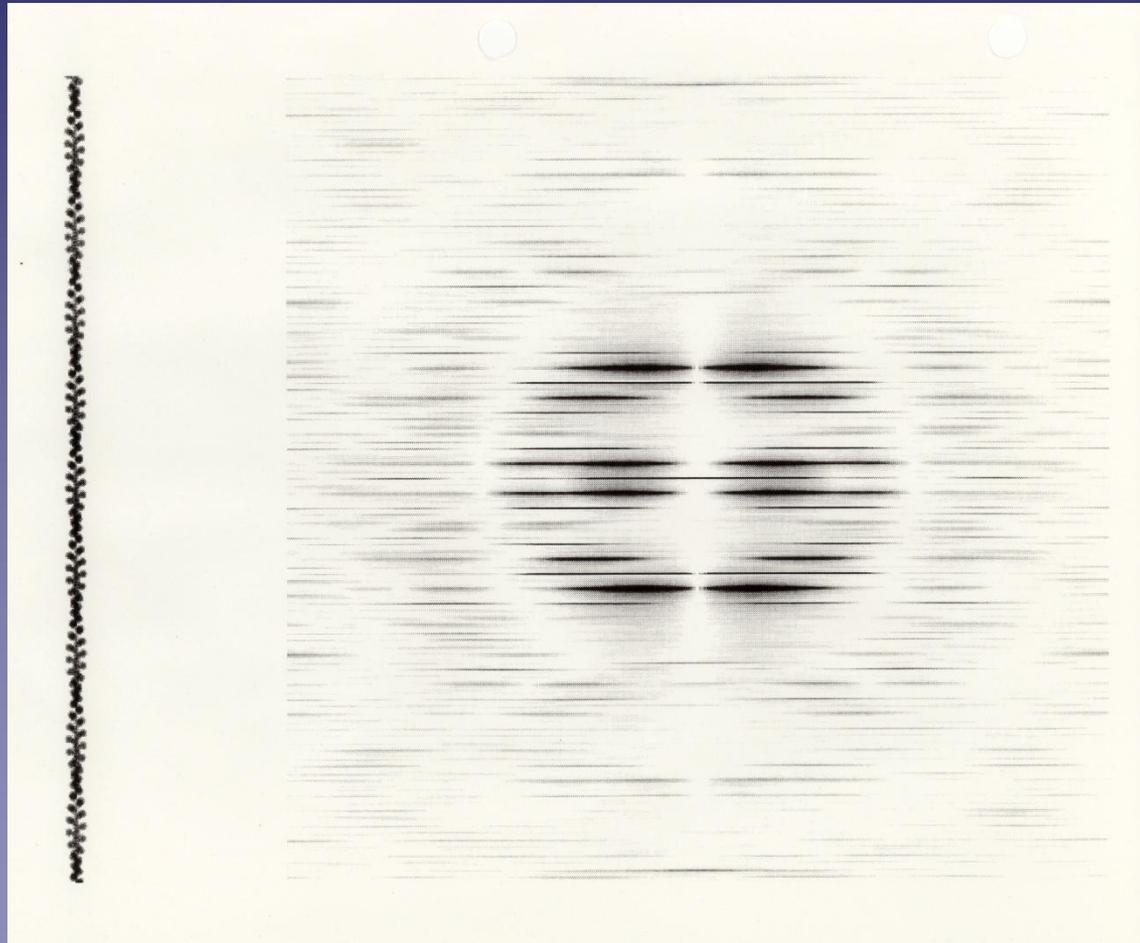
u : Num. of subunits/rep

m : Integer

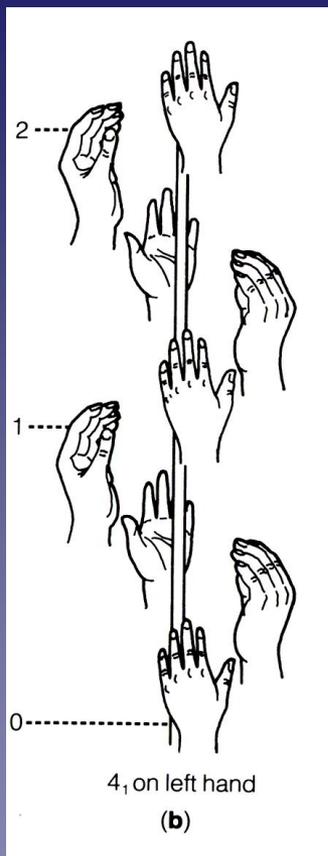
$$\text{SR: } l = 162 * n + 347 * m$$

$$\Phi : 168.07 \quad h = \text{rep} / 347$$

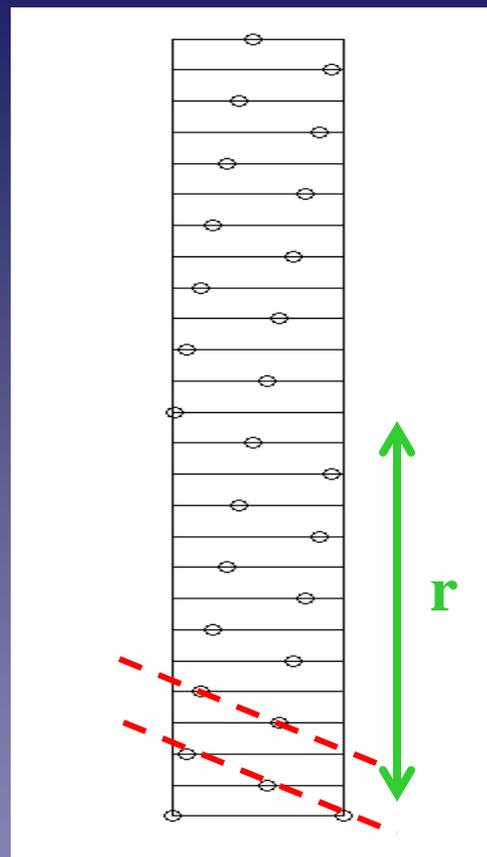
Finding Helical Symmetry Selection Rule Indexing the diffraction Pattern



The Selection Rule



4 units in 1 Turn (RH)



13 units in 6 Turns (LH)

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

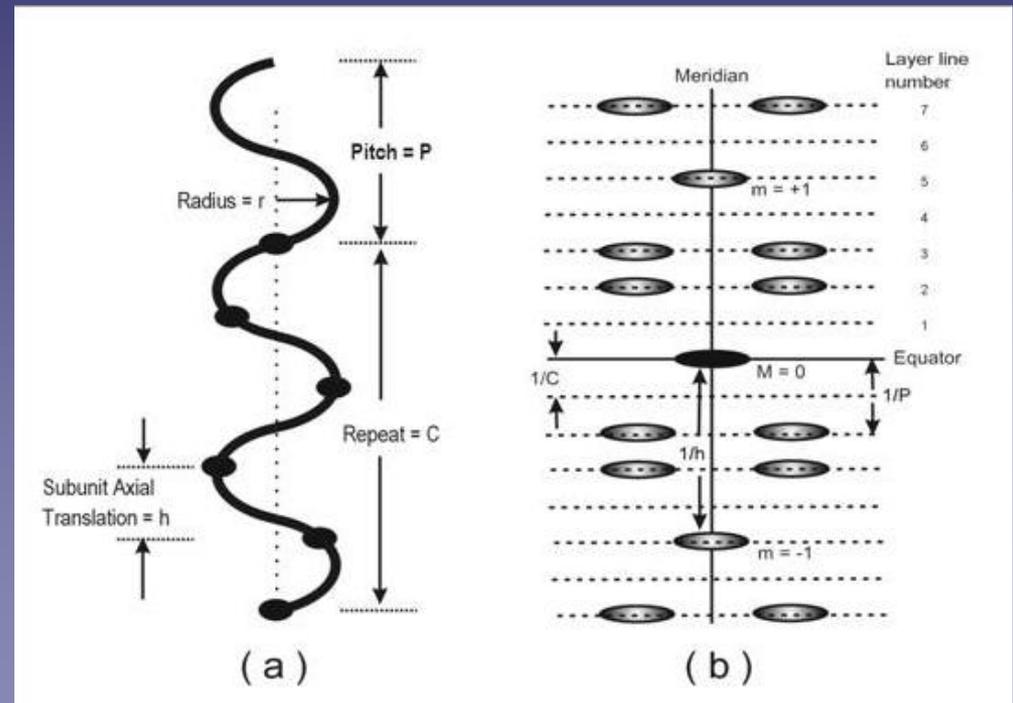
If k -fold rotational symmetry then:
 n must be multiple of k

$$Z = n(\phi/360^\circ) / h + m / h$$

Z: LL reciprocal spacing

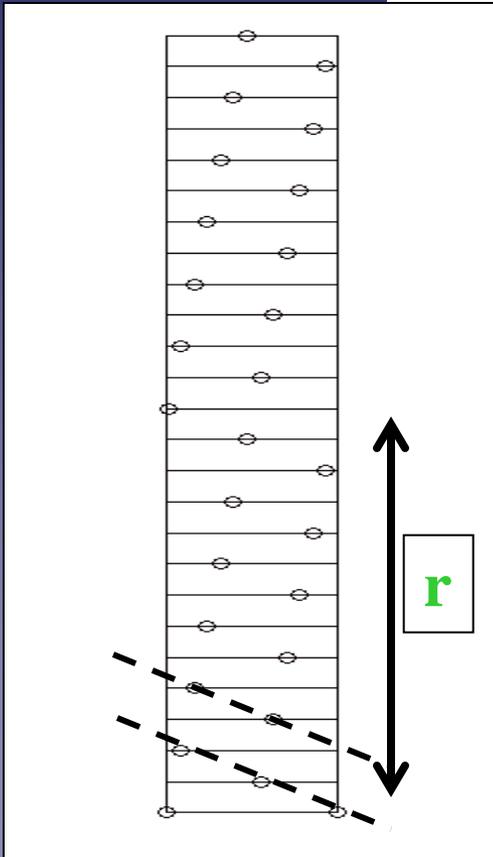
ϕ : twist angle.

h : rise distance.



Selection Rule Example

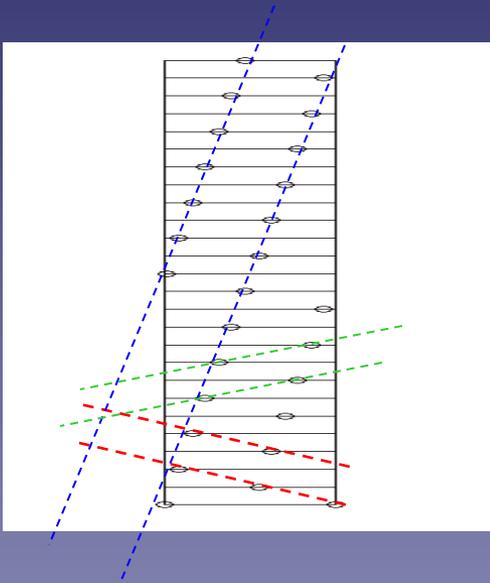
$$l = tn + um$$



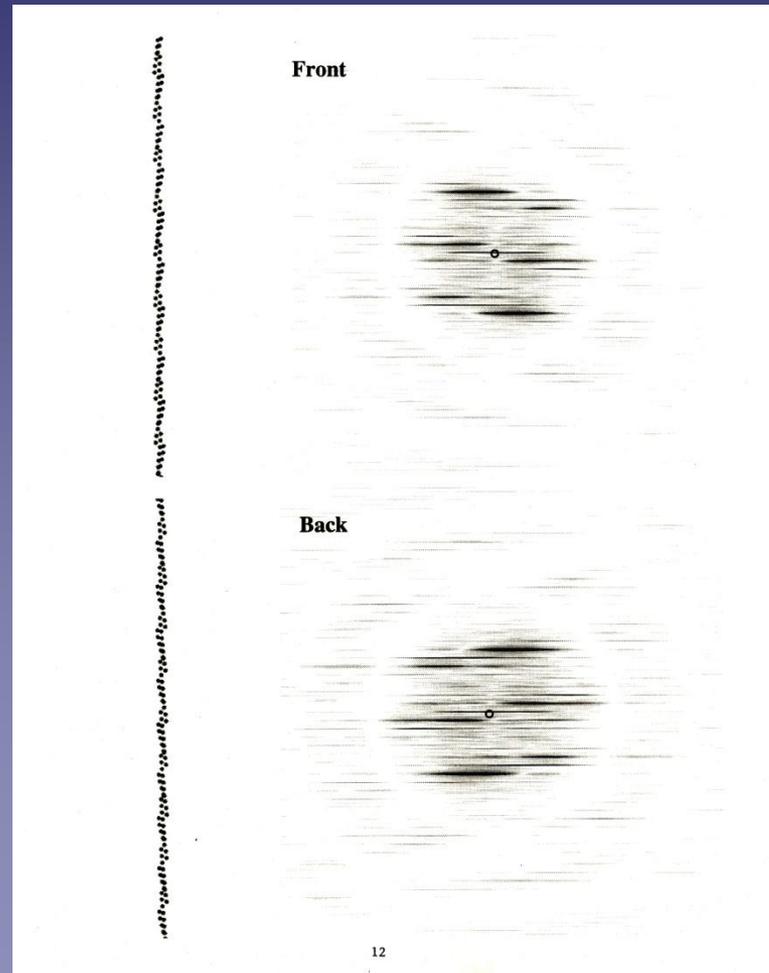
l	n ($ n \leq 10$)
0	0
1	2
2	4, -9
3	6
4	-5, 8
5	-3, 10
6	-1
7	1
8	3, -10
9	5, -8
10	-6, 7
11	-4, 9
12	-2
13	0

$$l = -6n + 13m$$

The Diffraction Pattern of a Helix Has Reflections From Planes in the Front and Back of the Helix



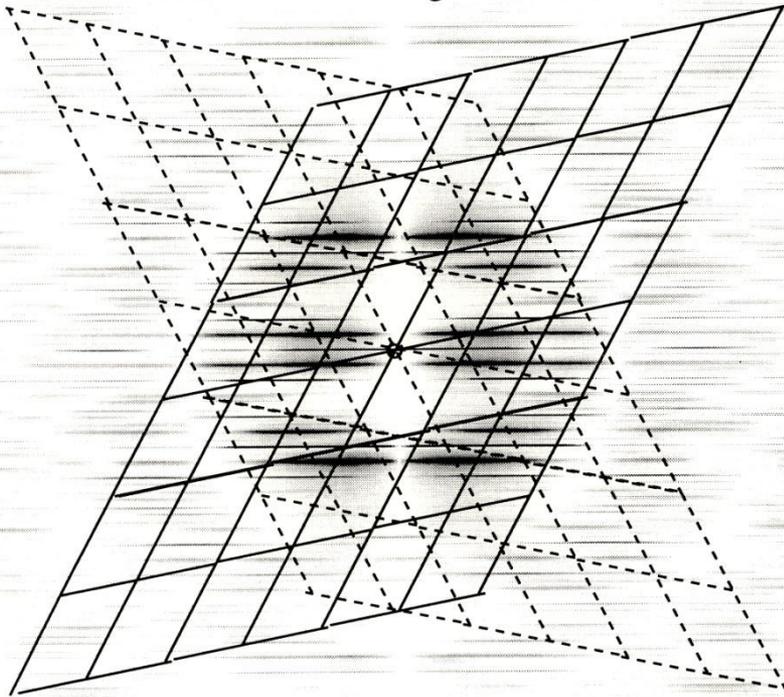
Different from the case of the transform of a 2D lattice where reflections from a set of planes form a spot in Fourier space. In the case of a helix the reflections are continuous Bessel function along “layer” lines.



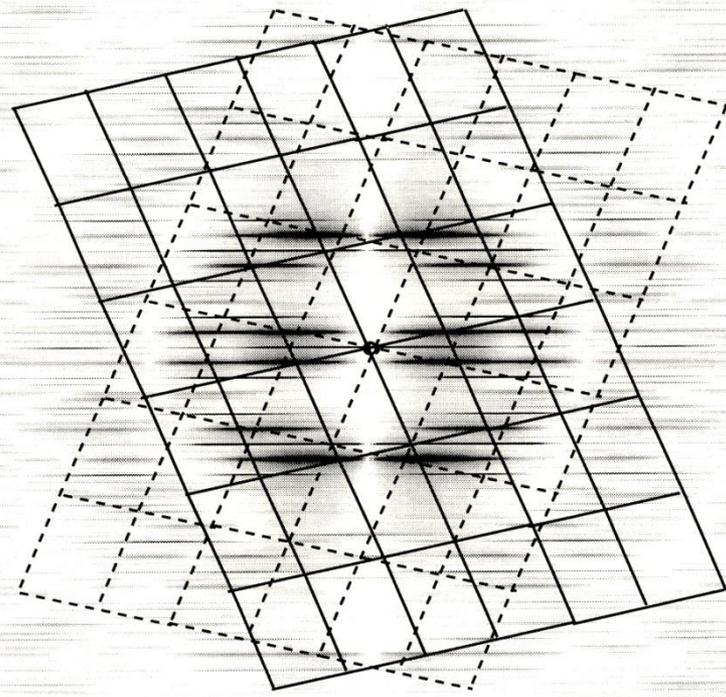
Drawing the Reciprocal Lattice

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.

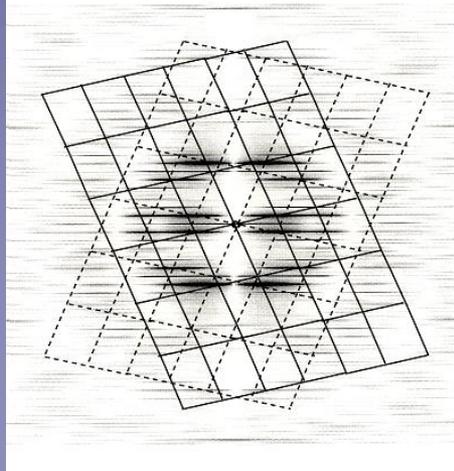
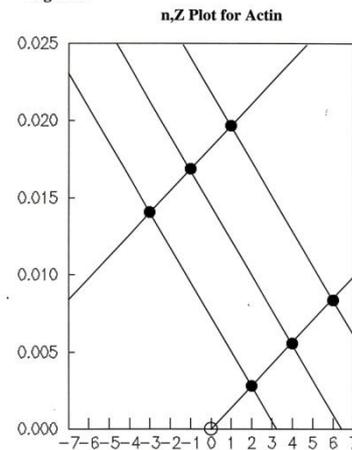
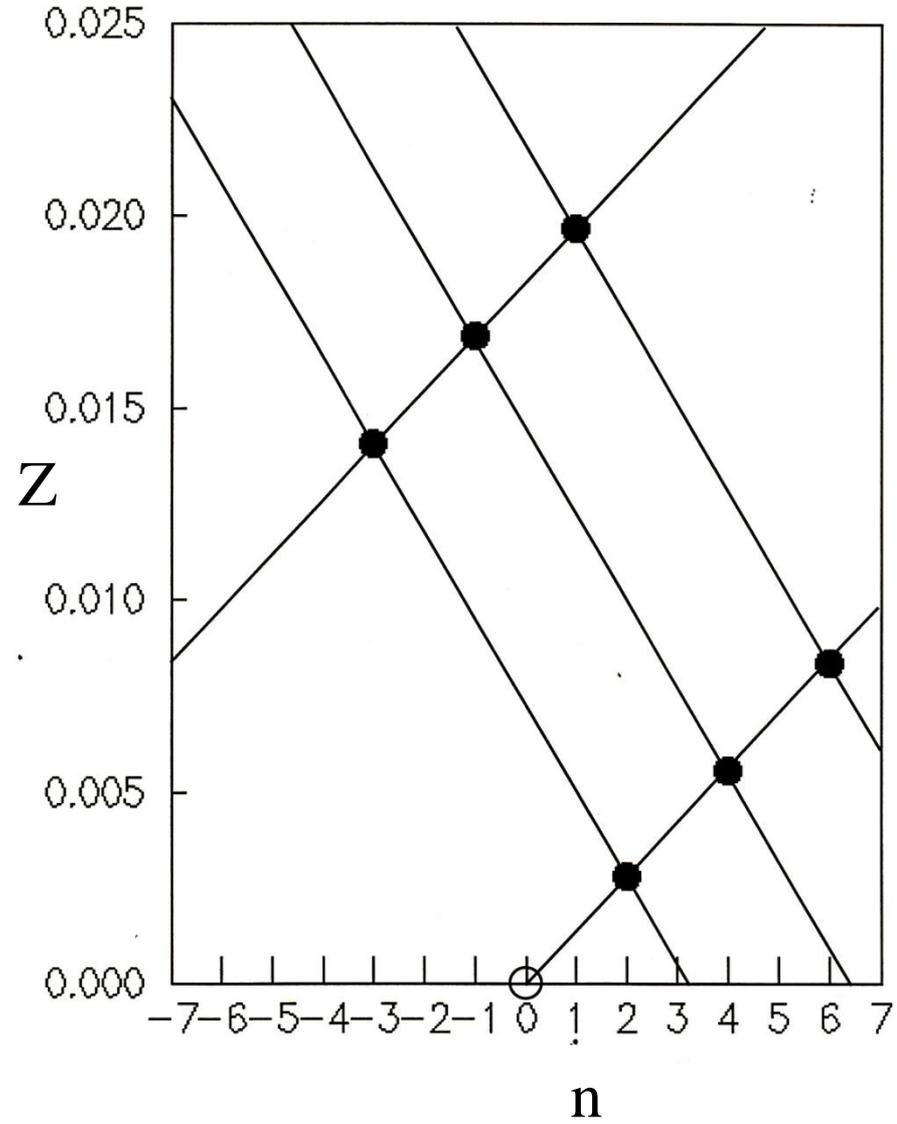


Figure 5



n,Z Plot for Actin



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)

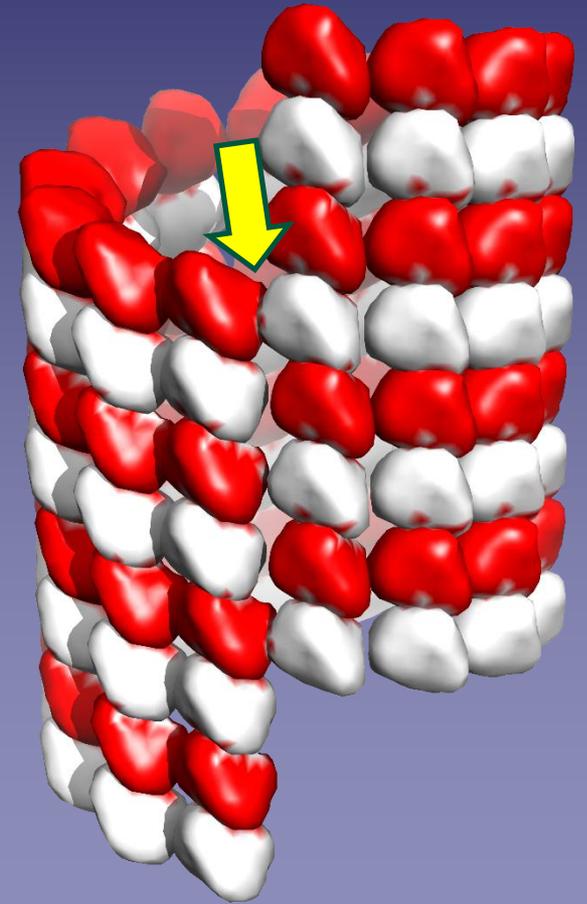
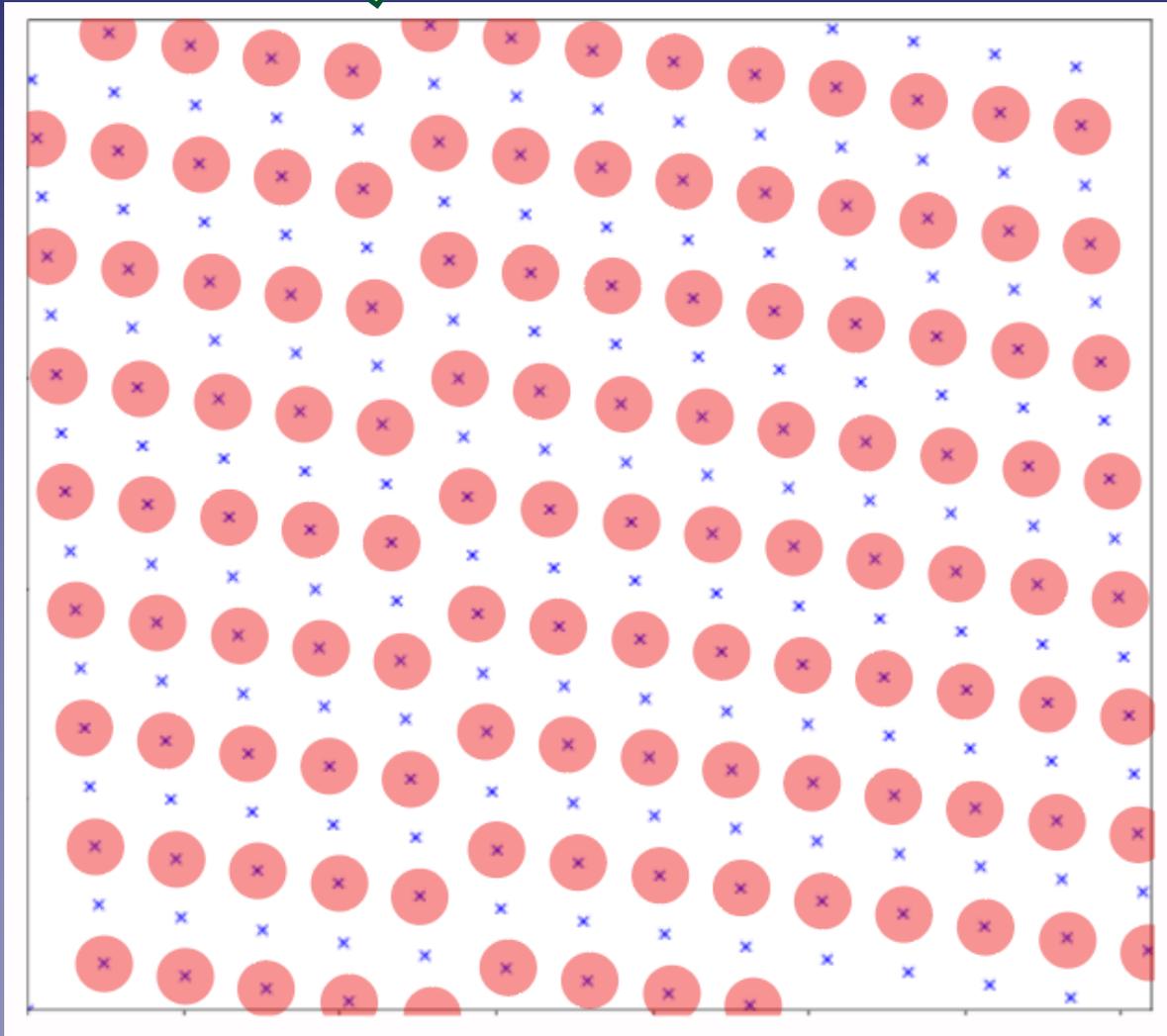
Real space or single-particle-like iterative refinement software

- FREALIGN (Grigorieff's lab)
 - IHRSR (Egelman's lab)
 - RELION (Scherer's lab)
 - SPRING (Sachse' lab)
 - CryoSPARC (Structure Biotechnology Inc.)
 - EMGlue (Sosa's lab)
- Spider
IHRSR
Frealign
Relion
EMAN

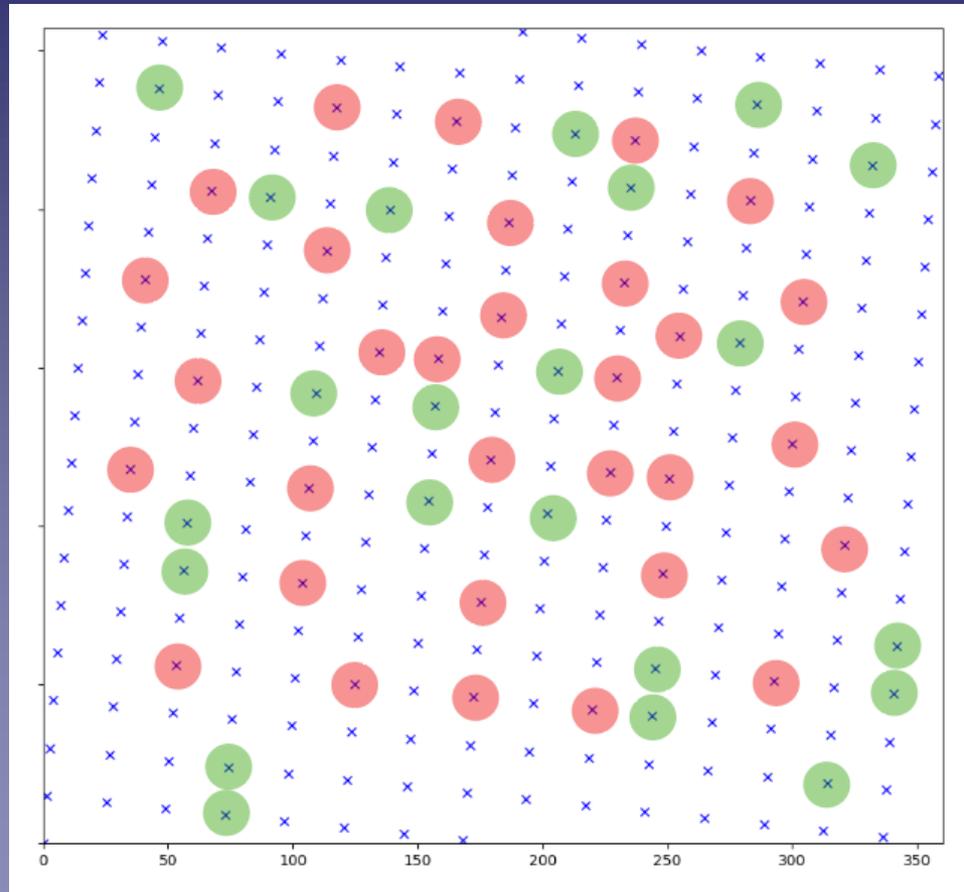
3D Helical Reconstruction Workflow

- Pick filaments & mark axis. (EMAN Boxer, Relion, CryoSPARC)
- Preliminary low resolution model (Custom)
- Classify filaments. (Custom, SPIDER, EMAN)
- Determine helical symmetry (ϕ , h) (Custom)
- Extract “single particle” boxes (Spider, Relion, CryoSPARC)
- Determine 3D orientation of single particle boxes (projection matching) & make 3D reconstruction (Spider, Custom, Relion, CryoSPARC)
- Refine 3D map (IHRSR, Relion, CryoSPARC).
- Model Building (UCSF-Chimera, Coot, Phenix, Direx, Modeller)

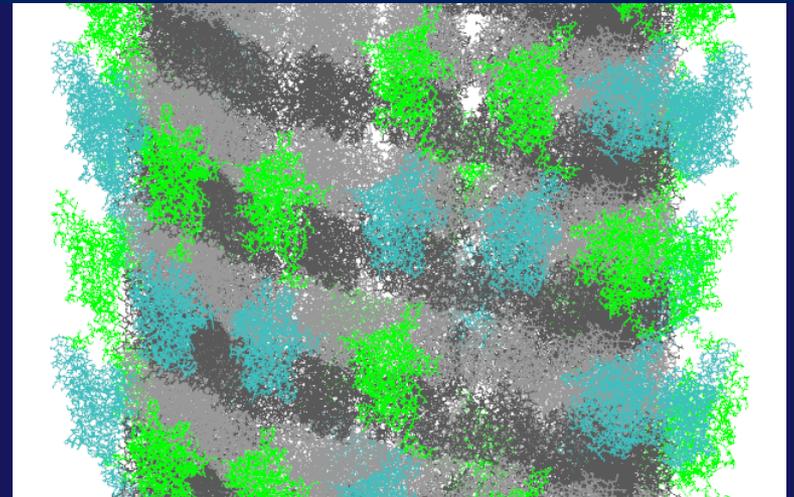
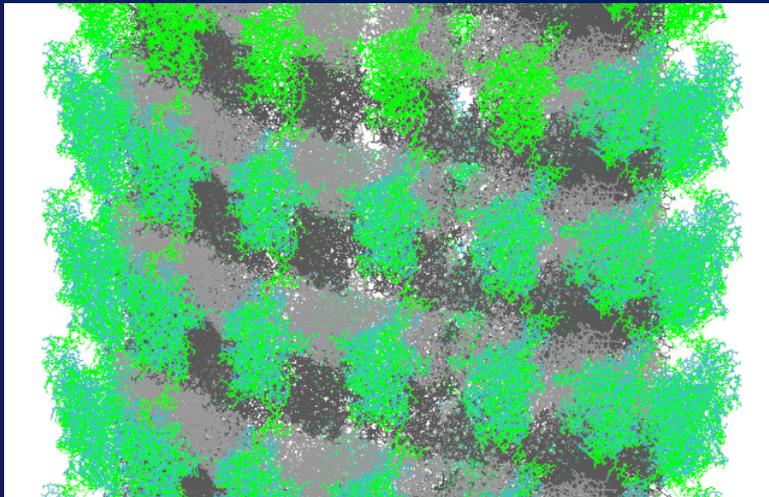
Helical Discontinuity, Seam



Resolving asymmetric features in helical assemblies



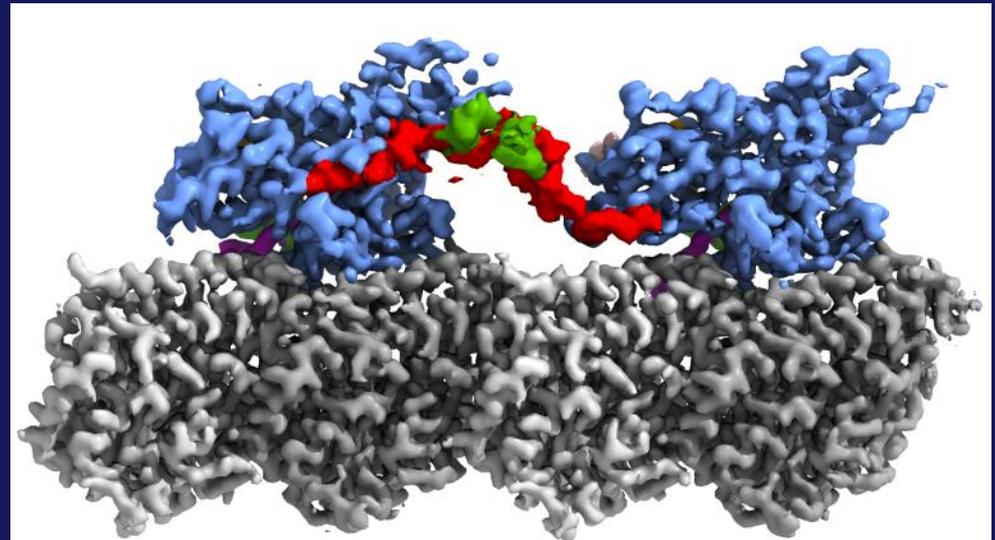
Resolving asymmetric features in helical assemblies



Helical Assembly Subunit Refinement and Classification (HASRC)

1. Helical reconstruction
2. Symmetry expansion , Signal subtraction , Subunit isolation.
3. Subunit local refinement.
4. Focus 3D classification.

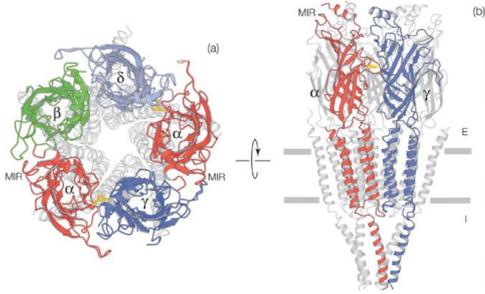
(Relion 3.0, CryoSPARC)



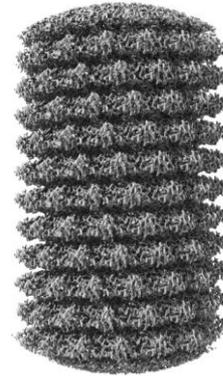
KIF14 Dimer MT complex (2.9 Å resol)
Benoit et al., *Nat. Comm.* 12:3637 (2021)

Examples of Helical Structures

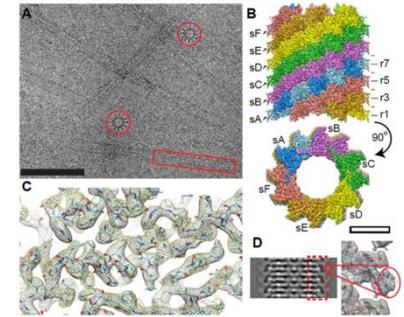
@ $\leq 4 \text{ \AA}$



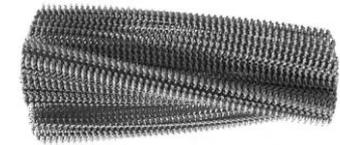
Ach Receptor (4Å)
Unwin N.(2005) JMB 346:976
(Fourier-Bessel Method)



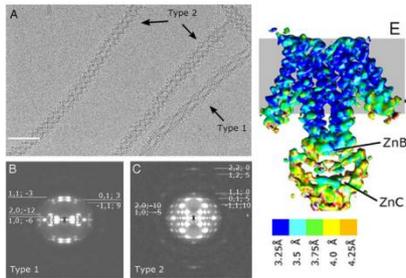
TMV (1.9Å)
Weis et. al., (2019)
EMBO Rep . 20 e48451



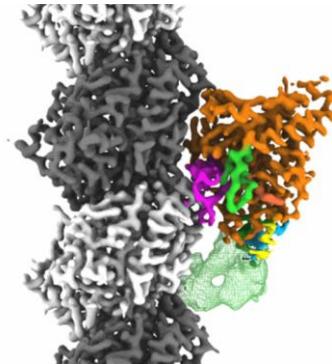
Bacteria type VI secretion system (3.5 Å)
Kudriashev et al., (2015)
Cell 26: 952



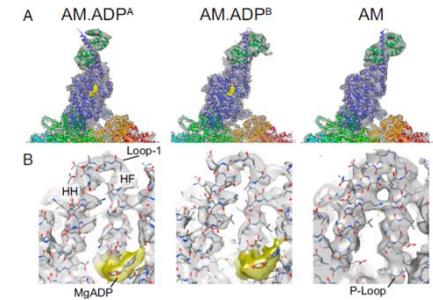
Tau Filaments (1.75 Å)
Flovestram et al., (2024).
Nature 625 : 119-125



YjiP Zinc Transporter (4.1 Å)
Lopez-Redondo et al., (2018)
PNAS 115: 3042



Kinesin-MT complexes (2.6 Å)
(EMD-26075). Hunter et.al., 2023
Nature Comm. 13:4198



Acto-myosin (3.2 Å)
Mentes et al., (2018). PNAS
<https://doi.org/10.1073/pnas.1718316115>

References & additional reading

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Single-Particle Approaches

IHRSR

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Grant, T., A. Rohou, and N. Grigorieff. 2018. cisTEM, user-friendly software for single-particle image processing. *eLife*. 7:e35383.

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CryoSPARC

<https://www.google.com/url?sa=t&source=web&rct=j&opi=89978449&url=https://guide.cryosparc.com/processing-data/all-job-types-in-cryosparc/helical-reconstruction-beta&ved=2ahUKEwiq8YuFz9yLAXrI4kEHc3hHM0QFnoECAgQAQ&usg=AOvVaw3s5eMGery2xRmUt4M1cDsS>

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Helical Reconstruction Tutorial (CryoSPARC)

<https://guide.cryosparc.com/processing-data/tutorials-and-case-studies/case-study-empiar-10031-mavs>