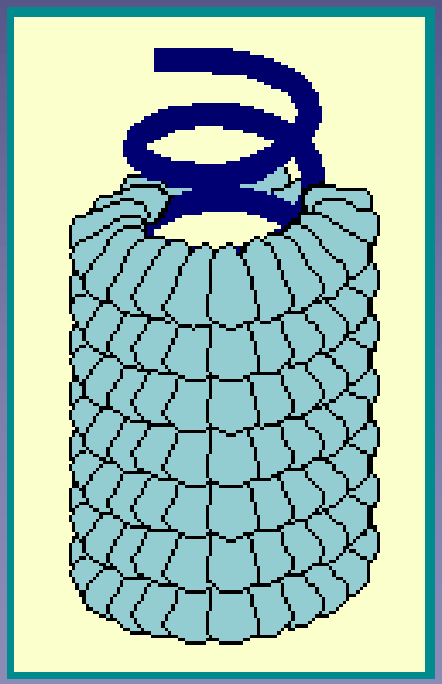
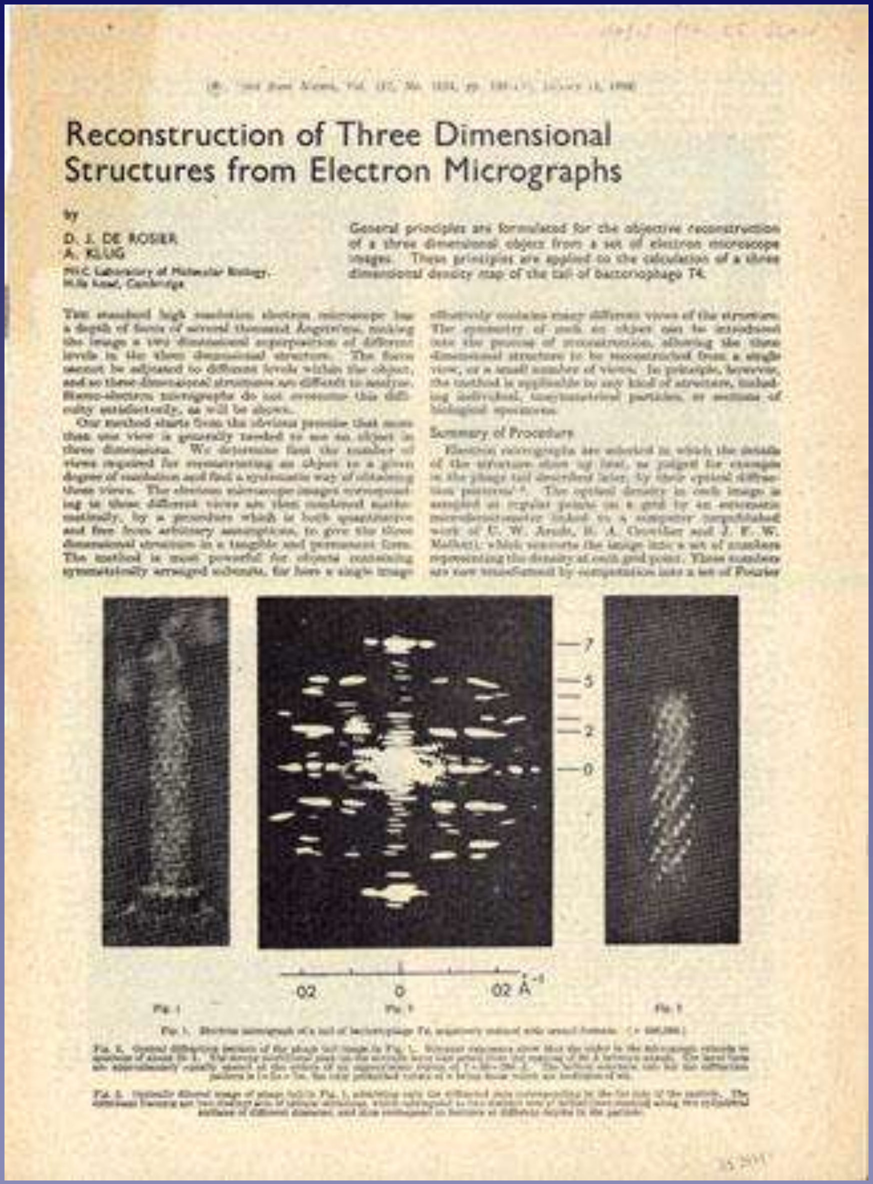
3D Reconstruction of Helical Specimens



Many Biological Specimens have helical symmetry

-DNA
-α-Helix

-Viruses (TMV)
-Actin filaments
-Myosin filaments
-Microtubules
-Bacterial Flagella
-Protein-lipid tubes



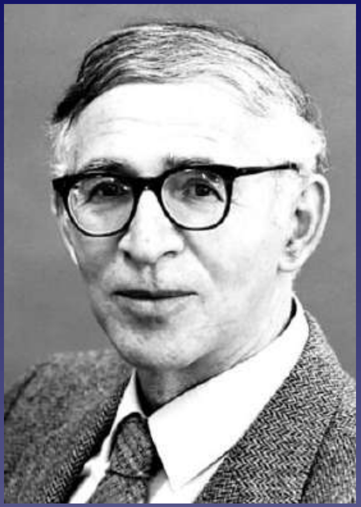
Reconstruction of Three Dimensional Structures from Electron Micrographs

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.

Summary of Procedure

Fig. 1
Fig. 2
Fig. 3



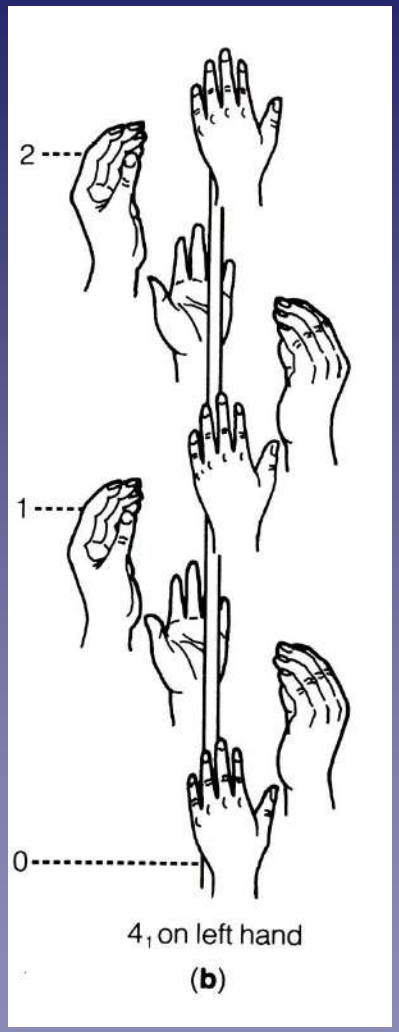
De Rosier & Klug. Nature 217: 130-134 1968

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**

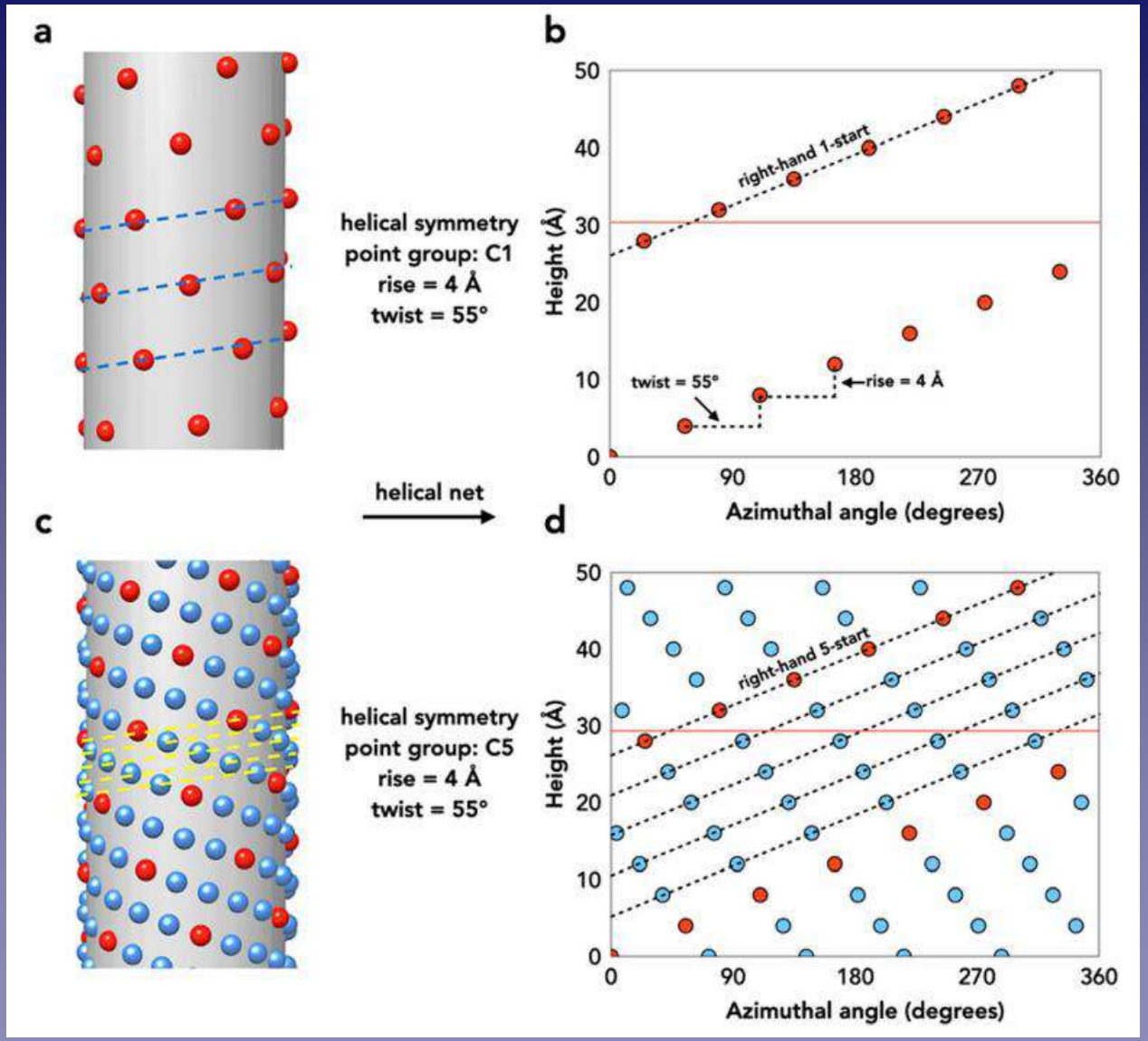


Possible Symmetry operations:

-Screw.

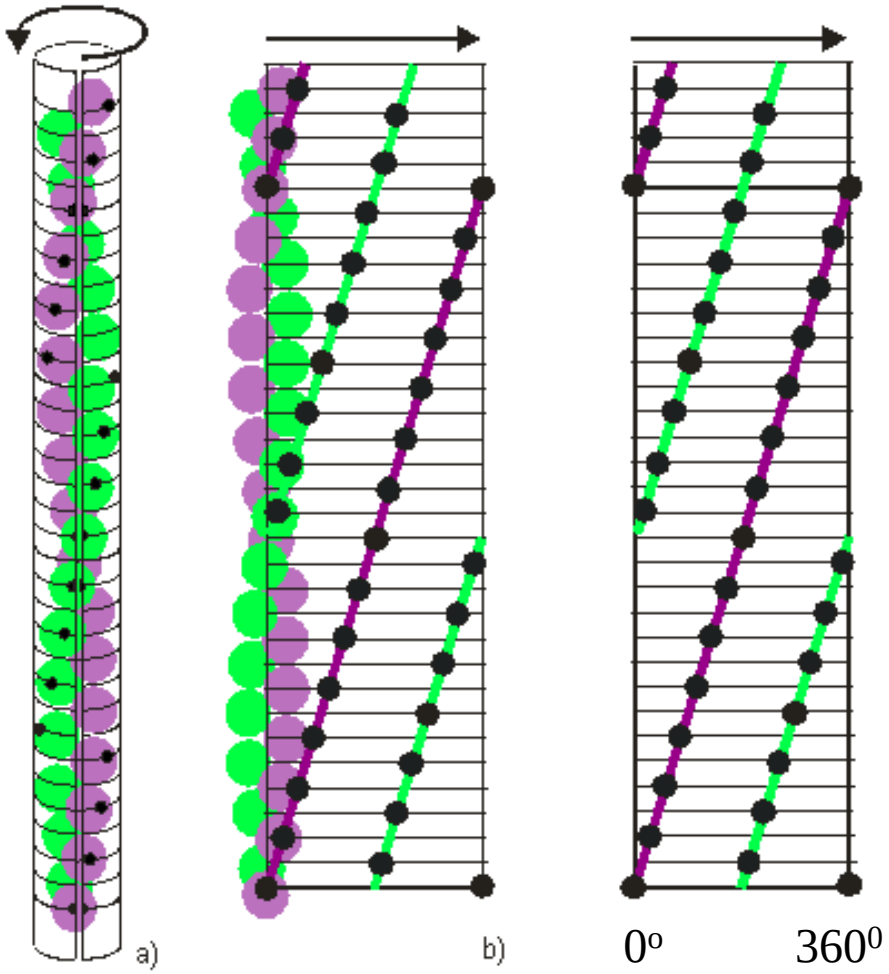
-n-fold rotation about axis.

-2-fold rotation perpendicular to axis.



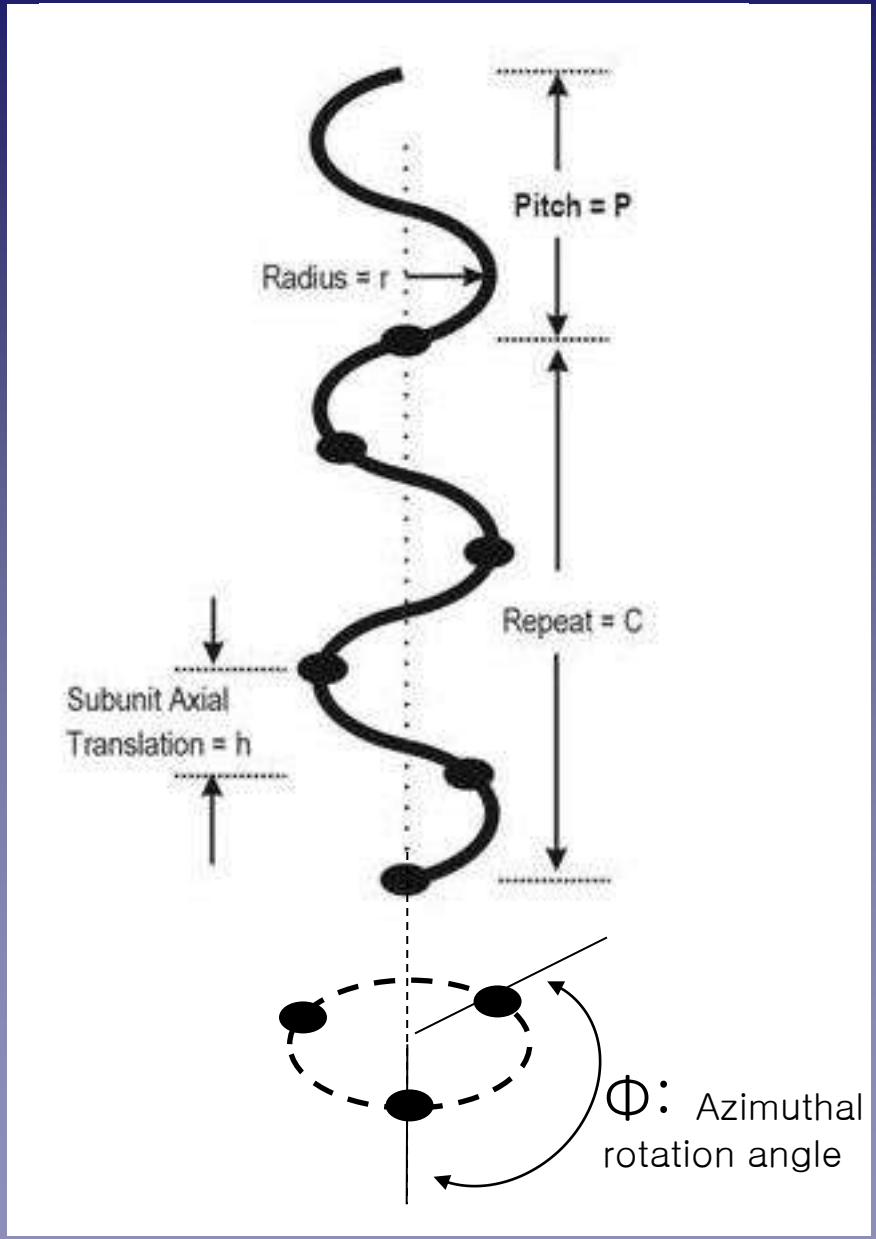
From: Wang et al., 2022

The Helical Lattice Radial Projection

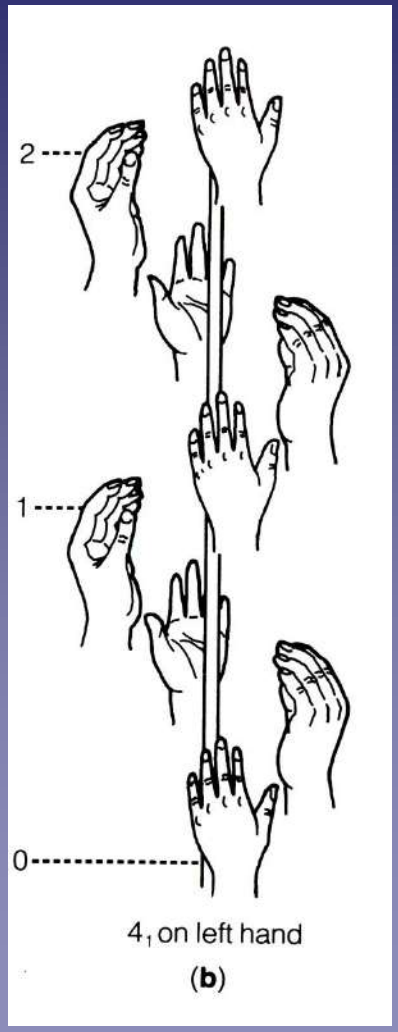


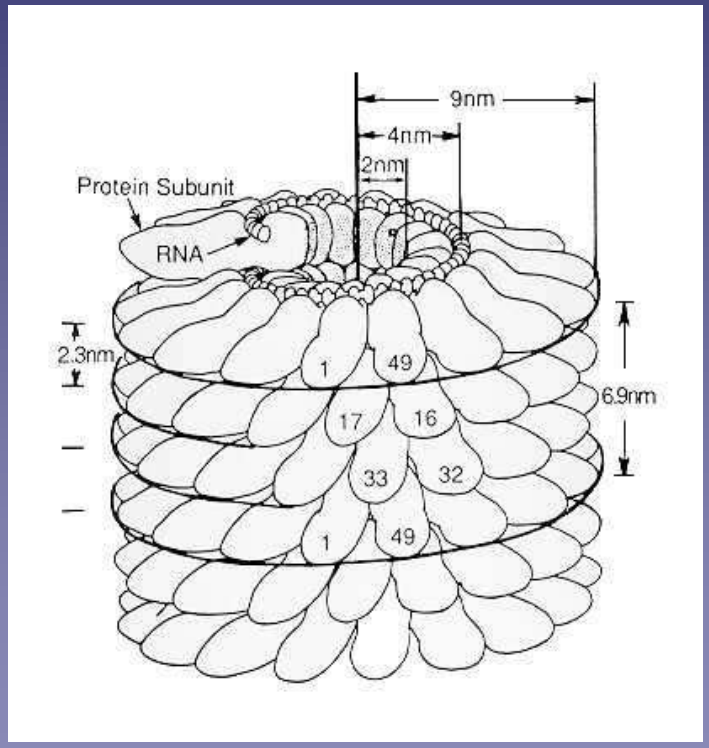
Helical Net

Parameters of a Helix



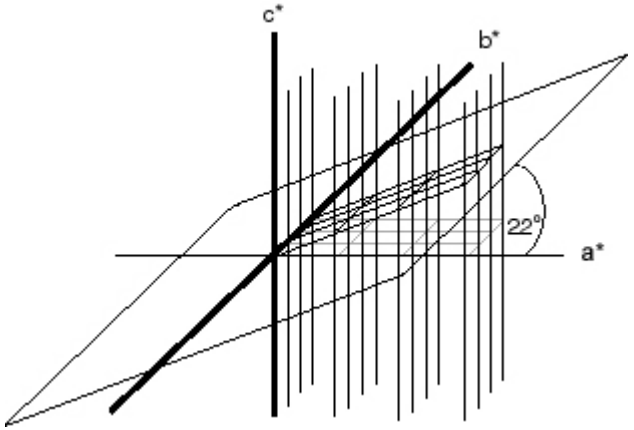
Helices give several orientation views of the asymmetric unit from a single view direction



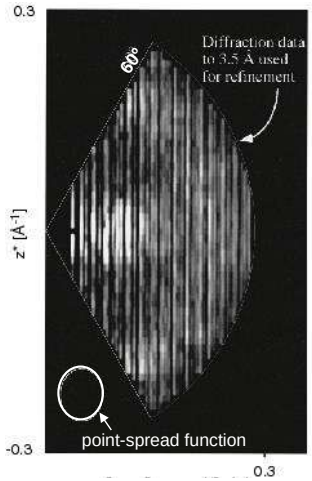


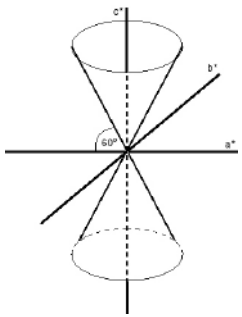
No Missing Cone !

2D crystals

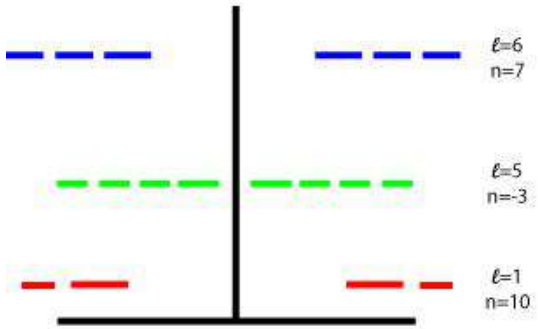


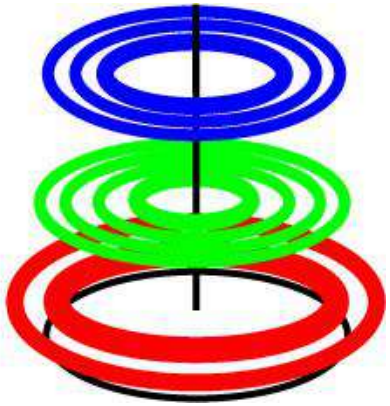
missing cone



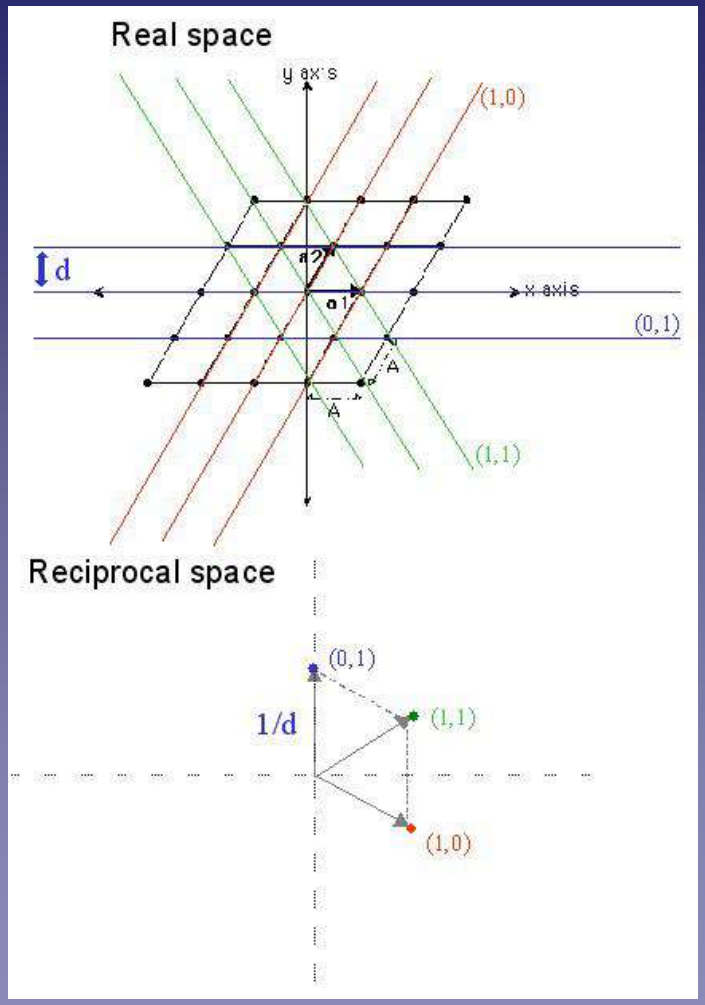


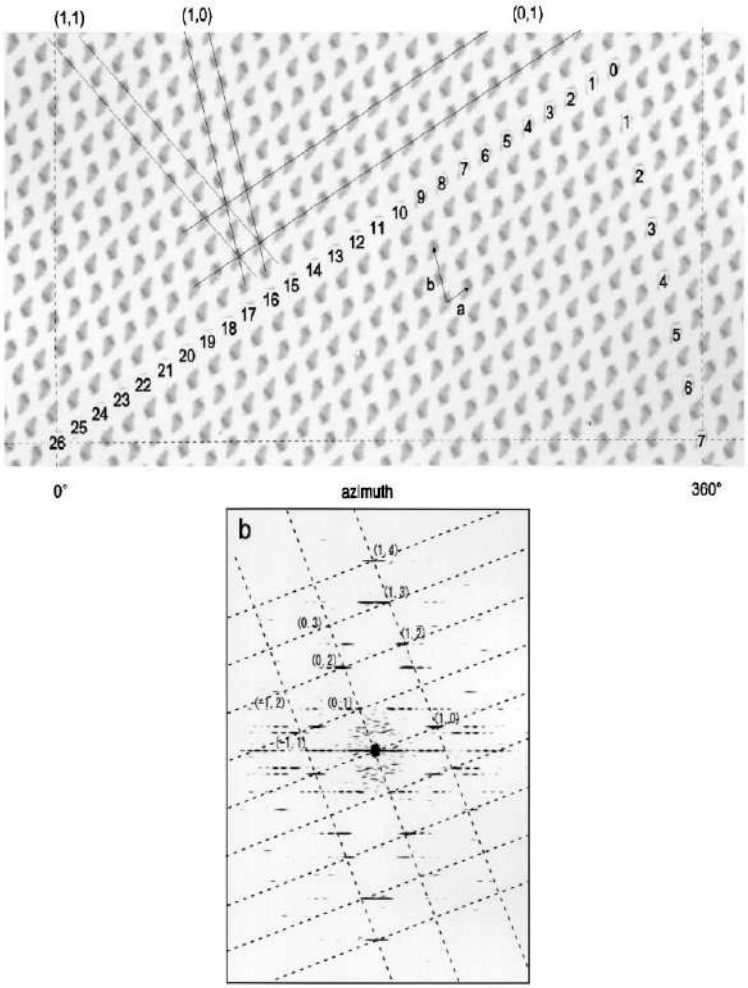
Helical crystals





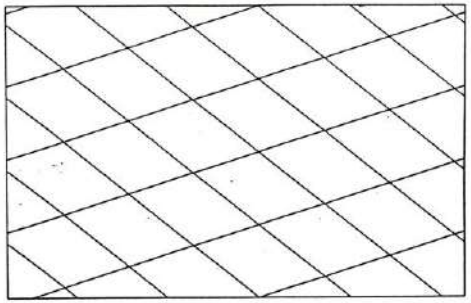
Analogy between 2D lattices and Helical Lattices

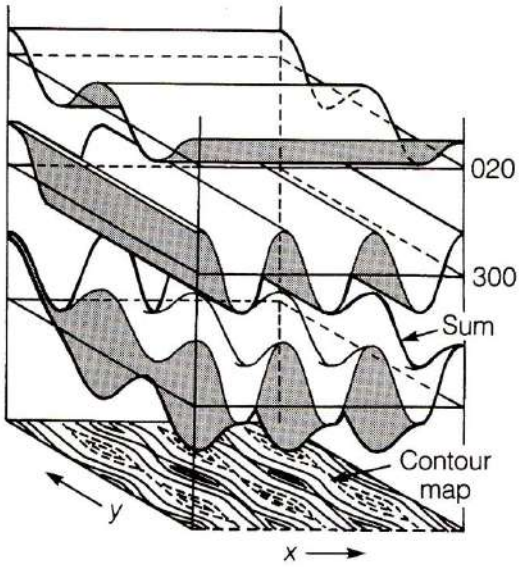




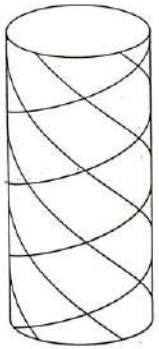
From: Toyoshima (2000) Ultramicroscopy 84: 1-14

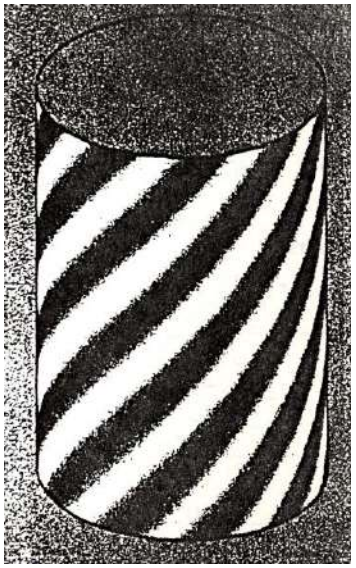
Analogy between 2D Fourier synthesis and Fourier-Bessel helical synthesis





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





A helical wave

The Fourier Transform of a Helix

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

Cochran, Crick & Vand 1952

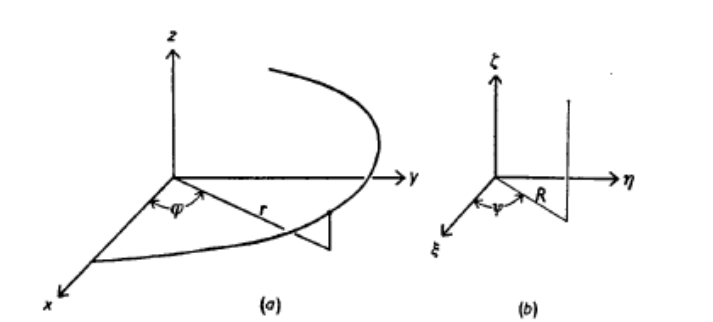
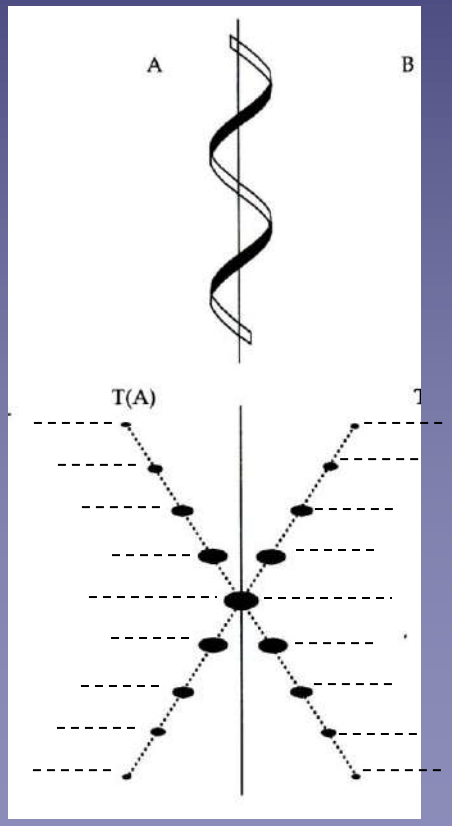
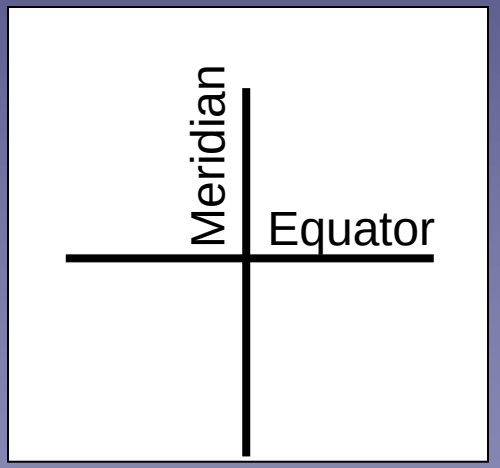


Fig. 1. (*a*) Cartesian (*x*, *y*, *z*) and cylindrical-polar (*r*, *φ*, *z*) co-ordinates of a point on a helix. (*b*) Corresponding coordinates of a point in reciprocal space.





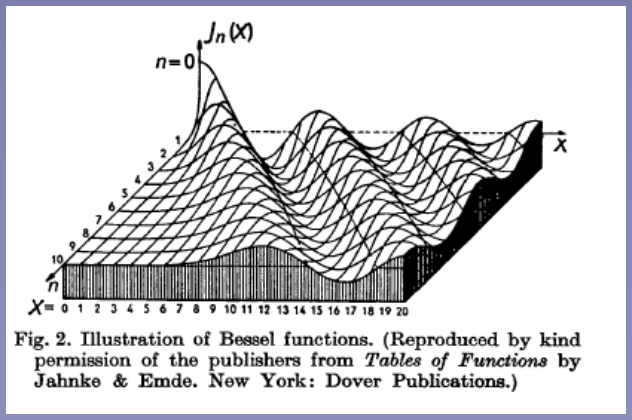
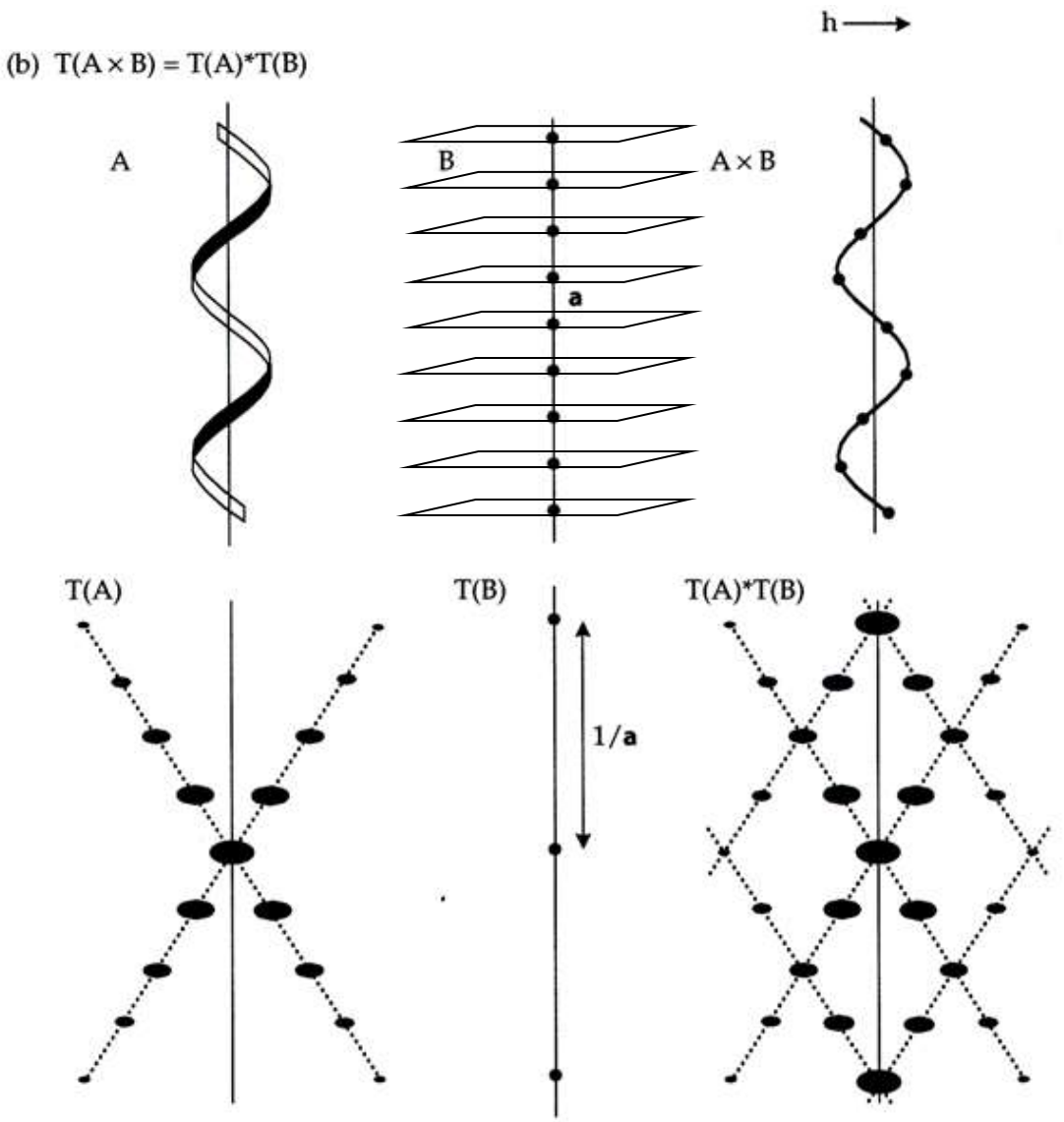
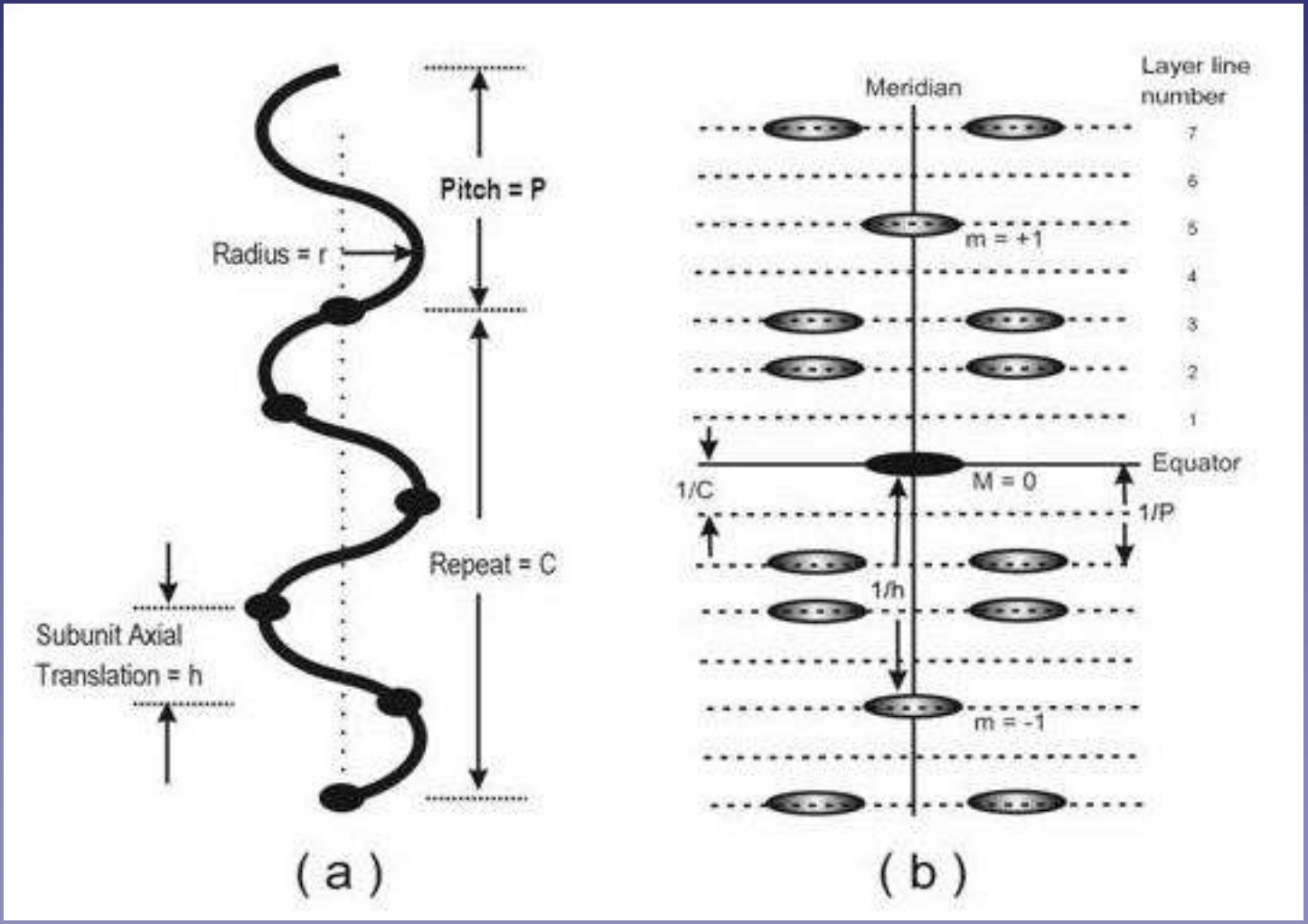


Fig. 2. Illustration of Bessel functions. (Reproduced by kind permission of the publishers from *Tables of Functions* by Jahnke & Emde. New York: Dover Publications.)

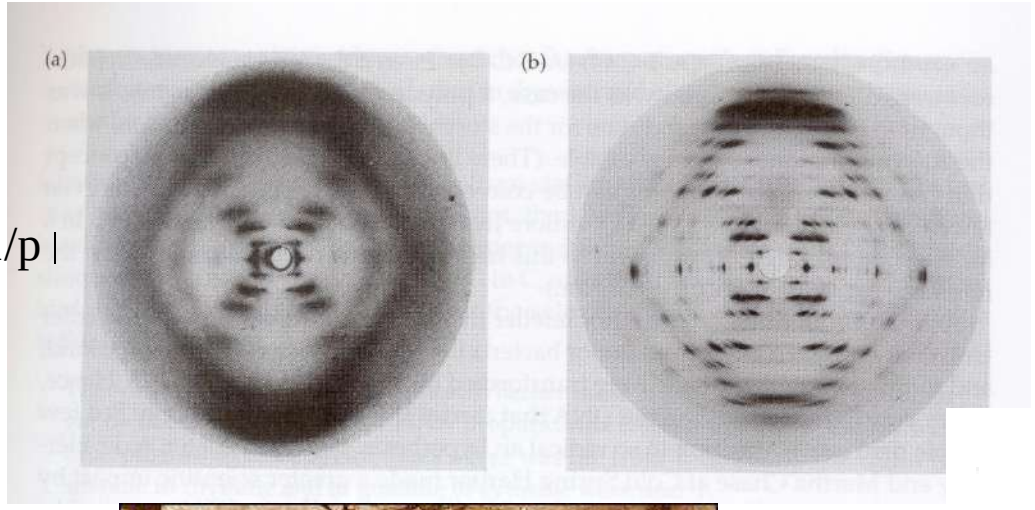
The Transform of a discontinuous helix



A helix and its corresponding Fourier Transform (Power Spectrum)

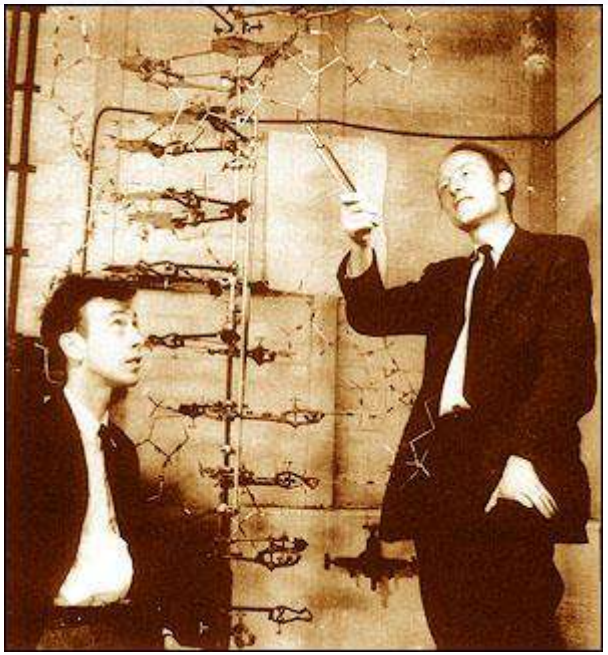


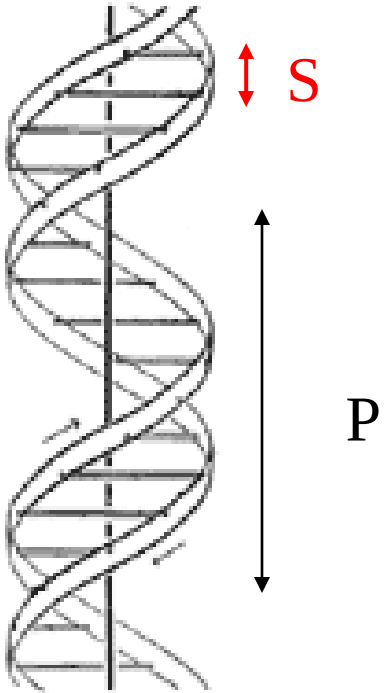
The DNA Structure



1/s

1/p





Helical 3D reconstruction Using the Fourier-Bessel Method

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

Fourier Transform
Reciprocal Space Function

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

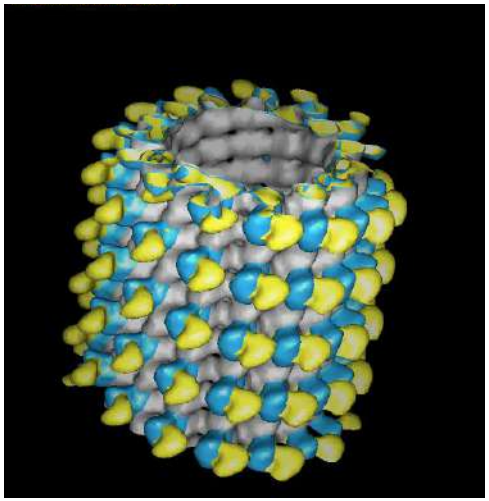
Selection rule

$$l = tn + um \quad (2)$$

Real space function
(structure)

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

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



Helical 3D reconstruction Using the Fourier-Bessel Method

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

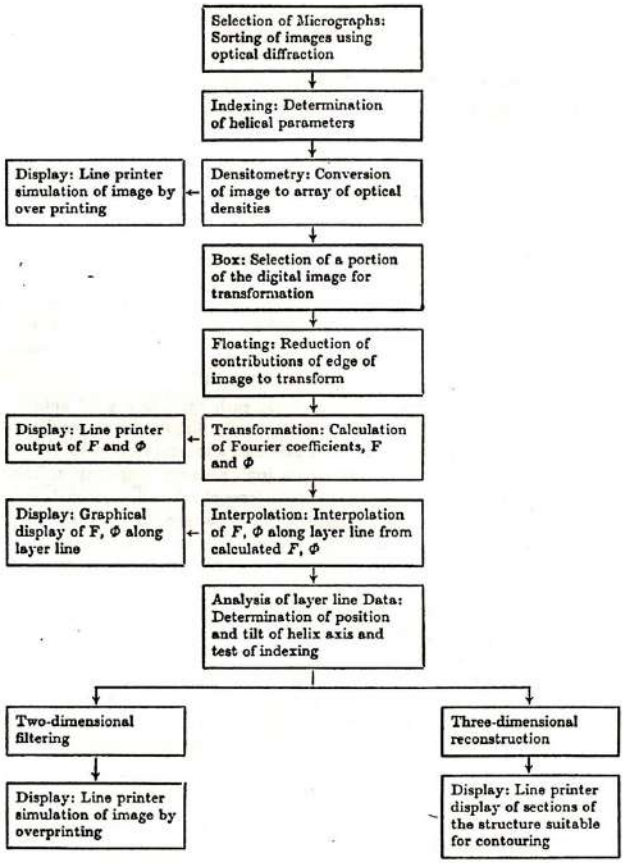


FIG. 1. The scheme presented shows the flow of data in the process of three-dimensional reconstruction.

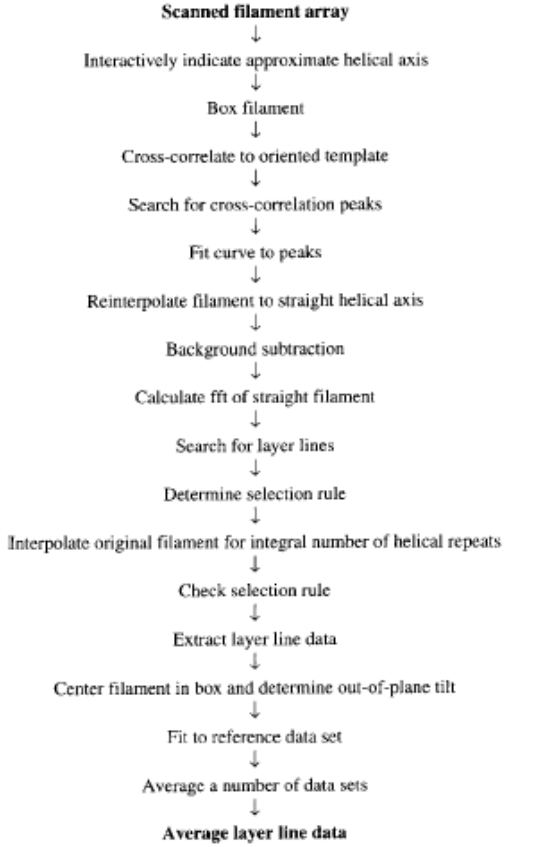
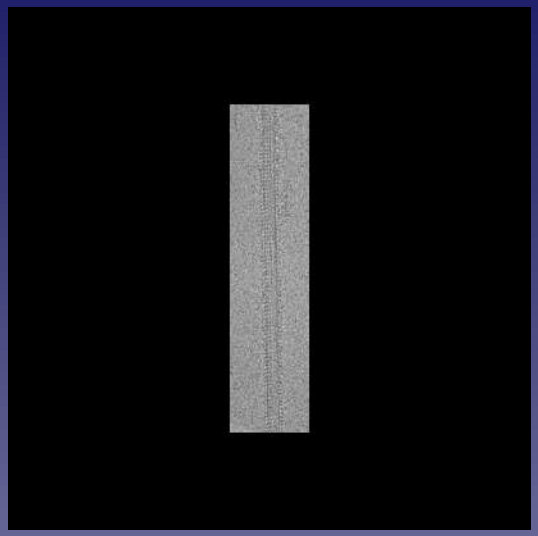


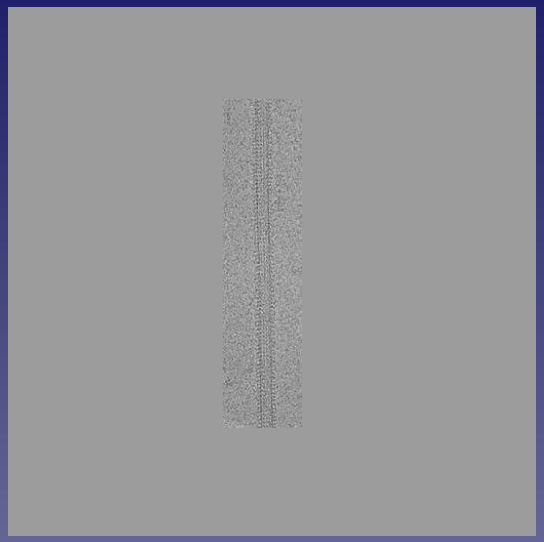
FIG. 1. Schematic diagram of the PHOELIX helical processing package. A detailed description of each step and the programs used is available as part of the PHOELIX distribution.

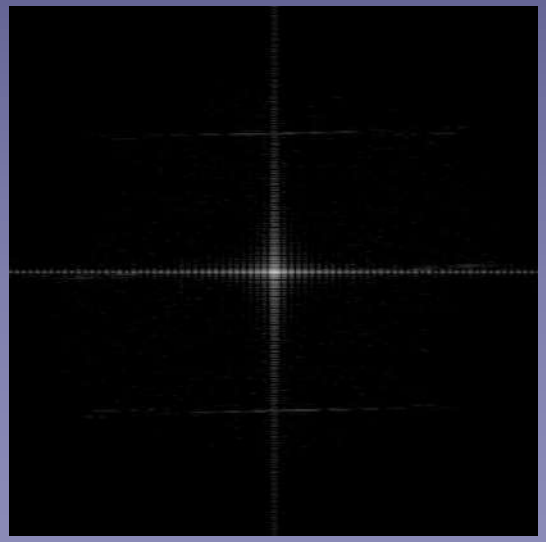
Boxing & Floating Image

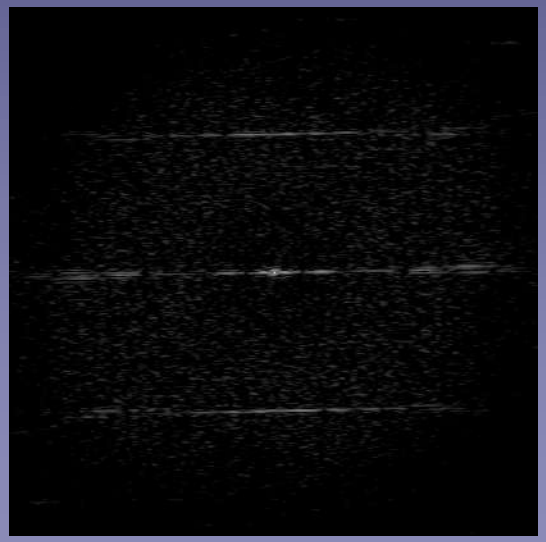
Image

FFT





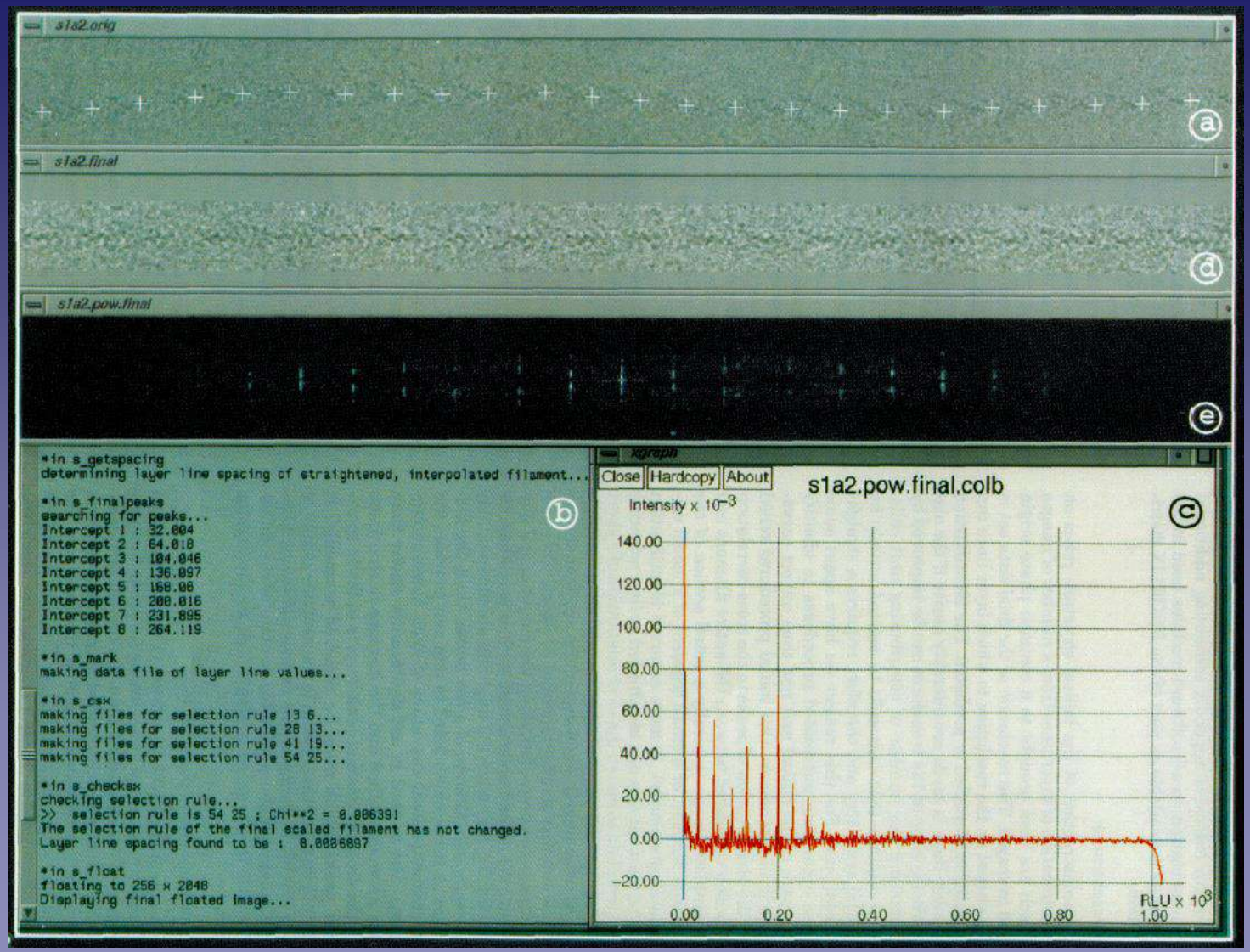




Non-Floated

Floated

Straightening

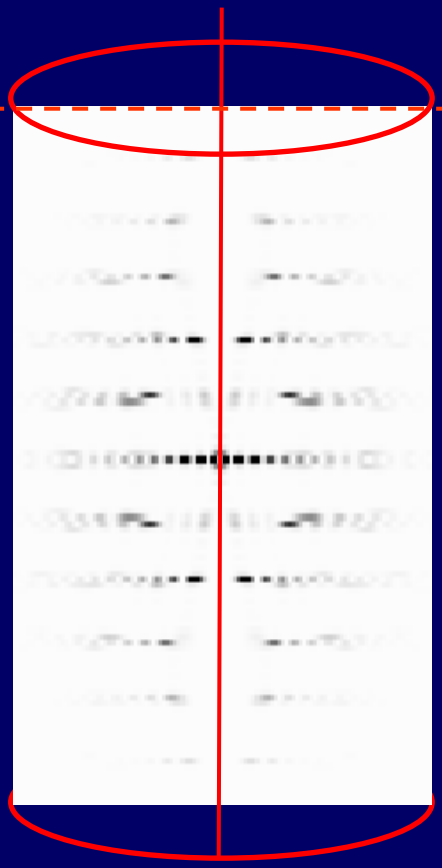


From: Carragher et al., JSB 116: 107-112 (1996)

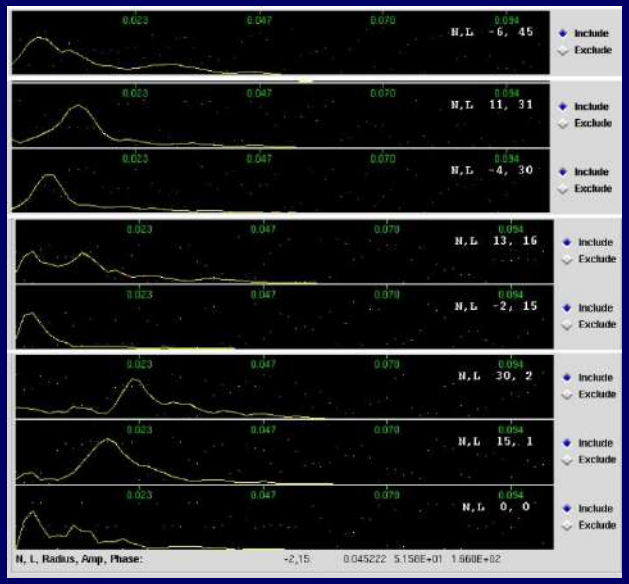
Gathering Amplitude and Phases

Image

FFT



Fourier Transform

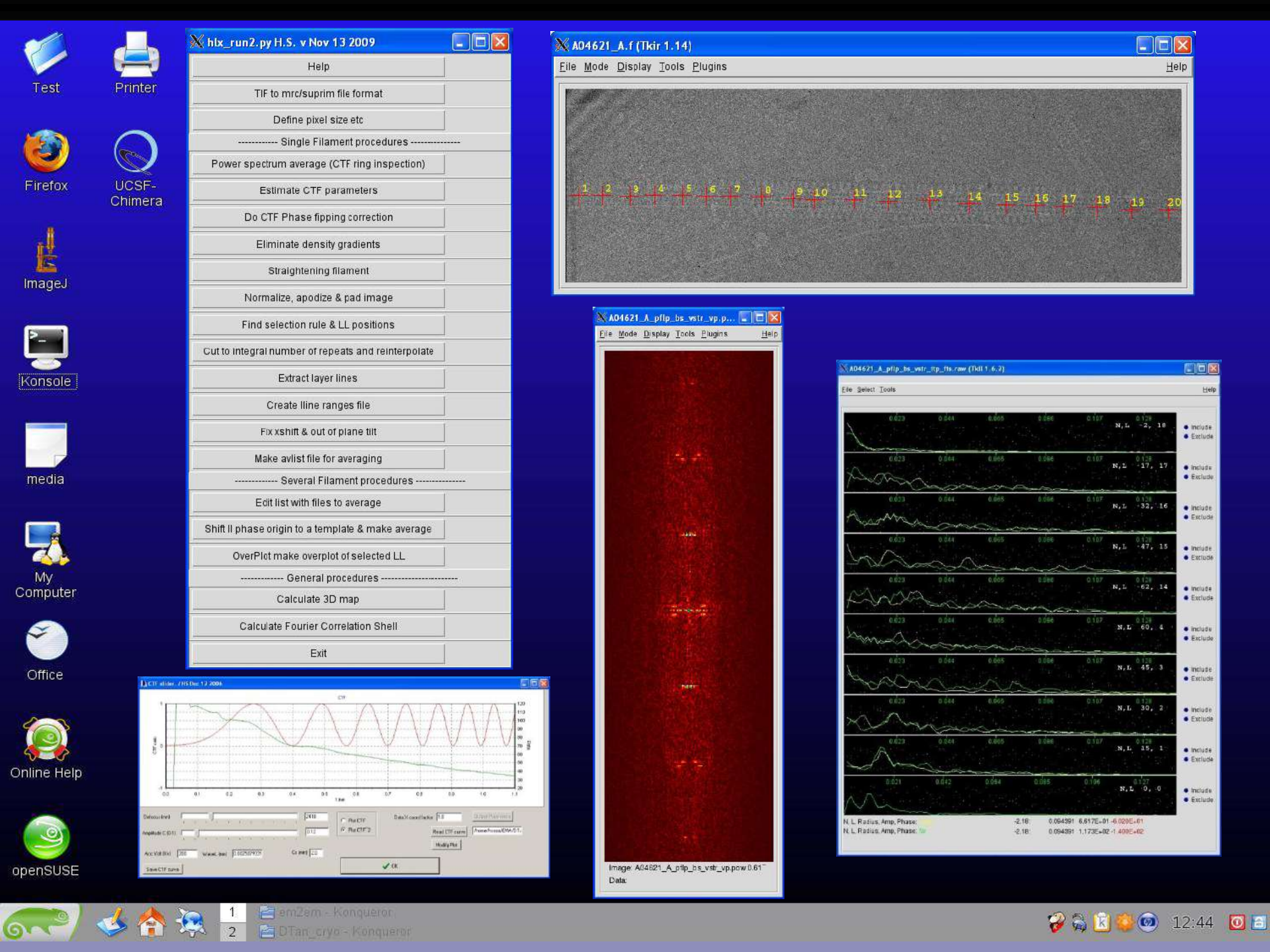


Layer Line Data Set

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

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

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

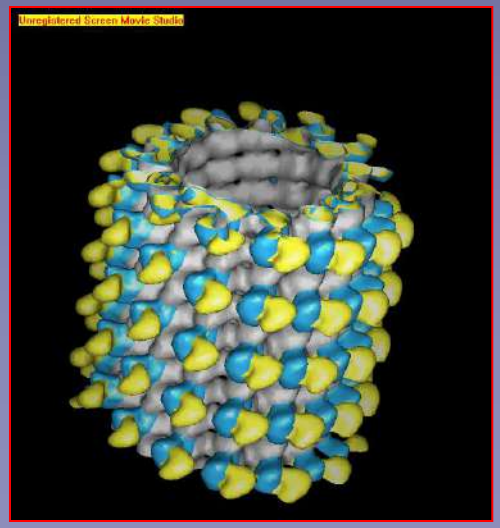

Test
Printer
Firefox
UCSF-Chimera
ImageJ
Konsole
media
My Computer
Office
Online Help
openSUSE
hlx_run2.py H.S. v Nov 13 2009
Help
TIF to mrc/suprim file format
Define pixel size etc
---------- Single Filament procedures ----------
Power spectrum average (CTF ring inspection)
Estimate CTF parameters
Do CTF Phase fipping 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
A04621_A.f (Tkir 1.14)
File Mode Display Tools Plugins Help
A04621_A_pflp_bs_vstr_vp.p...
File Mode Display Tools Plugins Help
Image: A04621_A_pflp_bs_vstr_vp.pow 0.61
Data:
A04621_A_pflp_bs_vstr_ltp_fts.raw (Tkil 1.6.2)
File Select Tools Help
em2em - Konqueror
DTan_cryo - Konqueror
12:44

3D Density Map Reconstruction
(Fourier Bessel inversion)

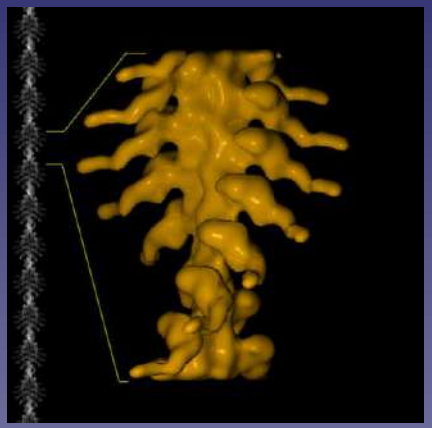
Average $G_{n,l}$ Data set.

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

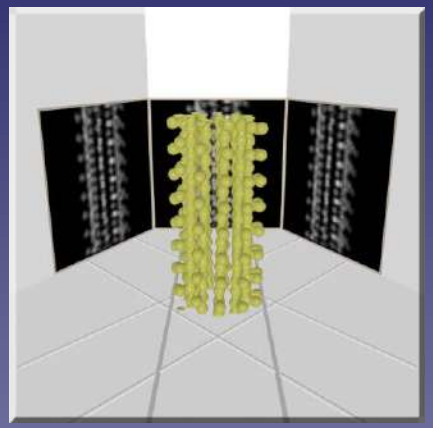
$$\rho(r, \phi, z) = \sum_l \sum_n g_{n,l}(r) \exp(in\phi) \exp(-2\pi i l z/c)$$



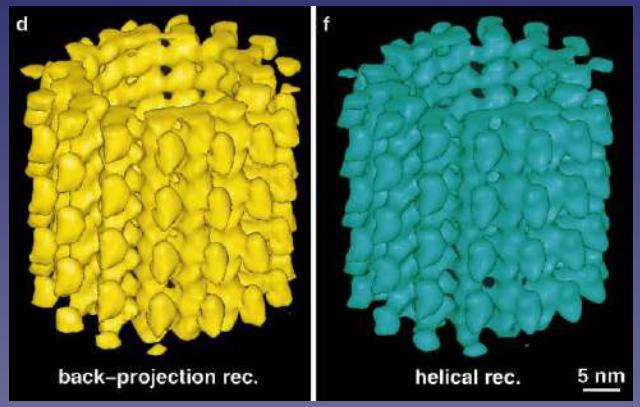
3D Helical Reconstruction Using Real Space Methods



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.



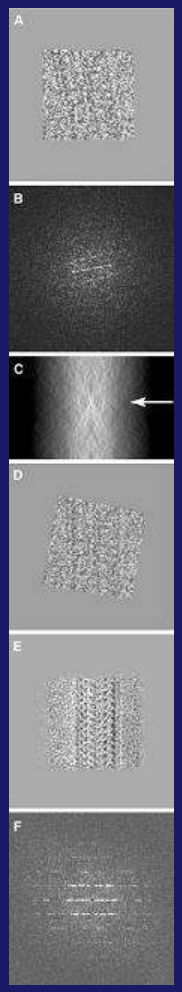
A 3D volume is obtained by back-projection of the boxed images.



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

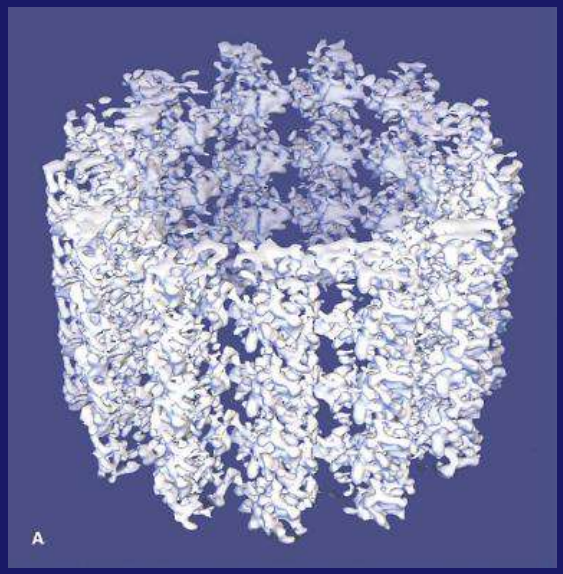
'Single Particle' Helical Reconstruction Methods

Box Filament segments



In-plane Rotational Alignment

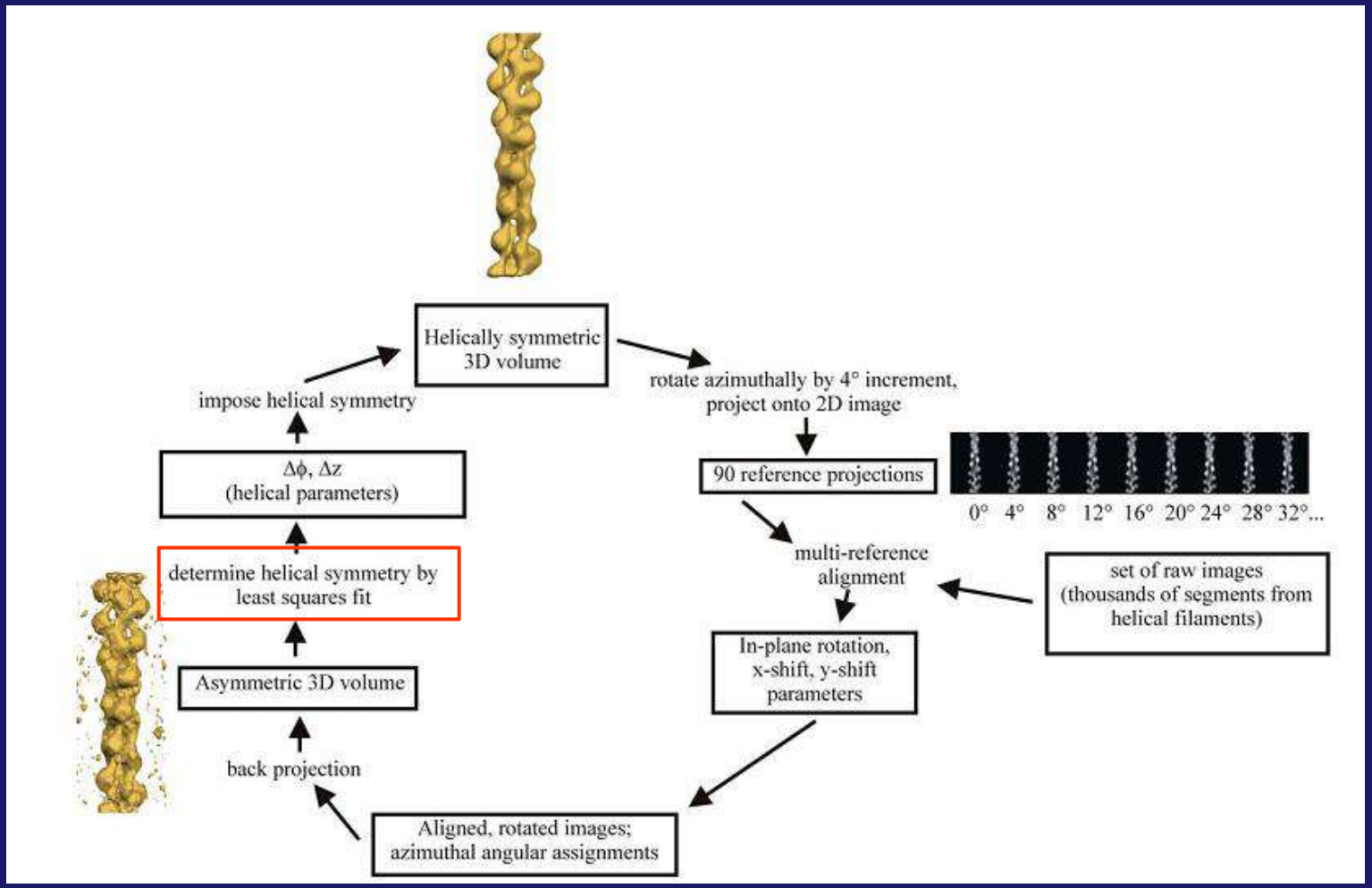
Multi-reference alignment with atomic models



8 Å resolution microtubule map

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

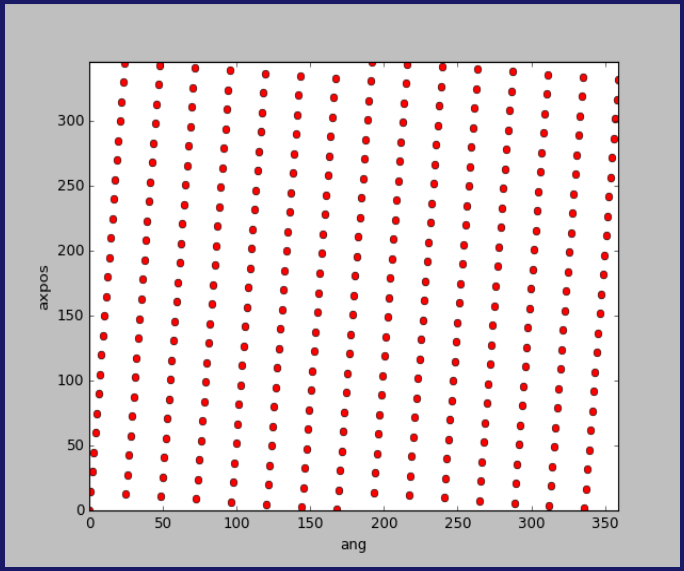
The Iterative Helical Real Space Reconstruction Method (IHRSR)



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

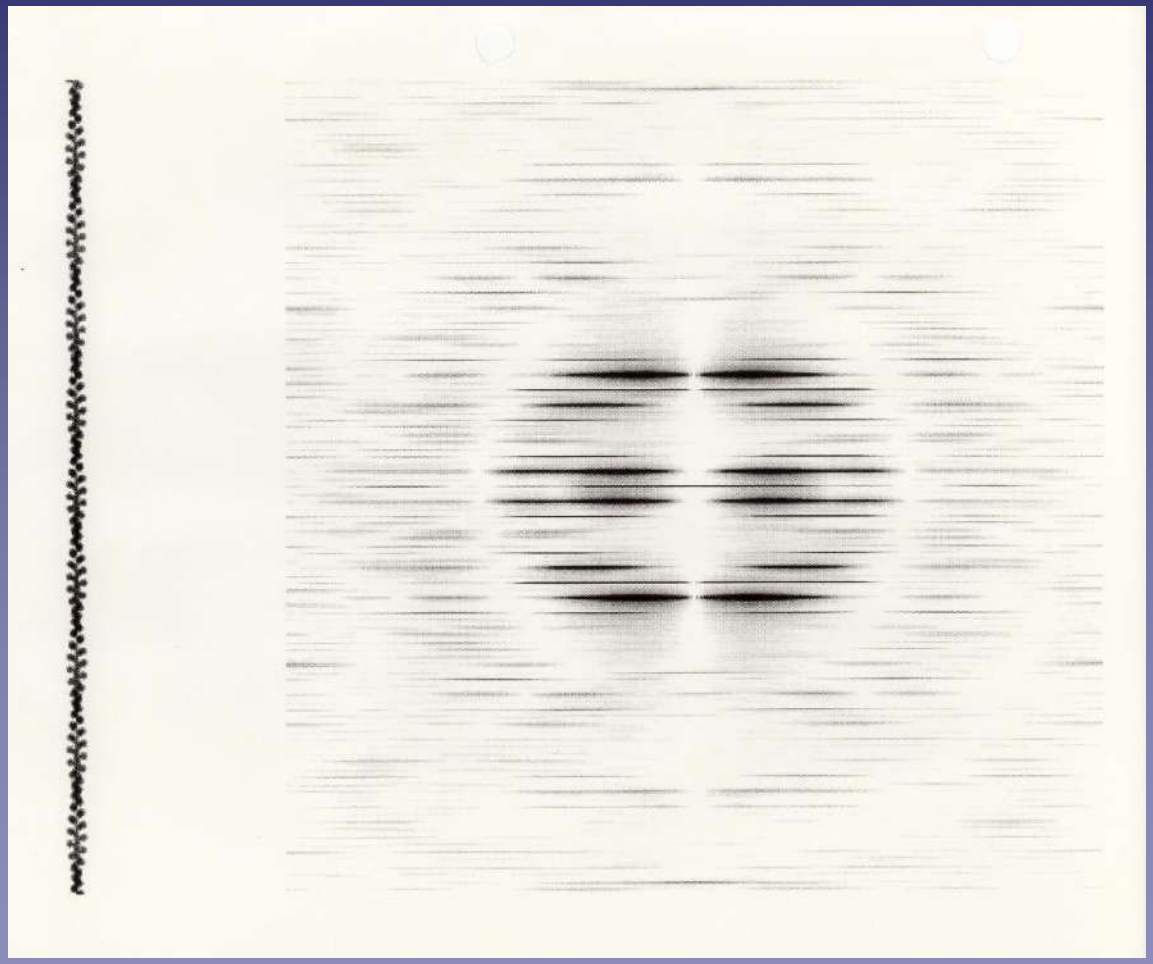
$$l = tn + um$$

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

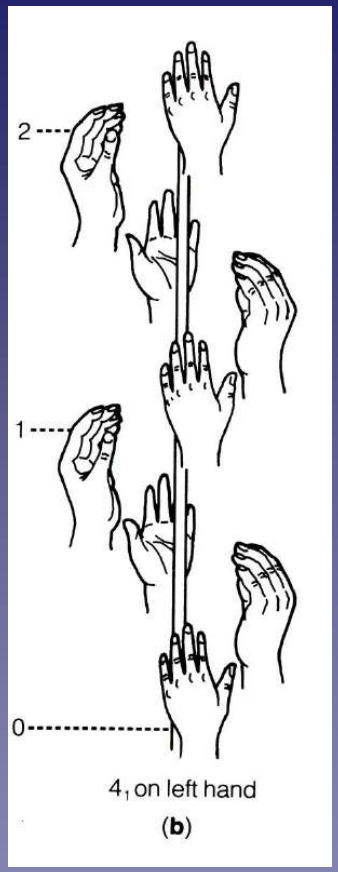
SR: $l = 162 * n + 347 * m$

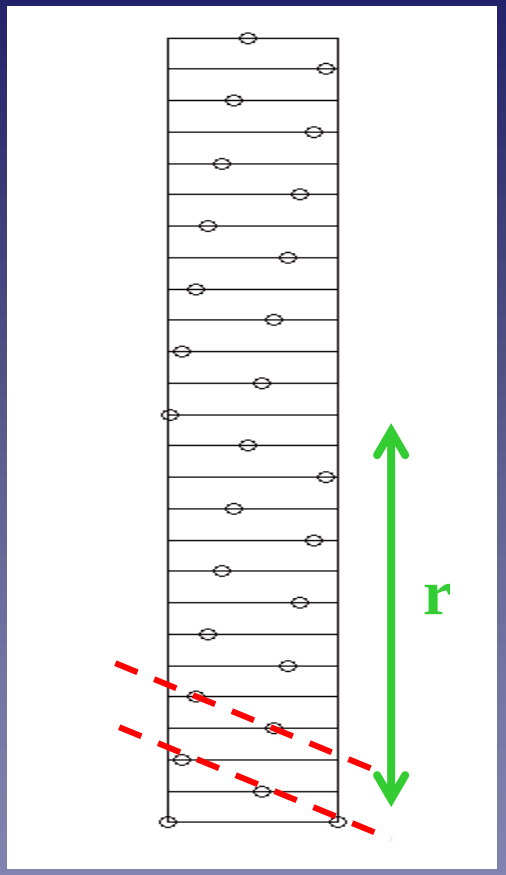
Φ : 168.07

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.

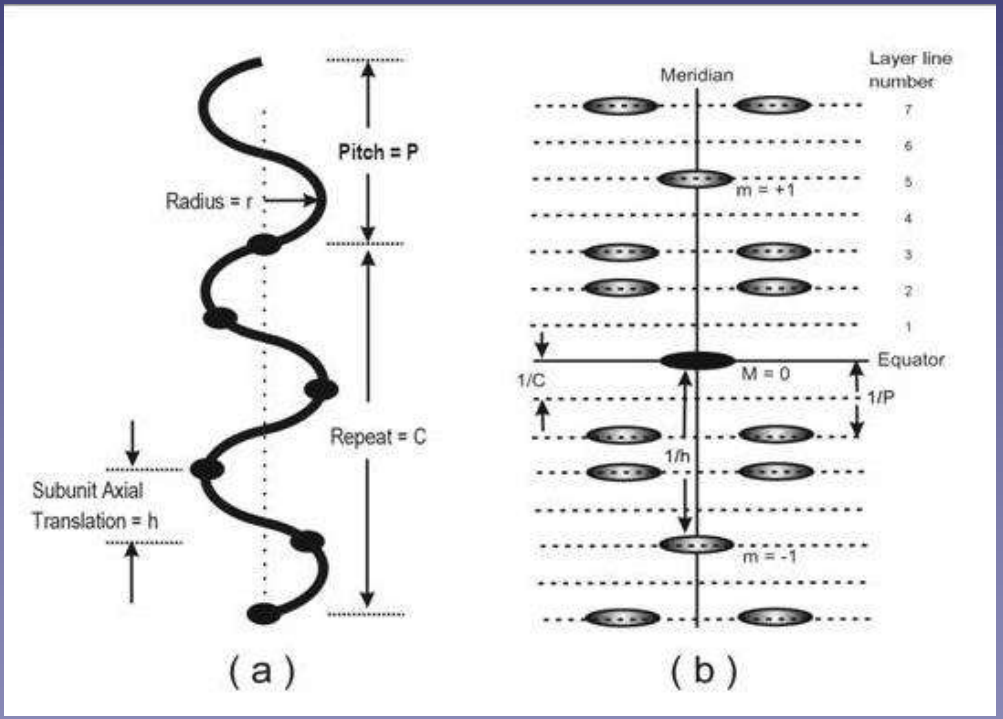
***u*:** Num. of subunits/rep

***m*:** Integer

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

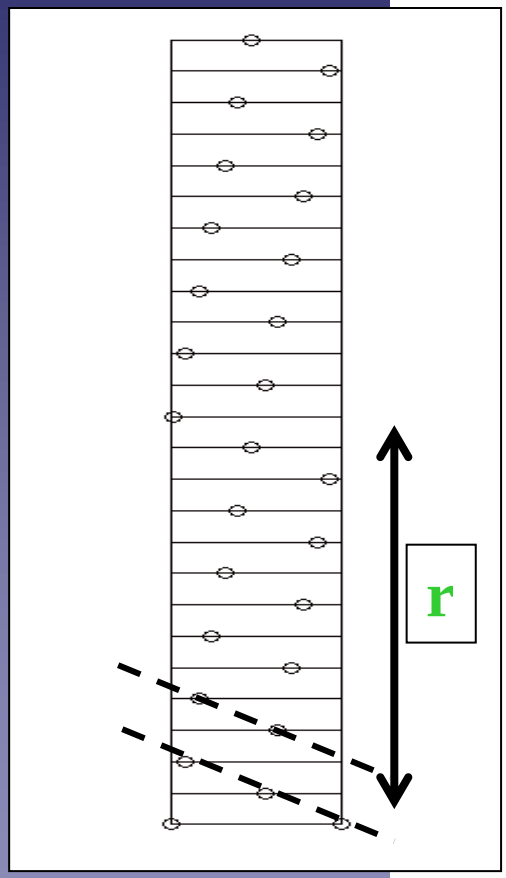
$$Z = n(\phi/360^0)/h + m/h$$

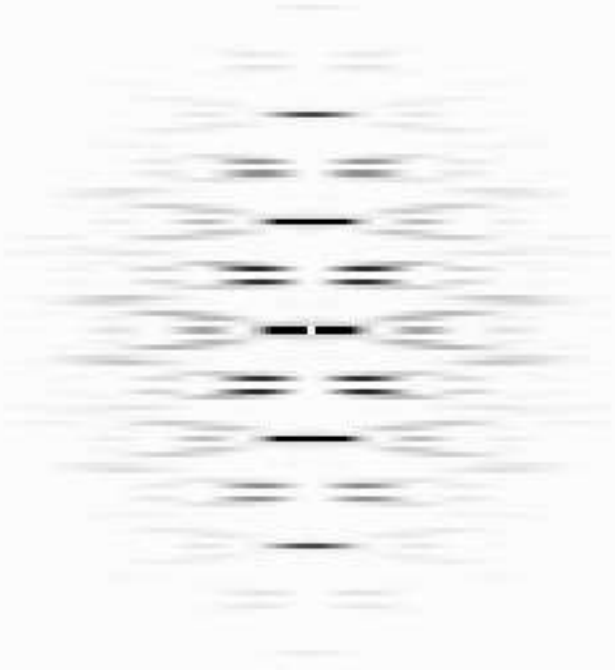
Z: LL reciprocal spacing
ϕ: twist angle.
h: rise distance.



Selection Rule Example

$$l = tn + um$$

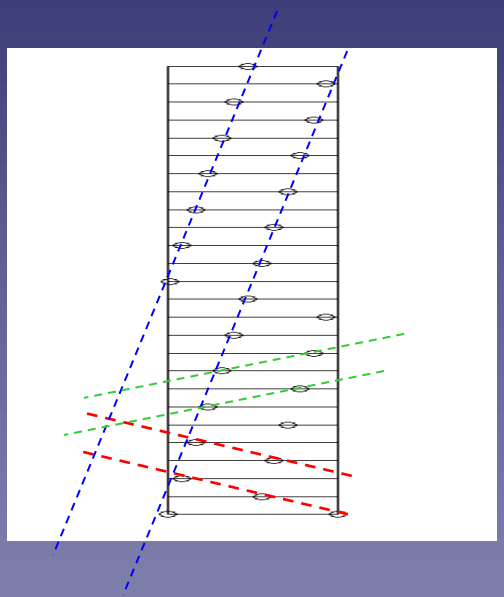




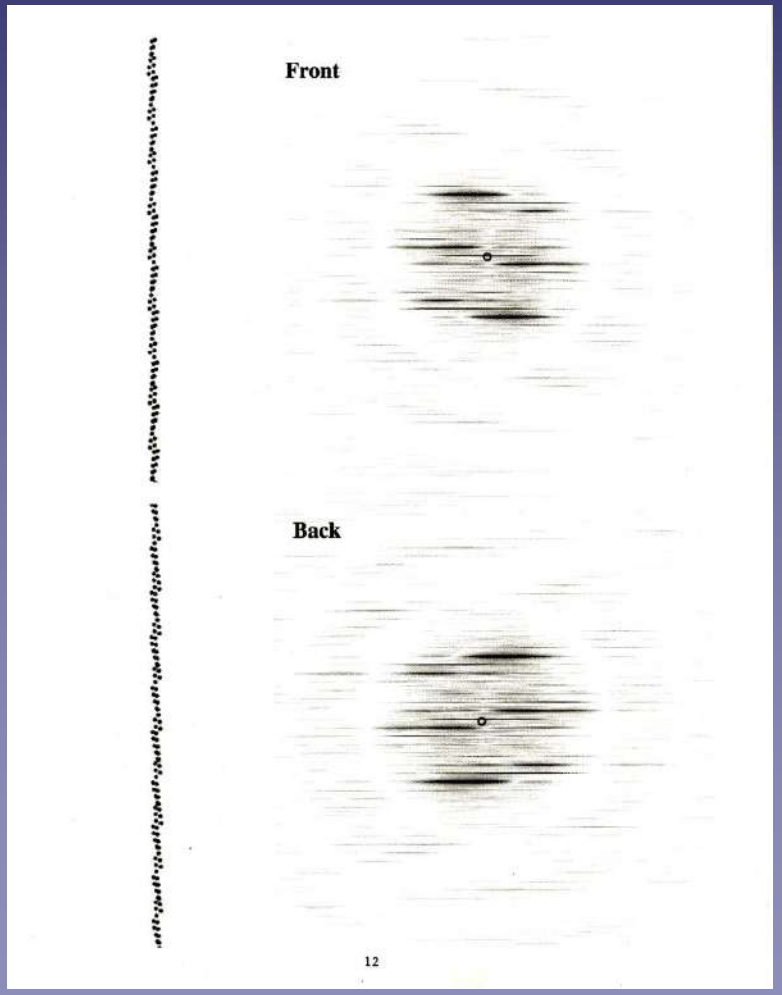
l	$n(\lvert n\rvert <= 10)$
0	0
1	2
2	4, -9
3	6
4	-5, 8
5	-3, 10
6	-1
7	1
8	3, -10
9	5, -8
10	-6, 7
11	-4, 9
12	-2
13	0

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

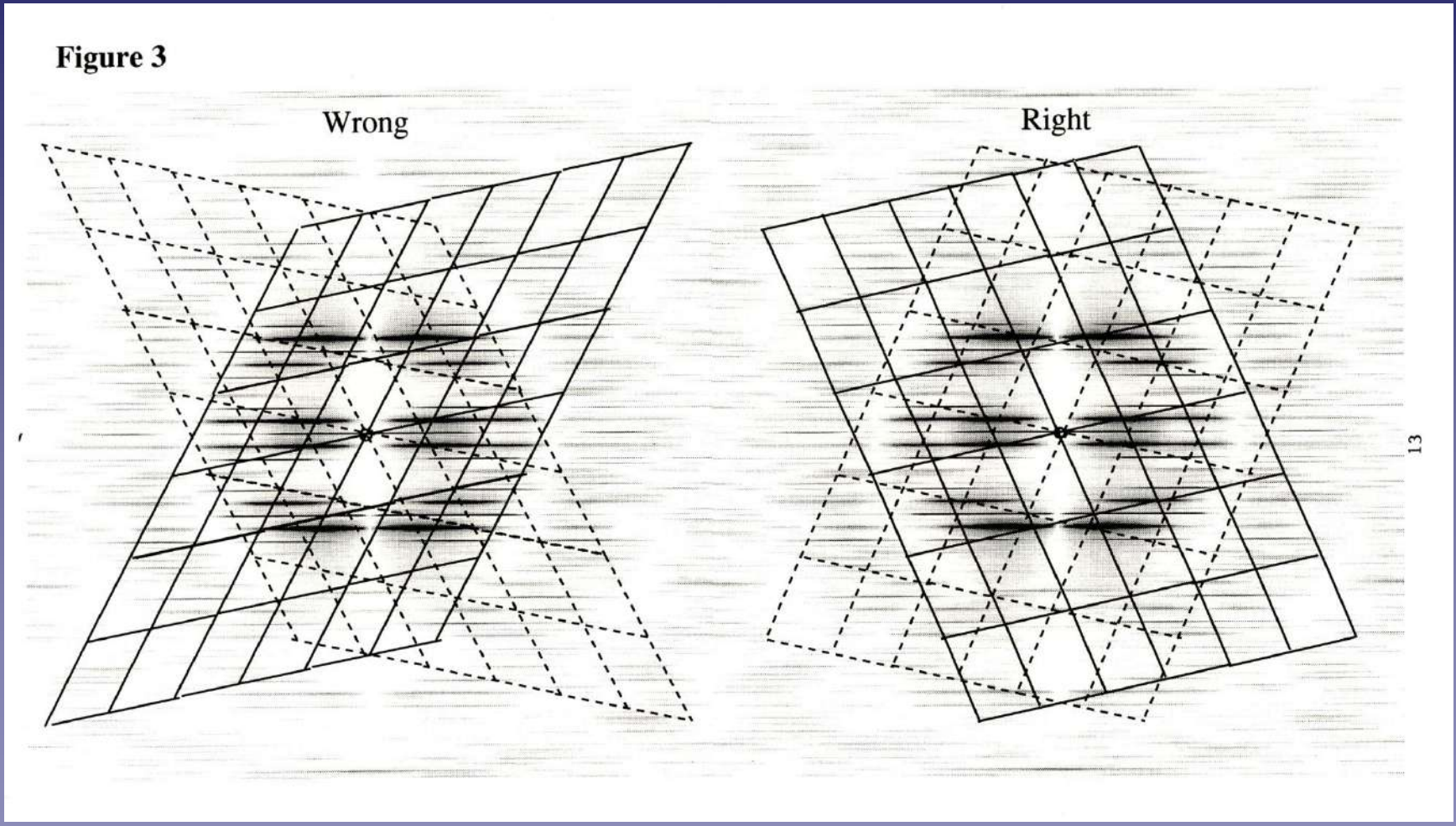
The Diffraction Pattern of a Helix Has Reflections From Planes in the Front and Back of the Helix



Different from the case of the 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



Clues to trace the Reciprocal Helical Lattice:

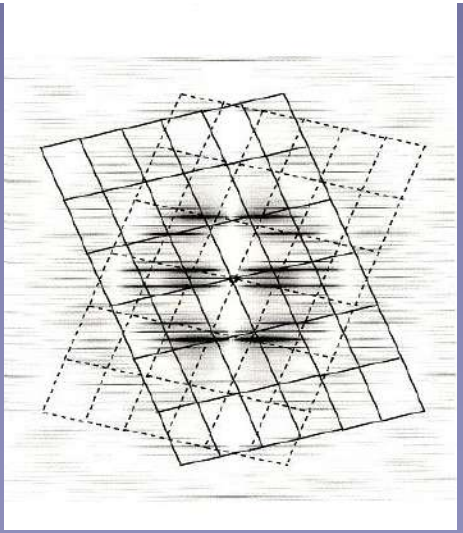
-The dimension of the unit vectors should be aproximately equal to the inverse of the subunits dimensions. $d=1.34(m)^{1/3}$ (d in Å, m in daltons) (e.g actin dimensions ~ 5 nm)

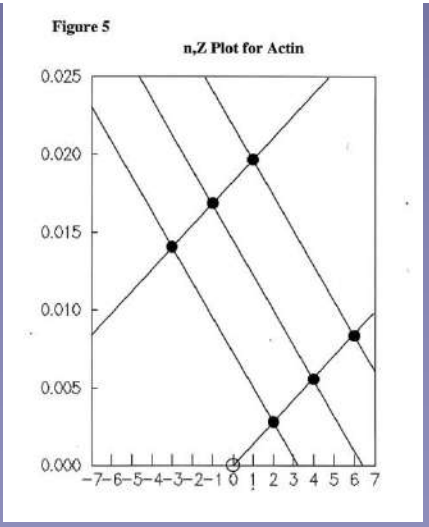
- Approximate value of |n| for each layer line is: $|n|+2 = 2\pi Rr$.
r: Helix radius, R: Reciprocal of layer line peak position to the meridian.

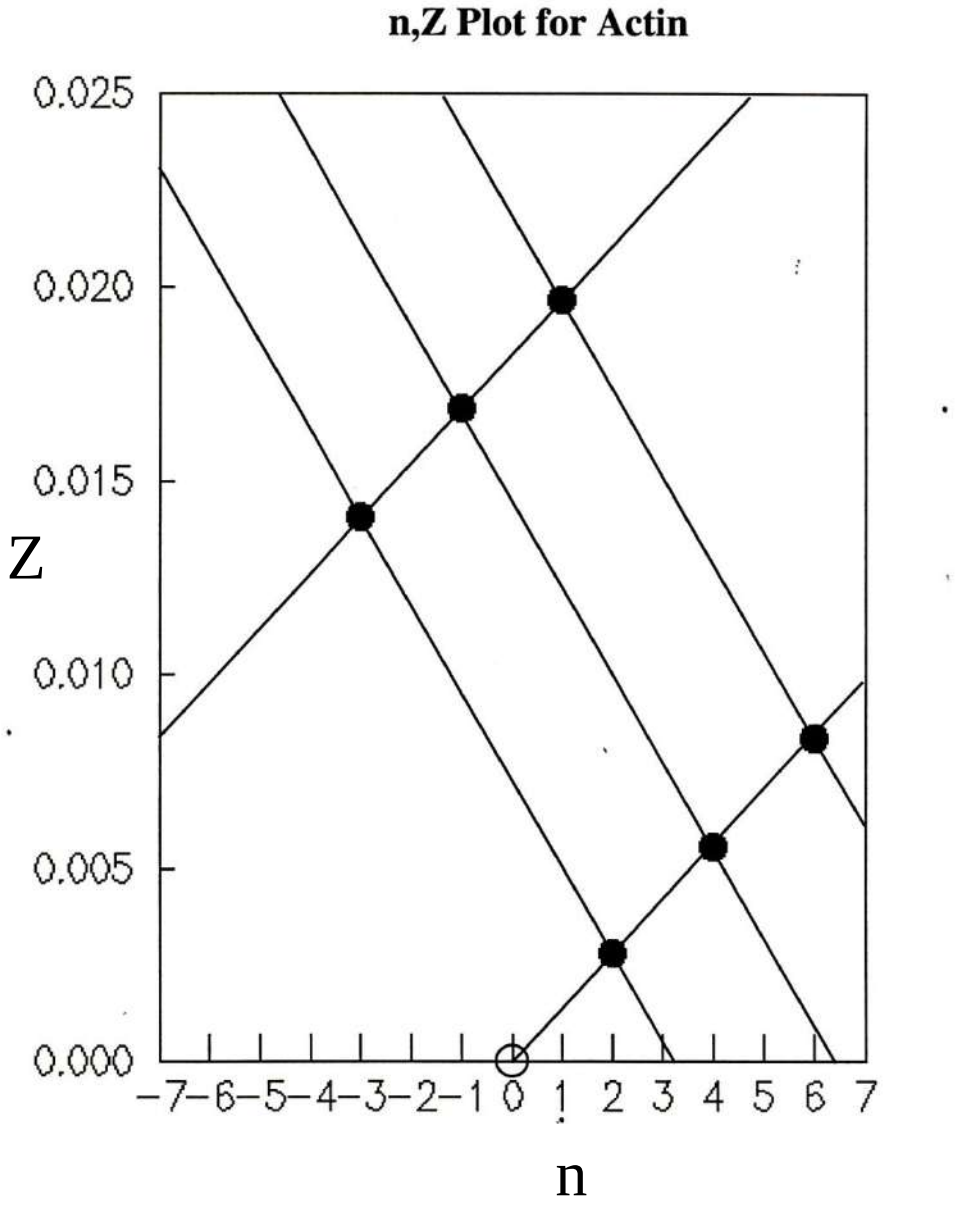
- Determine if n is odd or even by looking at mirror symmetric peaks from the meridian. Even if same phase, Odd if phase diff = 180^{o}.

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

- Draw n,Z plot.






n,Z Plot for Actin
0.025
0.020
0.015
0.010
0.005
0.000
Z
−7 −6 −5 −4 −3 −2 −1 0 1 2 3 4 5 6 7
n

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 (Schere'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, Others)

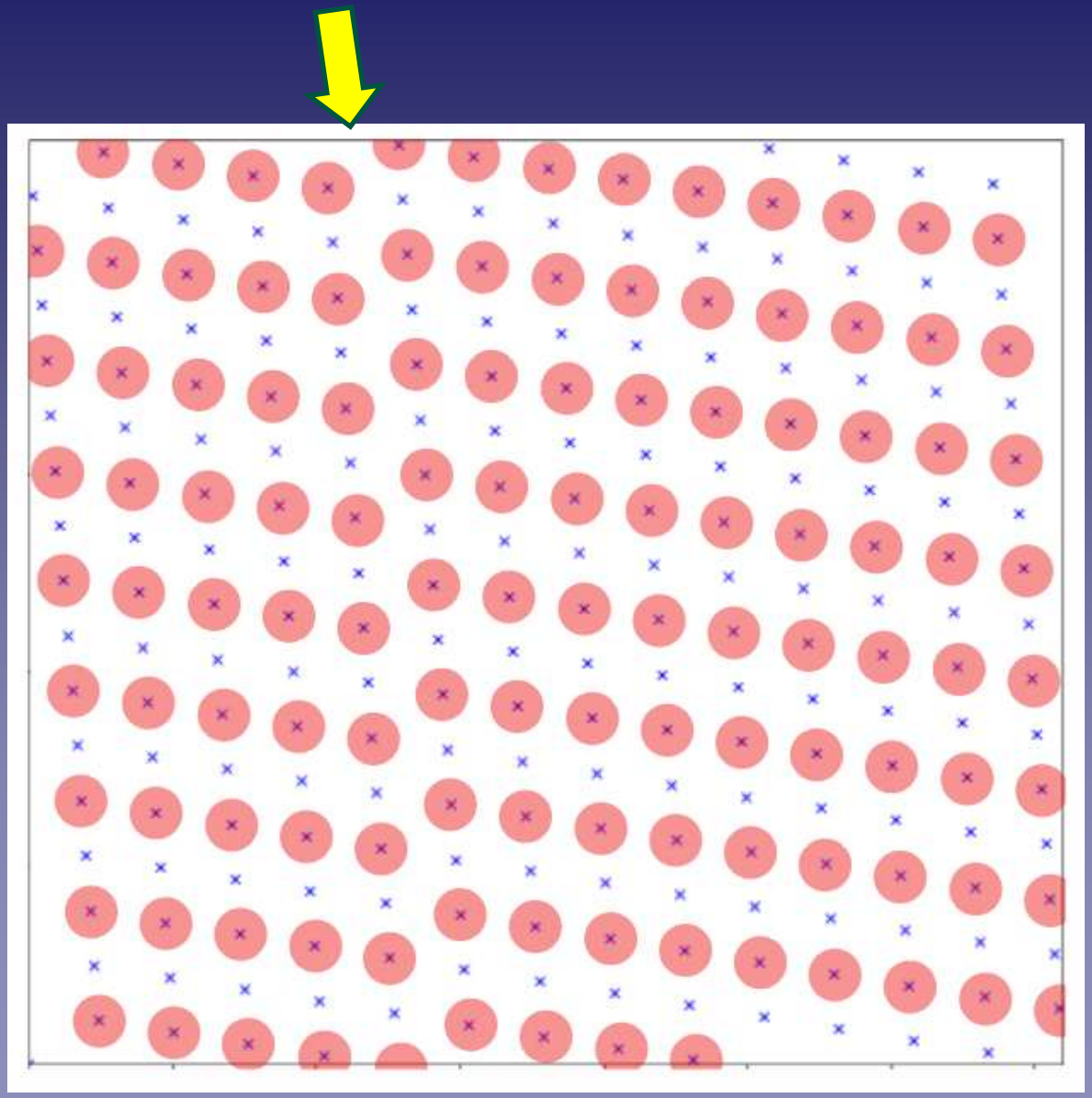
- Preliminary low resolution model (Custom)
- Classify filaments. (Custom, SPIDER, EMAN)
- Determine helical symmetry (ϕ, h) (Custom)

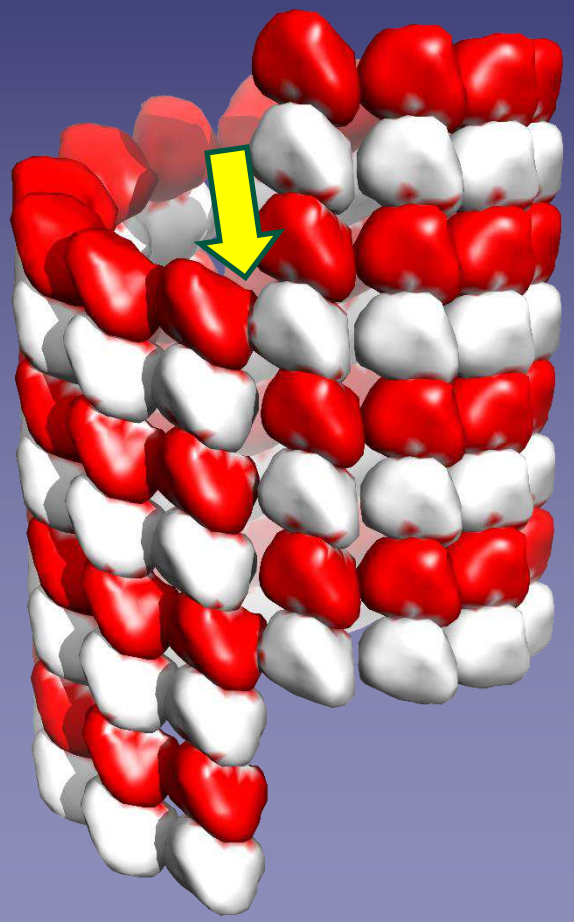
- Extract “single particle” boxes (Spider)
- Determine 3D orientation of single particle boxes (projection matching) & make 3D reconstruction (Spider, Custom)

- Refine 3D map (IHRSR).
- Refine 3D map (FREALIGN)
- Refine 3D map (RELION)

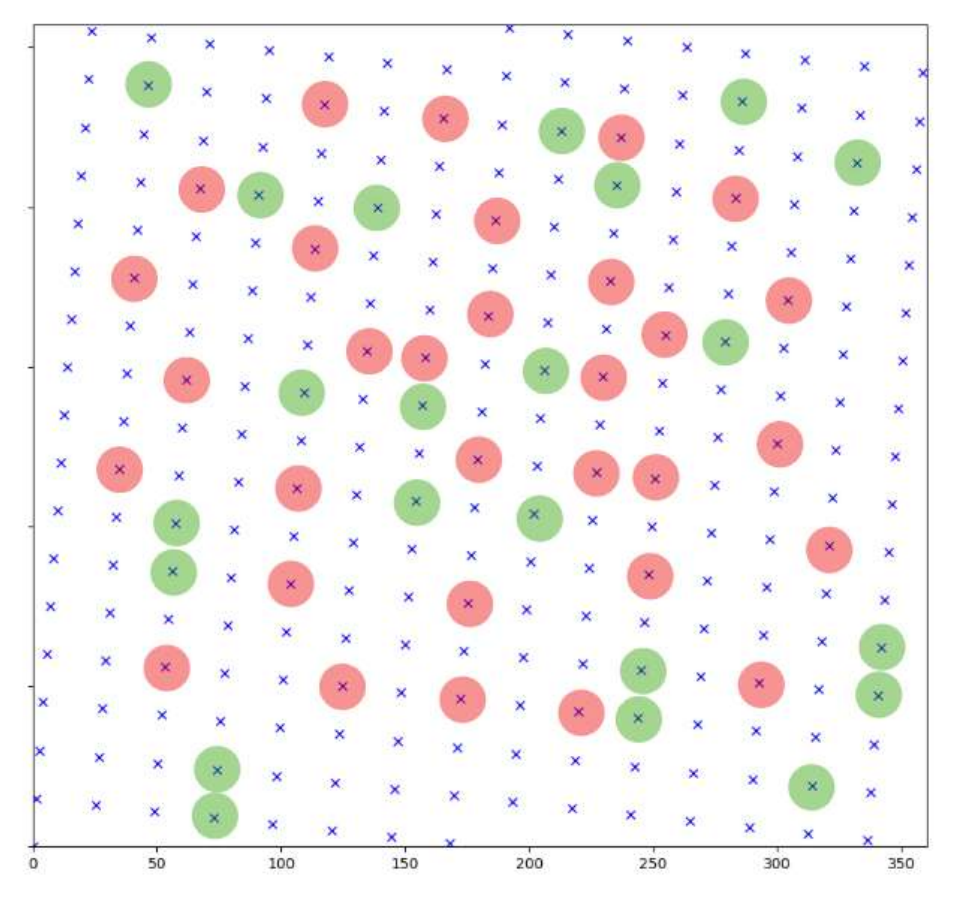
- Model Building (UCSF-Chimera, Coot, Phenix, Direx, Modeller)

Helical Discontiniuity, Seam

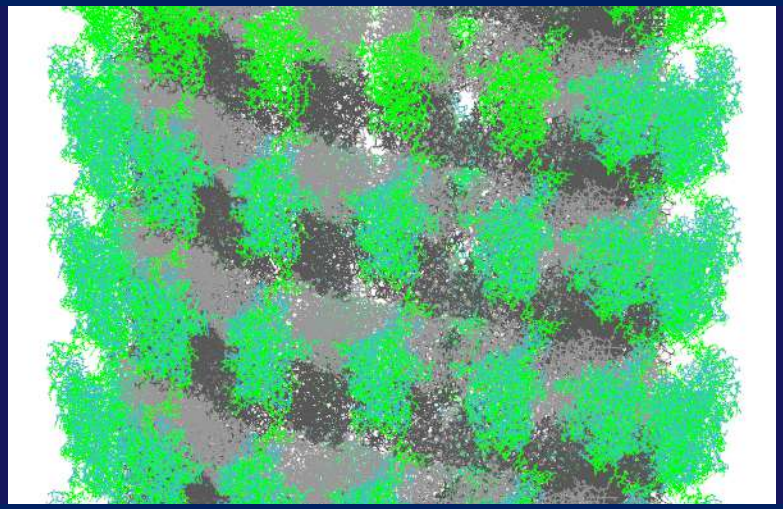


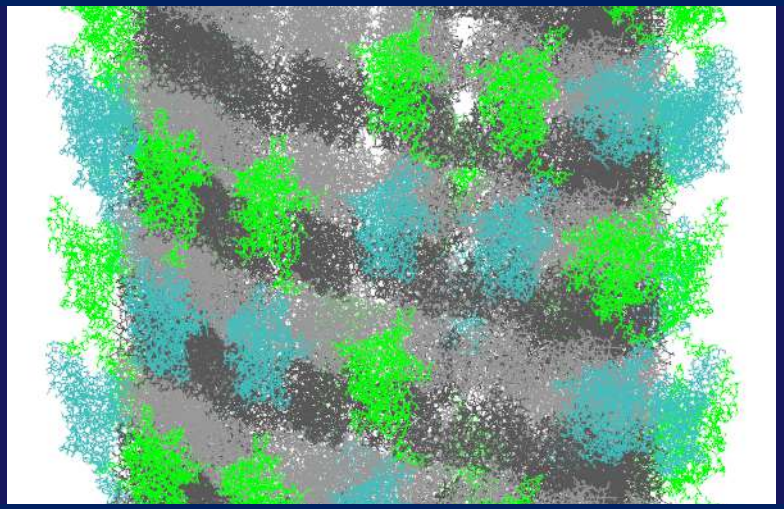


Resolving asymmetric features in helical assemblies



Resolving asymmetric features in helical assemblies





Helical Assembly Subunit Refinement and Classification (HASRC)

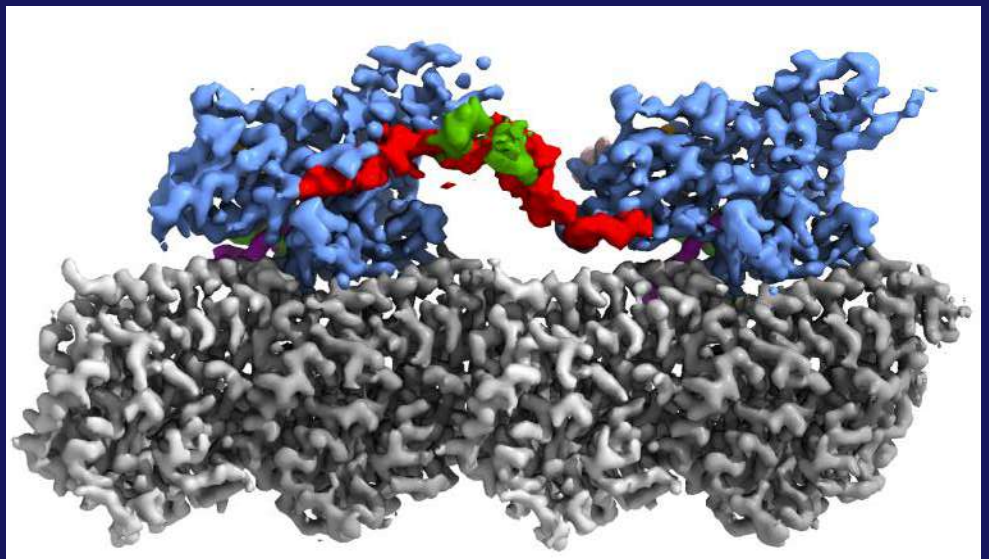
1. Helical reconstruction

2. Symmetry expansion , Signal subtraction , Subunit isolation.

3. Subunit local refinement.

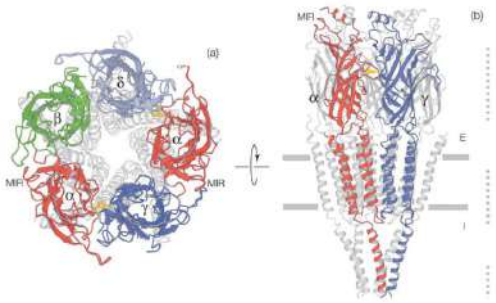
4. Focus 3D classification.

(Relion 3.0)

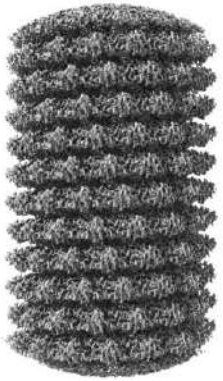


KIF14 Dimer MT complex (2.9 Å resol)
Benoit et al., *Nat. Comm*. 12:3637 (2021)

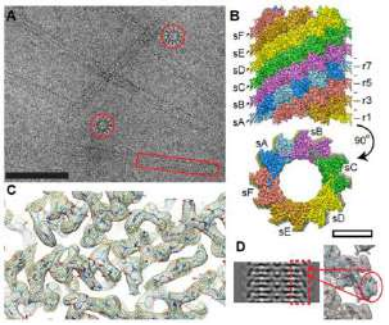
Examples of Helical Structures @ <= 4 Å



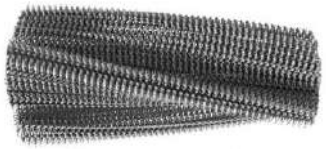
Ach Receptor (4Å)
Unwin N.(2005) JMB 346:976
(Fourier-Bessel Method)



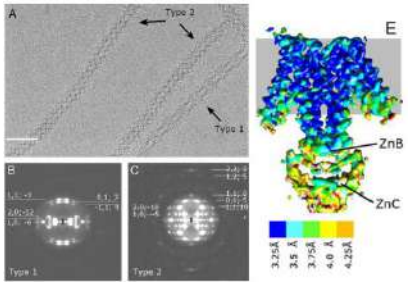
TMV (1.9Å)
Weis et. al., (2019)
EMBO Rep . 20 e48451



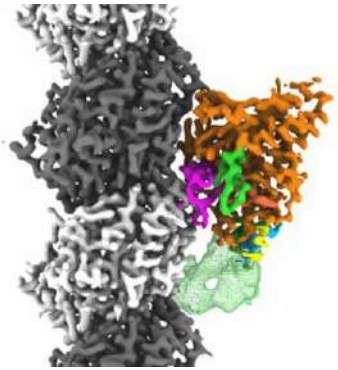
Bacteria type VI secretion system (3.5 Å)
Kudriashev et al., (2015)
Cell 26: 952



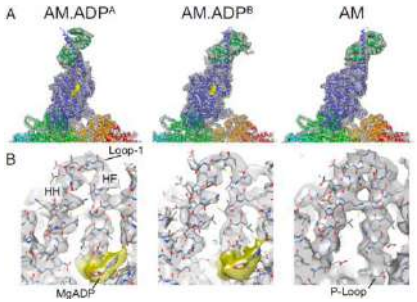
Tau Filaments (1.75 Å)
Flovestram et al., (2024).
Nature 625 : 119-125



YiiP 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

Recent Review

Wang, F., O. Gnewou, A. Solemanifar, V.P. Conticello, and E.H. Egelman. 2022. Cryo-EM of Helical Polymers. Chemical Reviews. 122:14055-14065.

Fourier-Bessel Method

DeRosier, D.J., and P.B. Moore. 1970. Reconstruction of three-dimensional images from electron micrographs of structures with helical symmetry. J Mol Biol. 52:355-369.

Diaz, R., W.J. Rice, and D.L. Stokes. 2010. Chapter Five - Fourier–Bessel Reconstruction of Helical Assemblies. In Methods in Enzymology. Vol. 482. G.J. Jensen, editor. Academic Press. 131-165.

Single-Particle approaches

IHRSR

Egelman, E.H. 2000. A robust algorithm for the reconstruction of helical filaments using single-particle methods. Ultramicroscopy. 85:225-234

Frealign/CisTEM

Grigorieff, N. 2016. Frealign: An Exploratory Tool for Single-Particle Cryo-EM. *Methods in Enzymology*. 579:191-226.

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

Relion

He, S., and S.H.W. Scheres. 2017. Helical reconstruction in RELION. Journal of Structural Biology. 198:163-176.

CryoSparc

https://guide.cryosparc.com/processing-data/all-job-types-in-cryosparc/helical-reconstruction-beta

Helical assembly subunit refinement and classification (HASRC)

Benoit, M.P.M.H., A.B. Asenjo, M. Paydar, S. Dhakal, B.H. Kwok, and H. Sosa. 2021. Structural basis of mechano-chemical coupling by the mitotic kinesin KIF14. *Nat. Commun.* 12:3637.