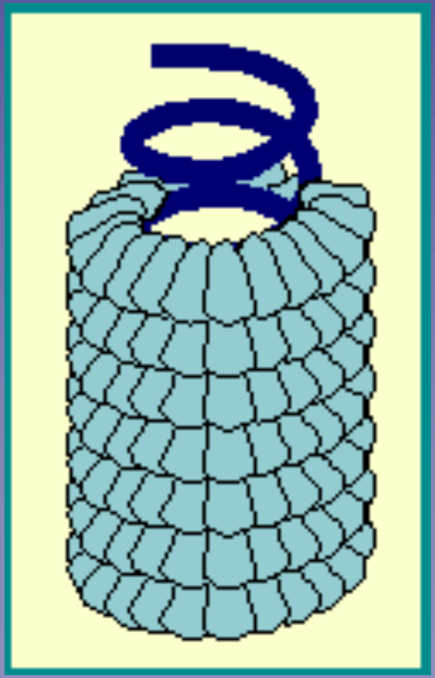
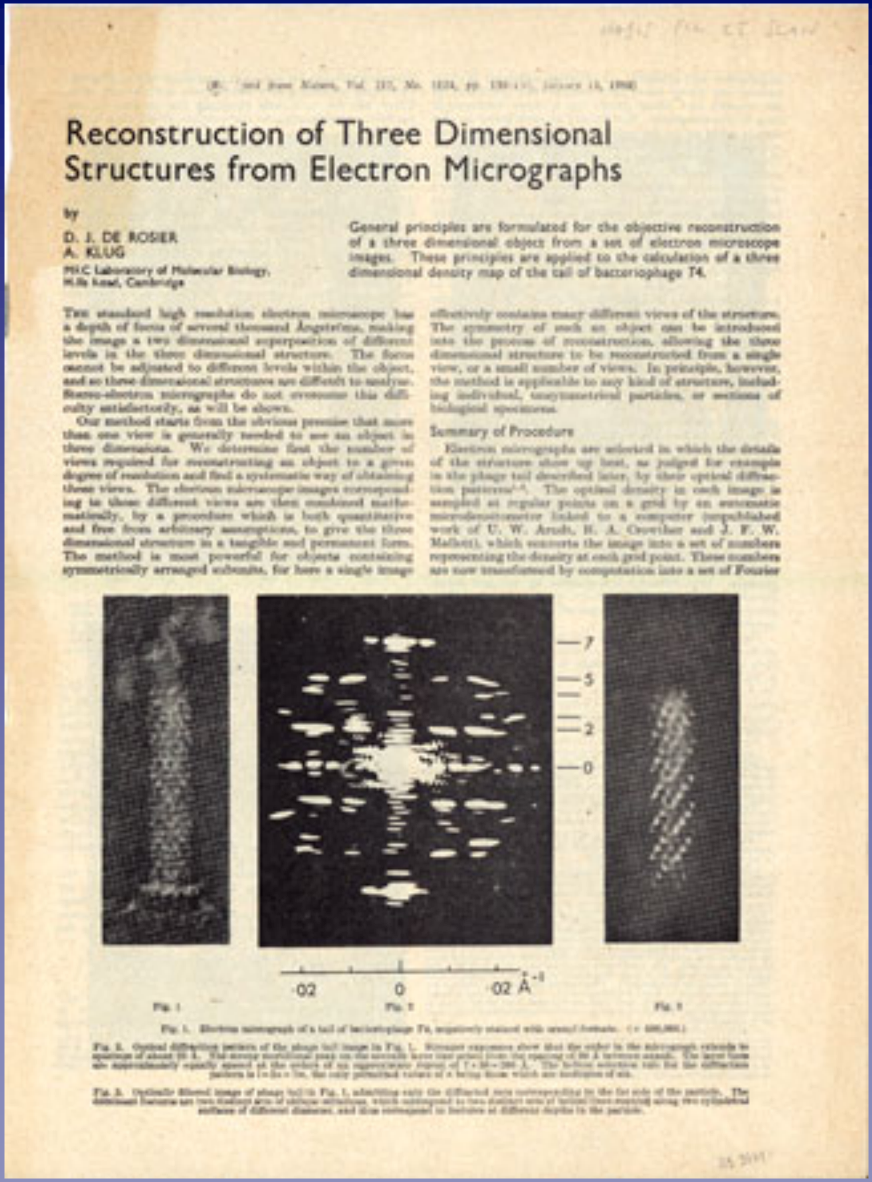
3D Reconstruction of Helical Specimens



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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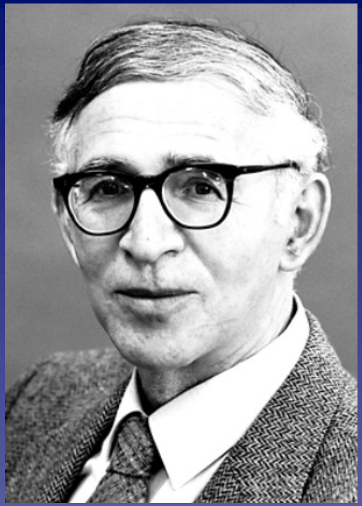
General principles are formulated for the objective reconstruction of a three dimensional object from a set of electron microscope images. These principles are applied to the calculation of a three dimensional density map of the tail of bacteriophage T4.

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[1-3]. 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



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

Topics

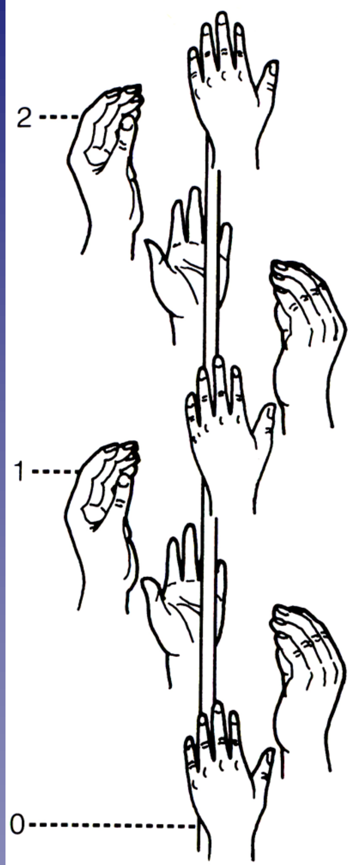
- Helix definition.
- Fourier Transform of a helix.
- Fourier-Bessel helical 3D reconstruction.
- Determining sample helical symmetry (twist & rise, selection rule).
- Real space/single particle helical 3D reconstruction.
- Some examples.

Helical Symmetry

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

Possible Symmetry operations:

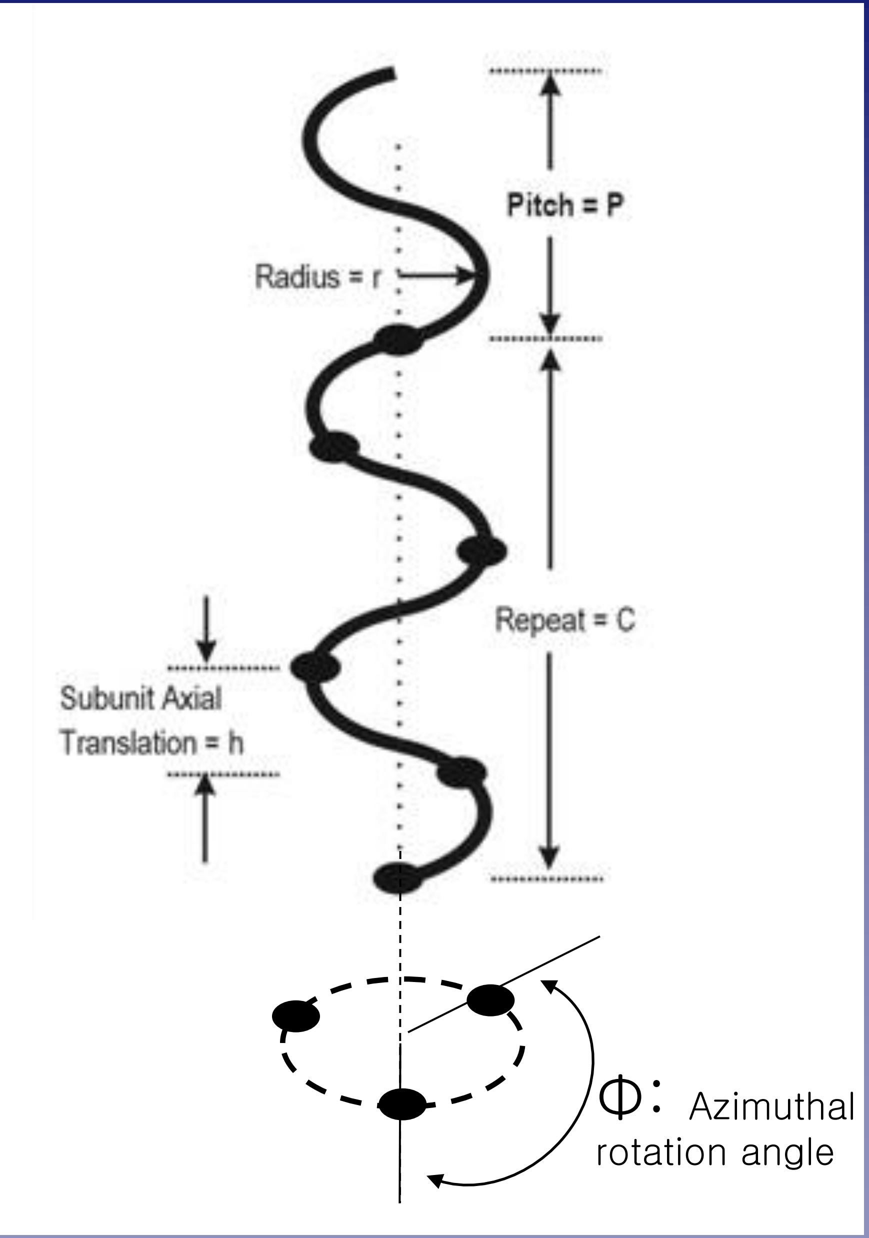
- -Screw.
- -n-fold rotation about axis.
- -2-fold rotation perpendicular to axis.



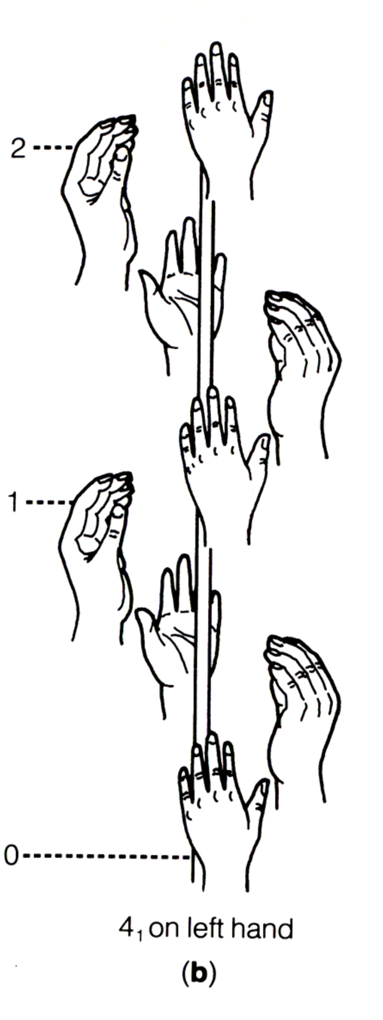
4_1 on left hand

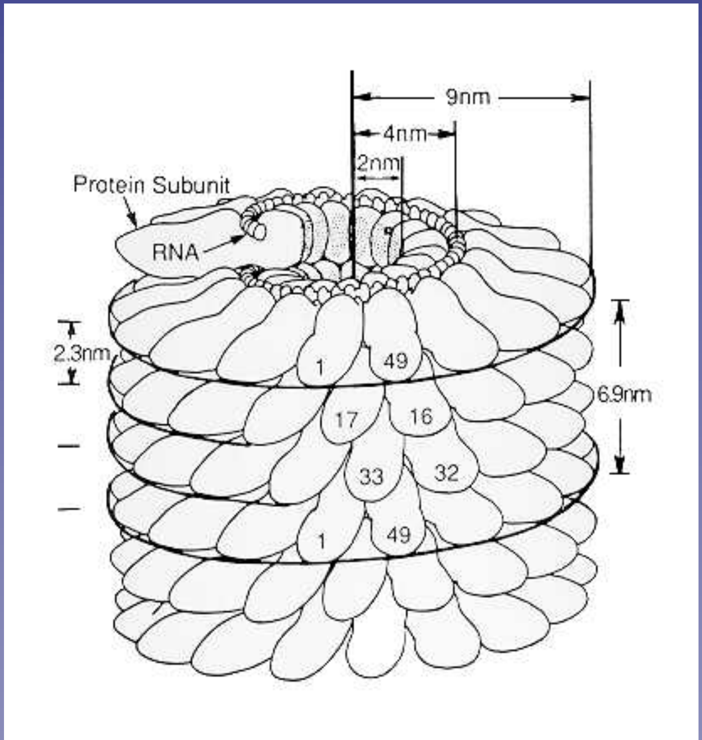
(**b**)

Parameters of a Helix



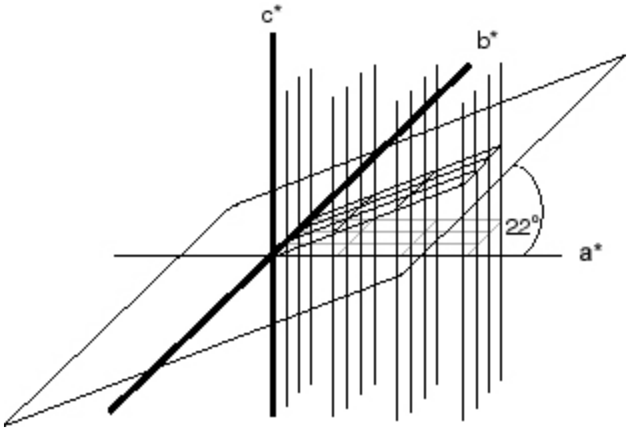
Helices give several orientation views of the asymmetric unit from a single view direction



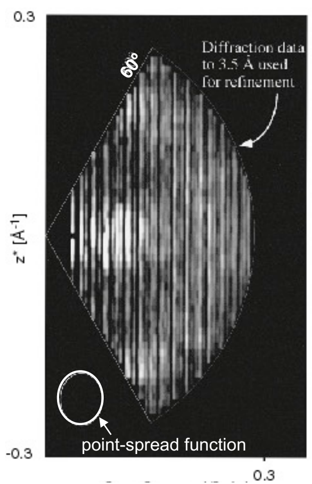


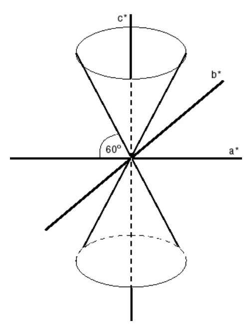
No Missing Cone !

2D crystals

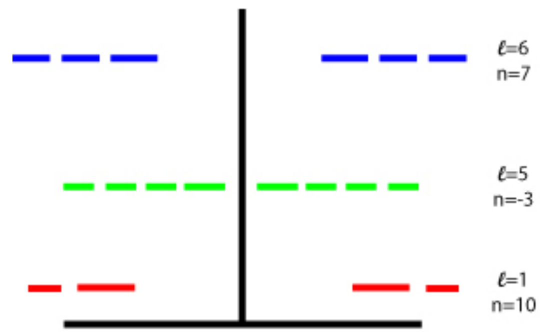


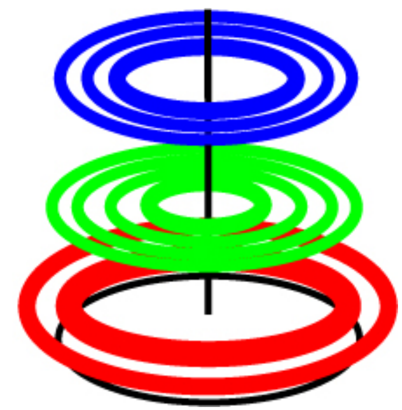
missing cone



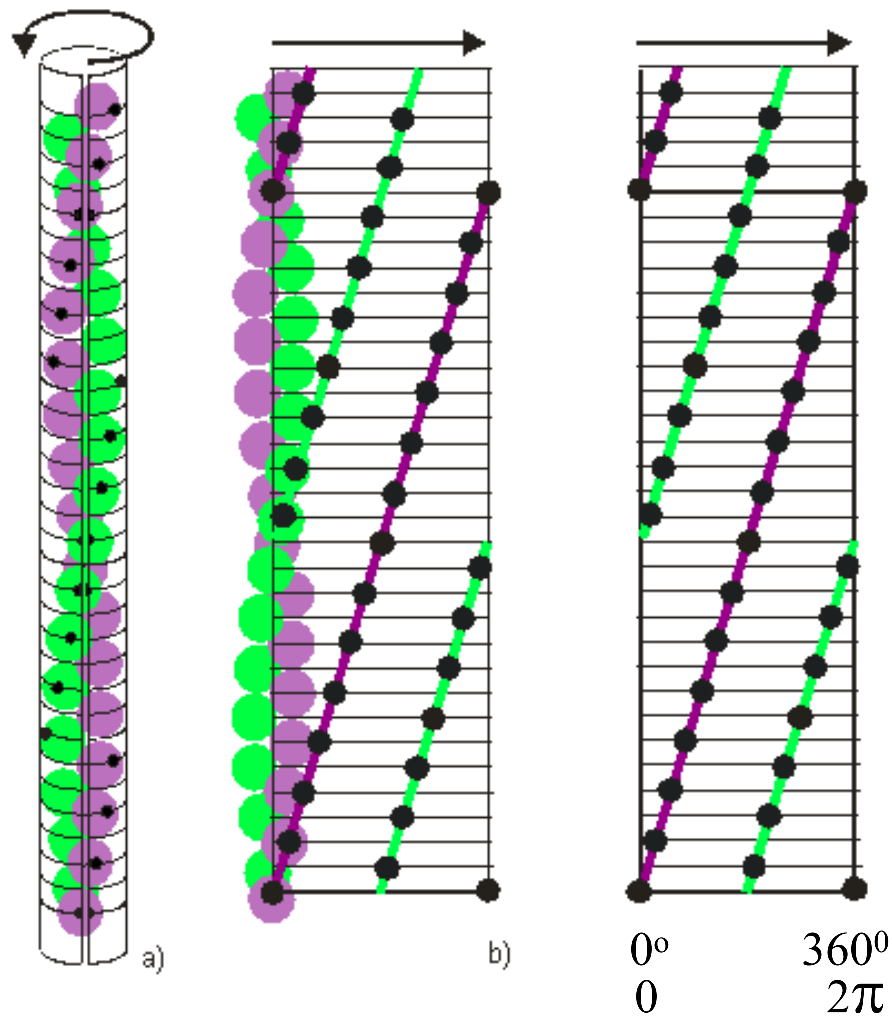


Helical crystals

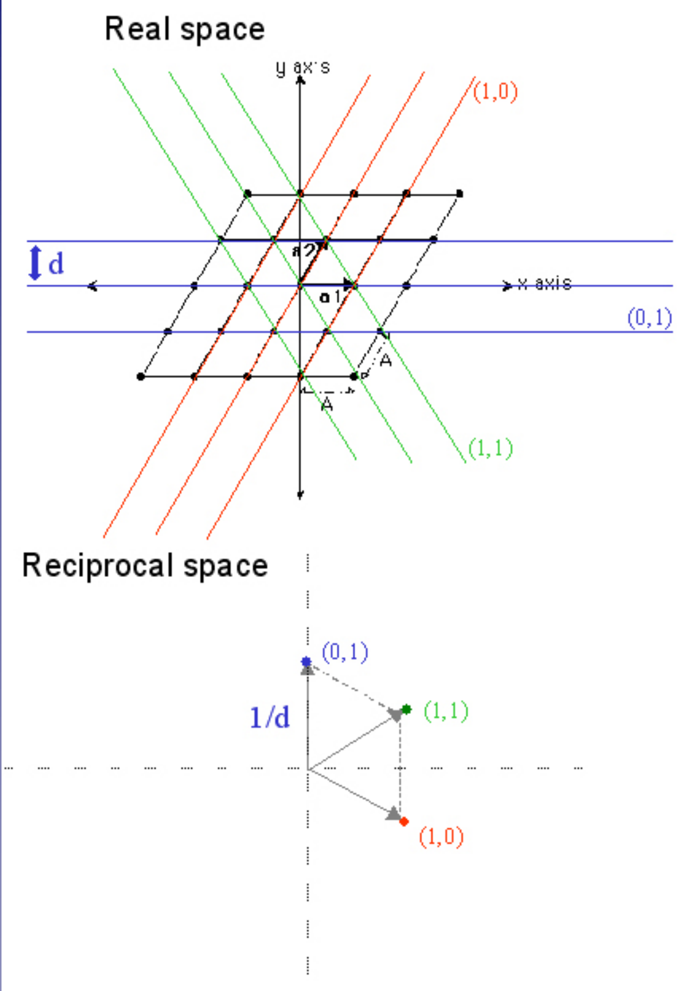


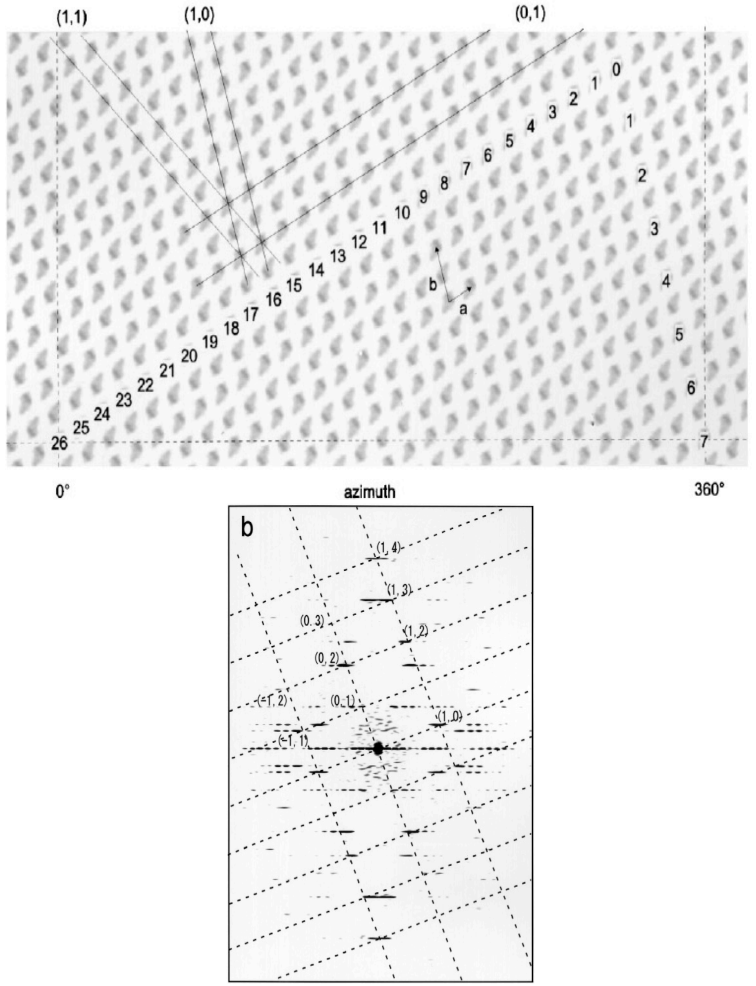


The Helical Lattice Radial Projection



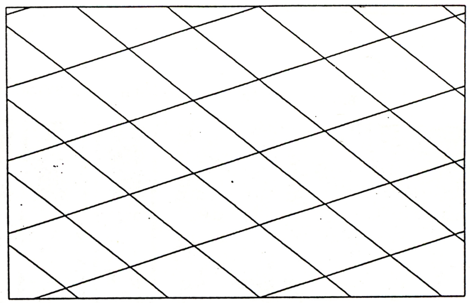
Analogy between 2D lattices and Helical Lattices

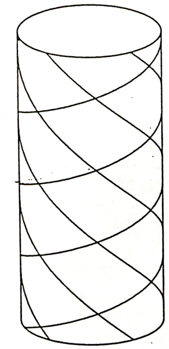


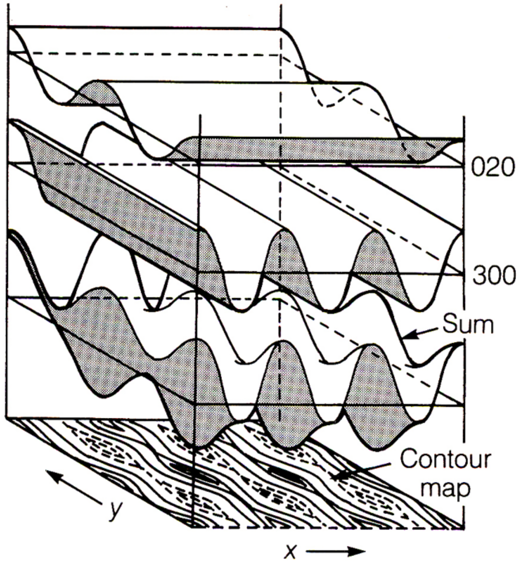


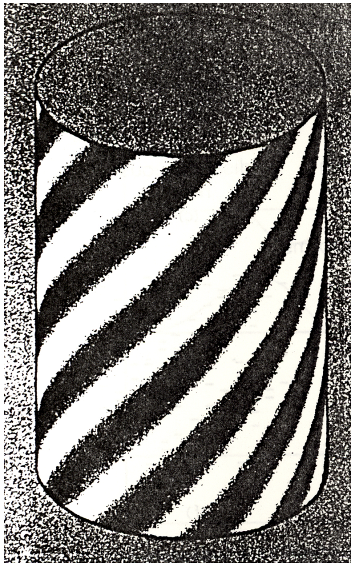
From: Toyoshima (2000) Ultramicroscopy 84: 1-14

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

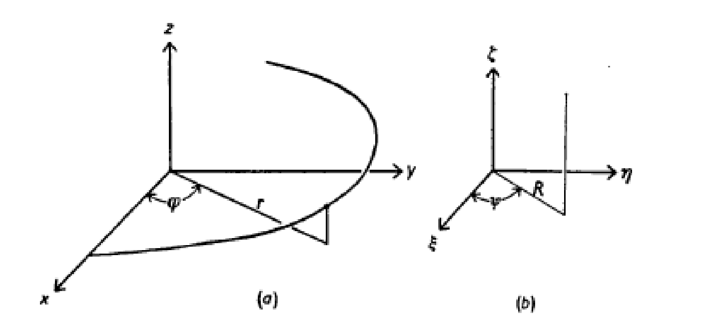


Fig. 1. (a) Cartesian (x, y, z) and cylindrical-polar (r, φ, z) coordinates of a point on a helix. (b) Corresponding coordinates of a point in reciprocal space.

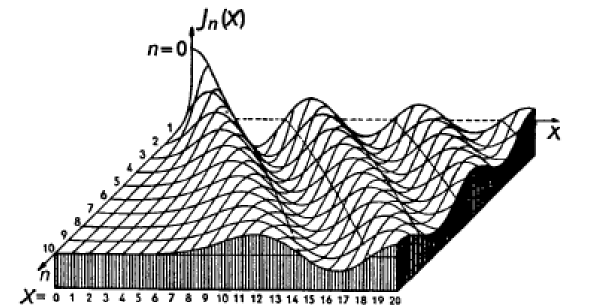
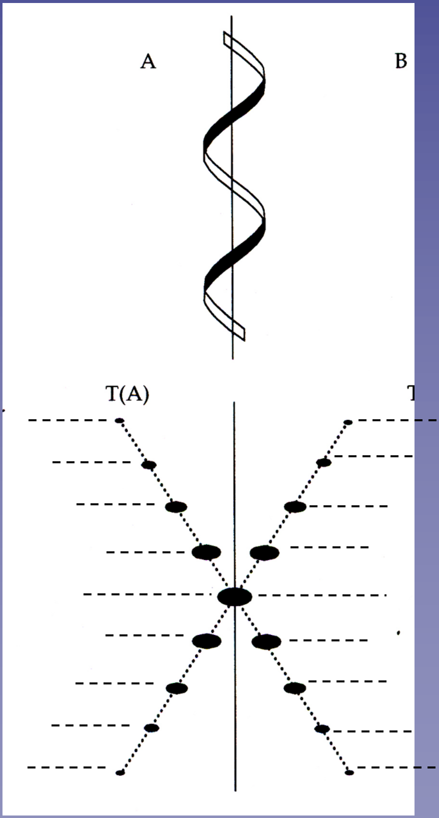
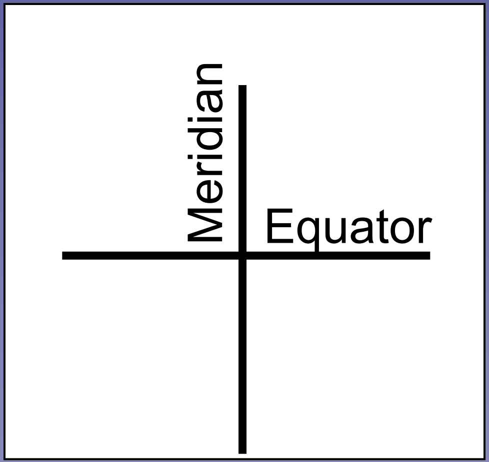
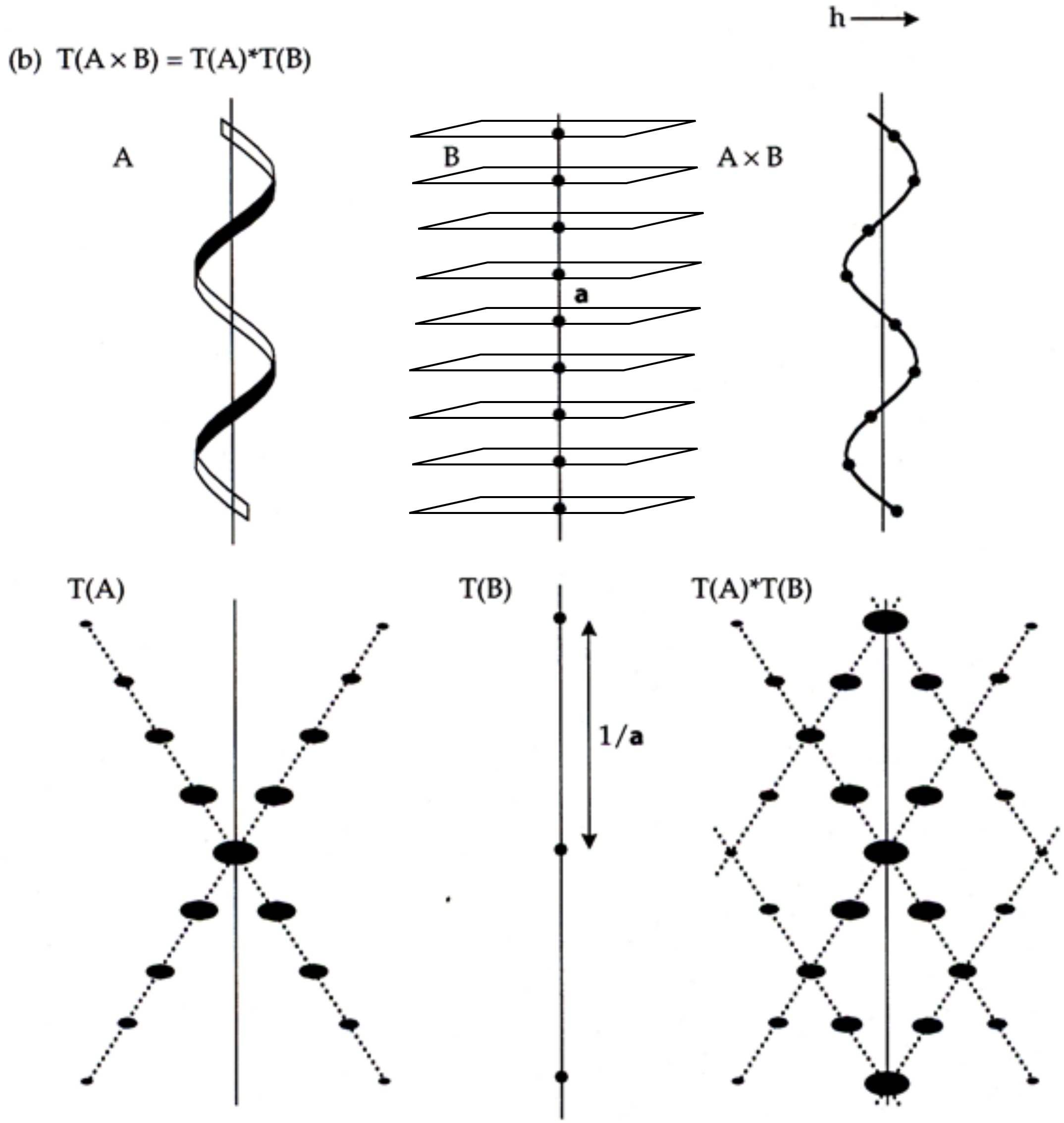


Fig. 2. Illustration of Bessel functions. (Reproduced by kind permission of the publishers from *Tables of Functions* by Jahnke & Emde. New York: Dover Publications.)

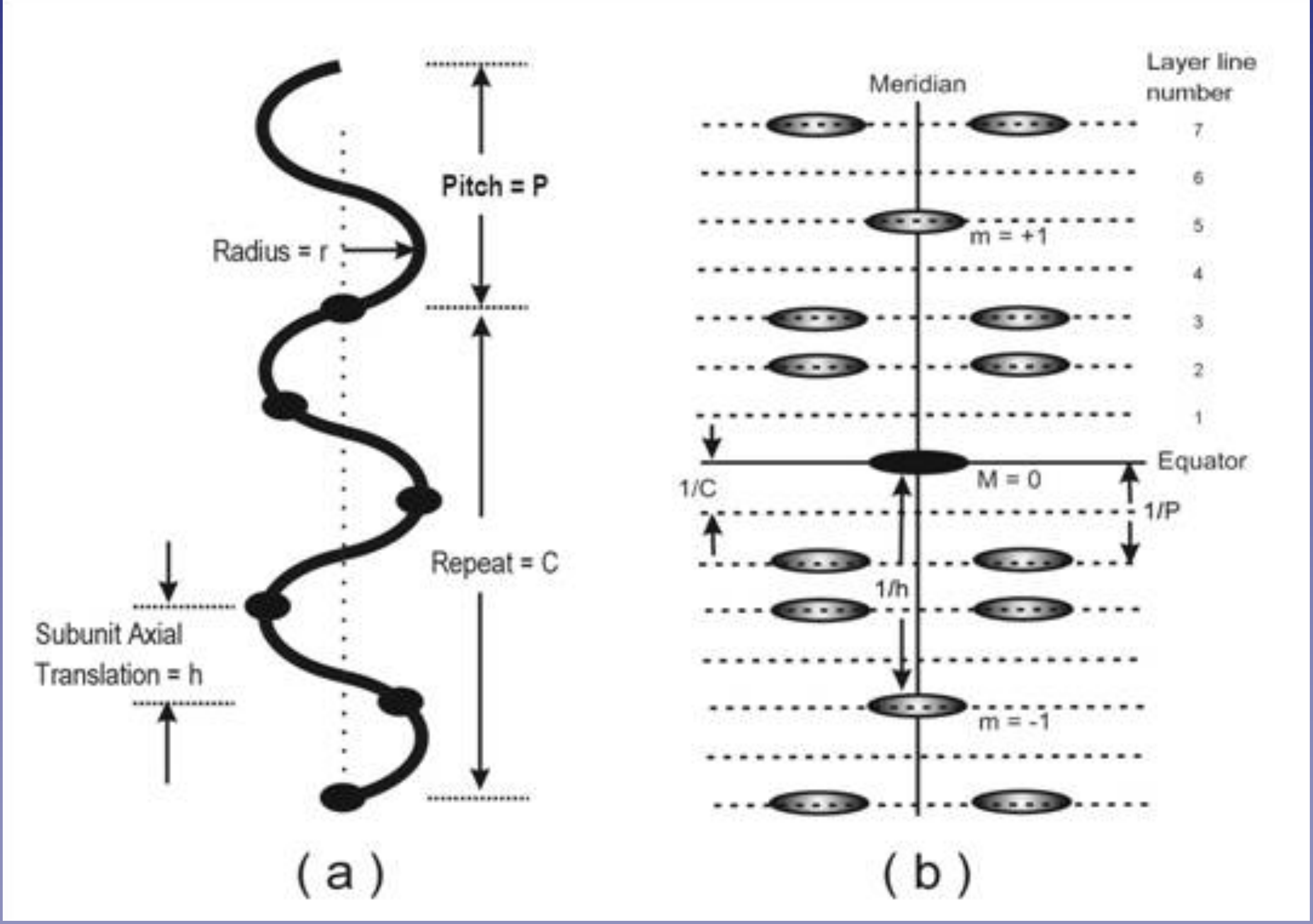




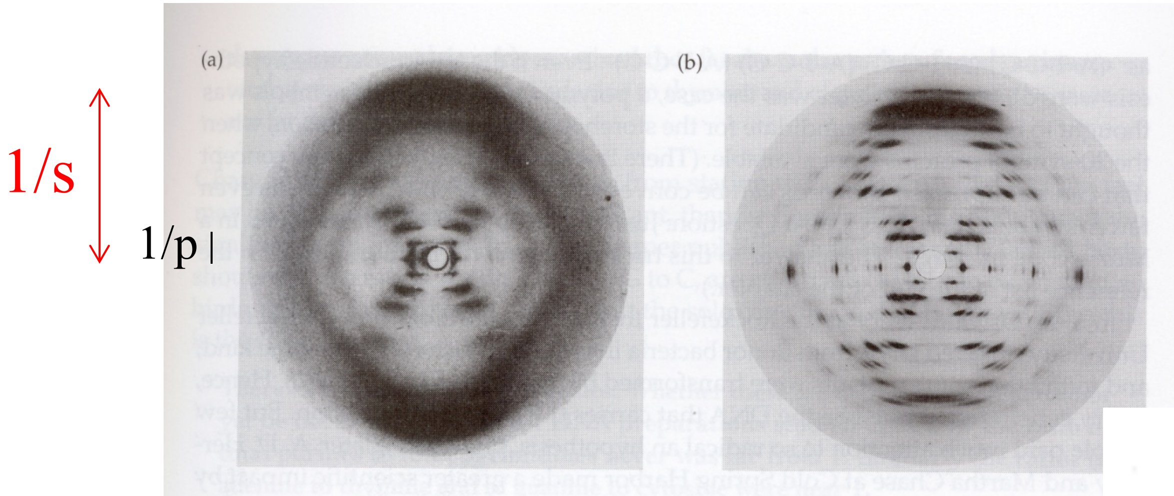
The Transform of a discontinuous helix

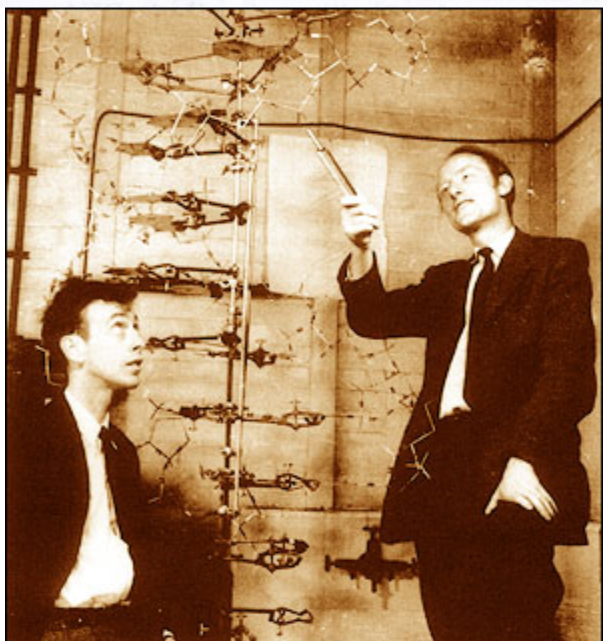


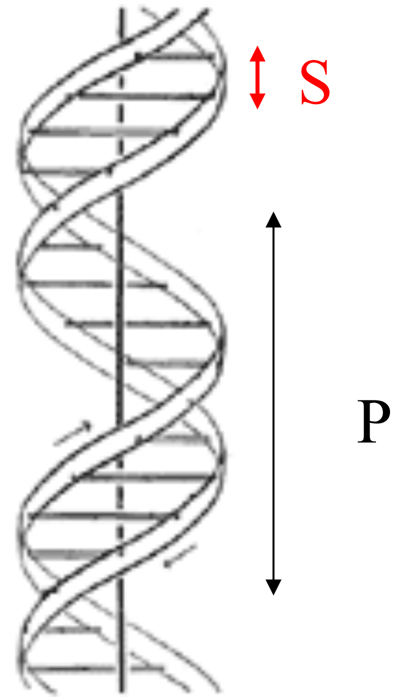
A helix and its corresponding Fourier Transform (Power Spectrum)



The DNA Structure







The Fourier Bessel Transform

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

Cochran, Crick & Vand 1952

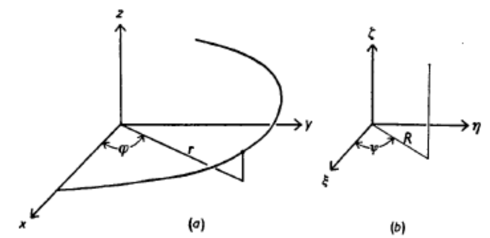


Fig. 1. (a) Cartesian (x, y, z) and cylindrical-polar (r, φ, z) coordinates of a point on a helix. (b) Corresponding coordinates of a point in reciprocal space.

Transform of group of j atoms at different radii

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

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

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

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

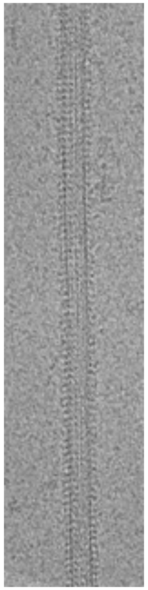
Selection rule

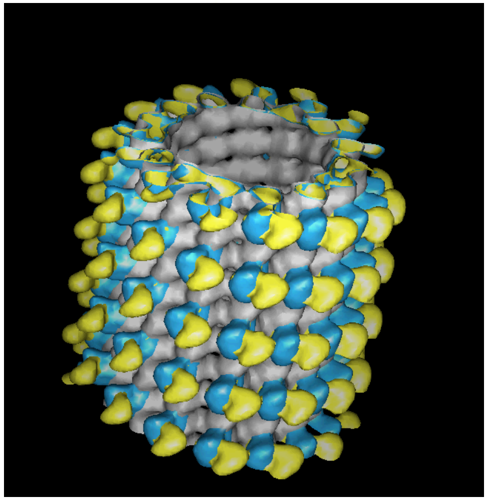
$$l = tn + um \quad (2)$$

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

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

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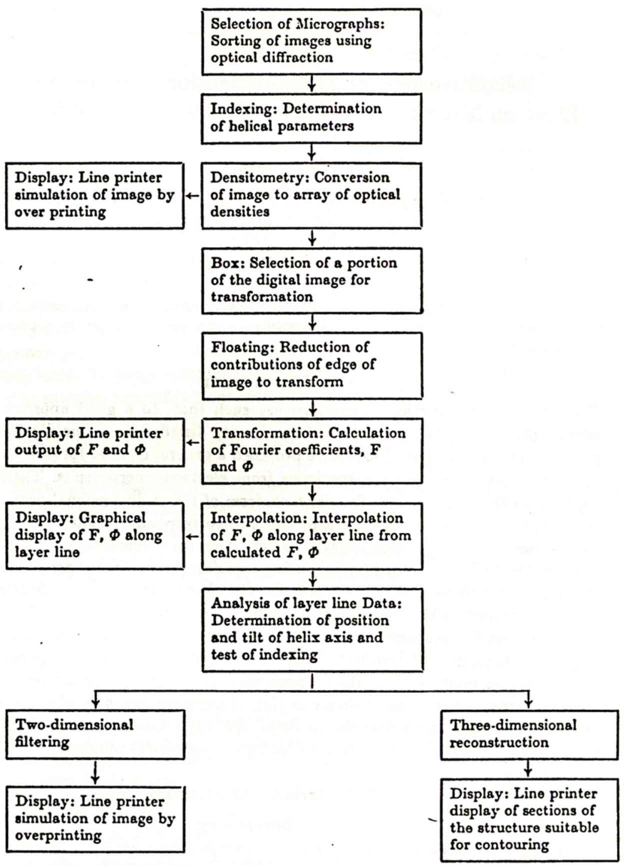


FIG. 1. The scheme presented shows the flow of data in the process of three-dimensional reconstruction.

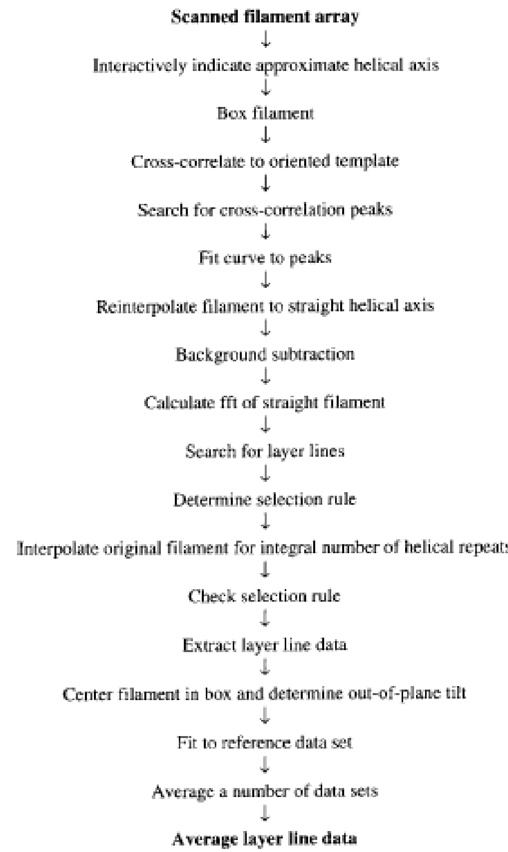
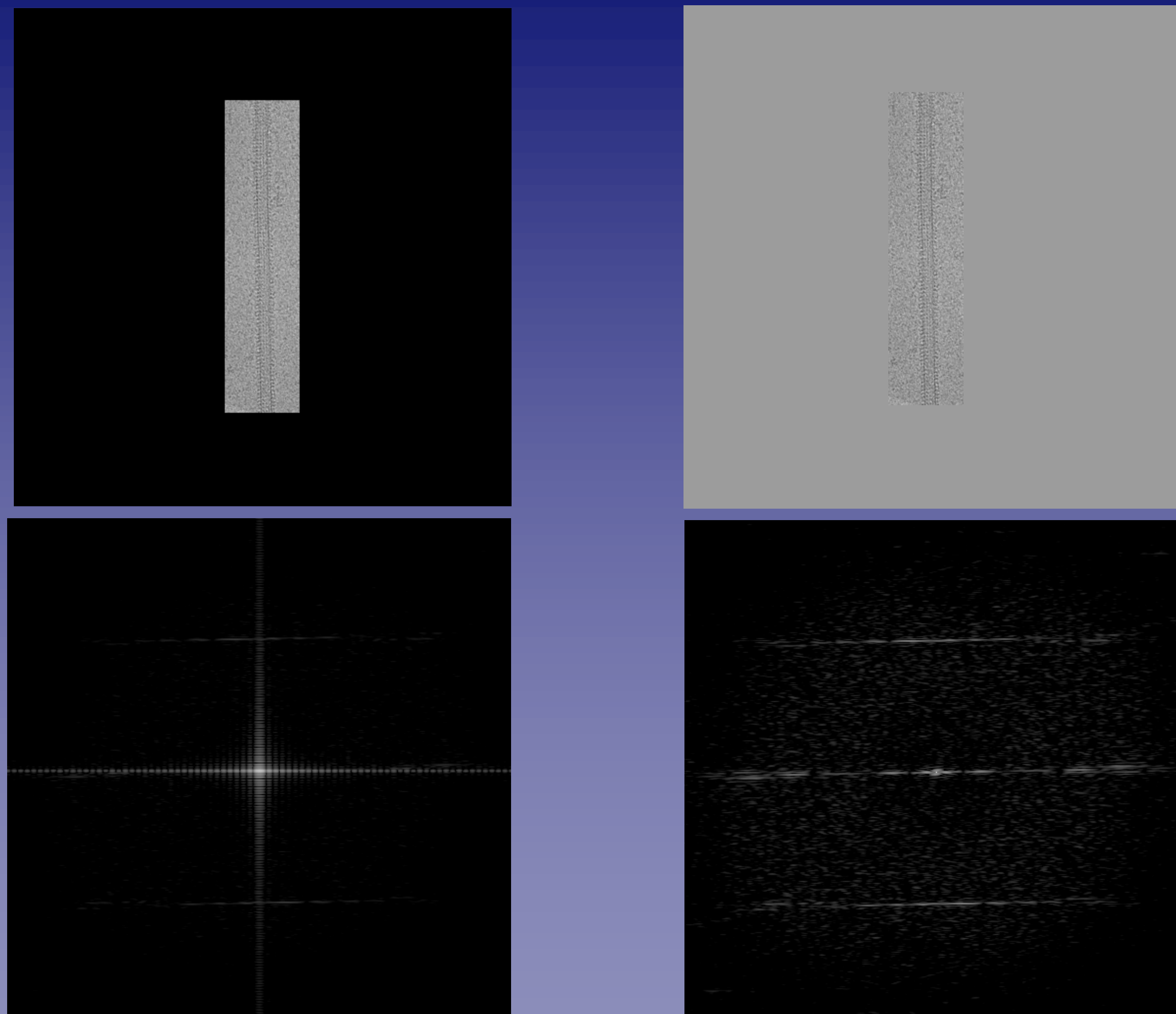


FIG. 1. Schematic diagram of the PHOELIX helical processing package. A detailed description of each step and the programs used is available as part of the PHOELIX distribution.

Boxing & Floating Image



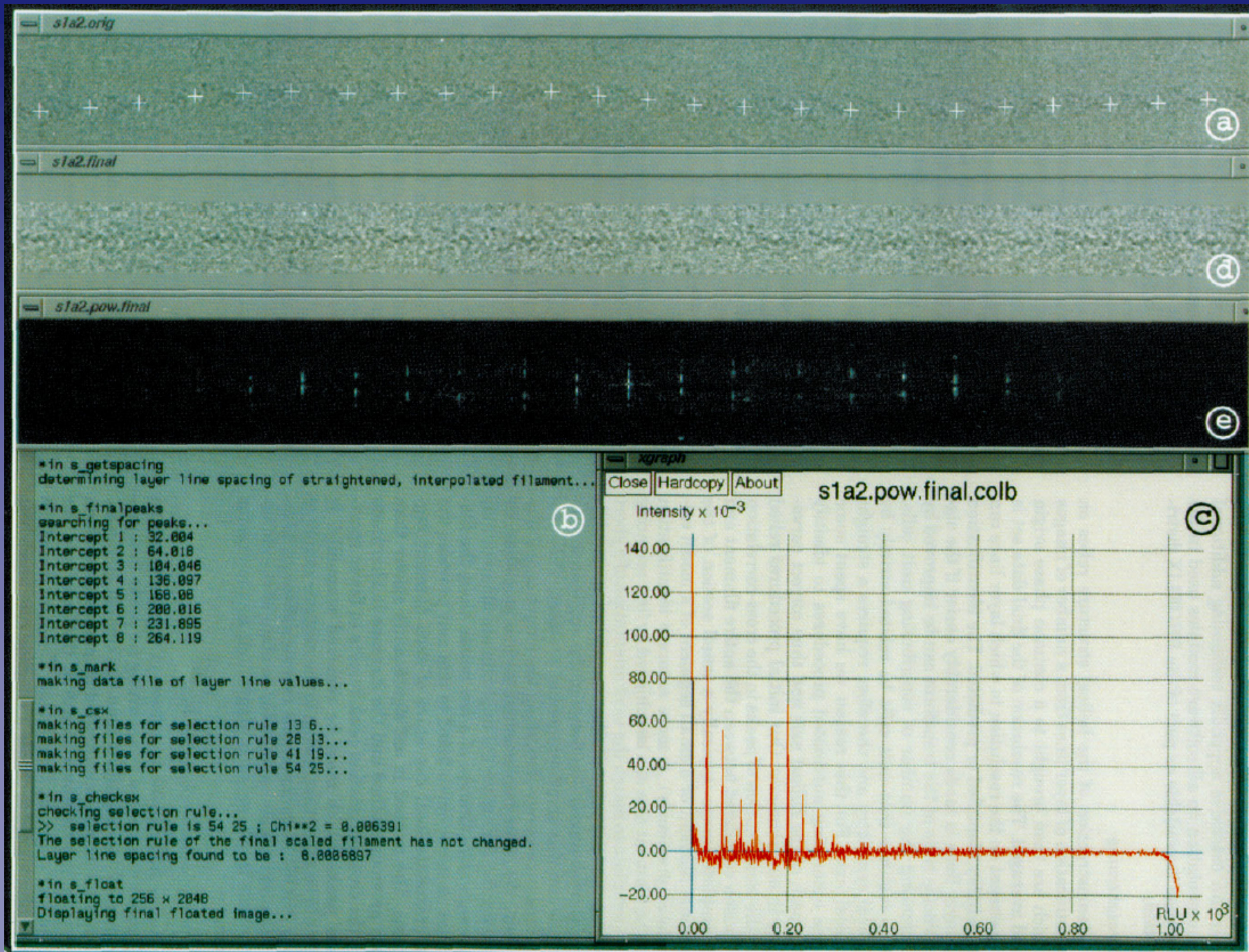
Image

FFT

Non-Floated

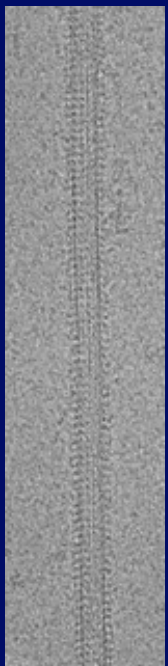
Floated

Straightening



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

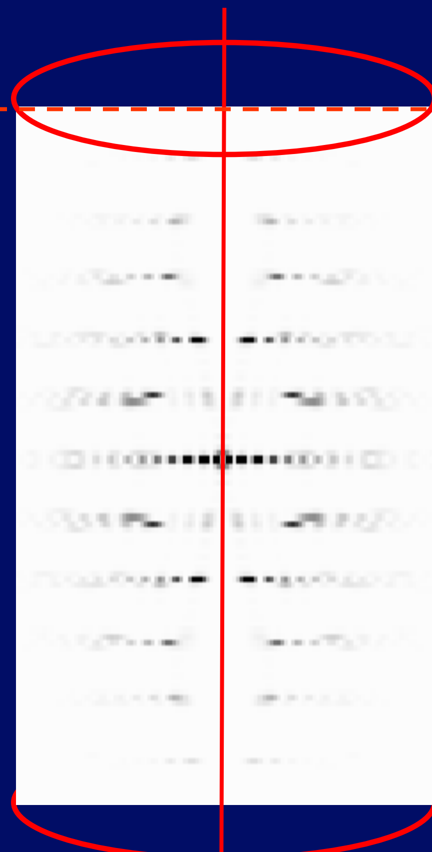
Gathering Amplitude and Phases



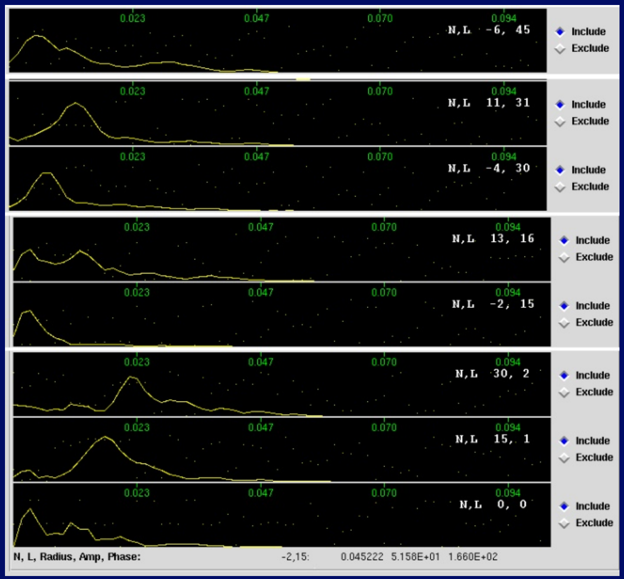
Image

FFT

$\Psi = -\pi/2$ $\Psi = \pi/2$



Fourier Transform

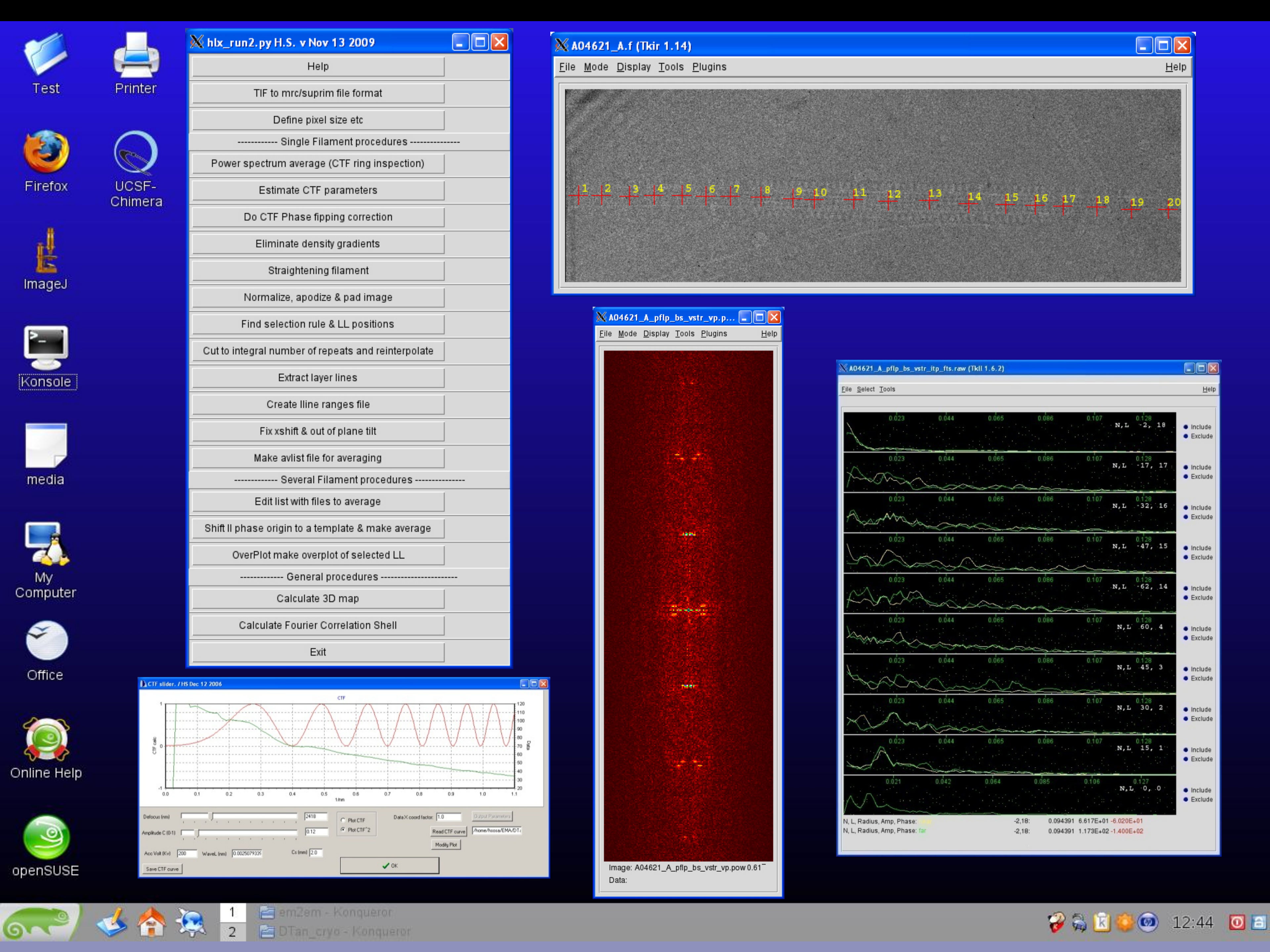


Layer Line Data Set

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

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

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


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
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_itp_fts.raw (Tkll 1.6.2)
File Select Tools Help
Include
Exclude
CTF slider. / HS Dec 12 2006
CTF
Test
Printer
Firefox
UCSF-Chimera
ImageJ
Konsole
media
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Online Help
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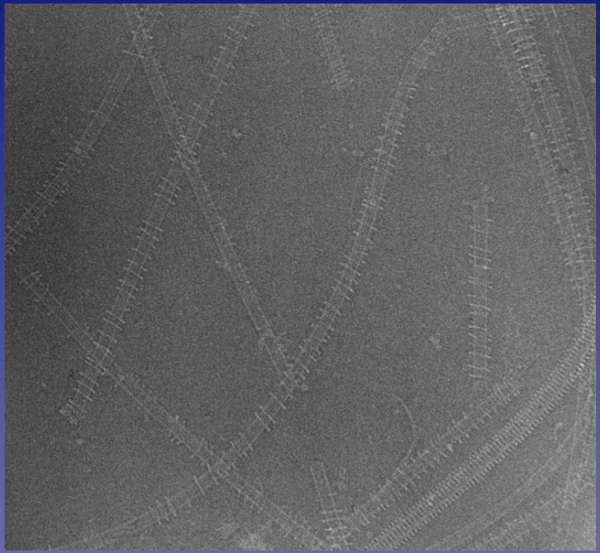
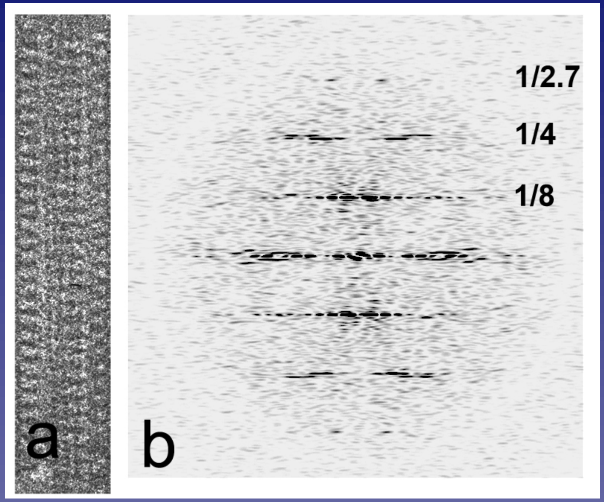
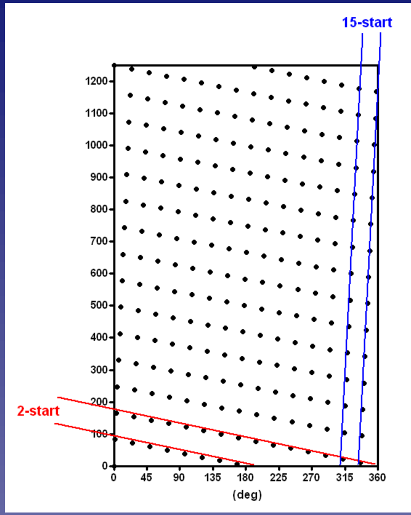
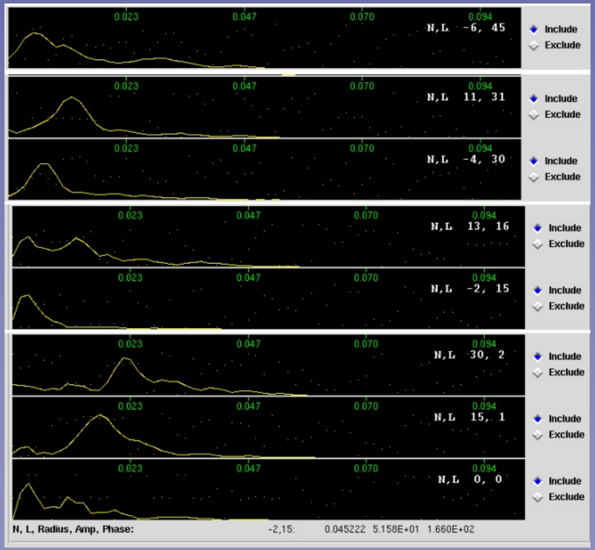
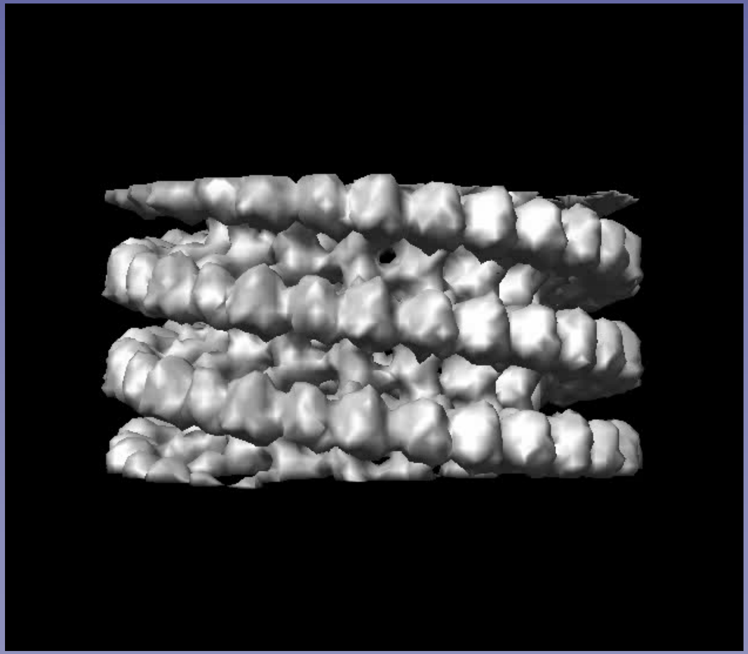
Kinesin13-Microtubule Ring Complex Helical Reconstruction









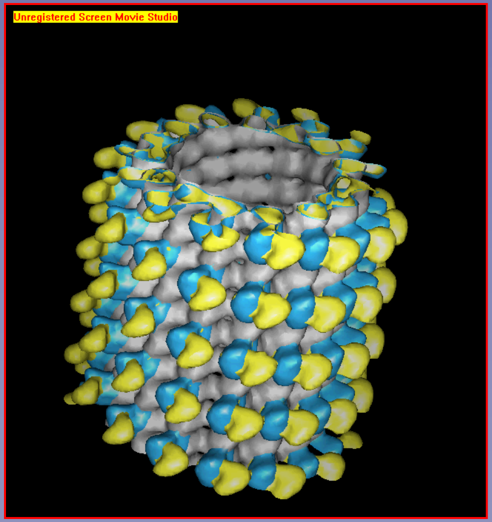


3D Density Map Reconstruction
(Fourier Bessel inversion)

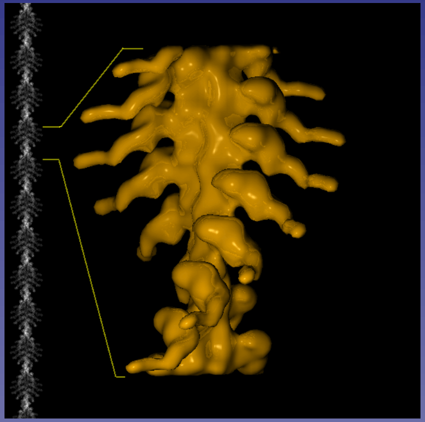
Average $G_{n,l}$ Data set.

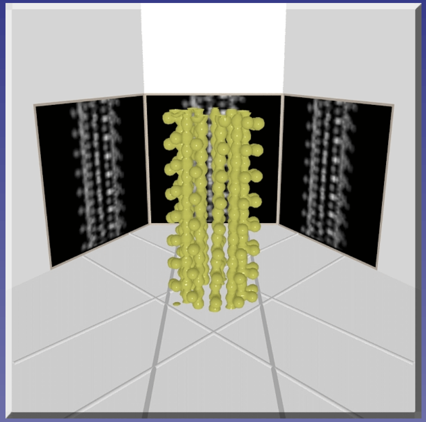
$$g_{n,l}(r) = \int G_{n,l}(R) J_n(2\pi Rr)\, 2\pi R \mathrm{d}R$$

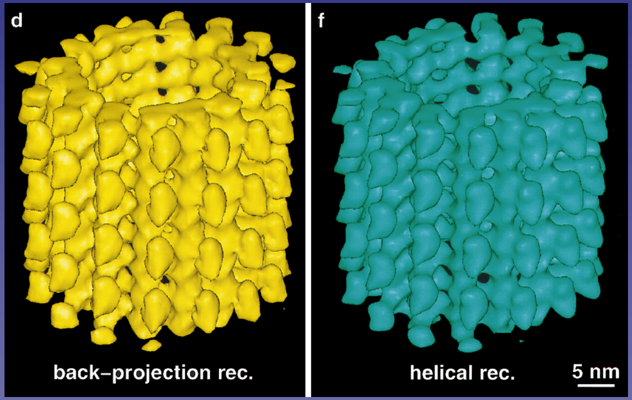
$$\rho(r, \phi, z) = \sum_l \sum_n g_{n,l}(r) \exp(in\,\phi) \exp(-2\pi i l z/c)$$



3D Helical Reconstruction Using Real Space Methods





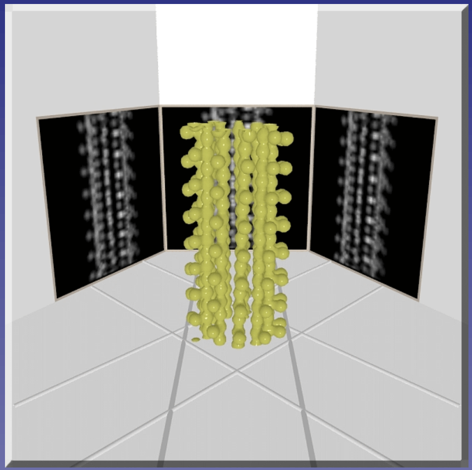


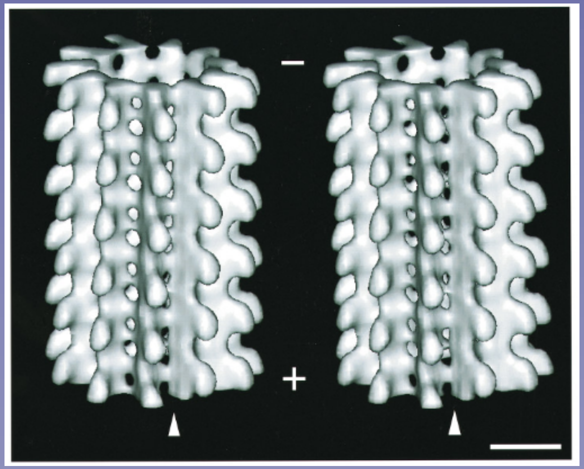
From Sosa et al. JSB 118: 149-158(1997)

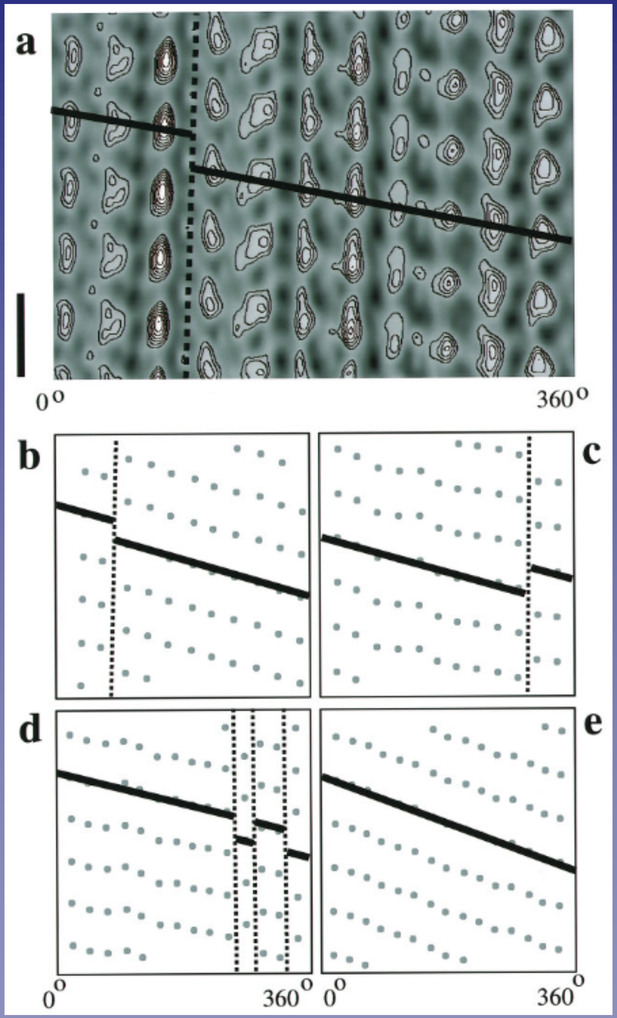
Individual images are boxed out of the filament at each asymmetric unit axial spacing and a view angle is assigned according to the helical symmetry of the filament.

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

3D reconstruction by helical -real-space back-projection

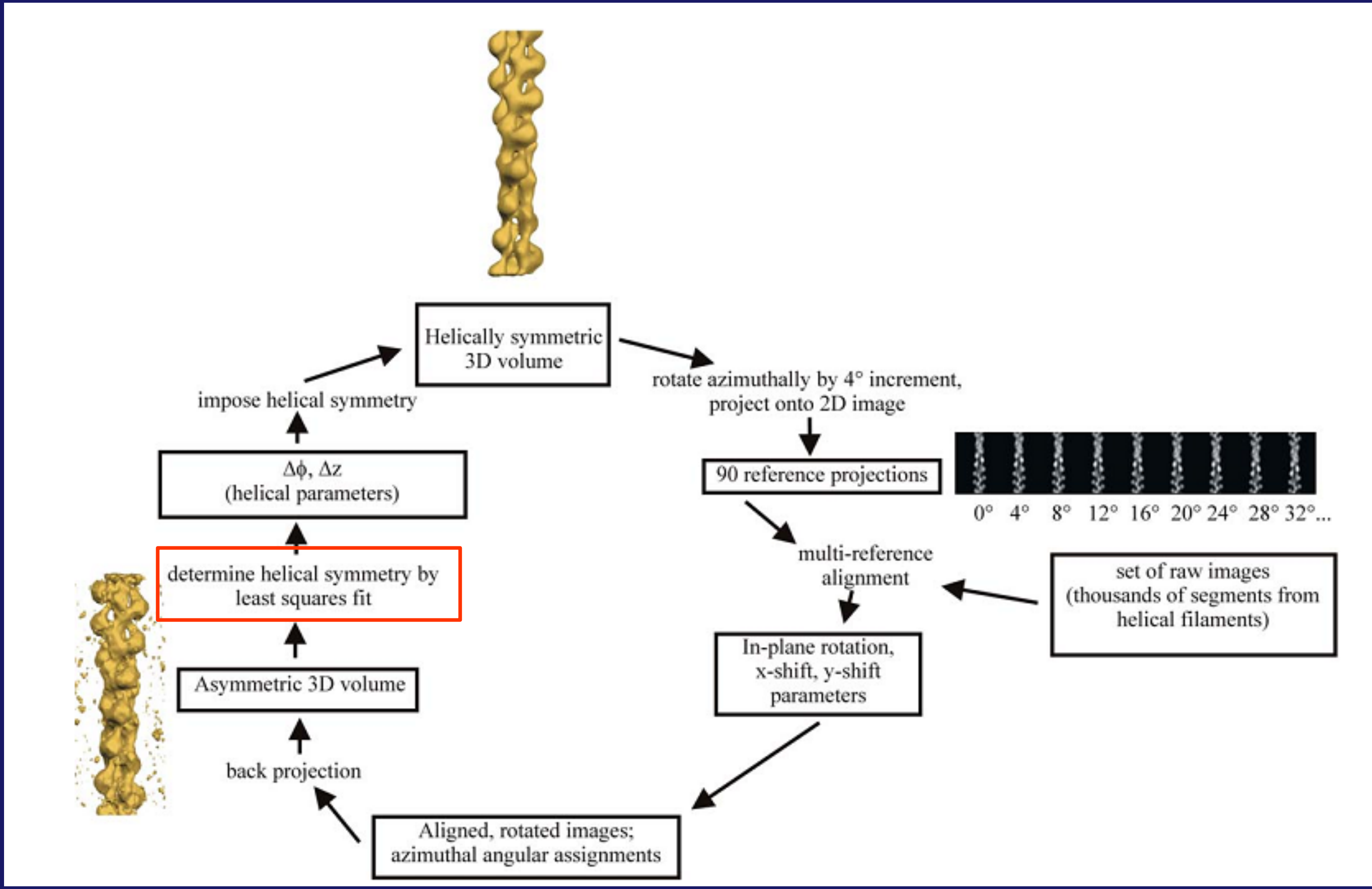






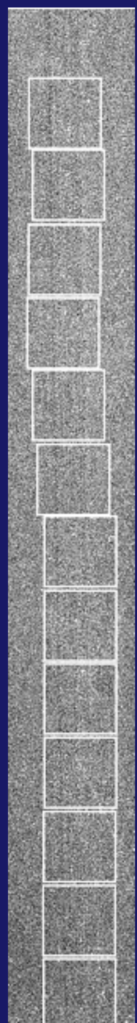
From :Sosa et al., (1996) JMB 260: 343

The Iterative Helical Real Space Reconstruction Method (IHRSR)

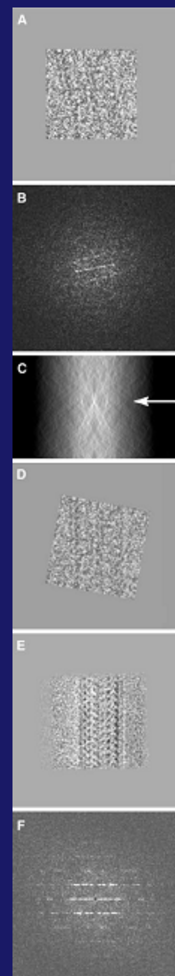


From: Egelman E.H. (2000) Ultramicroscopy 85: 225-234.

'Single Particle' Helical Reconstruction Methods

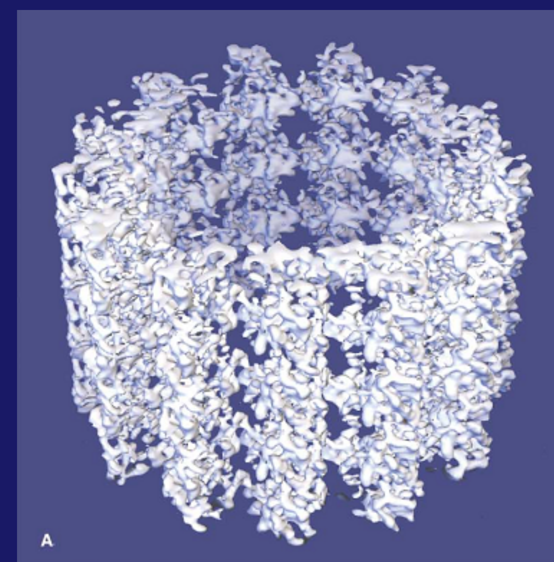


Box Filament segments



In-plane Rotational Alignment

Multi-reference alignment with atomic models



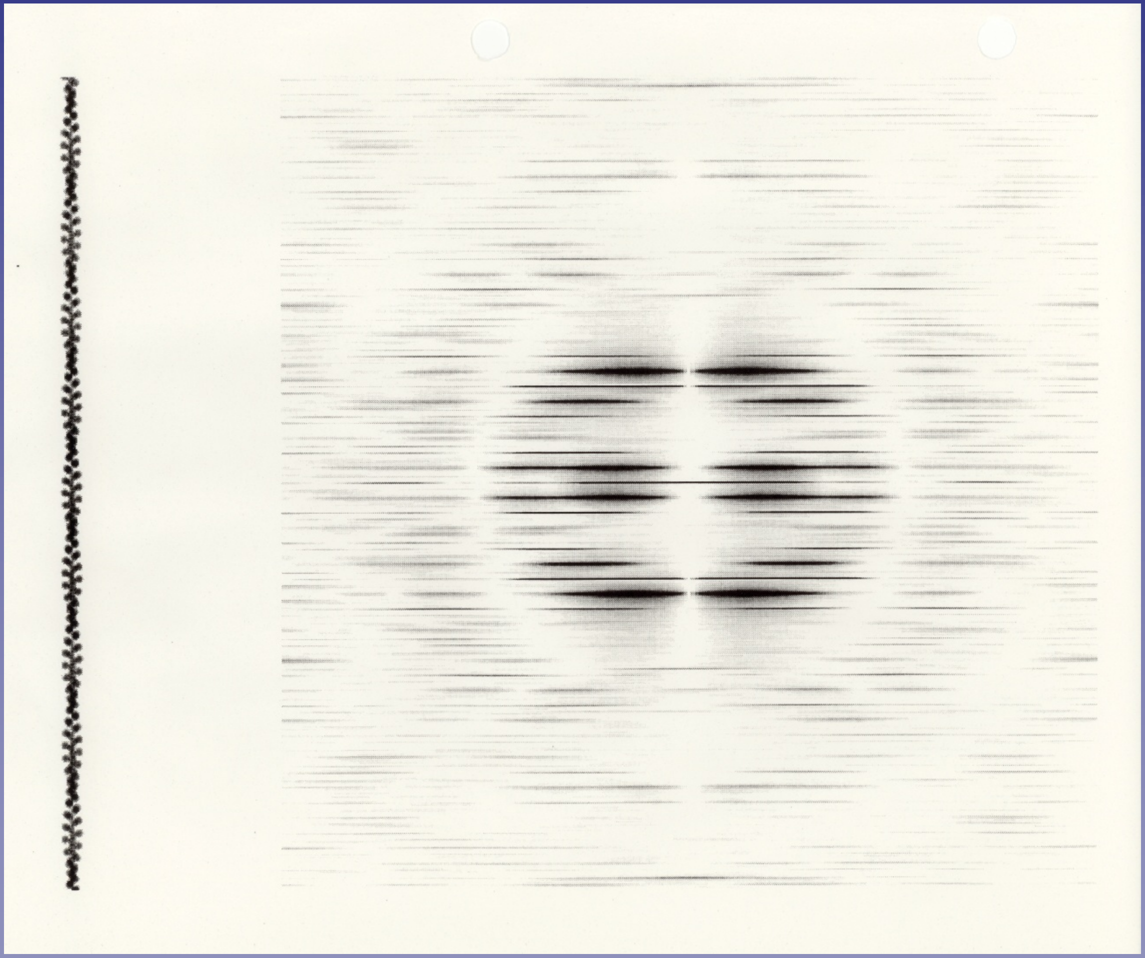
8 Å resolution microtubule map

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

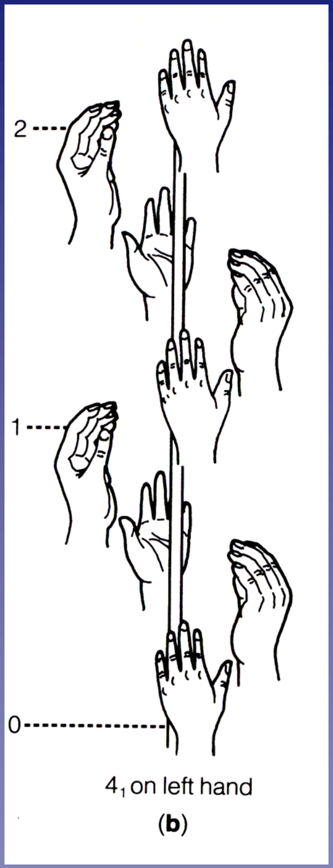
A first requirement for 3D reconstruction of a helical specimen, regardless of the method to be used (Fourier/Bessel or real space/single particle) is a good estimate of the helical parameters of the specimen:

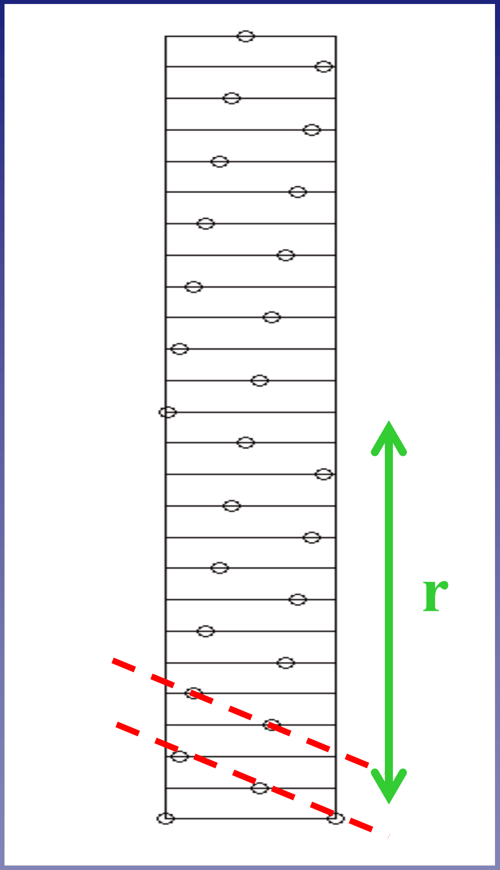
- Rise per repeating unit (h).
- Azimuthal rotation per repeating unit (Φ).

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

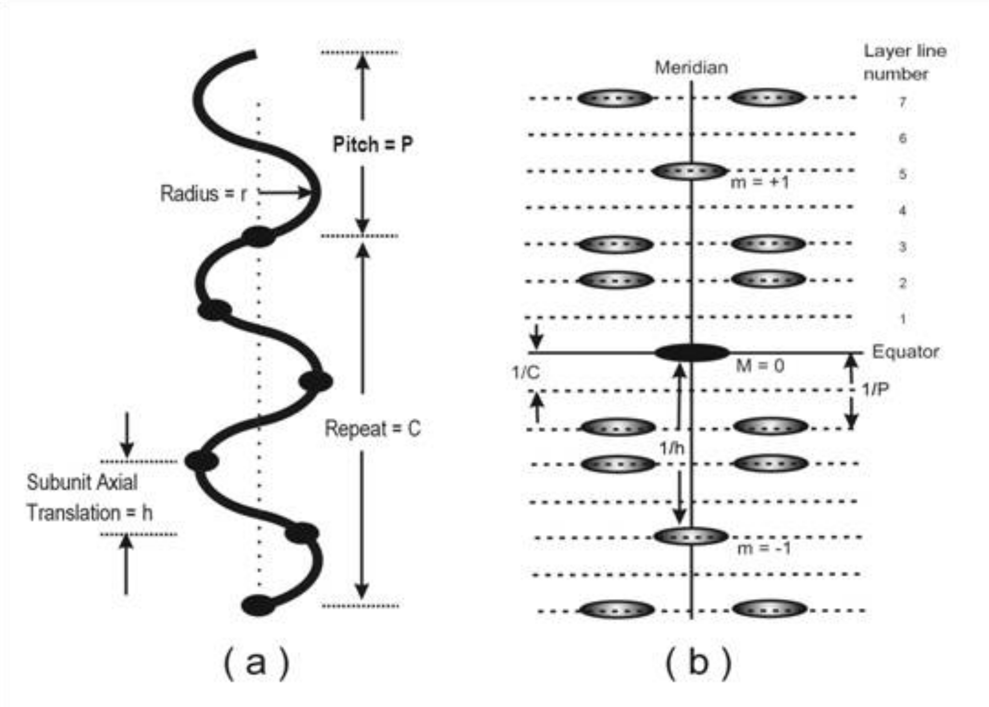
$$l = tn + um$$

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***

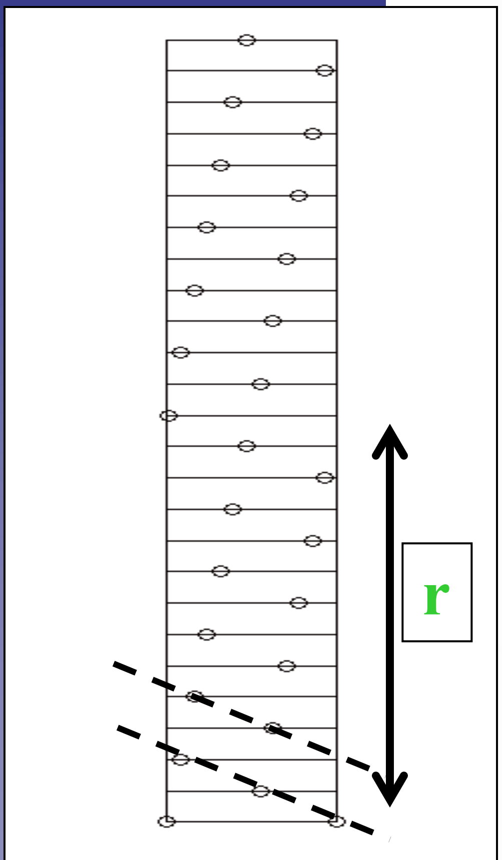
$$Z = n(\Omega/360^0)\ /h + m\ /h$$

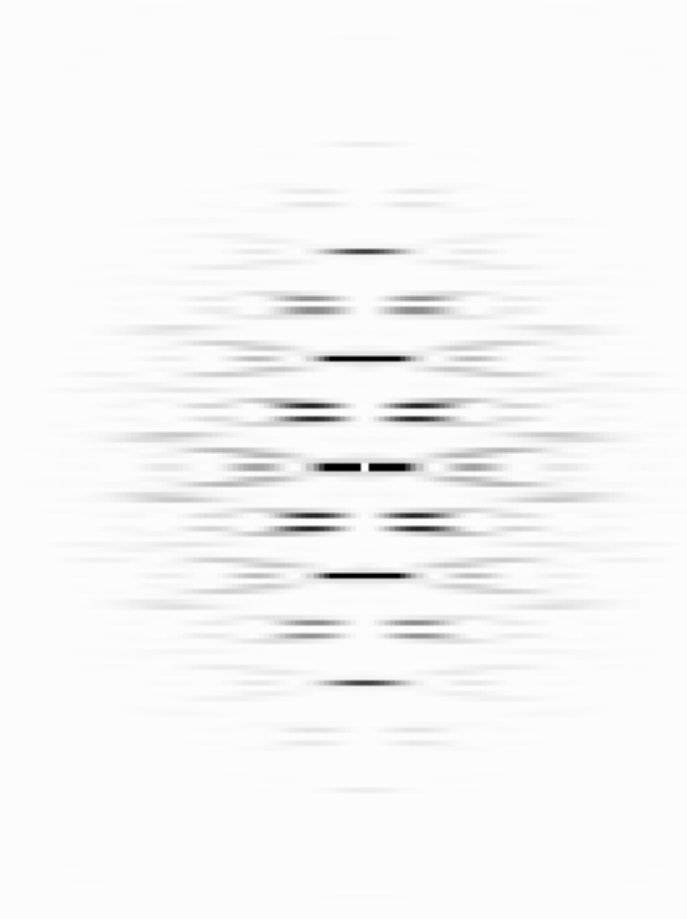
Z: LL reciprocal spacing
Ω: twist angle.
h: rise distance.



Selection Rule Example

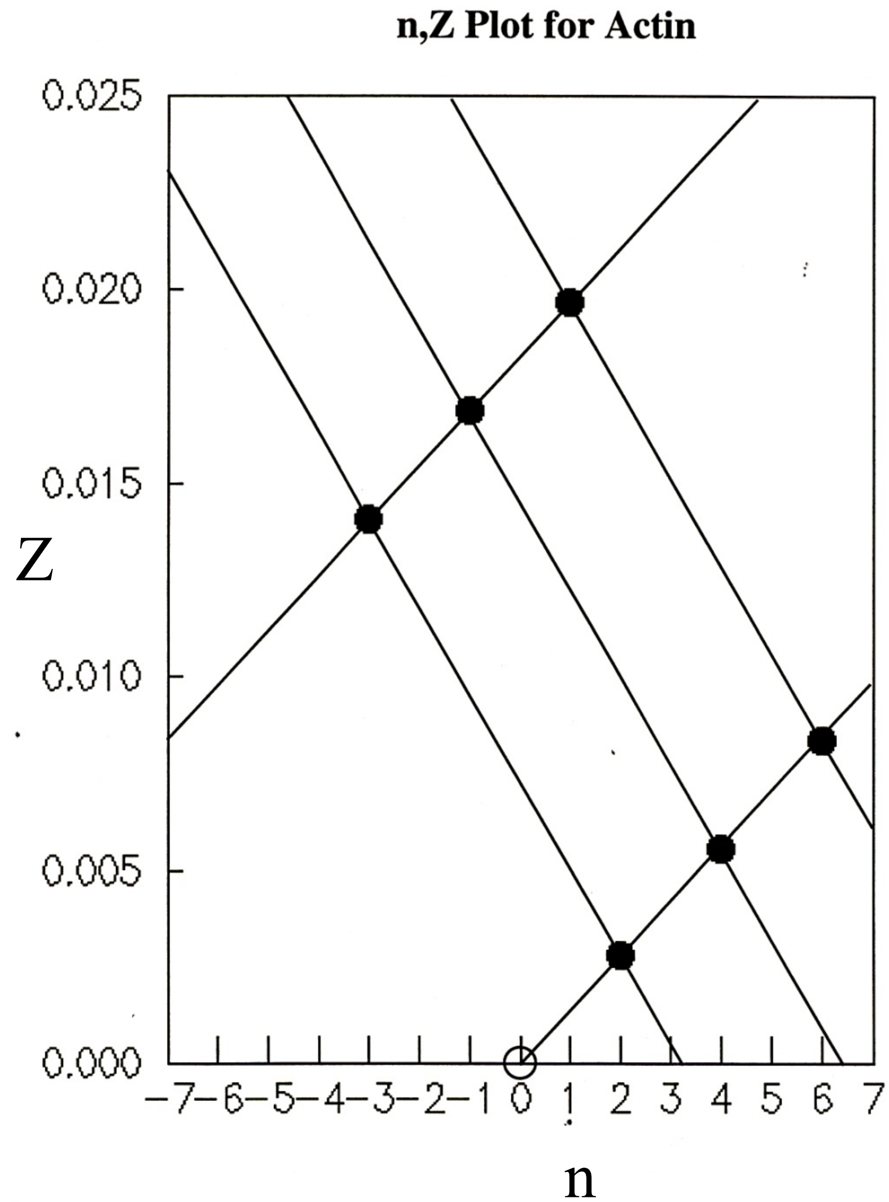
$$l = tn + um$$





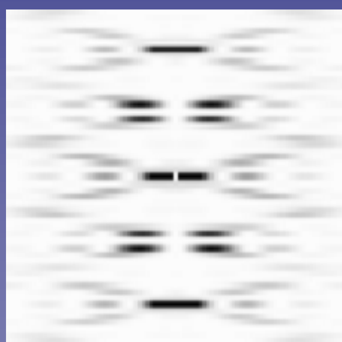
l	n ($\|n\|<=10$)
0	0
1	2
2	4, -9
3	6
4	-5, 8
5	-3, 10
6	-1
7	1
8	3, -10
9	5, -8
10	-6, 7
11	-4, 9
12	-2
13	0

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



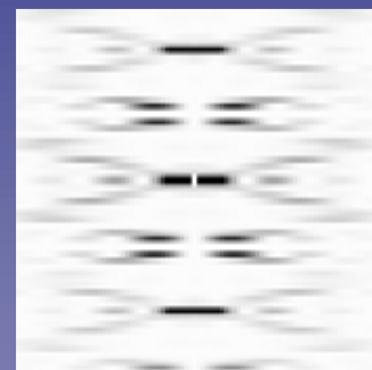
n,Z Plot for Actin
0.025
0.020
0.015
0.010
0.005
0.000
Z
−7 −6 −5 −4 −3 −2 −1 0 1 2 3 4 5 6 7
n

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



l	*n*(|*n*|<7)
0	0
1	2
2	4
3	6
4	-5
5	-3
6	-1
7	1
8	3
9	5
10	-6
11	-4,
12	-2
13	0

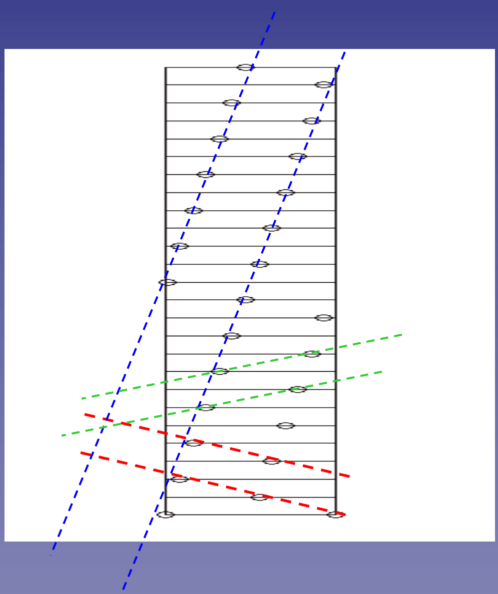
13 units in 6 Turns (LH)

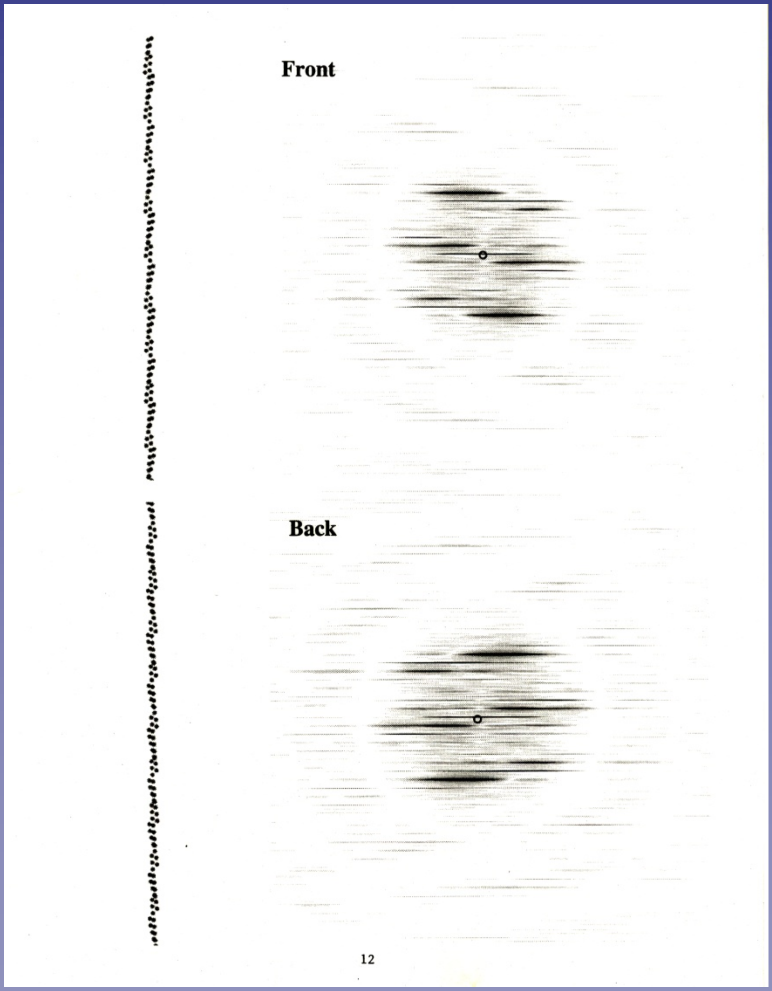


l	*n*(|*n*|<7)
0	0
4	2
8	4
12	6
17	-5
21	-3
25	-1
29	1
33	3
37	5
42	-6
46	-4
50	-2
54	0

54 units in 25 Turns (LH)

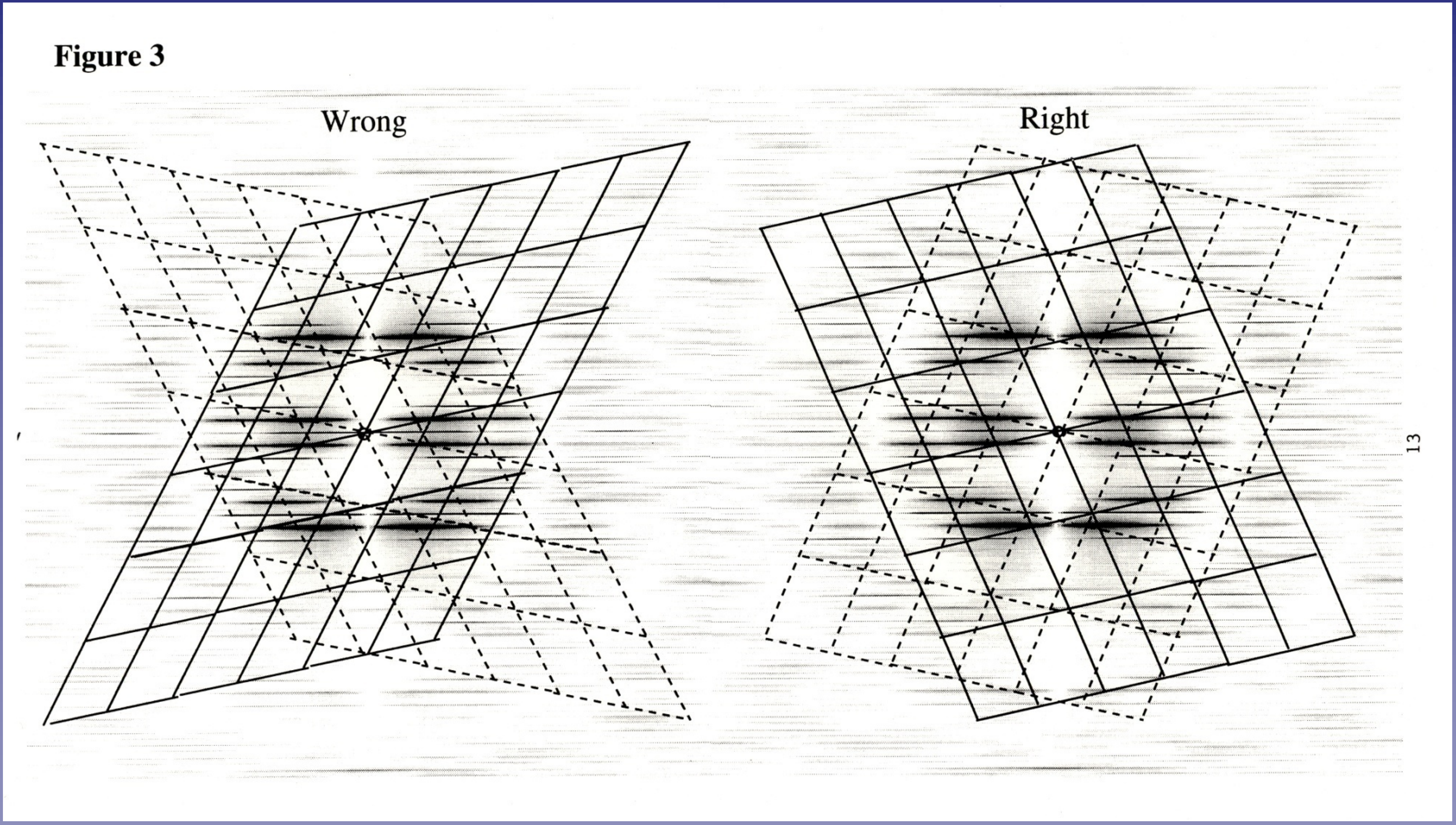
The Diffraction Pattern of a Helix Has Reflections From Planes in the Front and Back of the Helix



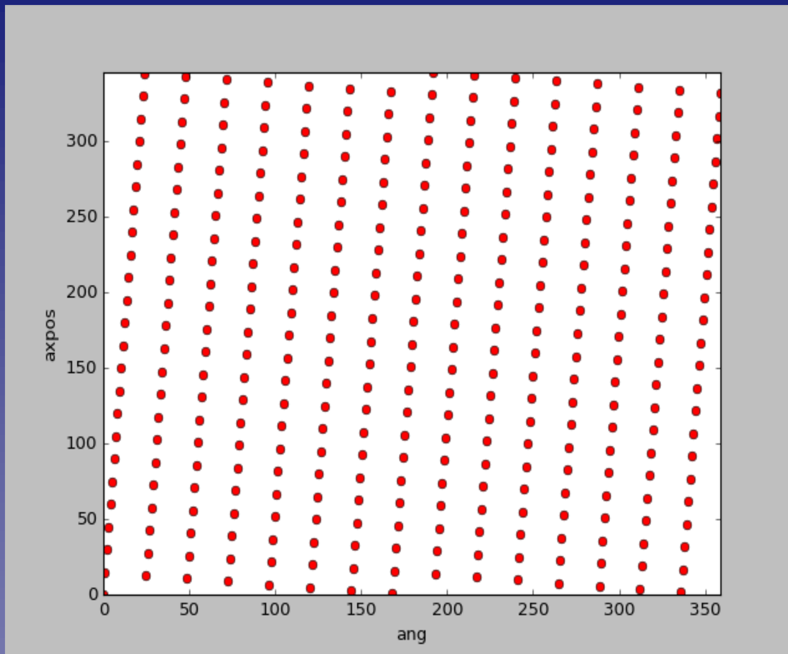


Different from the case of the transform of a 2D lattice where reflections from a set of planes form a spot in Fourier space. In the case of a helix the reflections are continuous Bessel function along “layer” lines.

Drawing the Reciprocal Lattice



Example 2

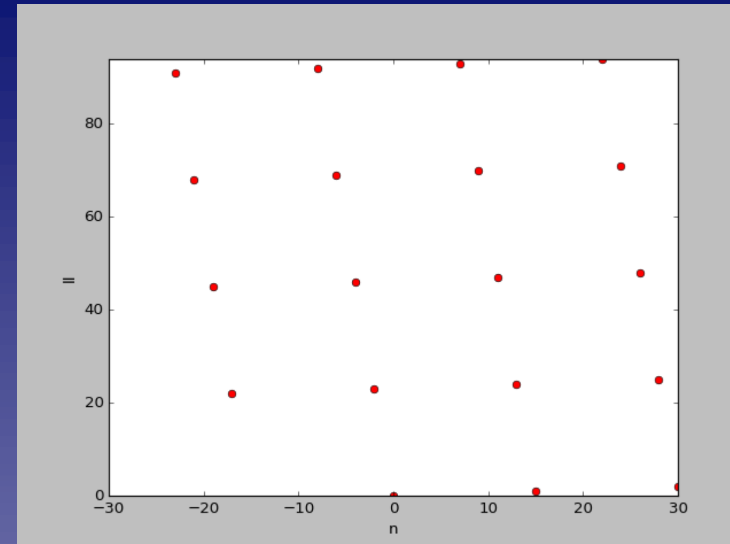


Helical Lattice

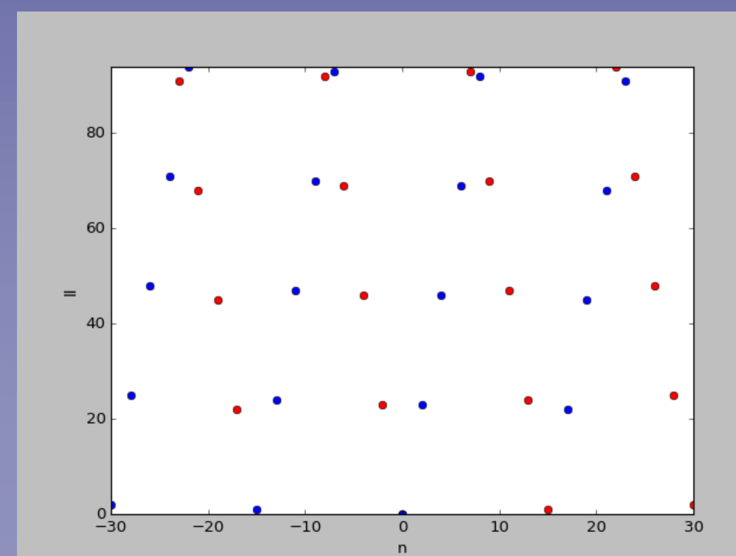
SR: $l = 162 * n + 347 * m$

Φ : 168.07

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 vs. n plot



Clues to trace the Reciprocal Helical Lattice:

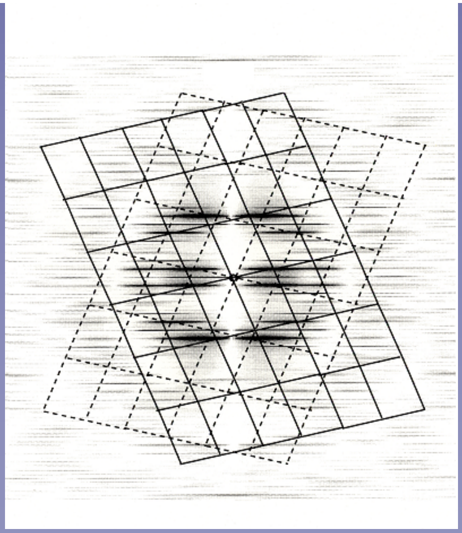
-The dimension of the unit vectors should be approximately equal to the inverse of the subunits dimensions. $d=1.34(m)^{1/3}$ (d in Å, m in Daltons) (e.g. actin dimensions ~ 5 nm)

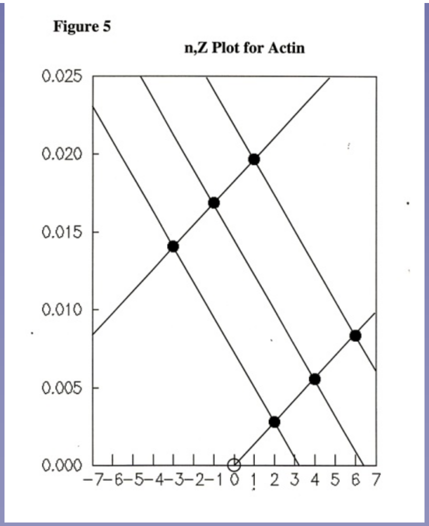
- Approximate value of $|n|$ for each layer line is: $|n|+2 = 2\pi Rr$.
r: Helix radius, R: Reciprocal of layer line peak position to the meridian.

- 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^o.

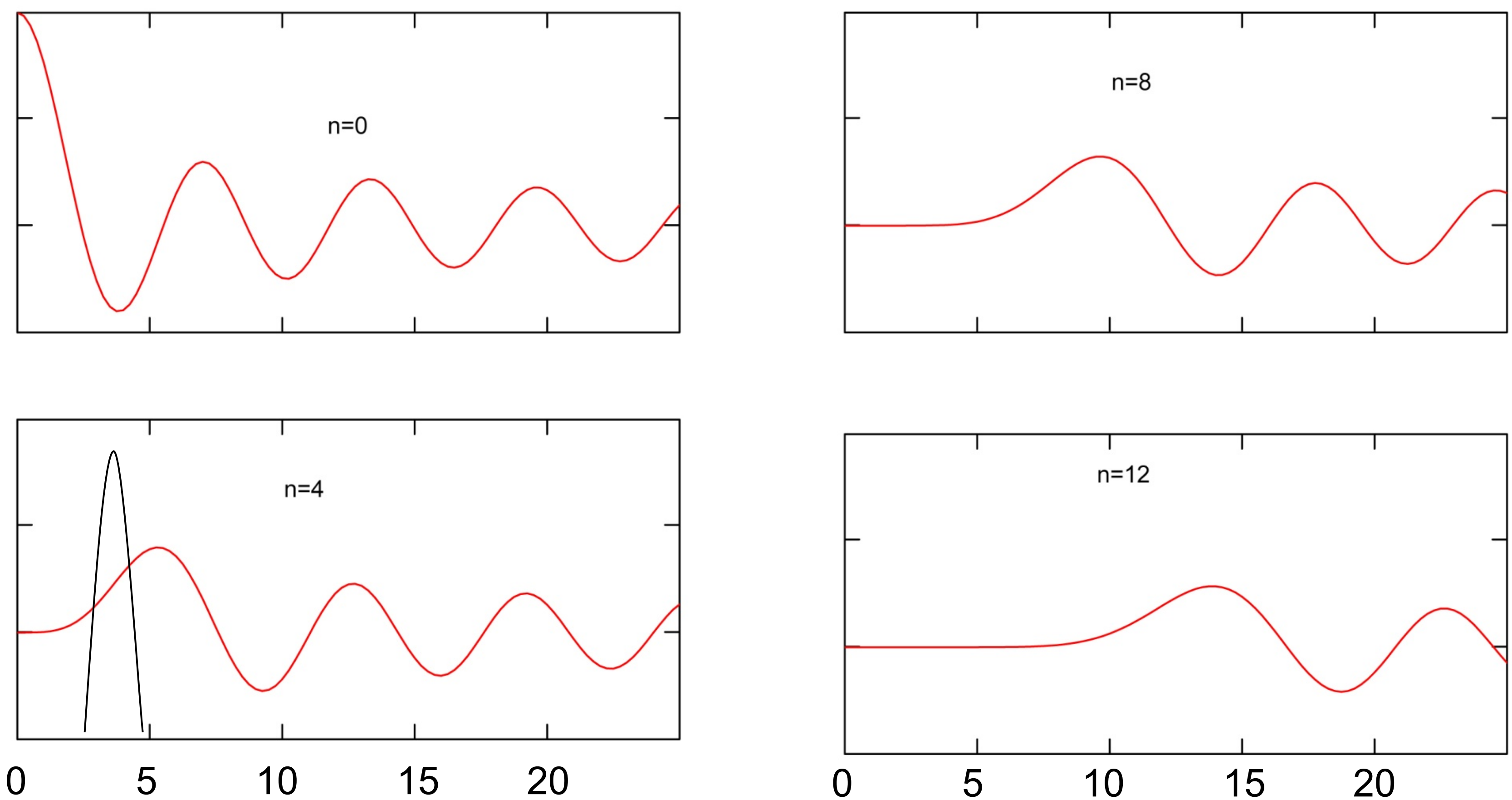
- Determine hand of helical paths (sign of n). Shadow or tilt specimens.

- Draw n,Z plot.



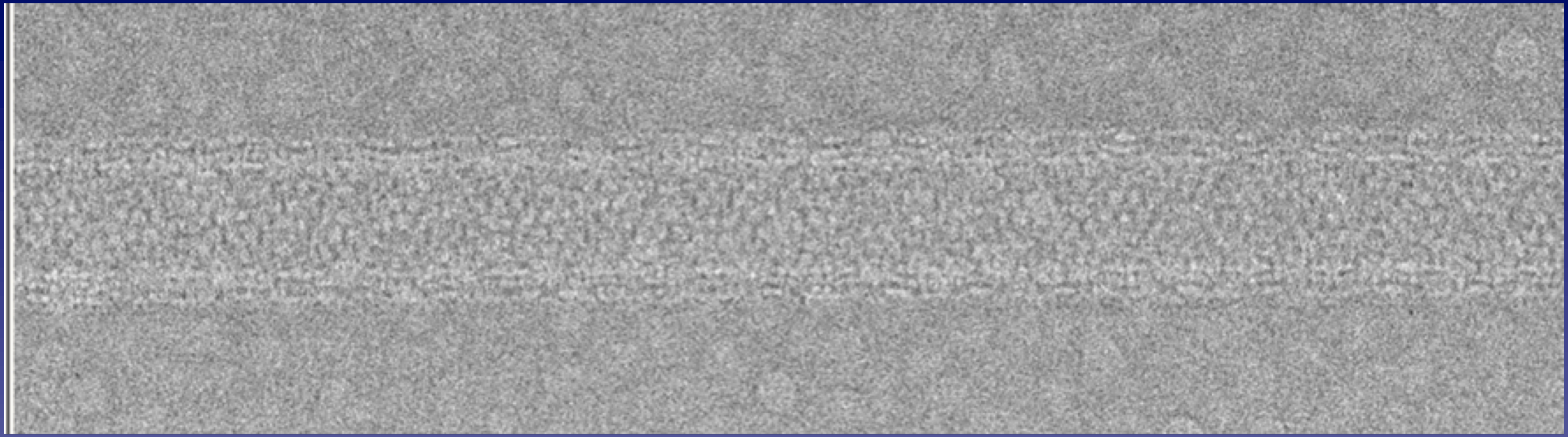
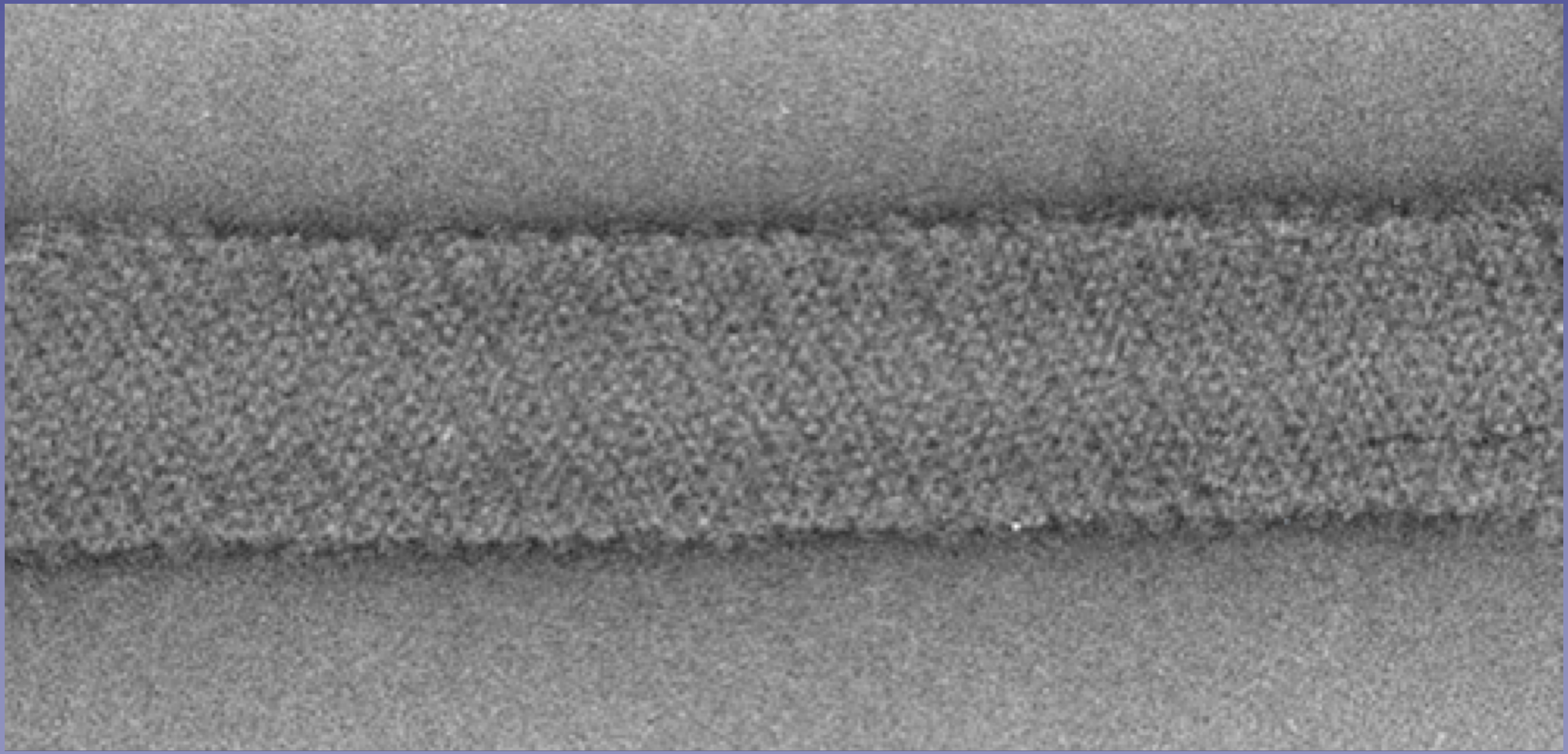


$J_n(2\pi Rr)$, 1st max at $R \approx (|n|+2)/(2\pi r)$



Use radial position to determine Bessel order (approximation)

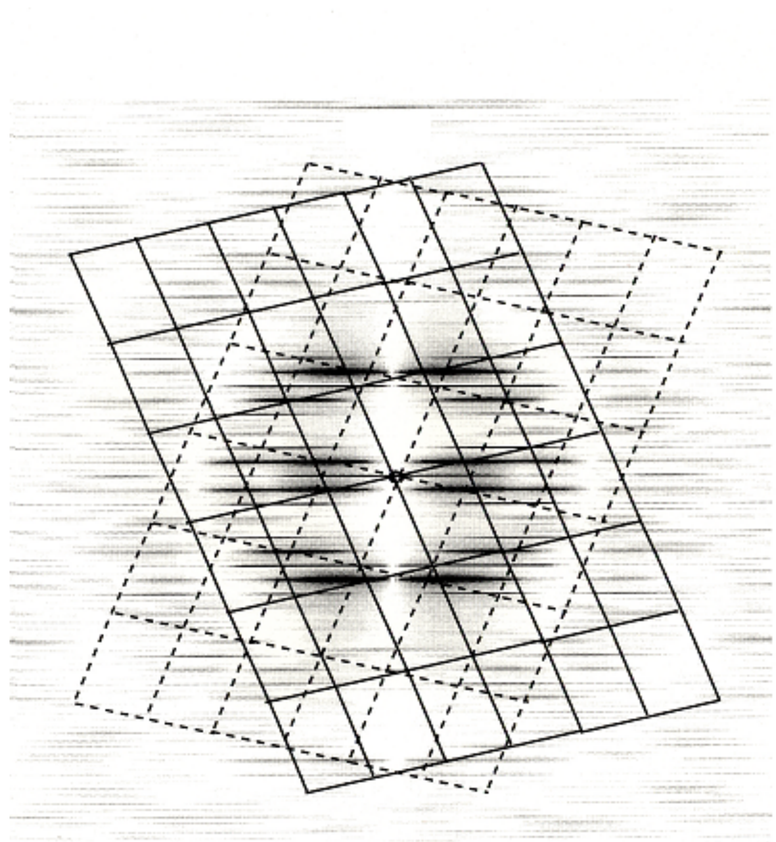
- radius hard to measure with defocus fringes
- different radii of contrast for different helical families
- particle may be flattened

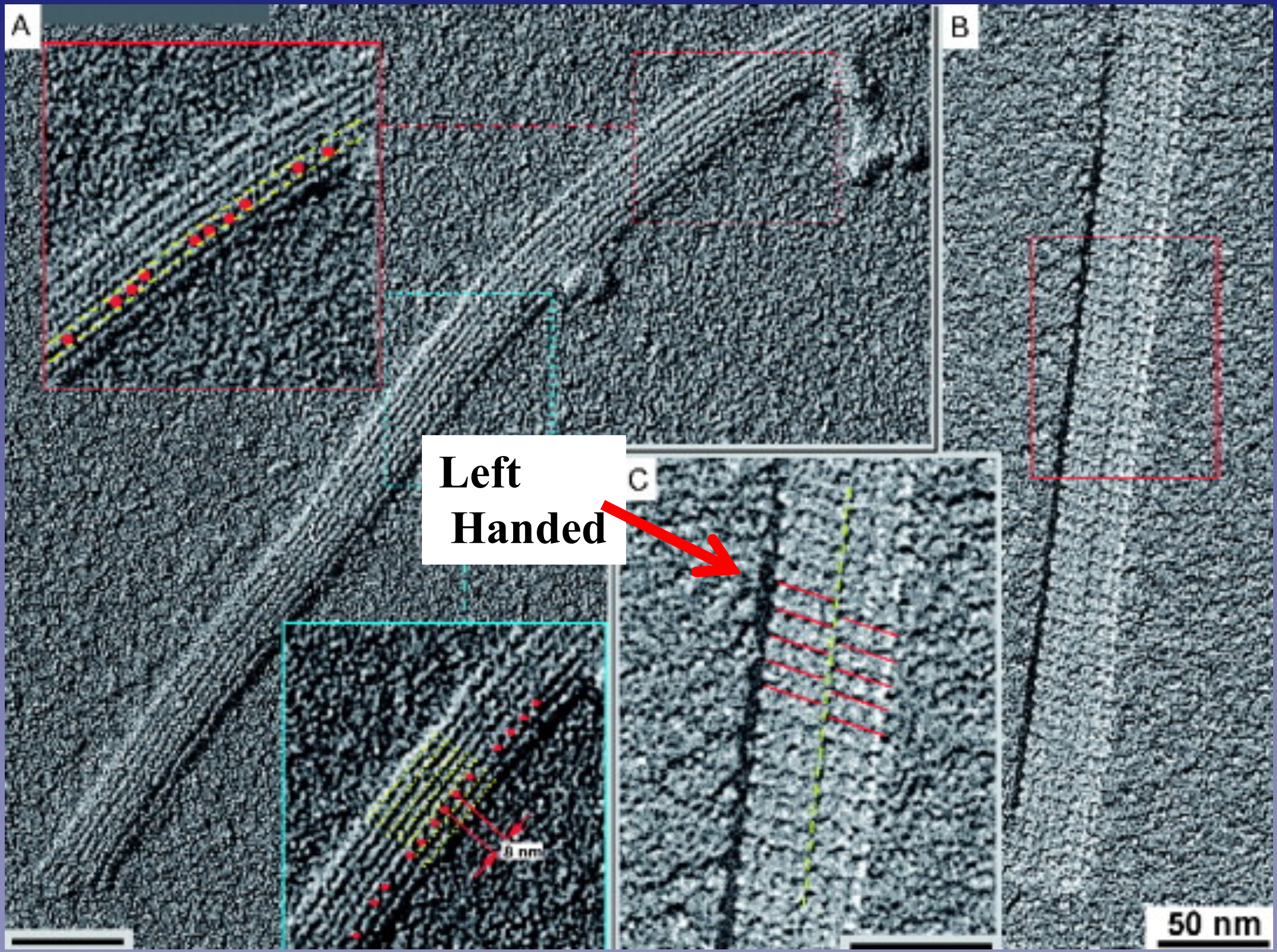
Determining the hand of the helical paths (bessel orders)

Convention: Right handed $n > 0$

Left handed $n < 0$



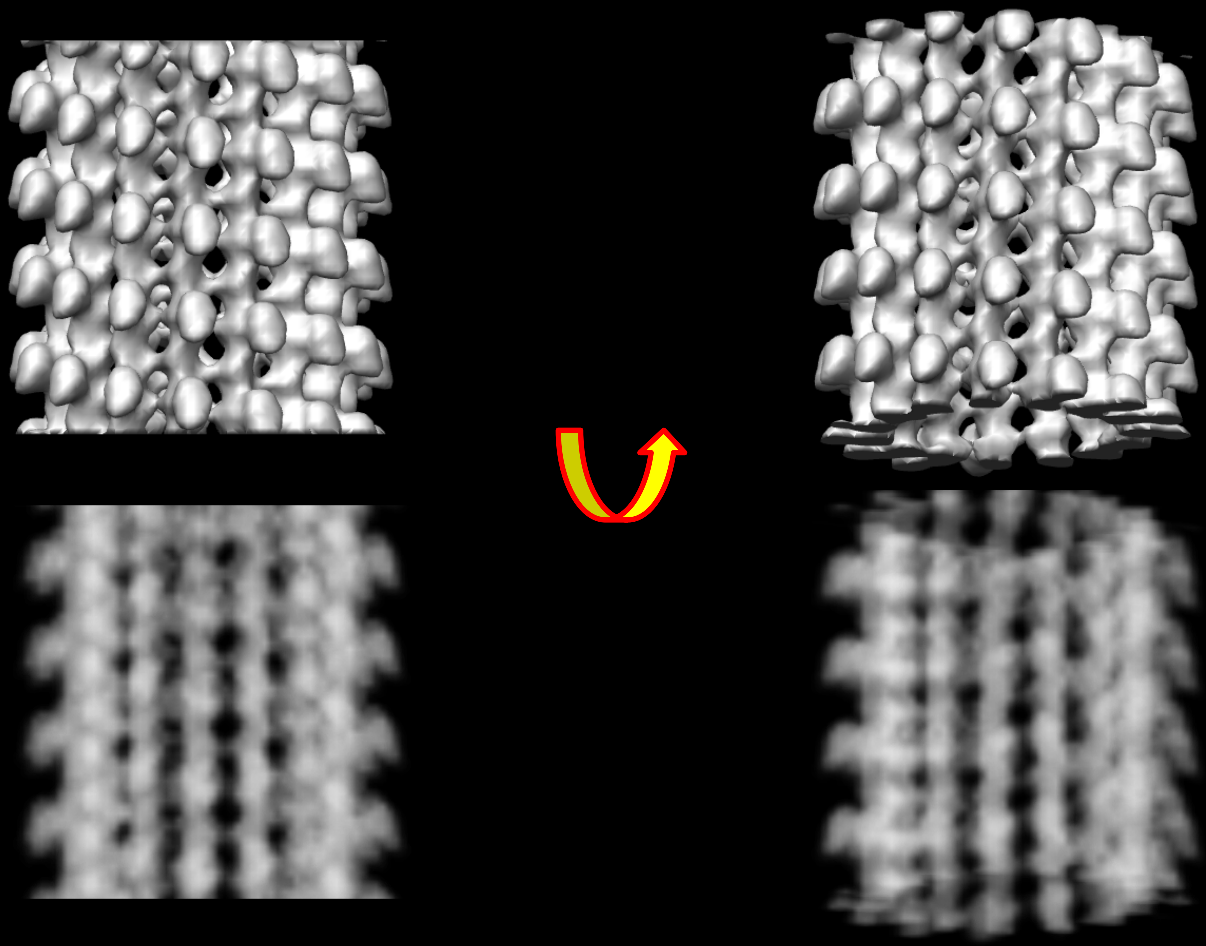
Determining the hand of the helical path by EM metal shadowing



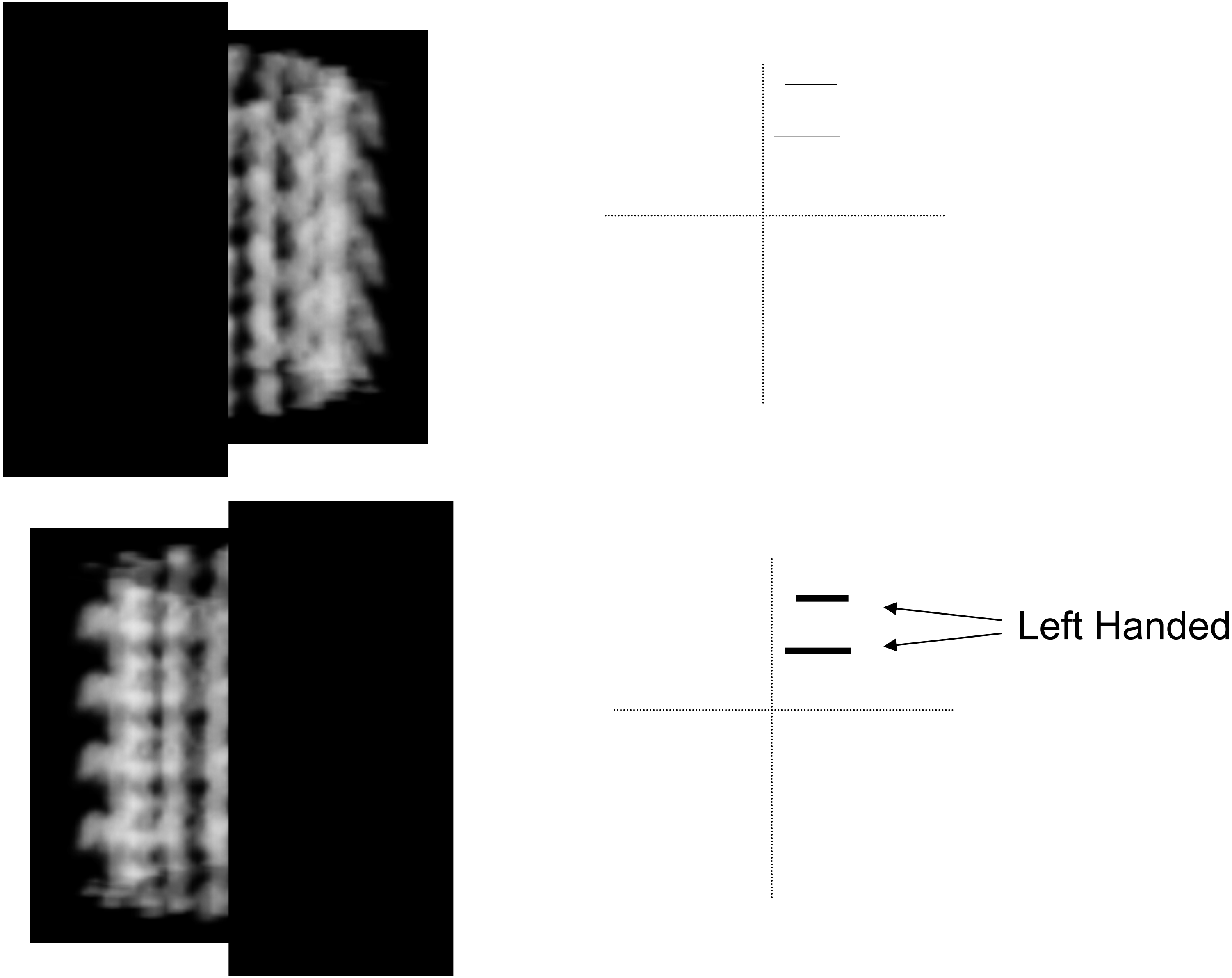
From: Hoenger & Gross J.Struct.Biol. 84: 425 (2008)

Determining the hand of the helical path by tilting specimen

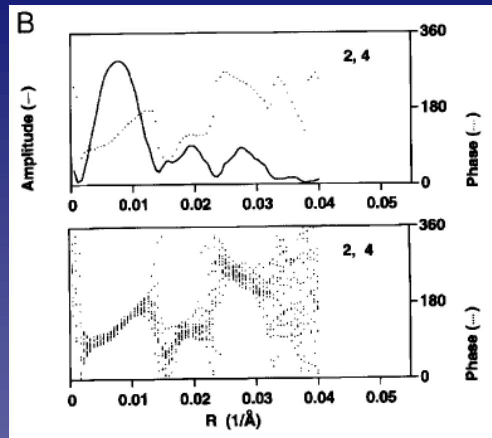
Left handed

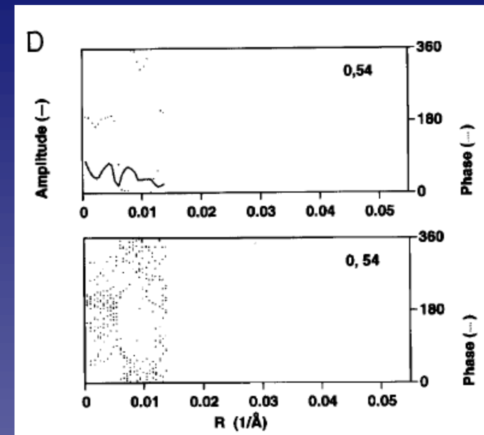


Serrated Pattern on Left

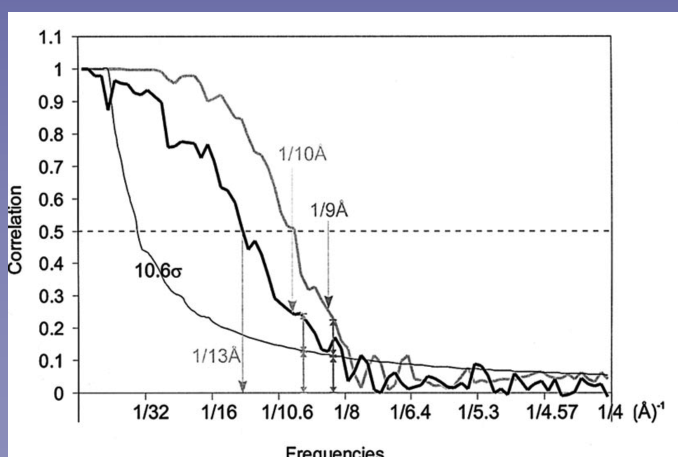

Left Handed

Resolution Criteria





Phase Residuals Along layer lines



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

Fourier Shell Correlation

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)
- EMGlue (Sosa's lab)
  - Spider
  - IHRSR
  - Frealign
  - Relion
  - EMAN

3D Helical Reconstruction Workflow

-Pick filaments & mark axis. (EMAN Boxer, Others)

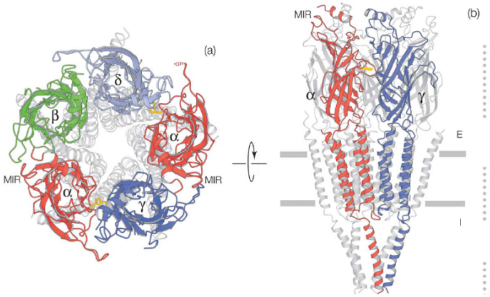
- Preliminary low resolution model (Custom)
- Classify filaments. (Custom, SPIDER, EMAN)
- Determine helical symmetry (ϕ, h) (Custom)

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

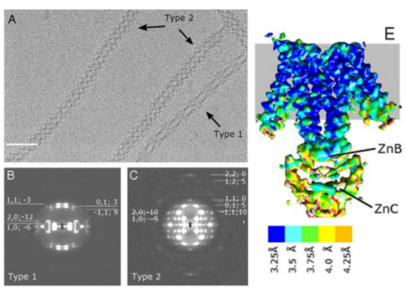
- Refine 3D map (IHRSR).
- Refine 3D map (FREALIGN)
- Refine 3D map (RELION)

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

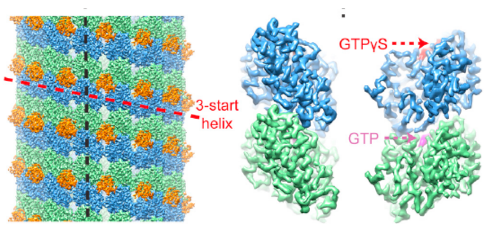
Examples of Helical Structures
@ <= 4 Å



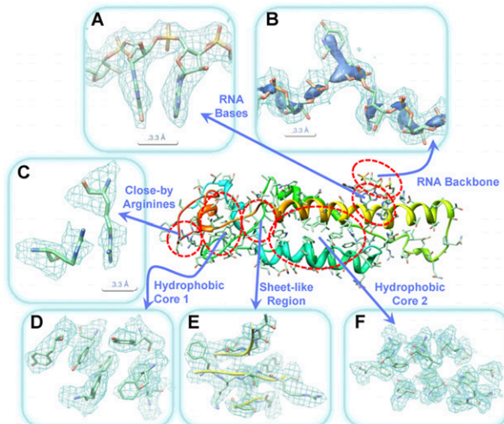
Ach Receptor (4Å)
Unwin N.(2005) JMB 346:976
(Fourier-Bessel Method)



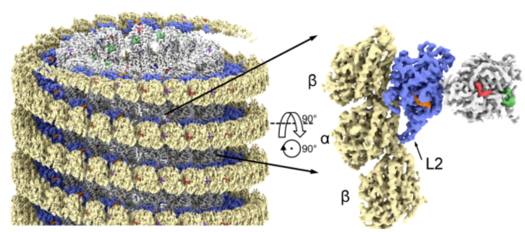
YiiP Zinc Transporter (4.1 Å)
Lopez-Redondo et al., (2018)
PNAS 115: 3042



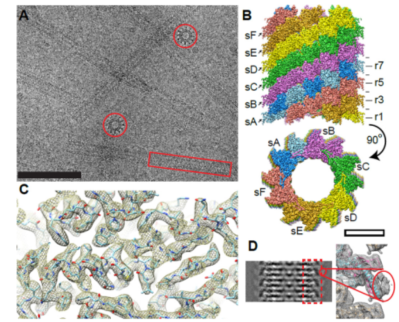
Microtubules (3.3 Å)
Zhang et al., (2015)
Cell 162: 849



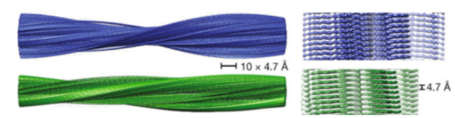
TMV (3.3 Å)
Ge & Zhou. (2011)
PNAS 108: 9637.



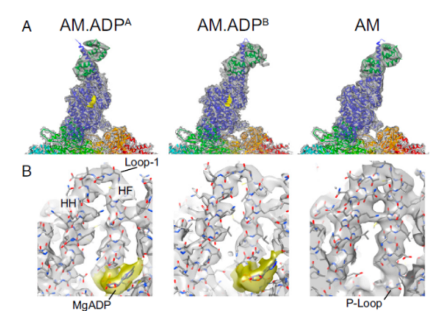
Kinesin-Microtubule complexes (3.5 Å)
Benoit et al., (2018) Nature Comm.



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

1. DeRosier DJ, Moore PB. Reconstruction of three-dimensional images from electron micrographs of structures with helical symmetry. *Journal of Molecular Biology* **52**, 355-369 (1970).

2. Bluemke DA, Carragher B, Potel MJ, Josephs R. Real-space reconstructions of nonideal helical particles. *Journal of Electron Microscopy Technique* **5**, 141-151 (1987).

3. Sosa H, Hoenger A, Milligan RA. Three Different Approaches for Calculating the Three-Dimensional Structure of Microtubules Decorated with Kinesin Motor Domains. *Journal of Structural Biology* **118**, 149-158 (1997).

4. Egelman EH. A robust algorithm for the reconstruction of helical filaments using single-particle methods. *Ultramicroscopy* **85**, 225-234 (2000).

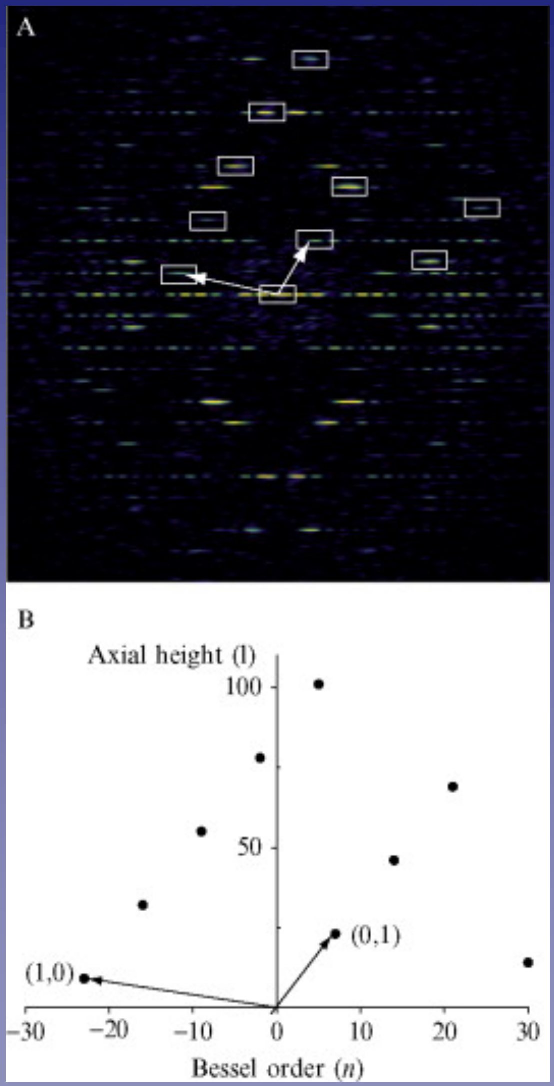
5. Sachse C, Chen JZ, Coureux P-D, Stroupe ME, Fändrich M, Grigorieff N. High-resolution Electron Microscopy of Helical Specimens: A Fresh Look at Tobacco Mosaic Virus. *Journal of Molecular Biology* **371**, 812-835 (2007).

6. Diaz R, Rice WJ, Stokes DL. Chapter Five - Fourier–Bessel Reconstruction of Helical Assemblies. In: *Methods in Enzymology* (ed^(eds Jensen GJ). Academic Press (2010).

7. Behrmann E, Tao G, Stokes DL, Egelman EH, Raunser S, Penczek PA. Real-space processing of helical filaments in SPARX. *Journal of Structural Biology* **177**, 302-313 (2012).

8. He S, Scheres SHW. Helical reconstruction in RELION. *Journal of Structural Biology* **198**, 163-176 (2017).

Indexing the diffraction Pattern



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