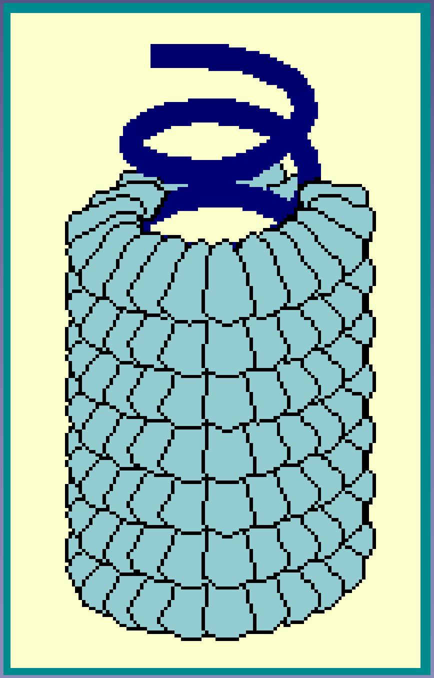
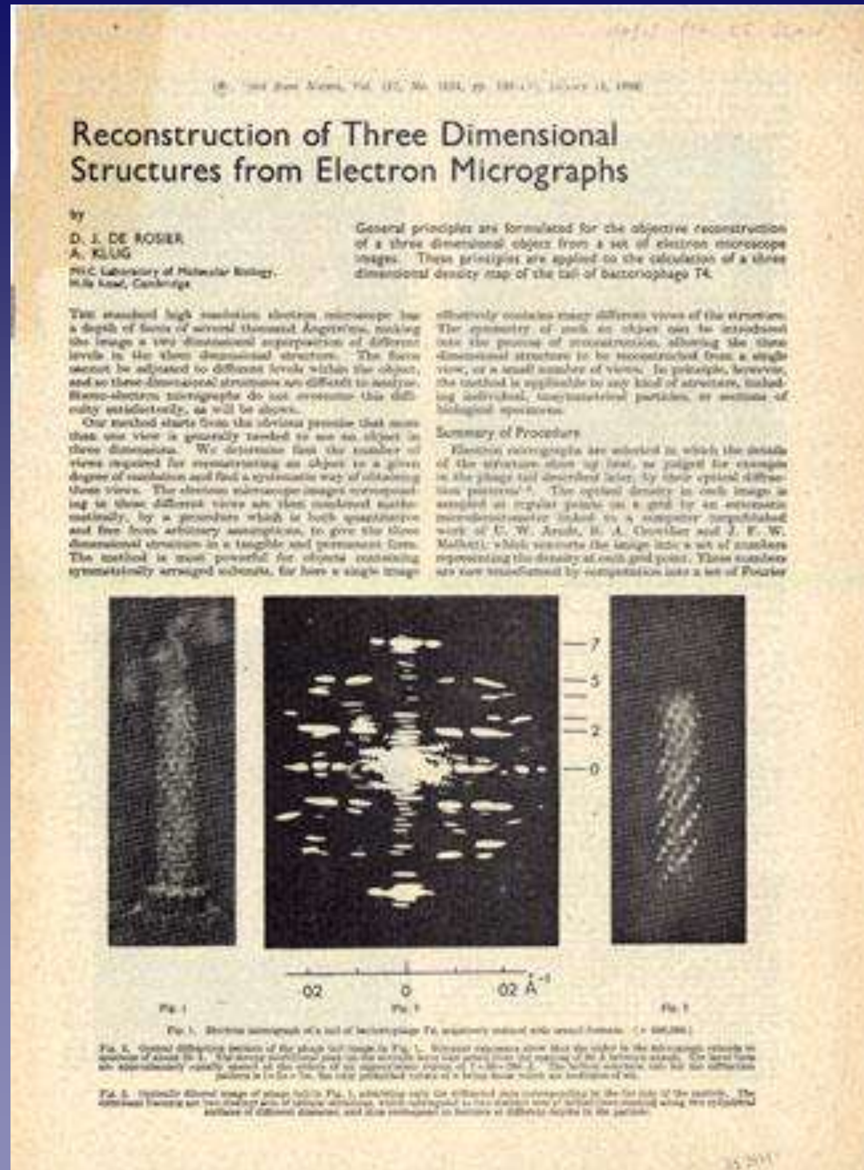
3D Reconstruction of Helical Specimens



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
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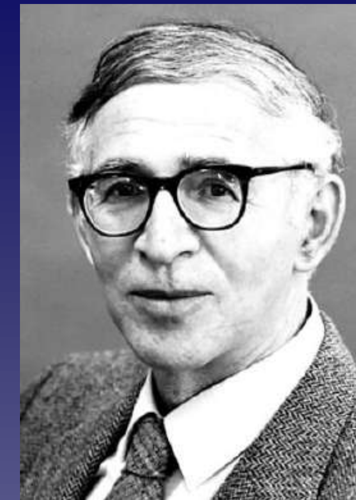
Hills Road, Cambridge

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



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

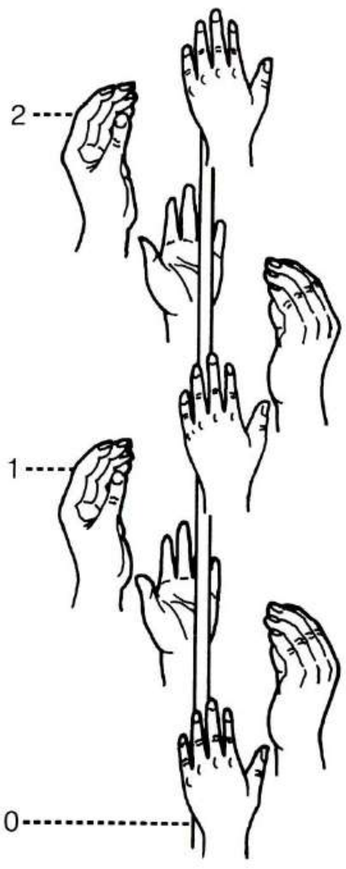
Combining the symmetry operation of **translation and rotation** (screw) produces a **helix**

Possible Symmetry operations:

-Screw.

-n-fold rotation about axis.

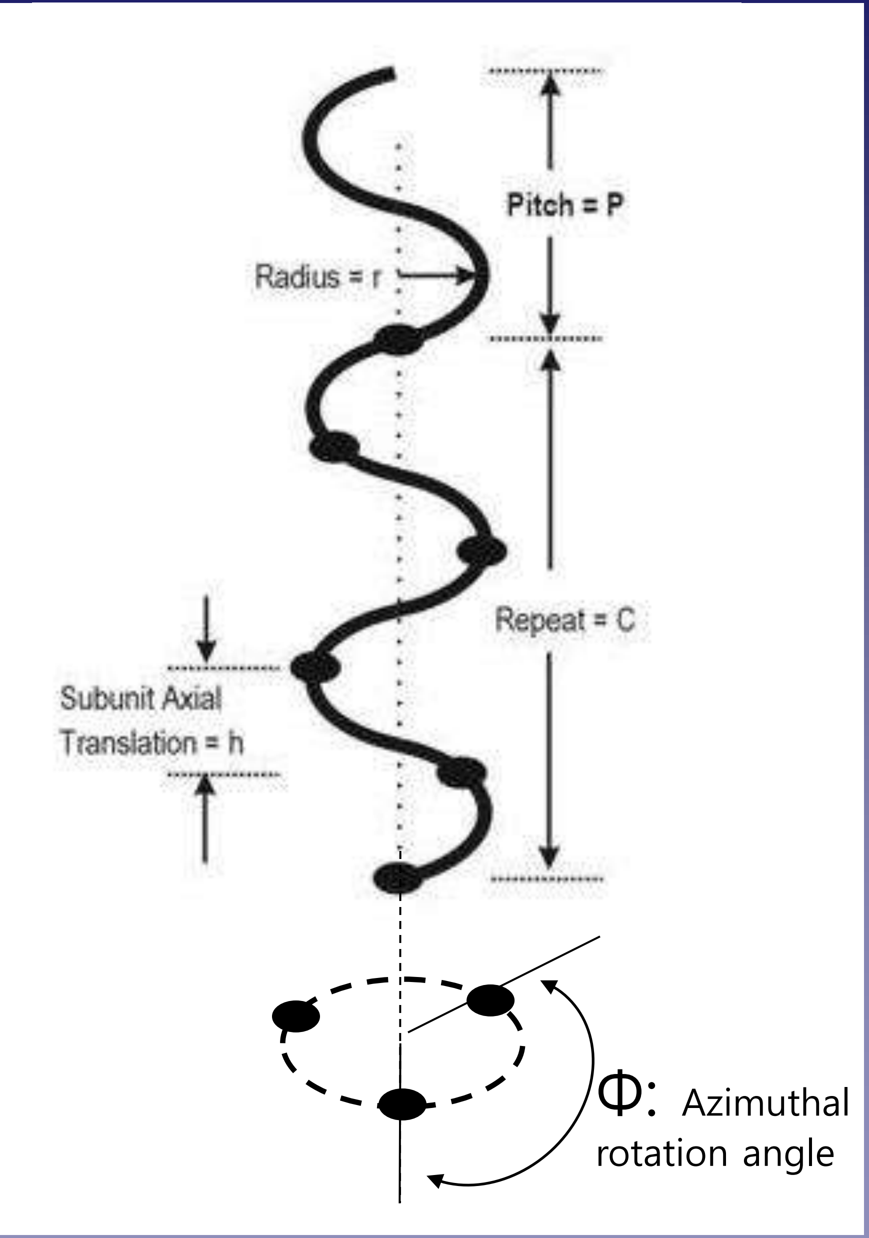
-2-fold rotation perpendicular to axis.



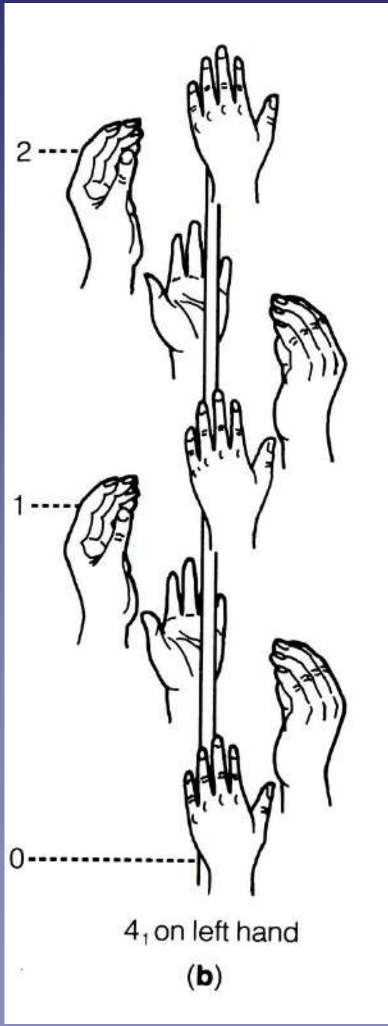
4_1 on left hand

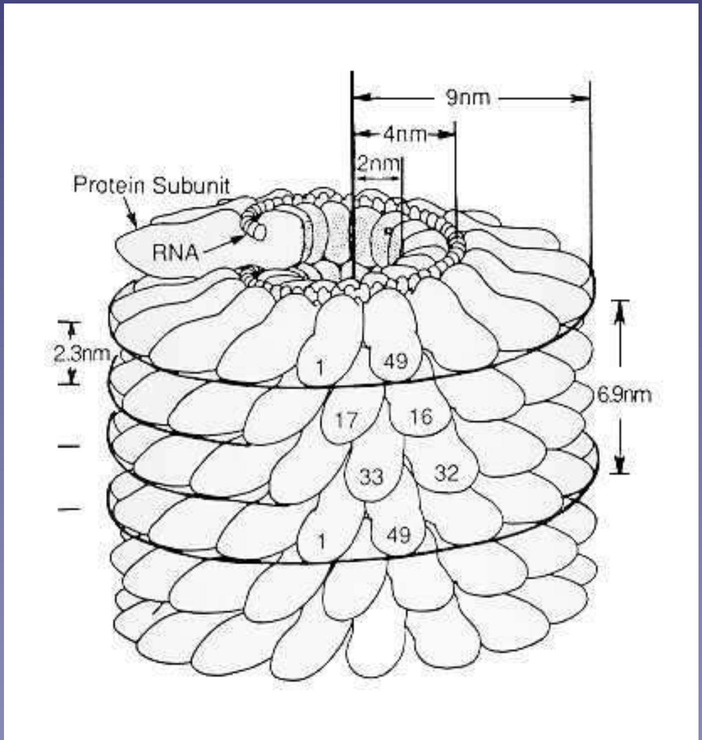
(b)

Parameters of a Helix



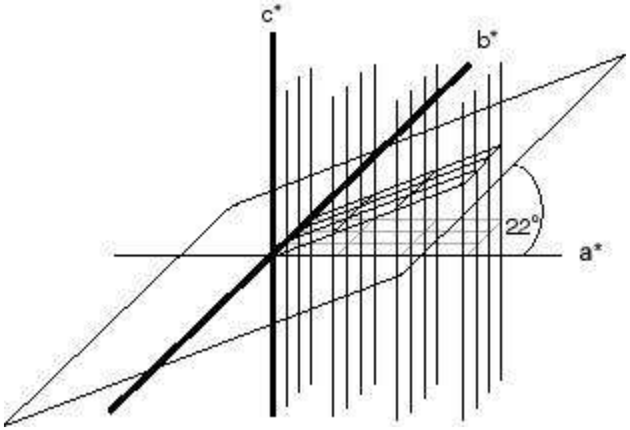
Helices give several orientation views of the asymmetric unit from a single view direction

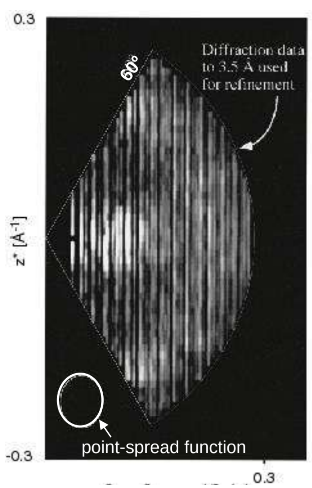




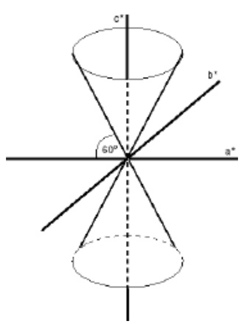
No Missing Cone !

2D crystals

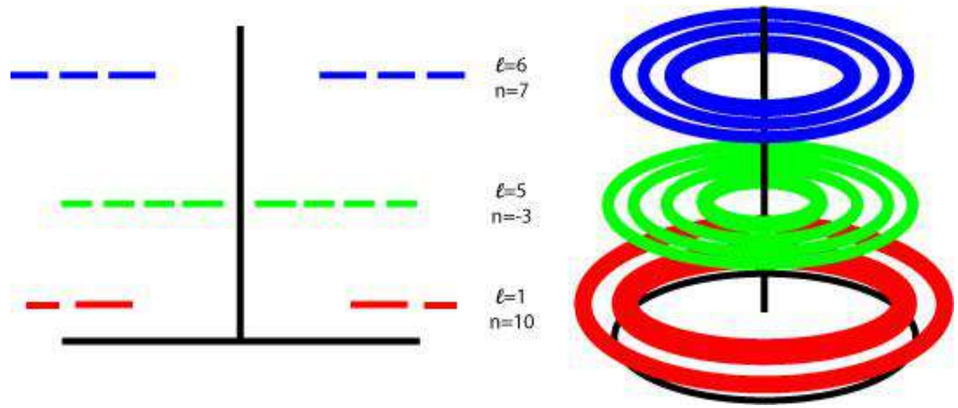




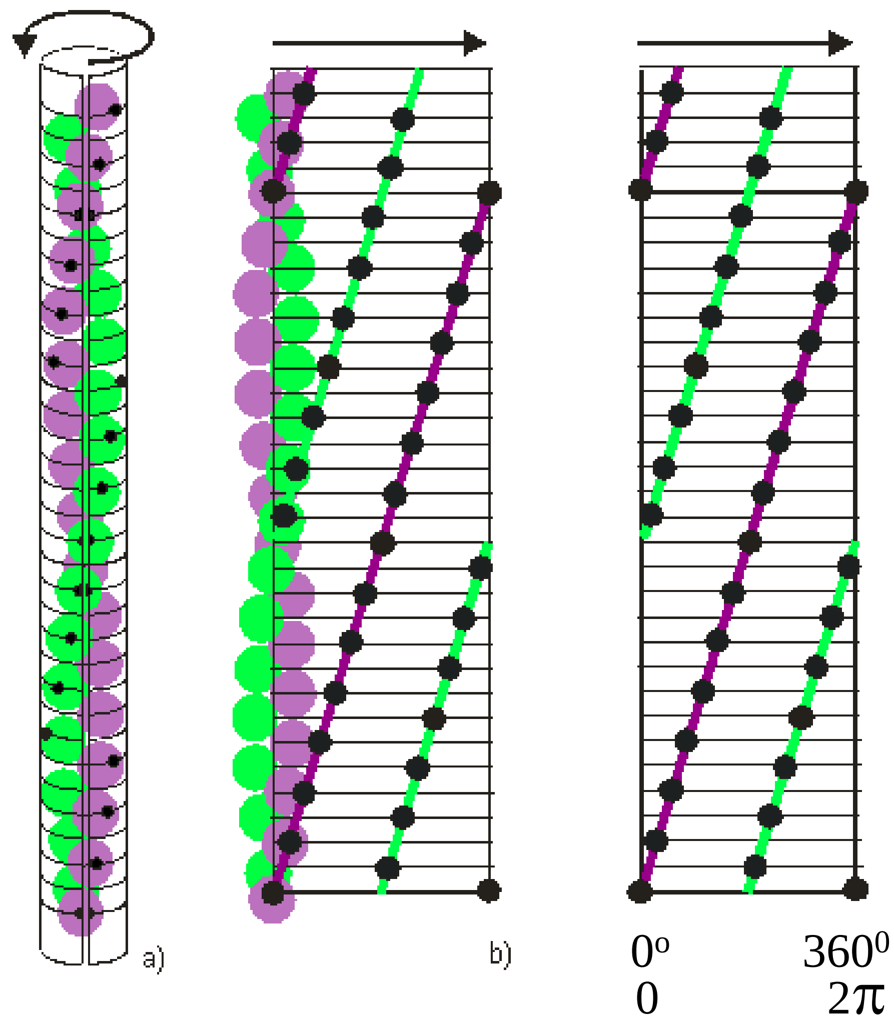
missing cone



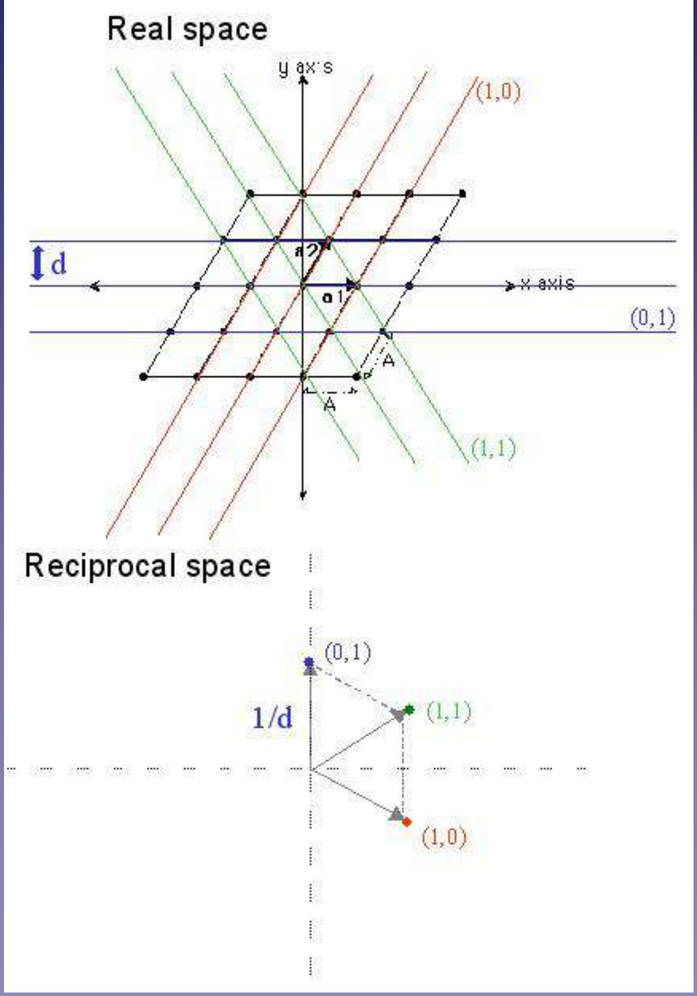
Helical crystals

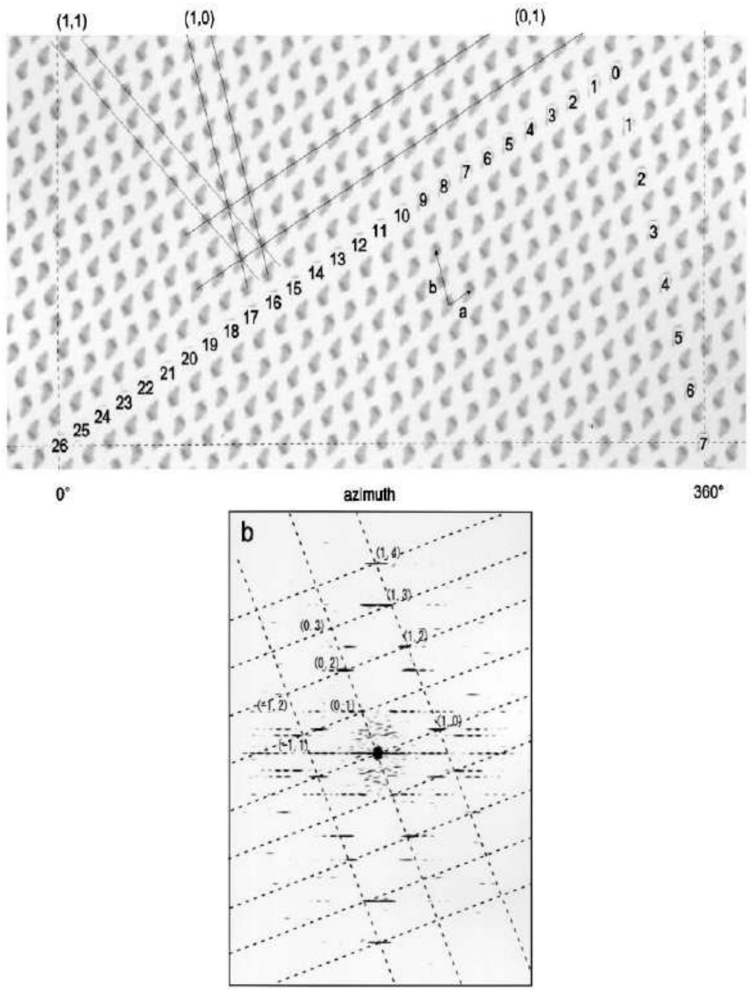


The Helical Lattice Radial Projection



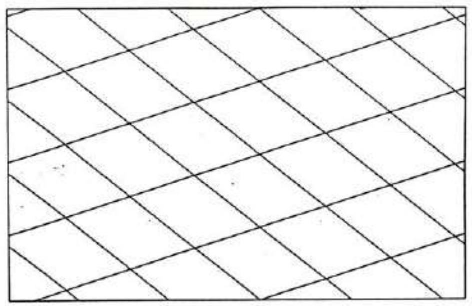
Analogy between 2D lattices and Helical Lattices

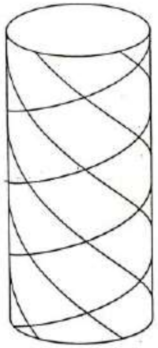


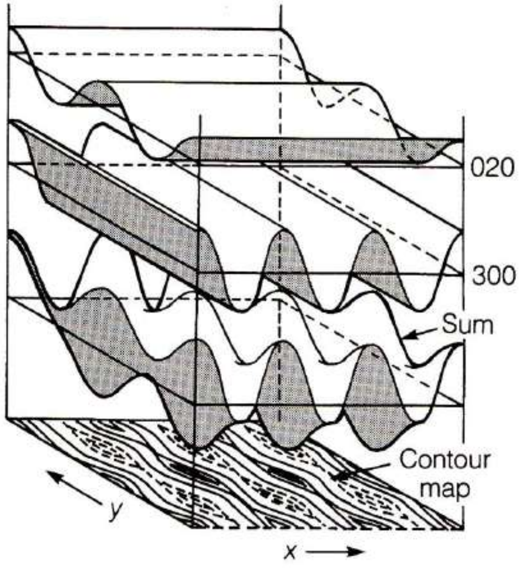


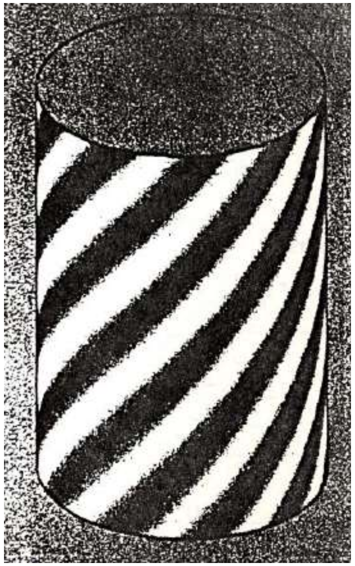
From: Toyoshima (2000) Ultramicroscopy 84: 1-14

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

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

Cochran, Crick & Vand 1952

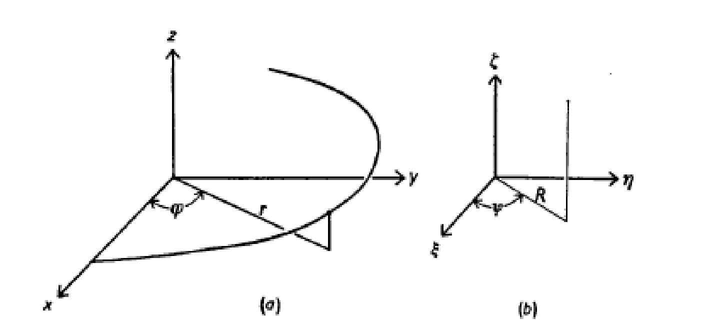
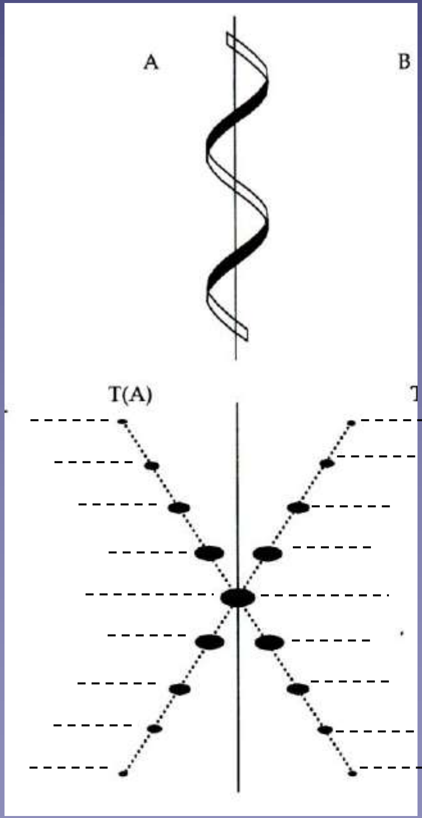
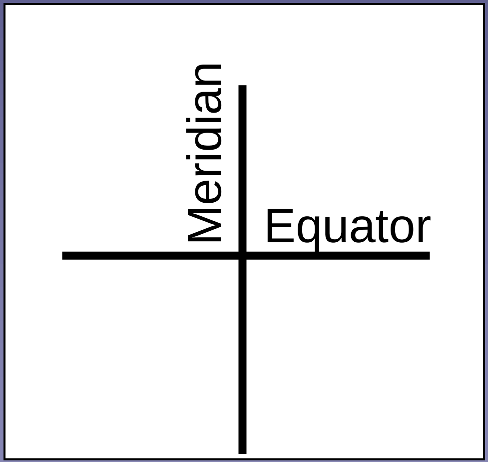


Fig. 1. (a) Cartesian (x, y, z) and cylindrical-polar (r, φ, z) co-ordinates of a point on a helix. (b) Corresponding coordinates of a point in reciprocal space.





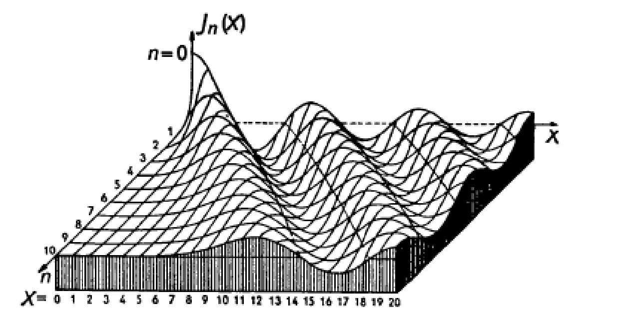
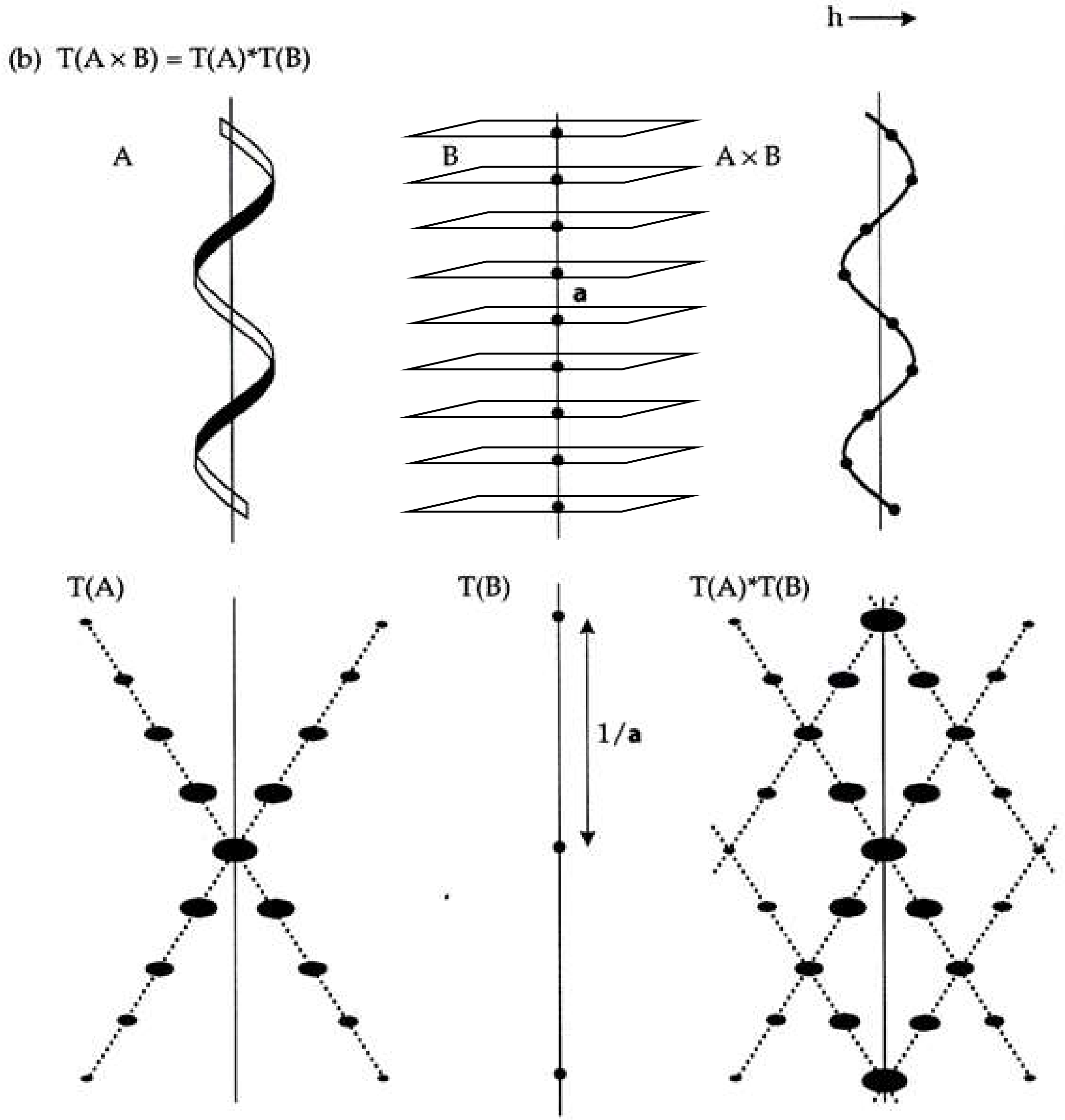
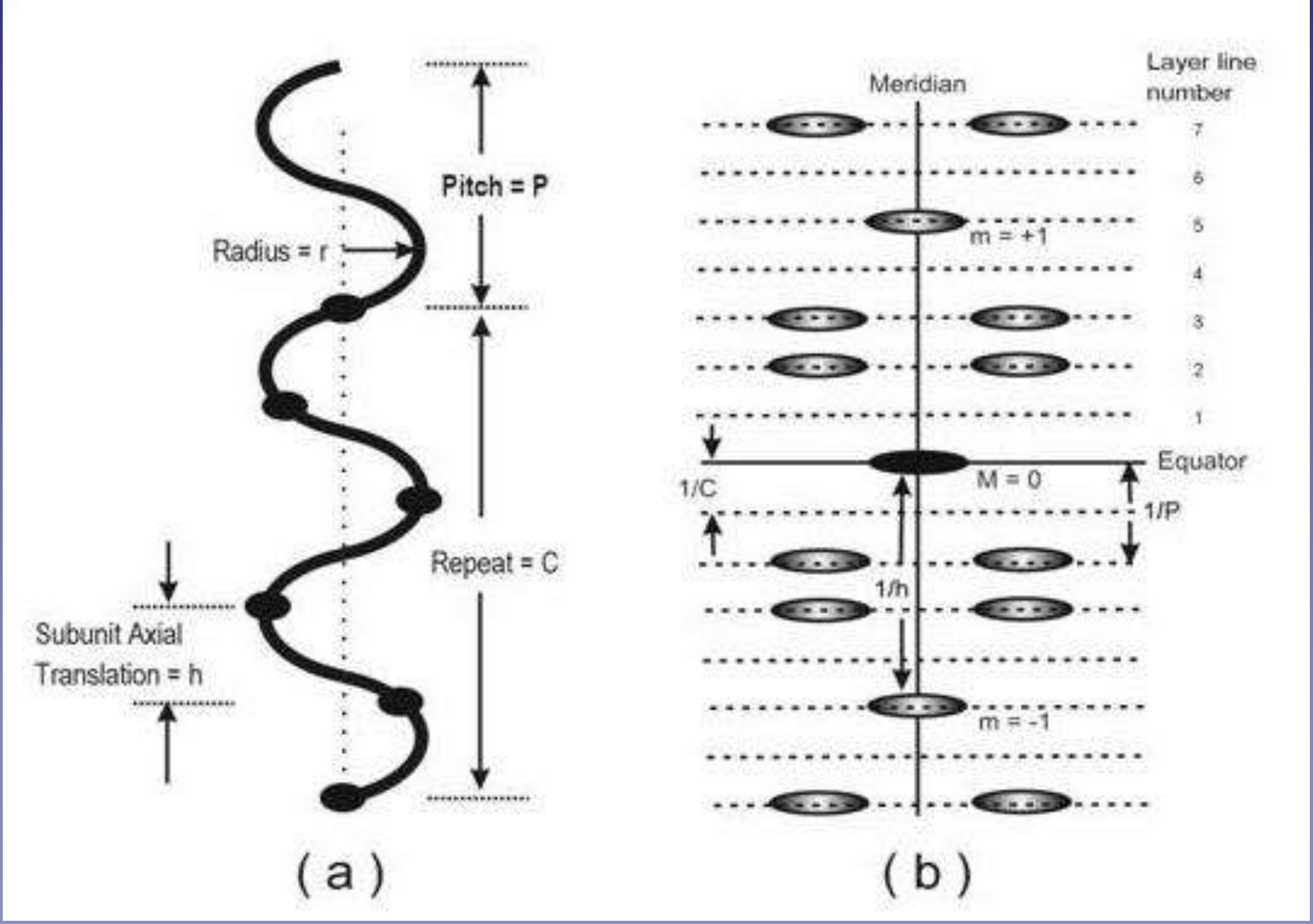


Fig. 2. Illustration of Bessel functions. (Reproduced by kind permission of the publishers from *Tables of Functions* by Jahnke & Emde. New York: Dover Publications.)

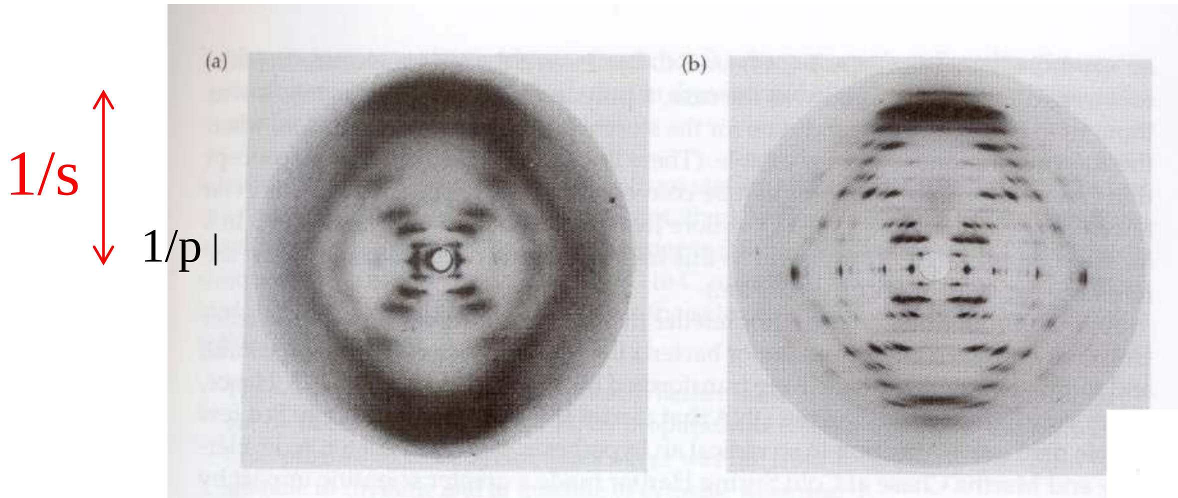
The Transform of a discontinuous helix

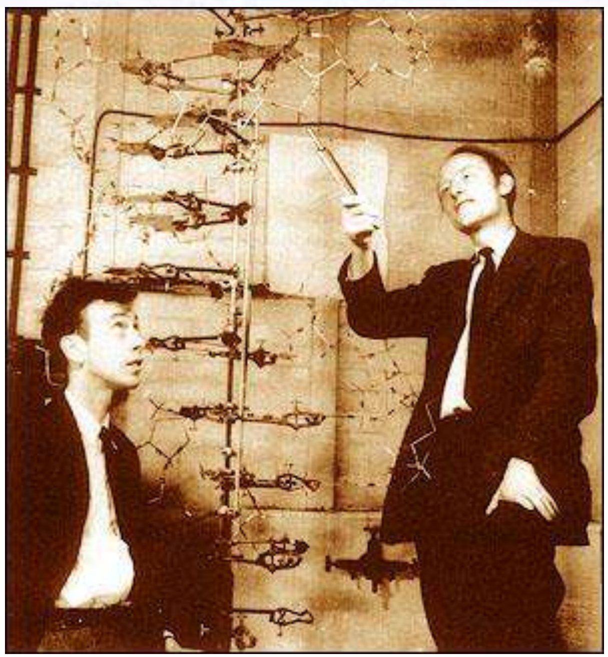


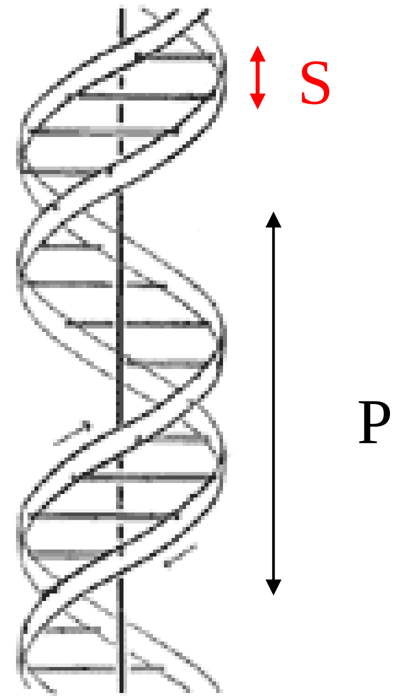
A helix and its corresponding Fourier Transform (Power Spectrum)



The DNA Structure







Helical 3D reconstruction Using the Fourier-Bessel Method

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

Fourier Transform
Reciprocal Space Function

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

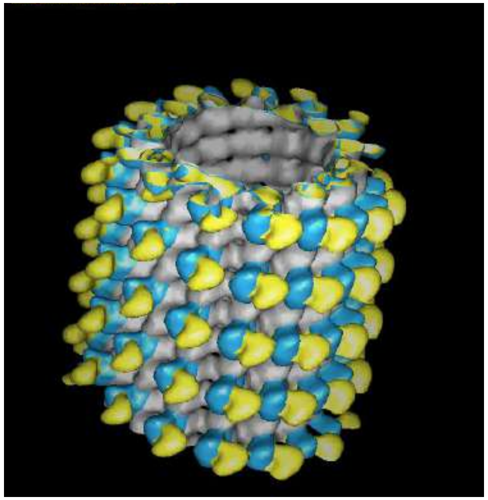
Selection rule

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

Real space function
(structure)

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

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



Helical 3D reconstruction Using the Fourier-Bessel Method

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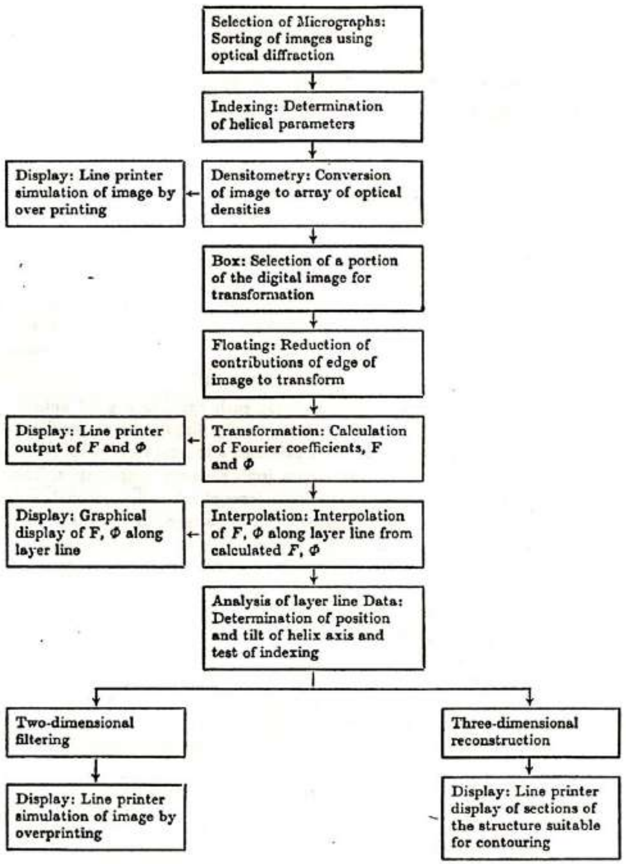


FIG. 1. The scheme presented shows the flow of data in the process of three-dimensional reconstruction.

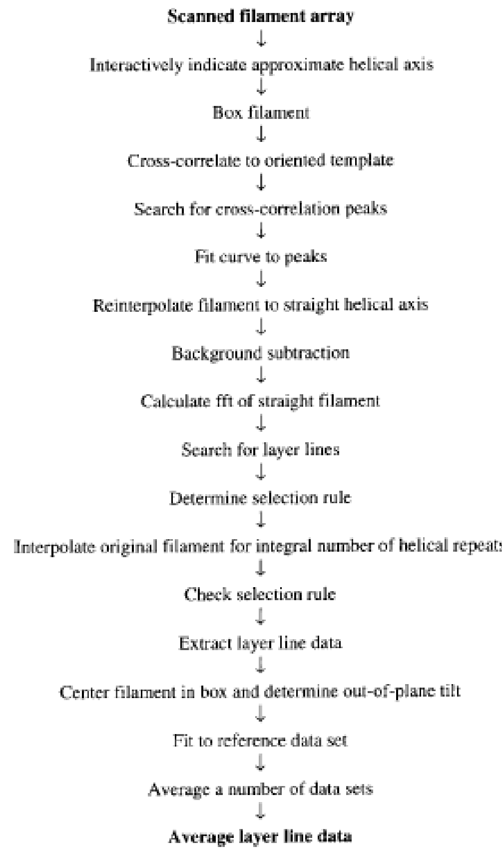
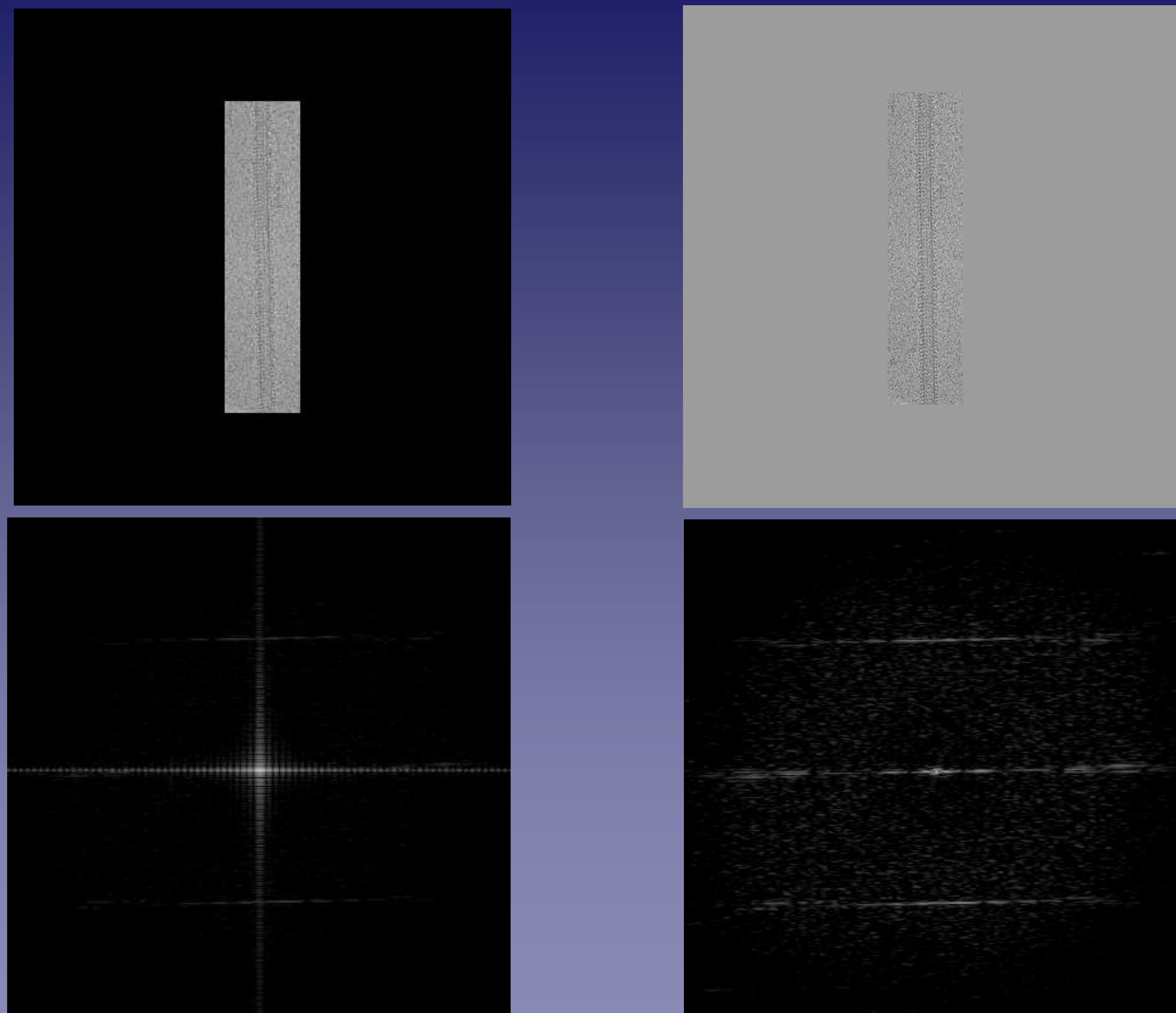


FIG. 1. Schematic diagram of the PHOELIX helical processing package. A detailed description of each step and the programs used is available as part of the PHOELIX distribution.

Boxing & Floating Image



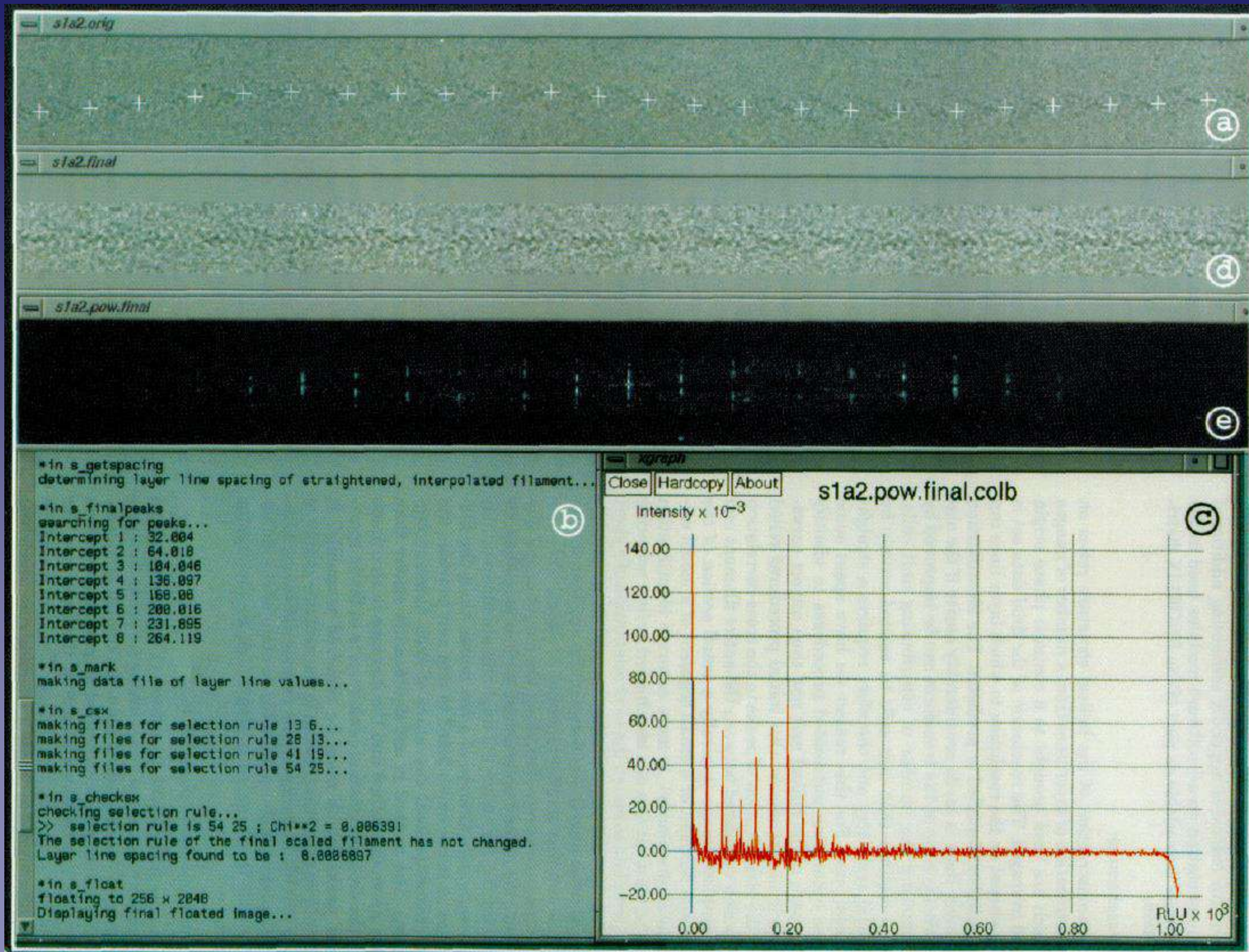
Image

FFT

Non-Floated

Floated

Straightening

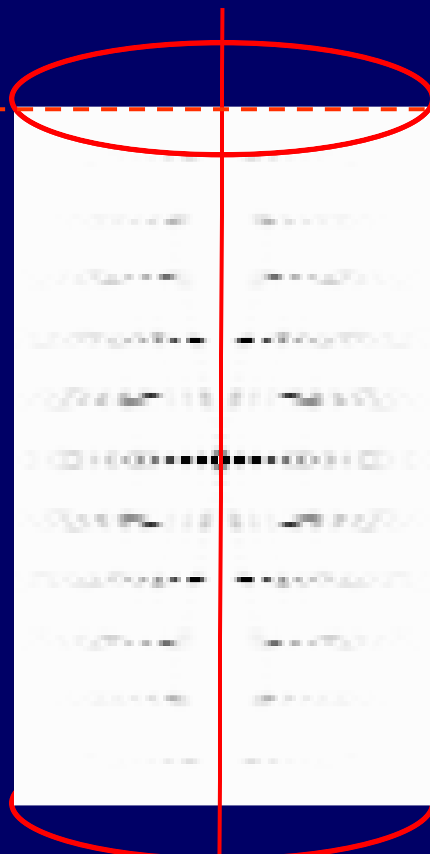


From: Carragher et al., JSB 116: 107-112 (1996)

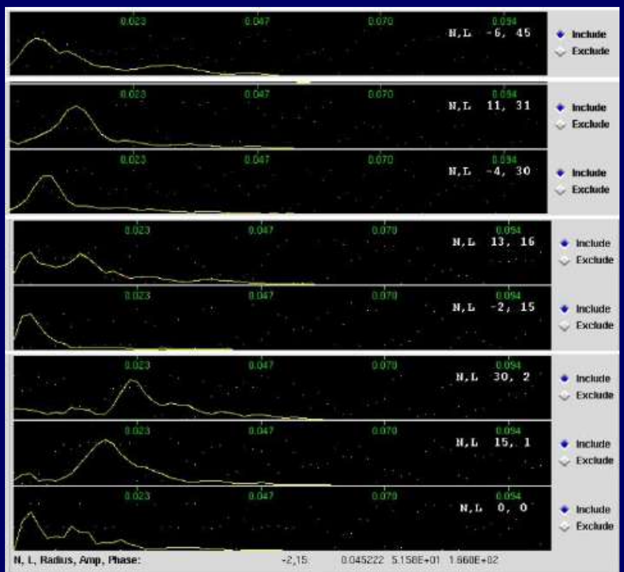
Gathering Amplitude and Phases

Image

FFT



Fourier Transform

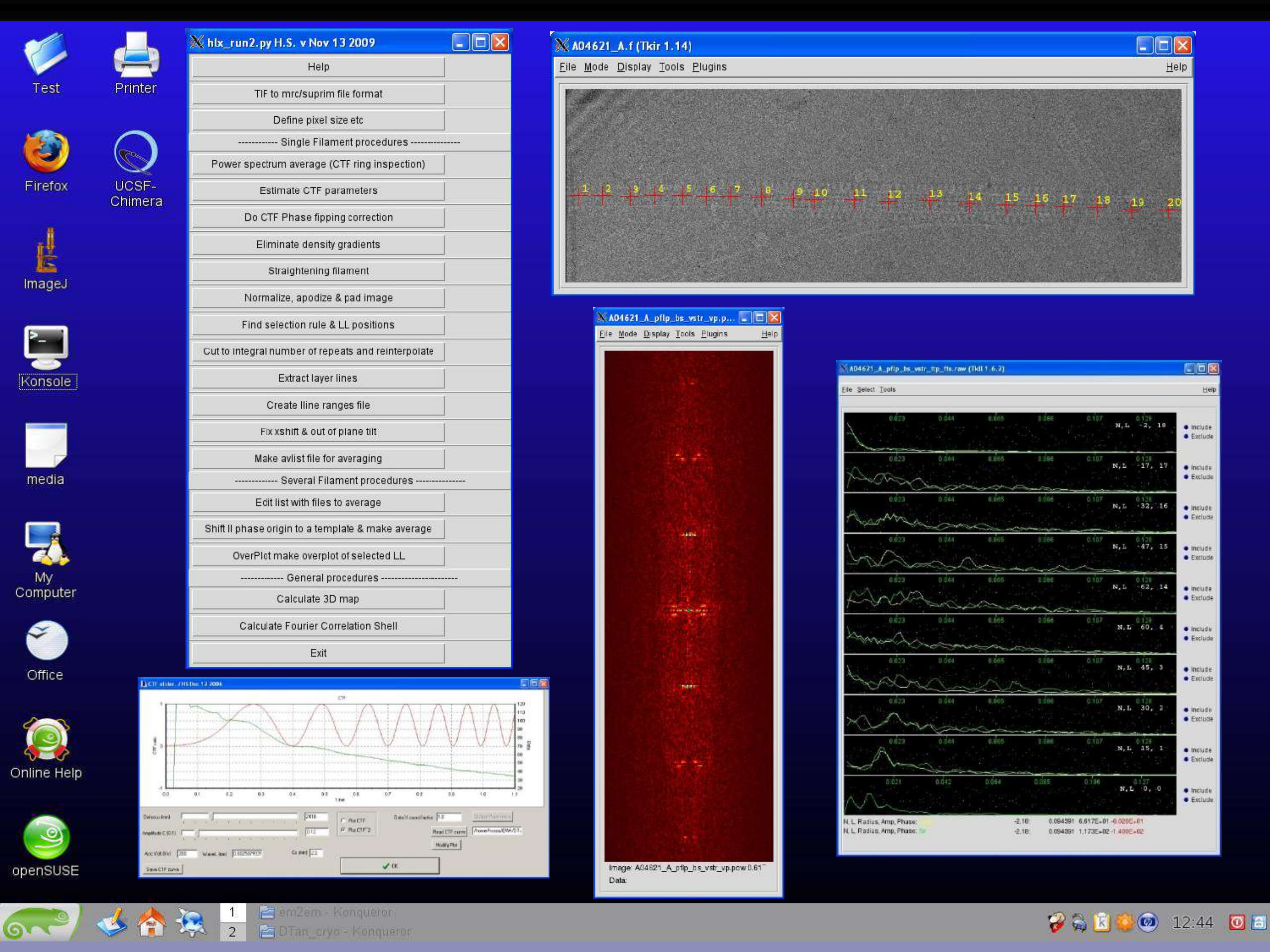


Layer Line Data Set

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

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

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

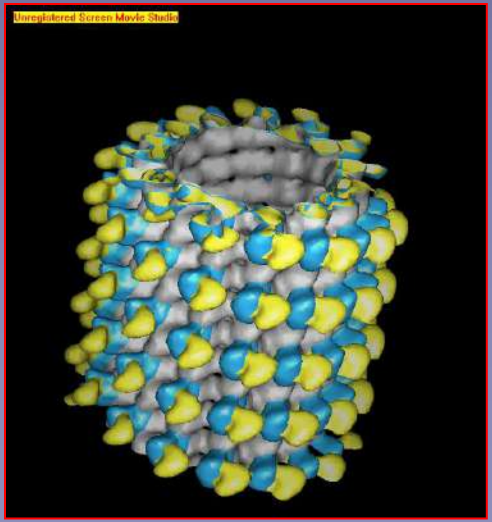

hlx_run2.py H.S. v Nov 13 2009
Help
TIF to mrc/suprim file format
Define pixel size etc
------------ Single Filament procedures --------------
Power spectrum average (CTF ring inspection)
Estimate CTF parameters
Do CTF Phase flipping correction
Eliminate density gradients
Straightening filament
Normalize, 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
A04621_A.f (Tkir 1.14)
File Mode Display Tools Plugins Help
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
A04621_A_pflp_bs_vstr_vp.p...
File Mode Display Tools Plugins Help
Image: A04621_A_pflp_bs_vstr_vp.pow 0.61
Data:
Test
Printer
Firefox
UCSF-Chimera
ImageJ
Konsole
media
My Computer
Office
Online Help
openSUSE
em2em - Konqueror
DTan_cryo - Konqueror
12:44

3D Density Map Reconstruction
(Fourier Bessel inversion)

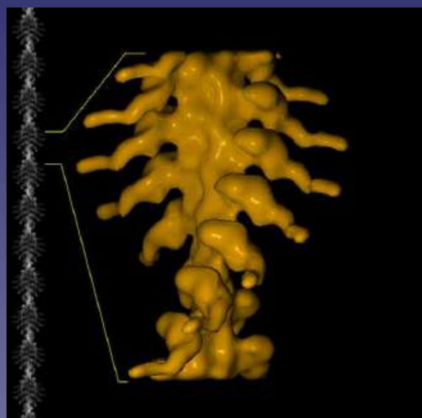
Average $G_{n,l}$ Data set.

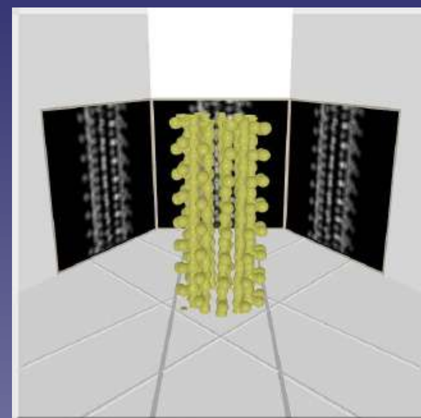
$$g_{n,l}(r) = \int G_{n,l}(R) J_n(2\pi R r)\, 2\pi R \,\mathrm{d}R$$

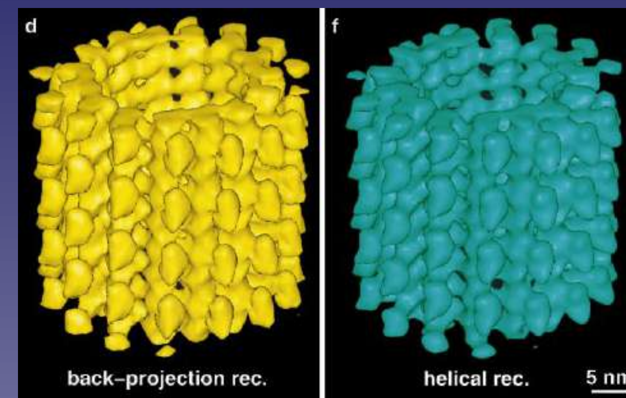
$$\rho(r, \phi, z) = \sum_l \sum_n g_{n,l}(r) \exp(in\,\phi) \exp(-2\pi i l z/c)$$



3D Helical Reconstruction Using Real Space Methods





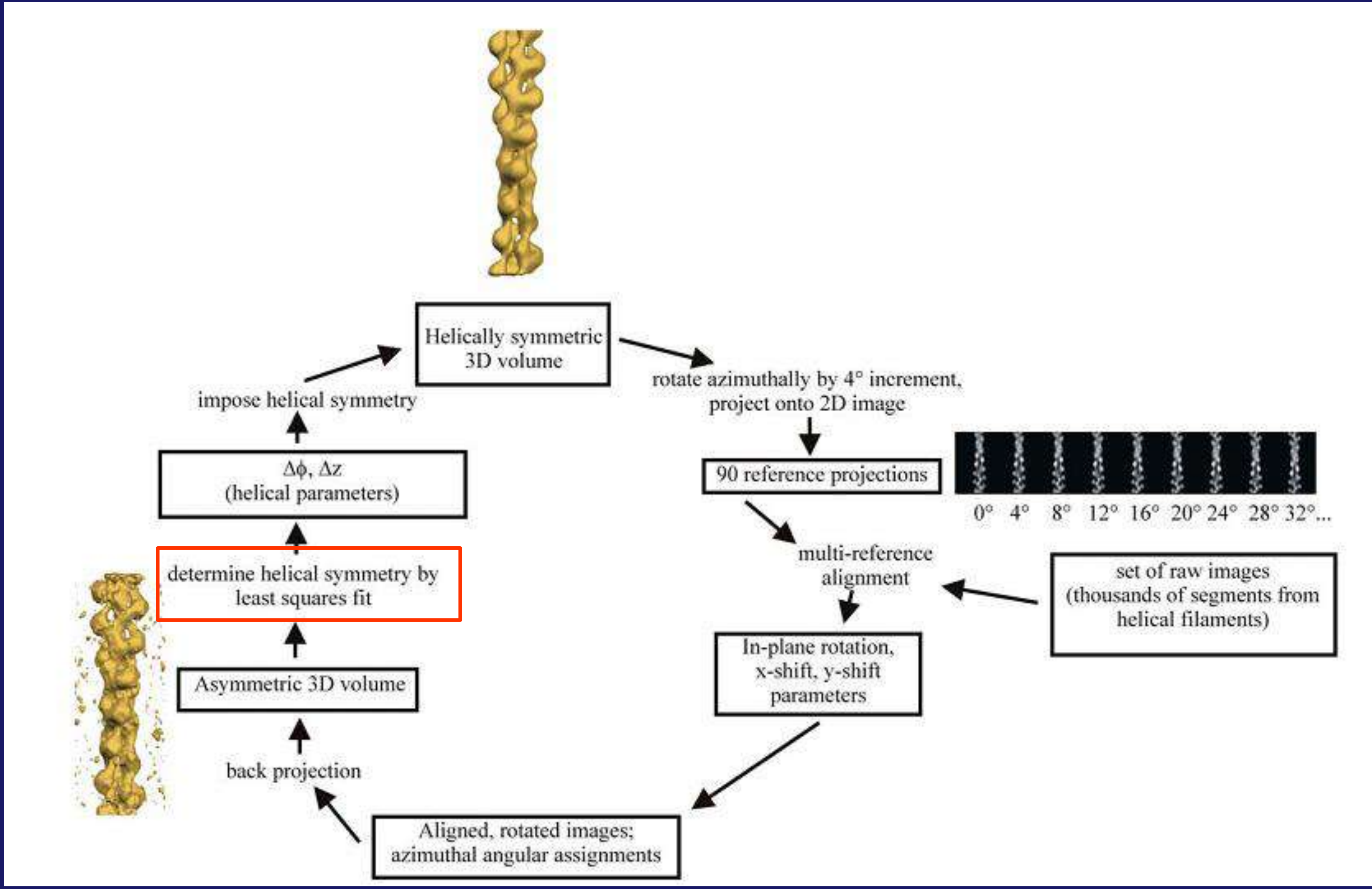


From Sosa et al. JSB 118: 149-158(1997)

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.

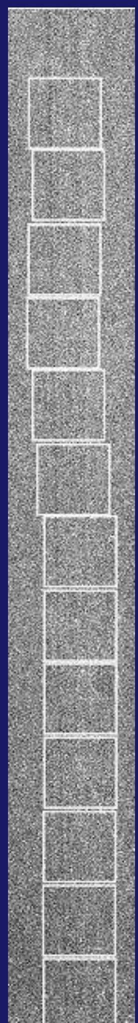
A 3D volume is obtained by back-projection of the boxed images.

The Iterative Helical Real Space Reconstruction Method (IHRSR)

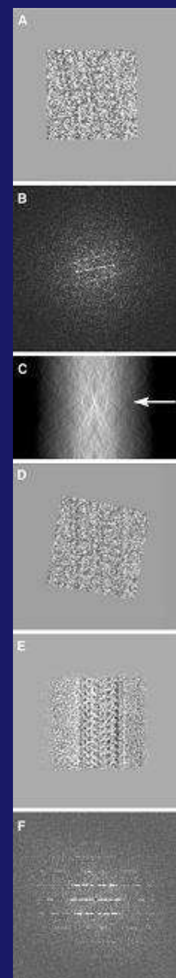


From: Egelman E.H. (2000) Ultramicroscopy 85: 225-234.

'Single Particle' Helical Reconstruction Methods

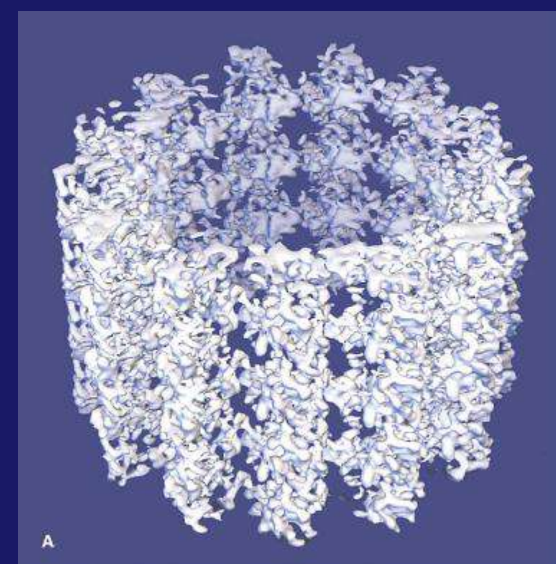


Box Filament segments



In-plane Rotational Alignment

Multi-reference alignment with atomic models

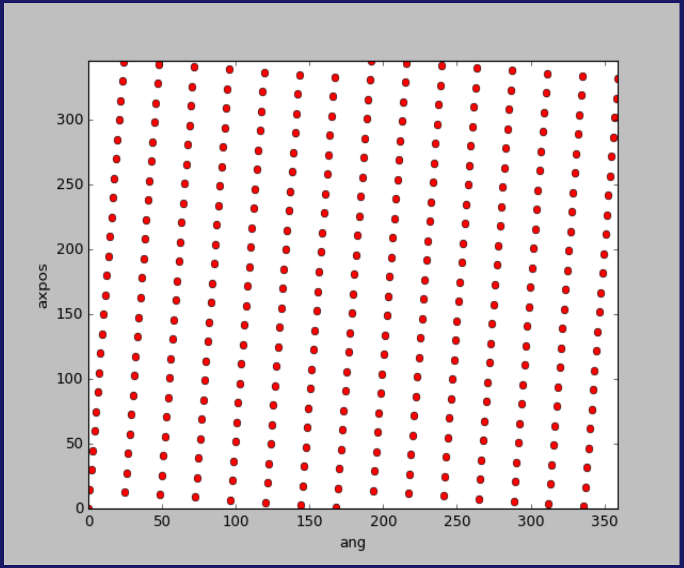


8 Å resolution microtubule map

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

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).



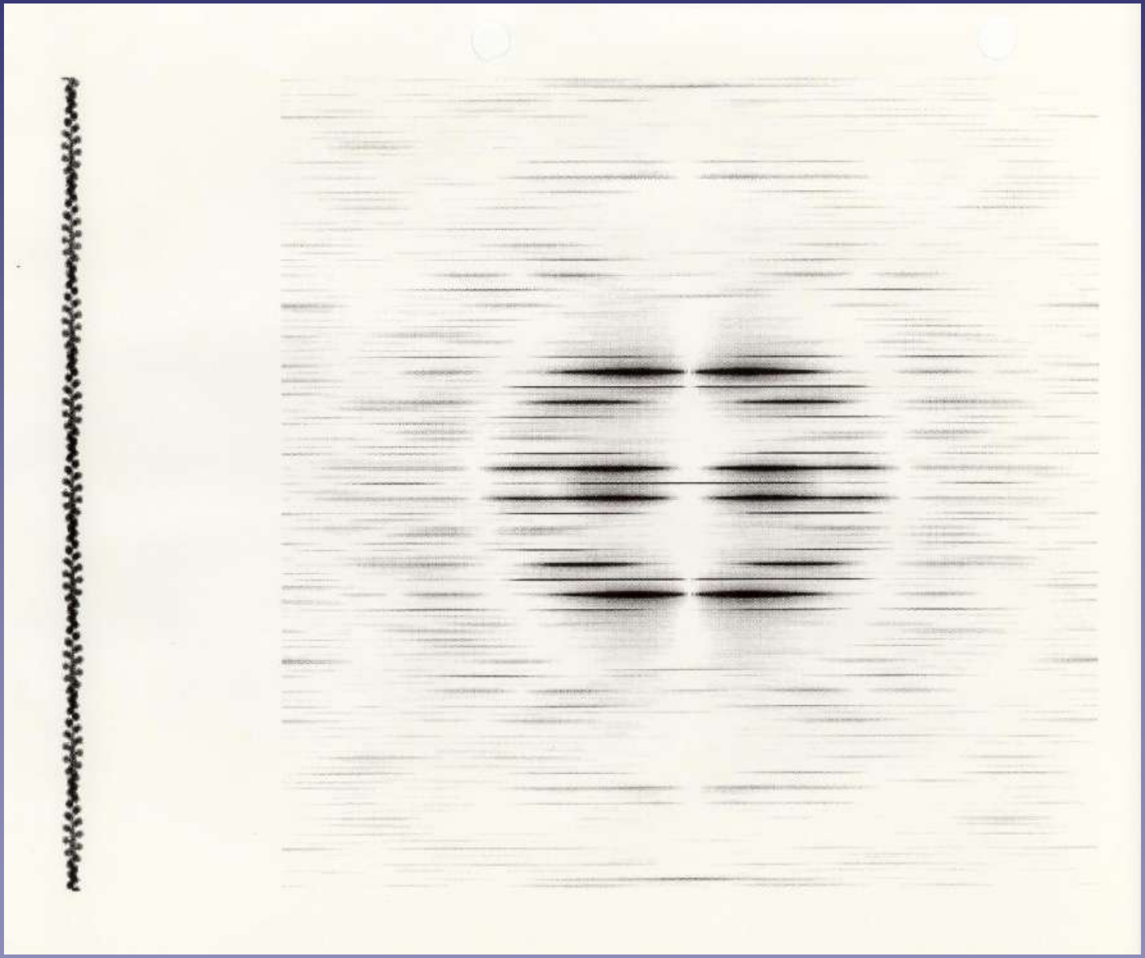
l	n
0	0
1	15
2	30
22	-17
23	-2
24	13
25	28
45	-19
46	-4
47	11
48	26
68	-21
69	-6
70	9
71	24
91	-23
92	-8
93	7
94	22

$$l = tn + um$$

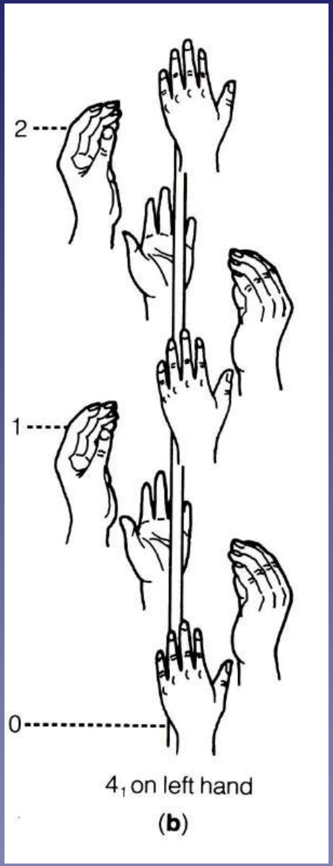
l **:** Layer line Number.
t **:** Num. of turns/rep.
***n*:** Num of Helical starts & bessel order.
***u*:** Num. of subunits/rep
***m*:** Integer

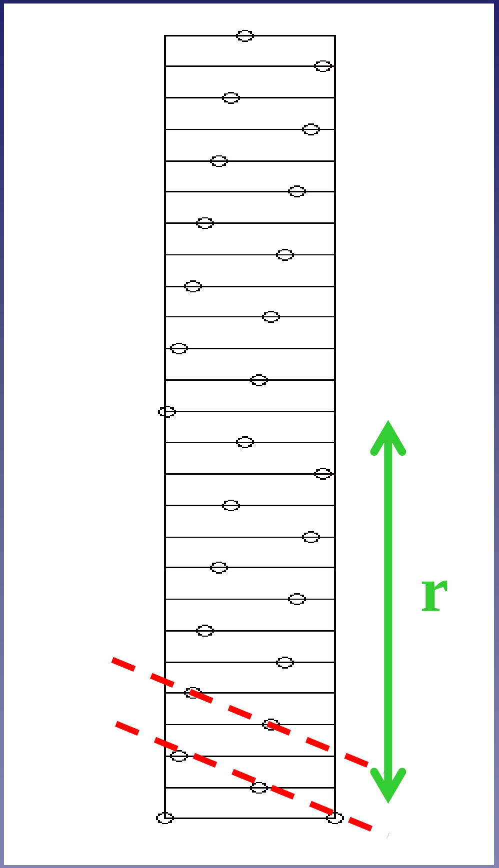
SR: l = 162 * n + 347 * m
Φ : 168.07

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

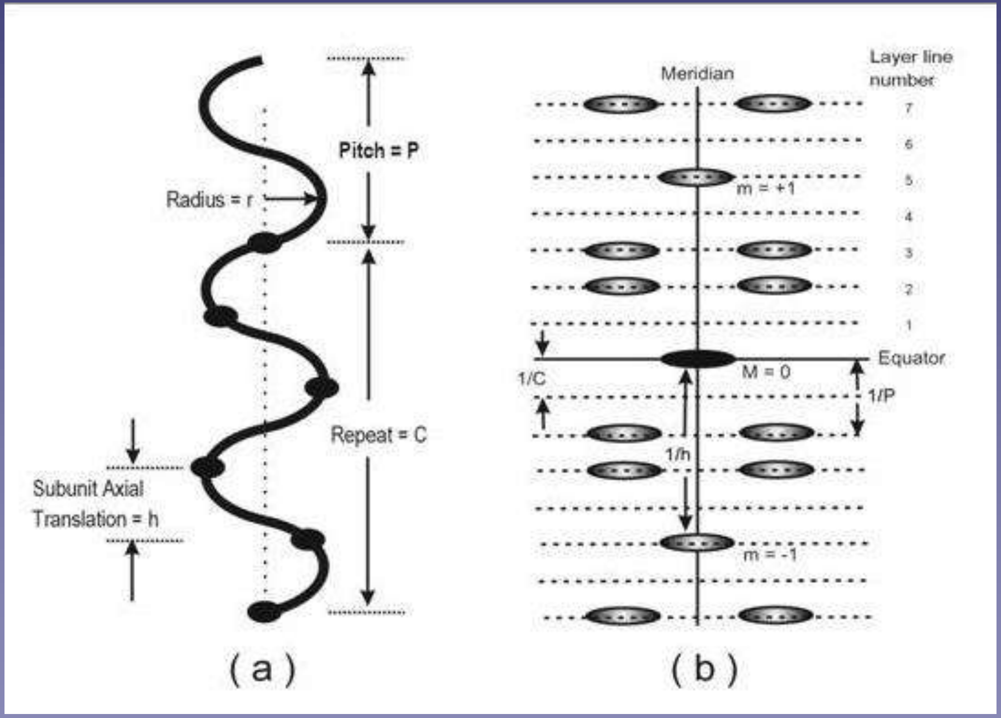
If ***k***-fold rotational symmetry then: *n* must be multiple of ***k***

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

Z: LL reciprocal spacing

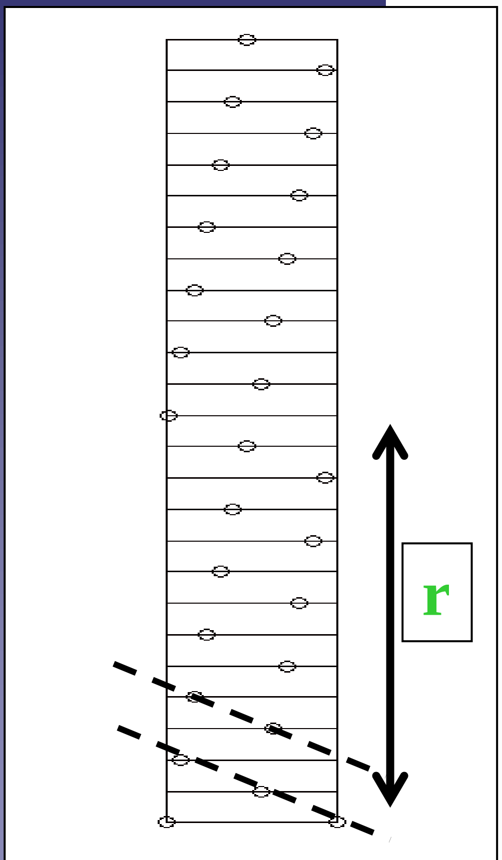
ϕ: twist angle.

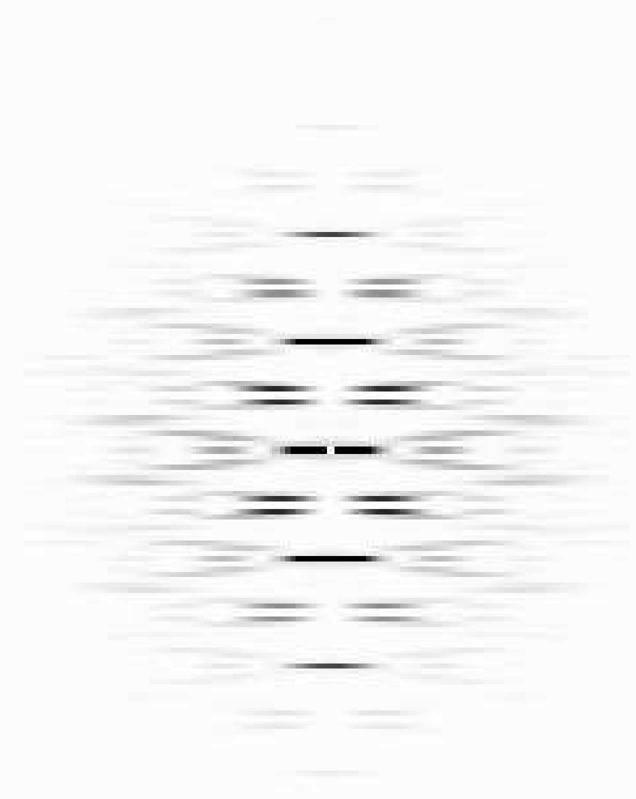
h: rise distance.



Selection Rule Example

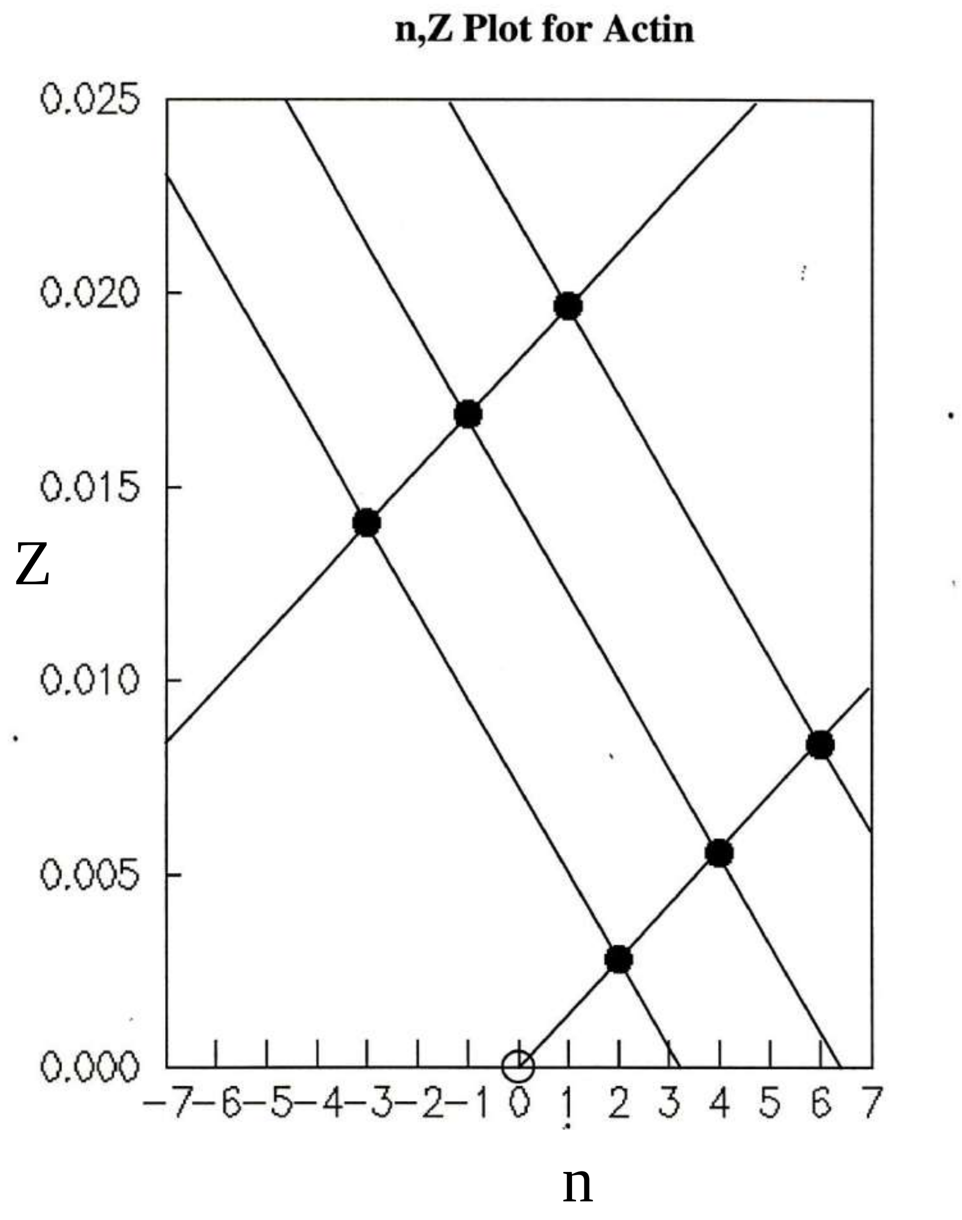
$$l = tn + um$$





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

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


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)

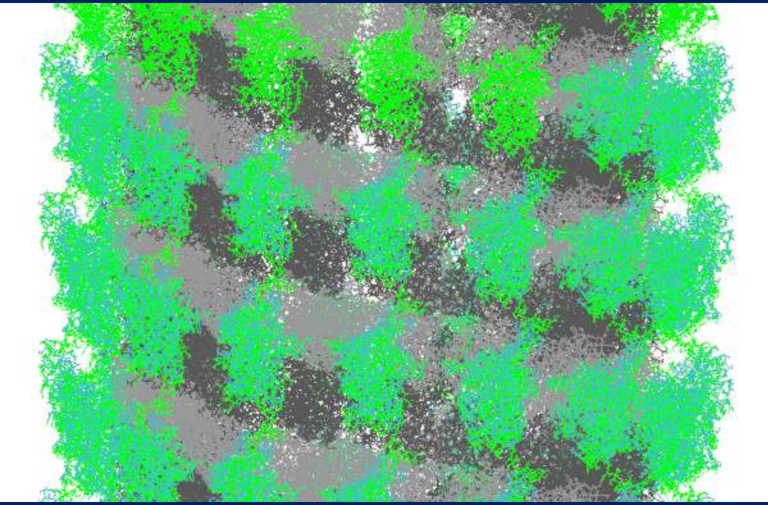
- Preliminary low resolution model (Custom)
- Classify filaments. (Custom, SPIDER, EMAN)
- Determine helical symmetry (ϕ, *h*) (Custom)

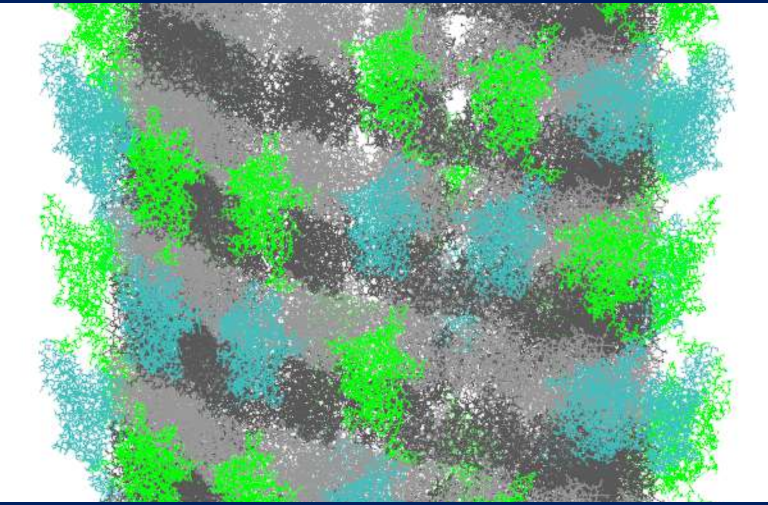
- Extract "single particle" boxes (Spider)
- Determine 3D orientation of single particle boxes (projection matching) & make 3D reconstruction (Spider, Custom)

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

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

Resolving asymmetric features in helical assemblies





Helical Assembly Subunit Refinement and Classification (HASRC)

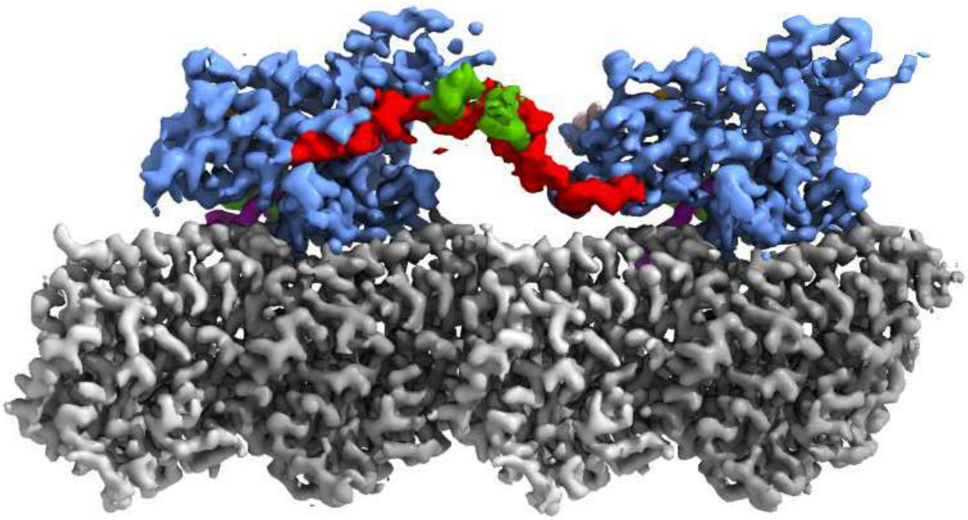
1. Helical reconstruction

2. Symmetry expansion , Signal subtraction , Subunit isolation.

3. Subunit local refinement.

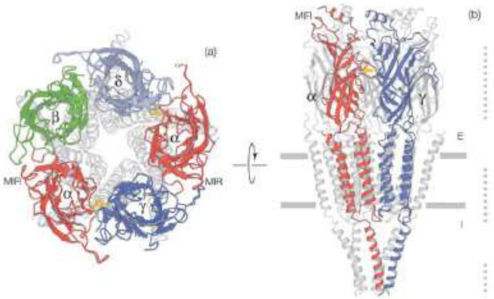
4. Focus 3D classification.

(Relion 3.0)

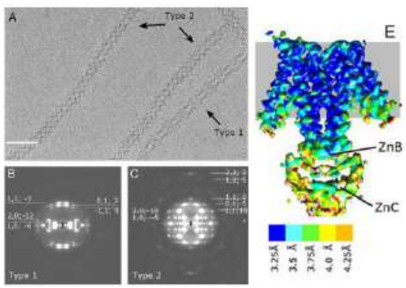


KIF14 Dimer MT complex (2.9 Å resol)
Benoit et al., *Nat. Comm*. 12:3637 (2021)

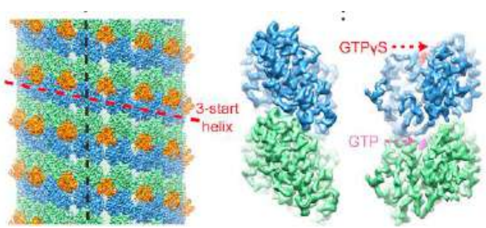
Examples of Helical Structures @ <= 4 Å



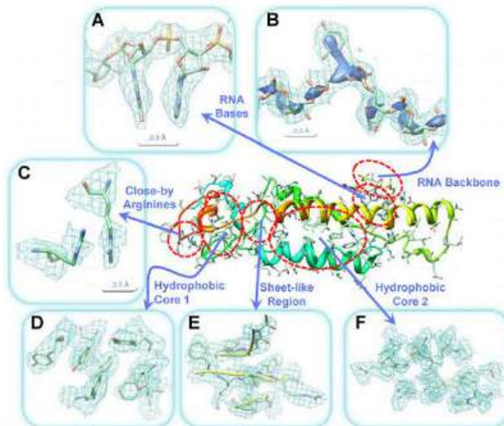
Ach Receptor (4Å)
Unwin N.(2005) JMB 346:976
(Fourier-Bessel Method)



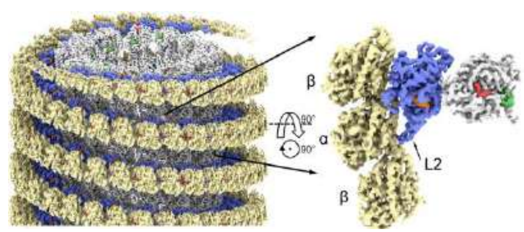
YiiP Zinc Transporter (4.1 Å)
Lopez-Redondo et al., (2018)
PNAS 115: 3042



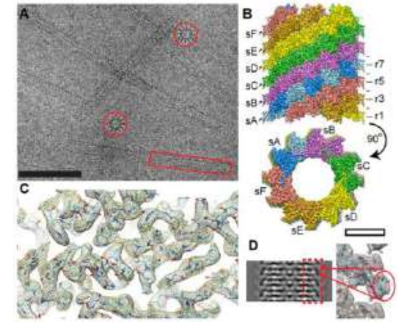
Microtubules (3.3 Å)
Zhang et al., (2015)
Cell 162: 849



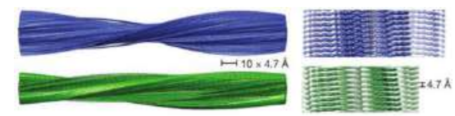
TMV (3.3 Å)
Ge & Zhou. (2011)
PNAS 108: 9637.



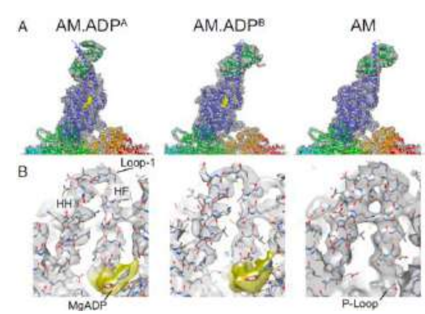
Kinesin-Microtubule complexes (3.5 Å)
Benoit et al., (2018) Nature Comm.



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



Tau Filaments (3.4Å)
Fitzpatrick et al., (2017).
Nature 547: 185



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

References & additional reading

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