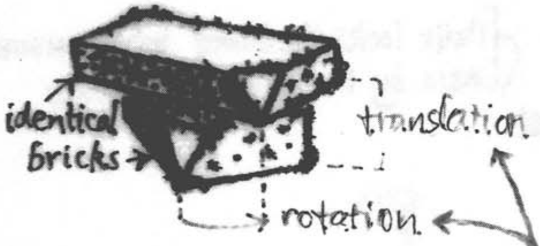
Michael Moody

Helical Reconstruction

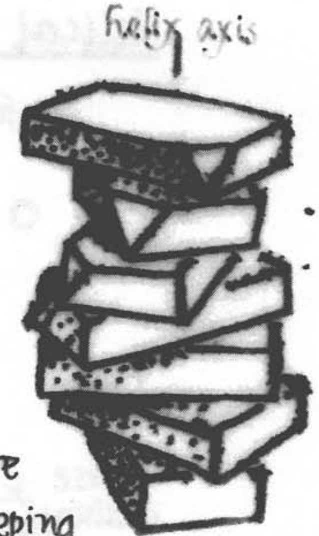
02/11/03

Types of helix

▷ Simplest



Starting configuration fixes screw displacement



Add more bricks, keeping same relation between all neighbours.

This helix is polar — different when upside-down (triangles point upward)

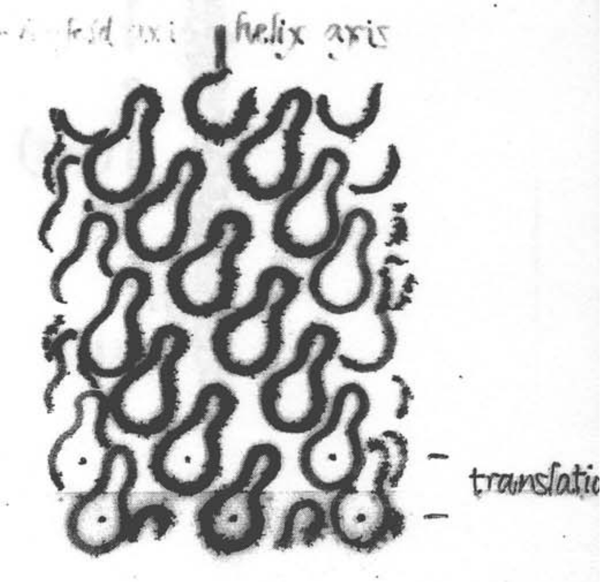
(These can be combined)

▷ Non-polar

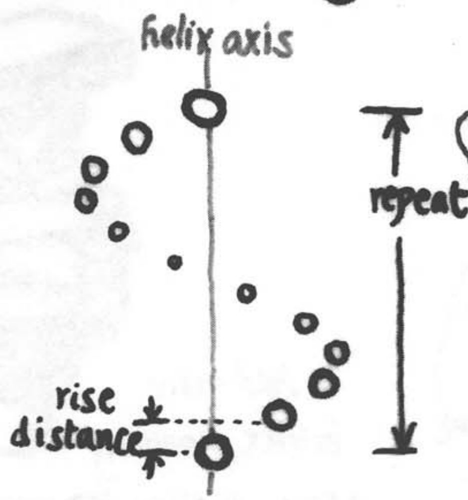
= same when turned upside-down.

▷ Rotation axis (parallel to helix axis)

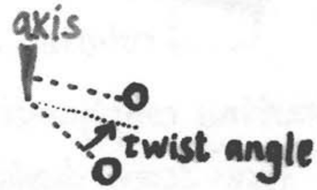
(Non-polar — different when upside down)



Helical Symmetry



[Helix looks the same when moved along axis by the repeat distance.]

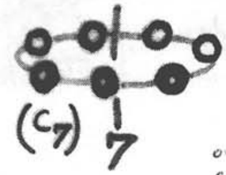


11 units/repeat, so 11(twist angle) = 360°
and 11(rise distance) = repeat

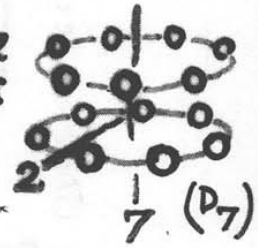
{rise distance, twist angle} fundamental quantities; repeat may be difficult to measure (& may not exist).

Pair of numbers form a screw

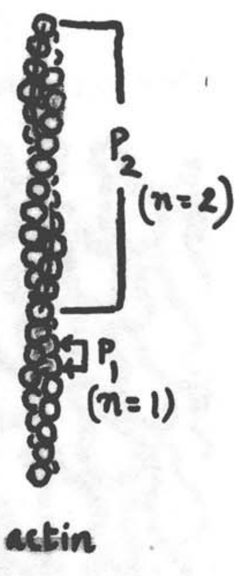
Besides screw symmetry, a helix can have cyclic or dihedral symmetry. I.e. units are rings



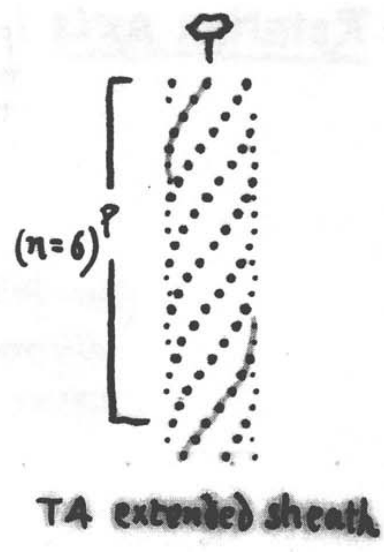
or double rings



only symmetries compatible with helix



actin

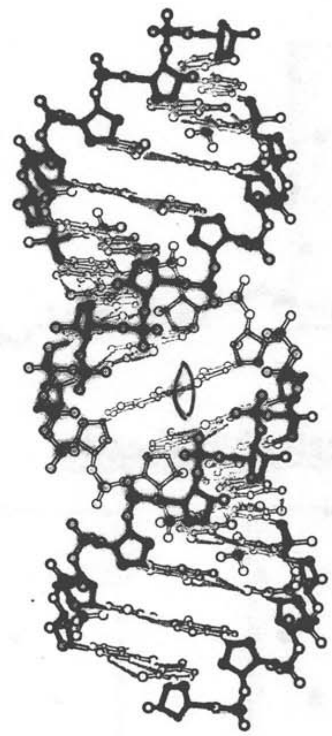


T4 extended sheath

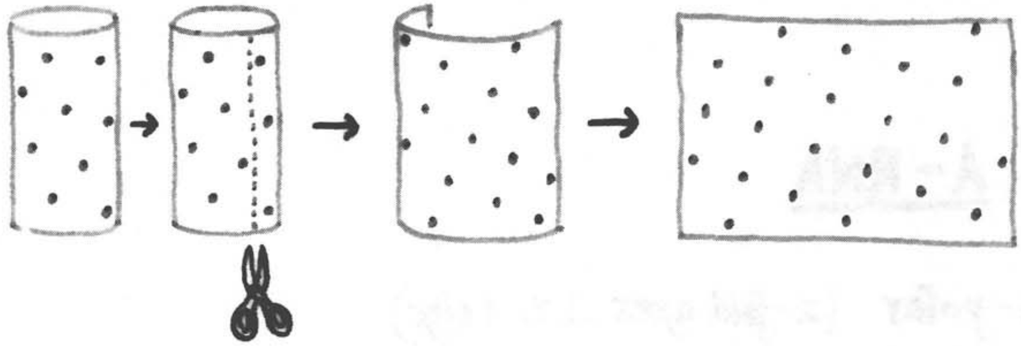
A-RNA

Non-polar (2-fold axes ⊥r. helix)
except for bases.

dihedral symmetry

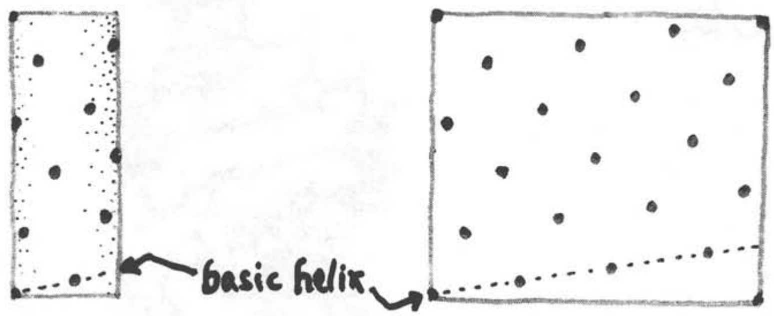


The Radial Projection

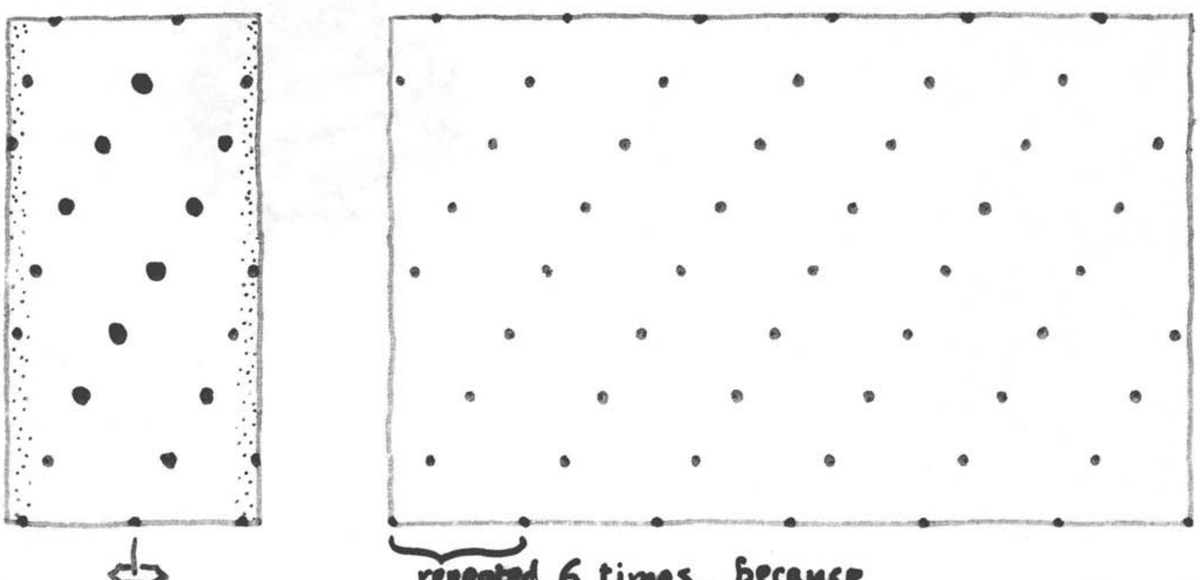


Examples:

α-helix

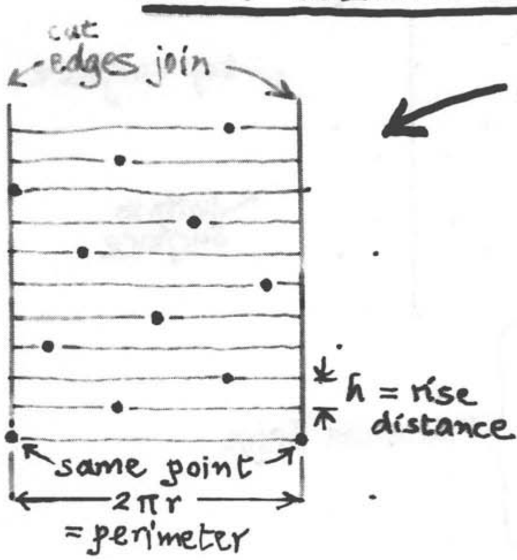


T4 extended sheath



repeated 6 times, because sheath has 6-fold axis (C_6)

FLAT HELICAL LATTICE

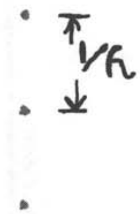


Lattice is unchanged if:

① ×

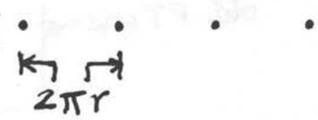
so F.T. is unchanged if

☆



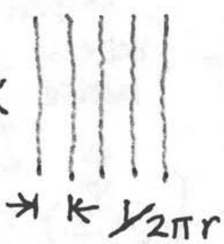
so F.T. lattice repeats when $Z \to Z + \frac{1}{h}$

② ☆



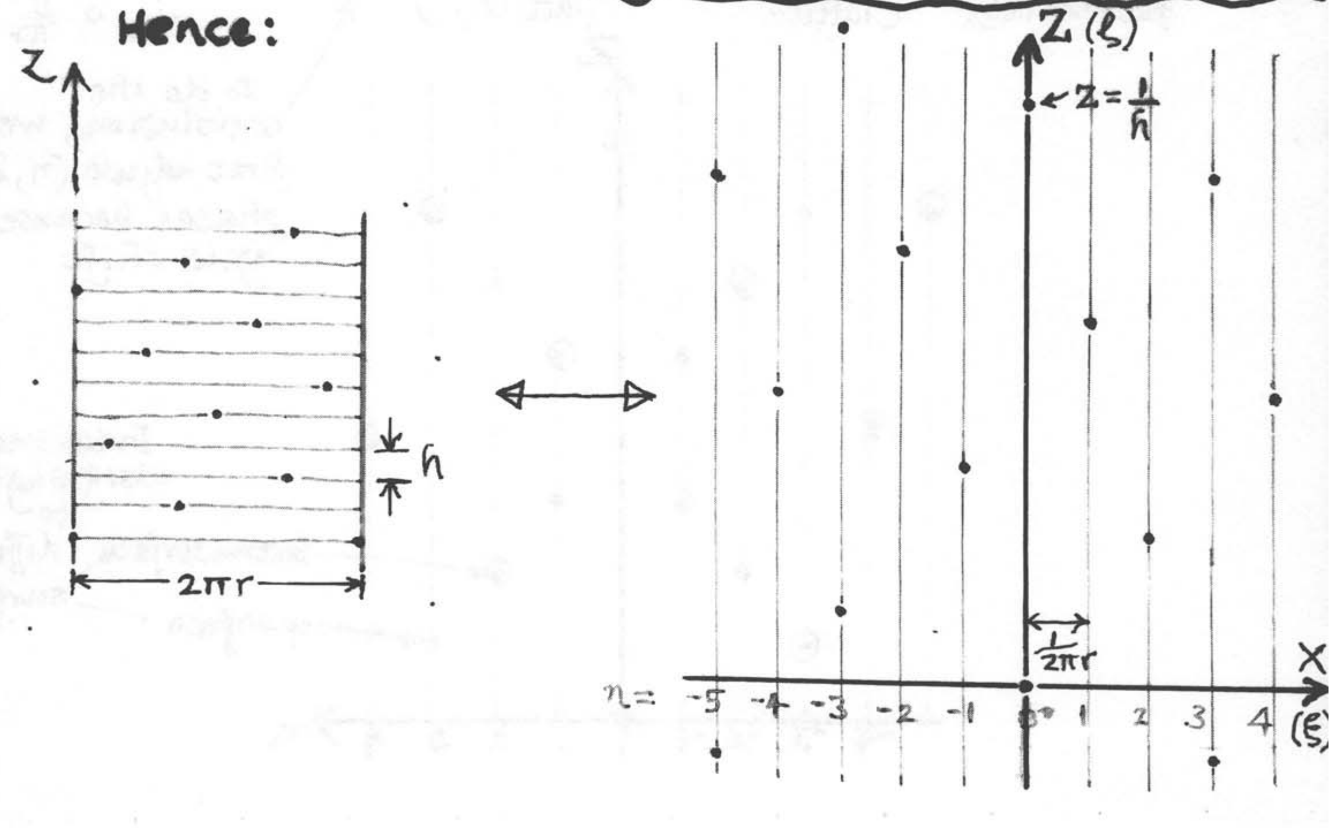
so F.T. is unchanged if

×

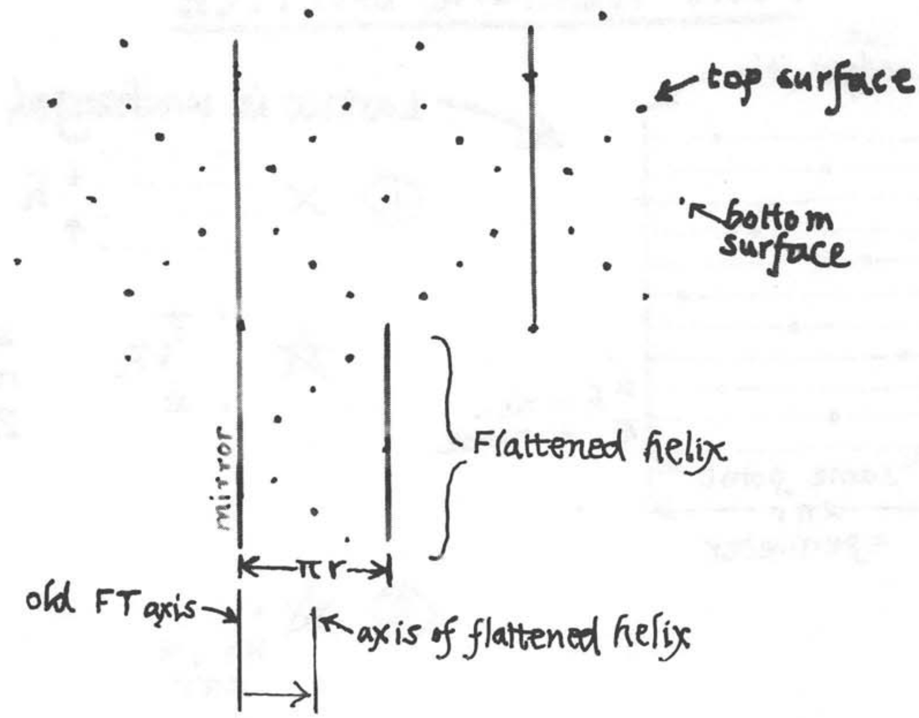


so F.T. lattice points are confined to $X = \pm \frac{n}{2\pi r}$

Hence:



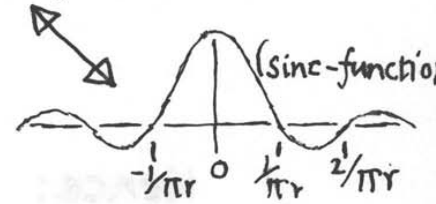
FLATTENED HELIX



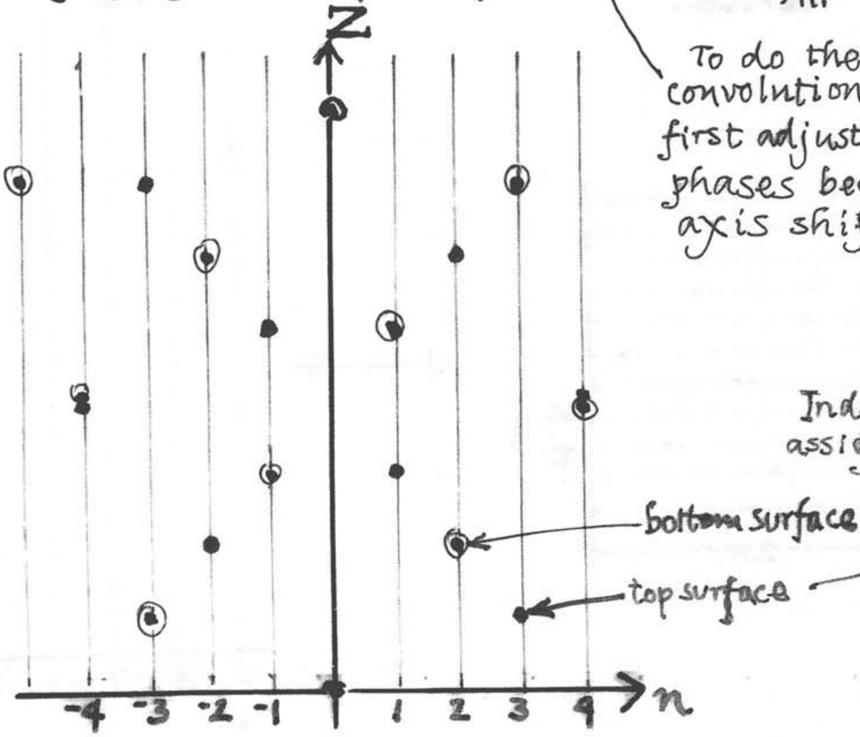
$$\text{Flattened helix} = \left\{ \begin{matrix}\text{shifted} \\ \text{Helical lattice}\end{matrix} + \text{mirror} \begin{pmatrix}\text{shifted} \\ \text{Helical lattice}\end{pmatrix} \right\} \times \text{slit, width} = \pi r$$

$$\Updownarrow$$

$$\text{F.T. of flattened helix} = \left\{ \begin{matrix}(n, Z) \\ \text{lattice}\end{matrix} + \text{mirror} \begin{pmatrix}(n, Z) \\ \text{lattice}\end{pmatrix} \right\} \star$$

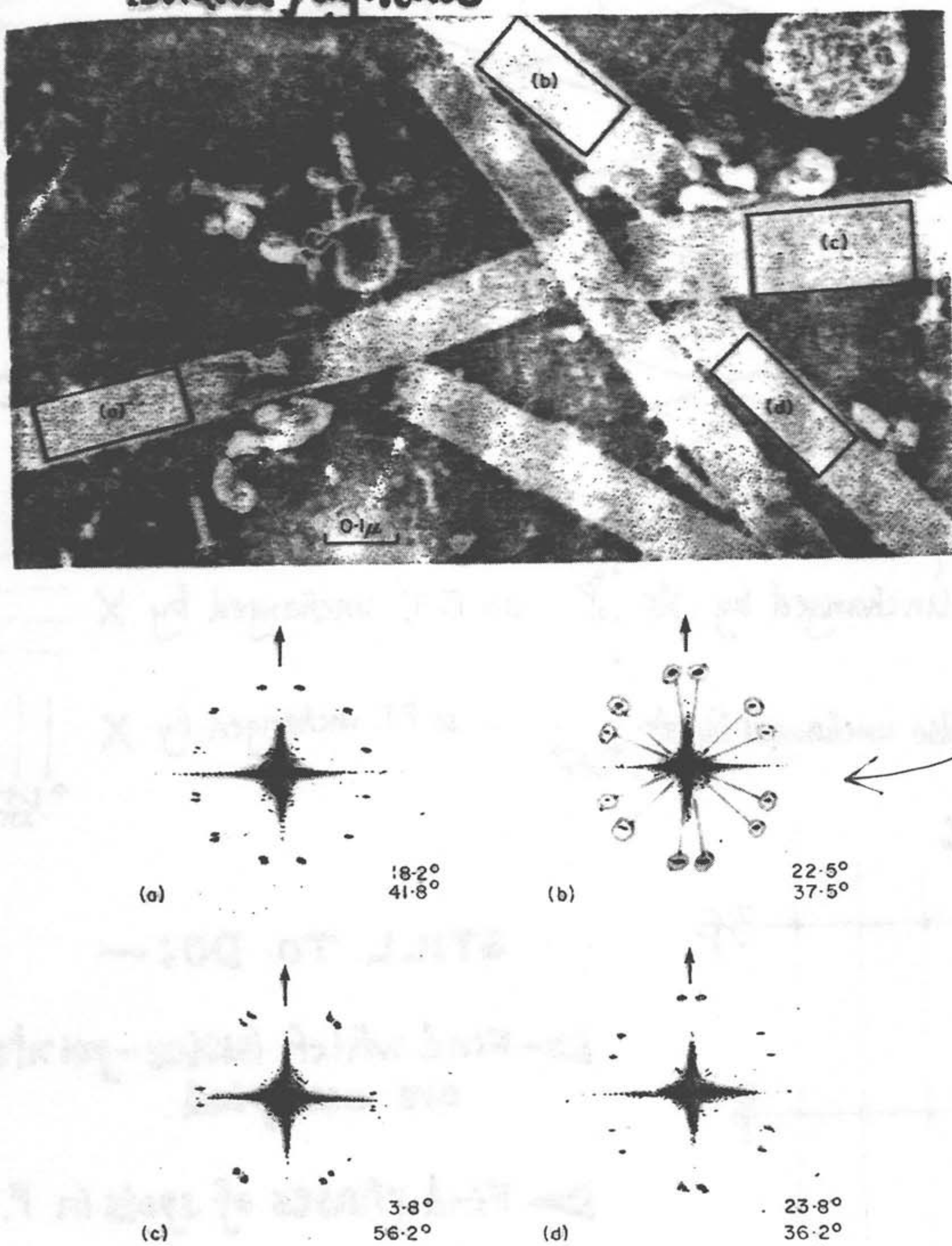


To do the convolution, we must first adjust (n, Z) phases because of axis shift



Indexing = assigning spots to different surfaces

Isolated polyheads

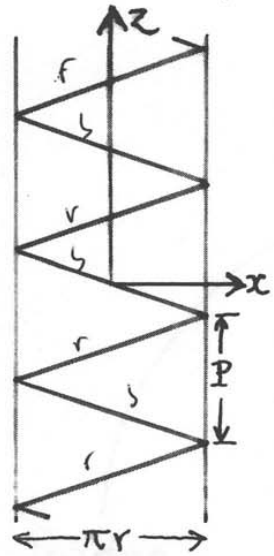


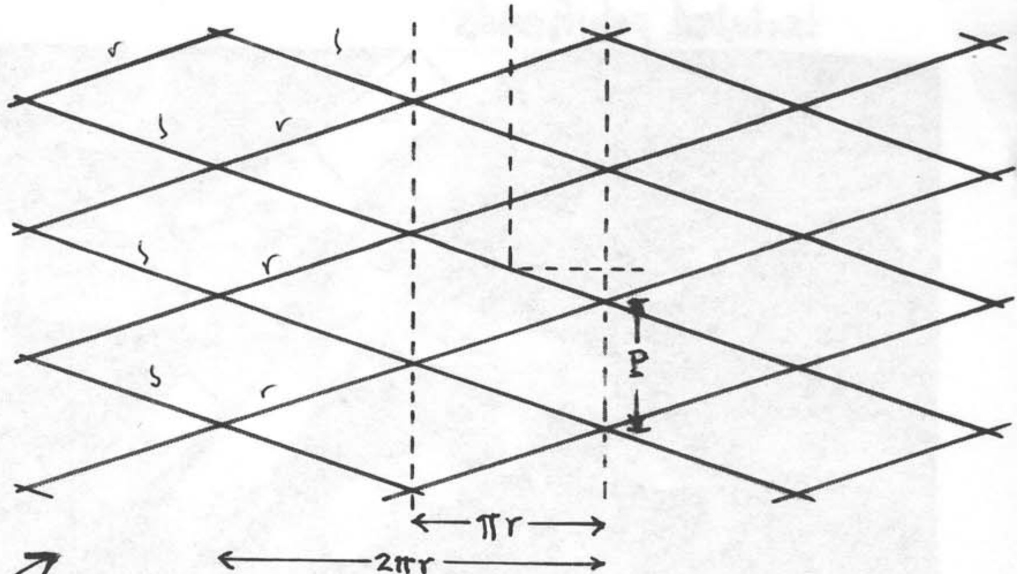
optical diffraction

Plate III. Negatively stained tubes and their optical diffraction patterns.

Micrographs of a preparation made from a formaldehyde-fixed lysate of mutant 22 (*am* B270), negatively stained with 1% sodium phosphotungstate. The tubular capsoids are completely flattened. Optical diffraction patterns from four of them are shown in (a) to (d). The observed pattern of twelve spots results from the superposition of two six-spot diffraction patterns, one coming from the upper layer of the flattened polyhead, and the other from the lower layer. The lattice lines ($\bar{1}2$), ($1\bar{1}$), and (21) are responsible for the most intense spots of the pattern (see Fig. 3(b) and Plate VI). The pitch, α, determined from these diffraction patterns as described in the text, has two possible values, $30° \pm \beta$, both of which are indicated in each diffraction pattern. Unambiguous values of α are obtained from diffraction patterns of shadowed polyheads (see Plate IX) to which only the upper layer contributes. The arrow in the diffraction patterns indicates the particle axis.

FLATTENED HELICAL LINE (p.1)

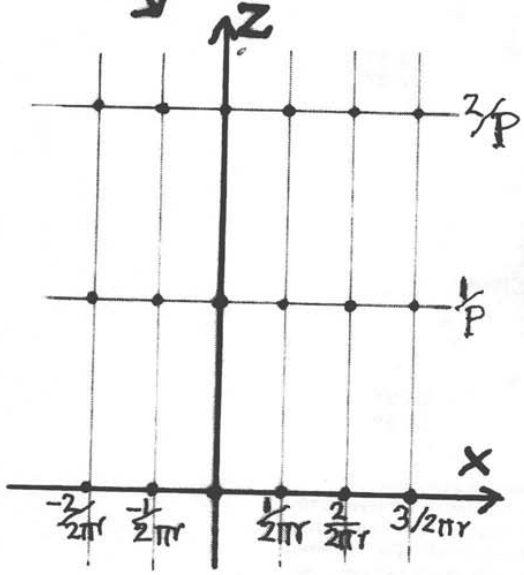




F.T. = ?

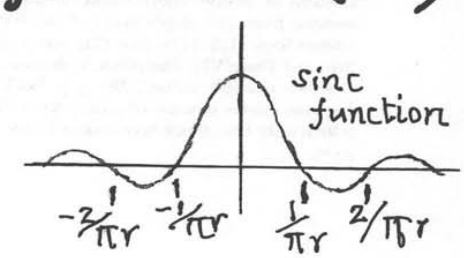
So F.T. exists only on lattice points

- Unchanged by ☆ (P) so F.T. unchanged by X
- Also unchanged by ☆ (2πr) so F.T. unchanged by X

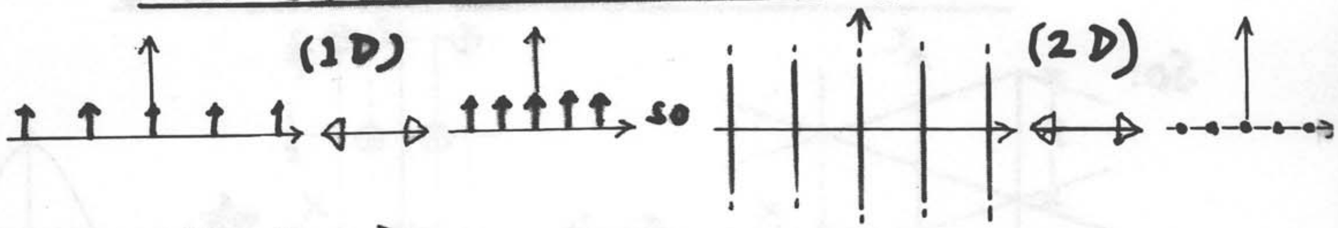


STILL TO DO:—

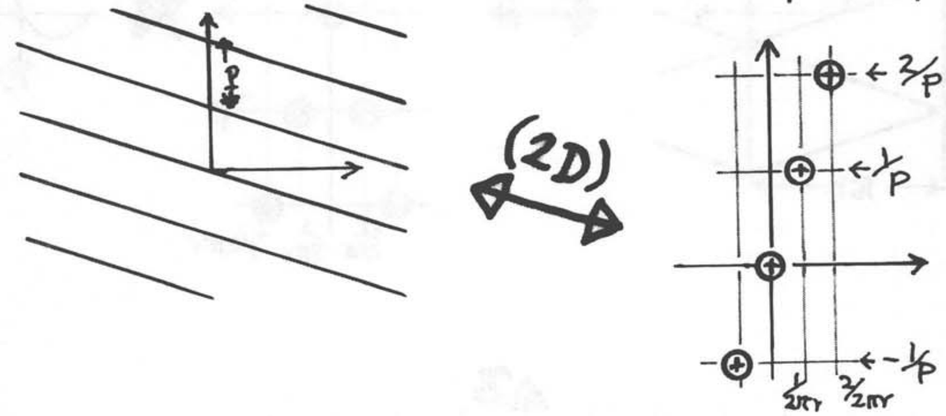
- ▷ Find which lattice-points are occupied.
- ▷ Find phases of spots in F.T.
- ▷ ☆ spots with F.T. (slit) =

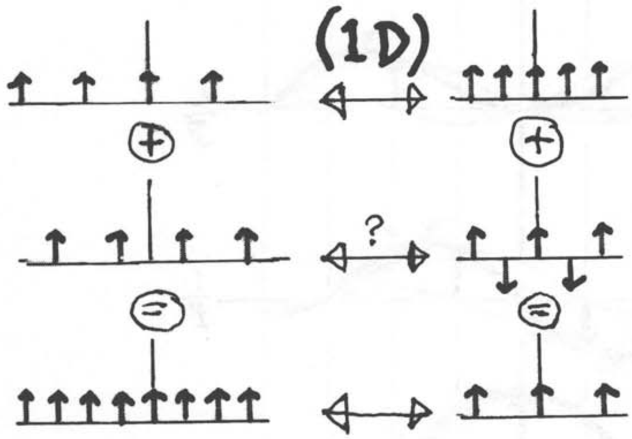


FLATTENED HELICAL LINE (p.2)



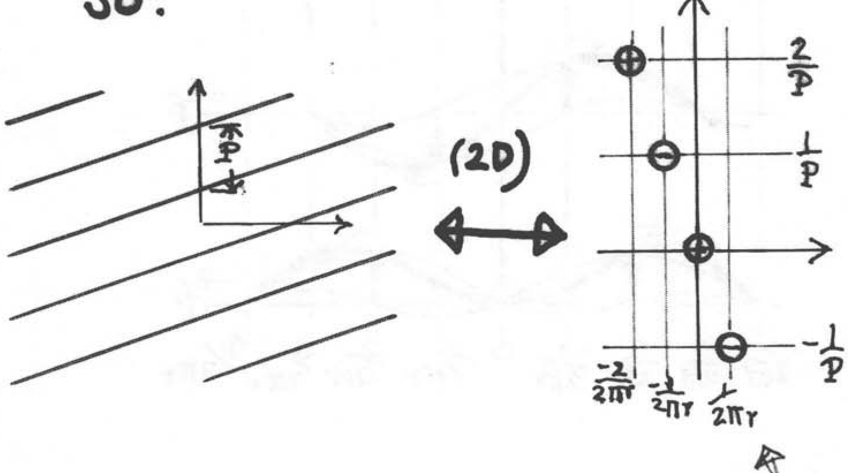
So





has to alternate signs to give

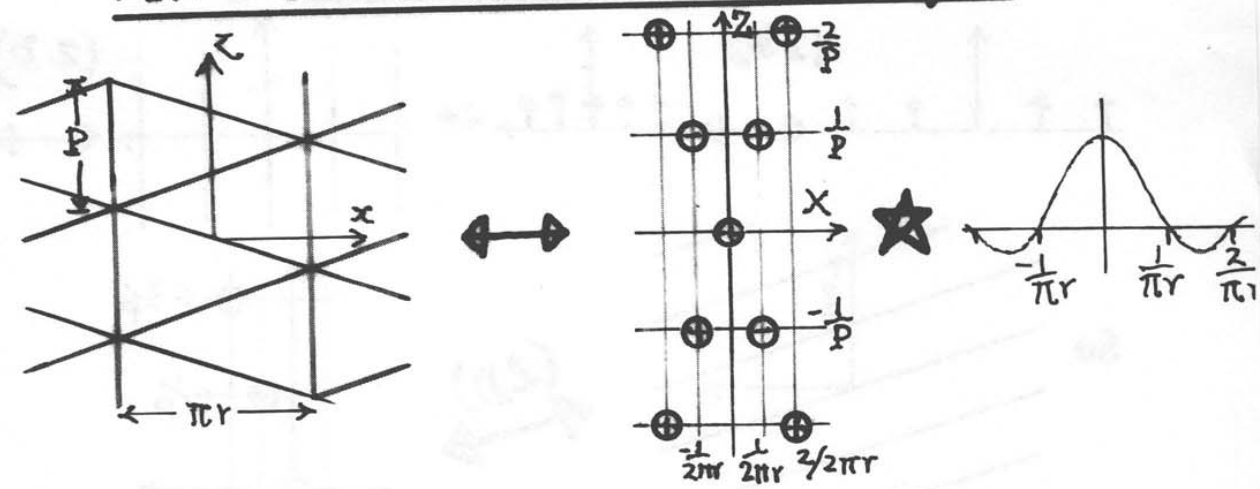
So:

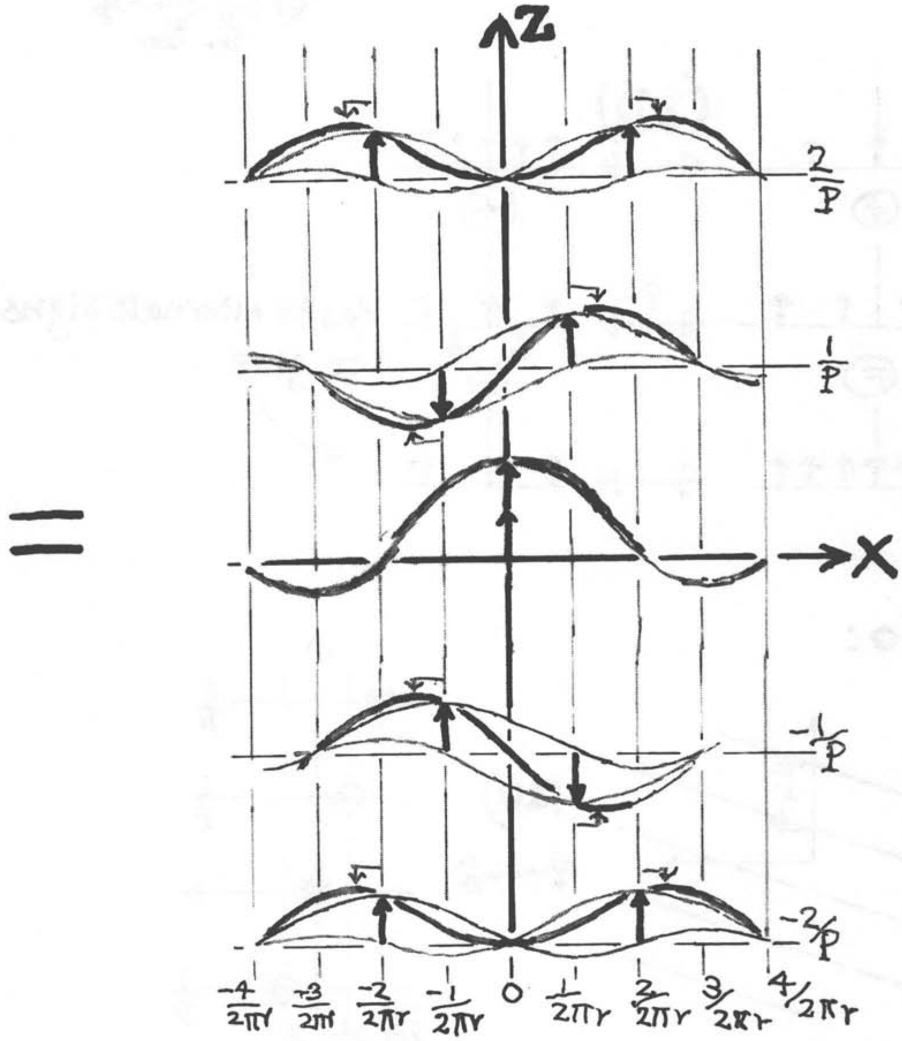


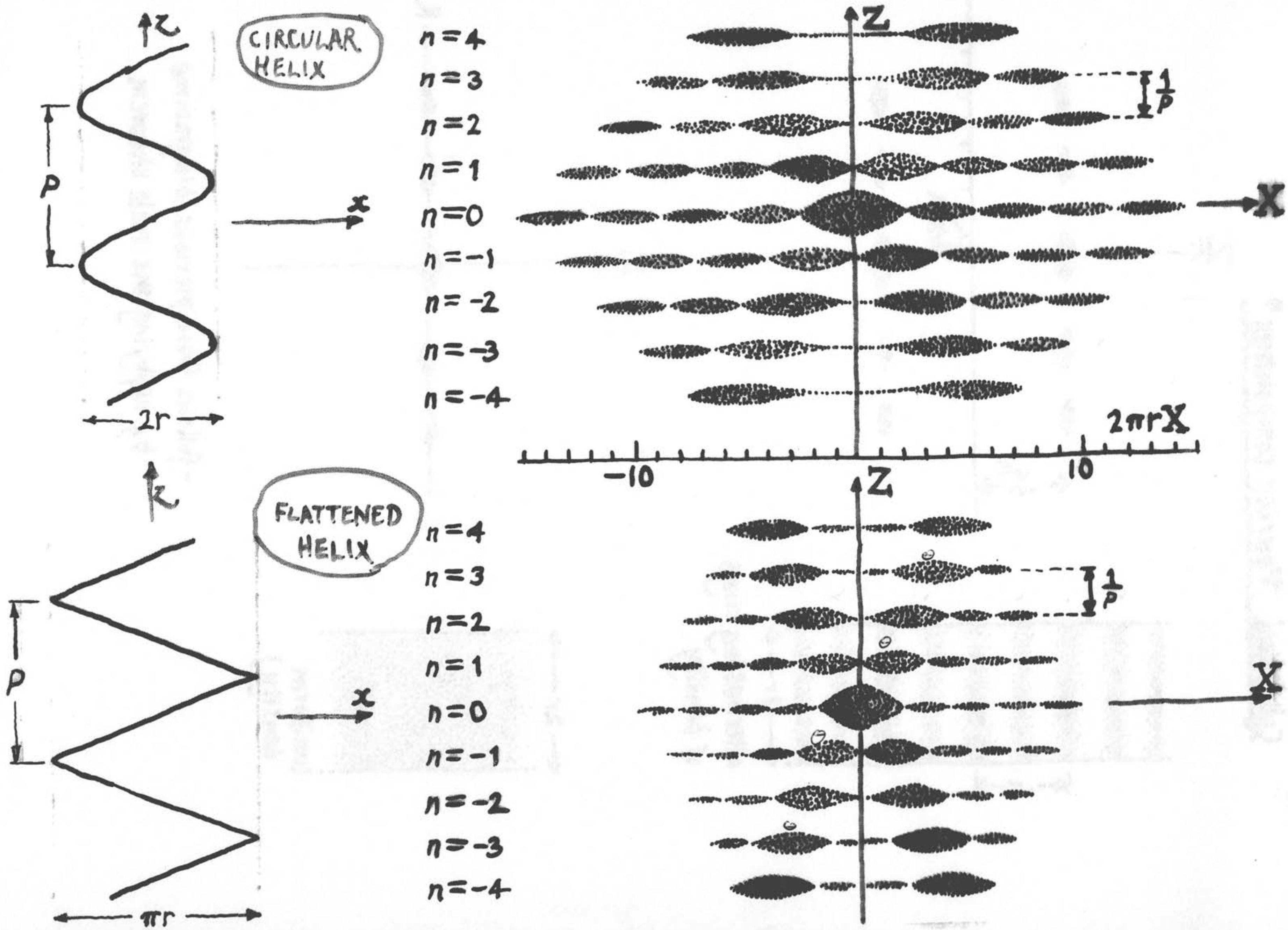
alternating +/-

FLATTENED HELICAL LINE (p.3)

So:

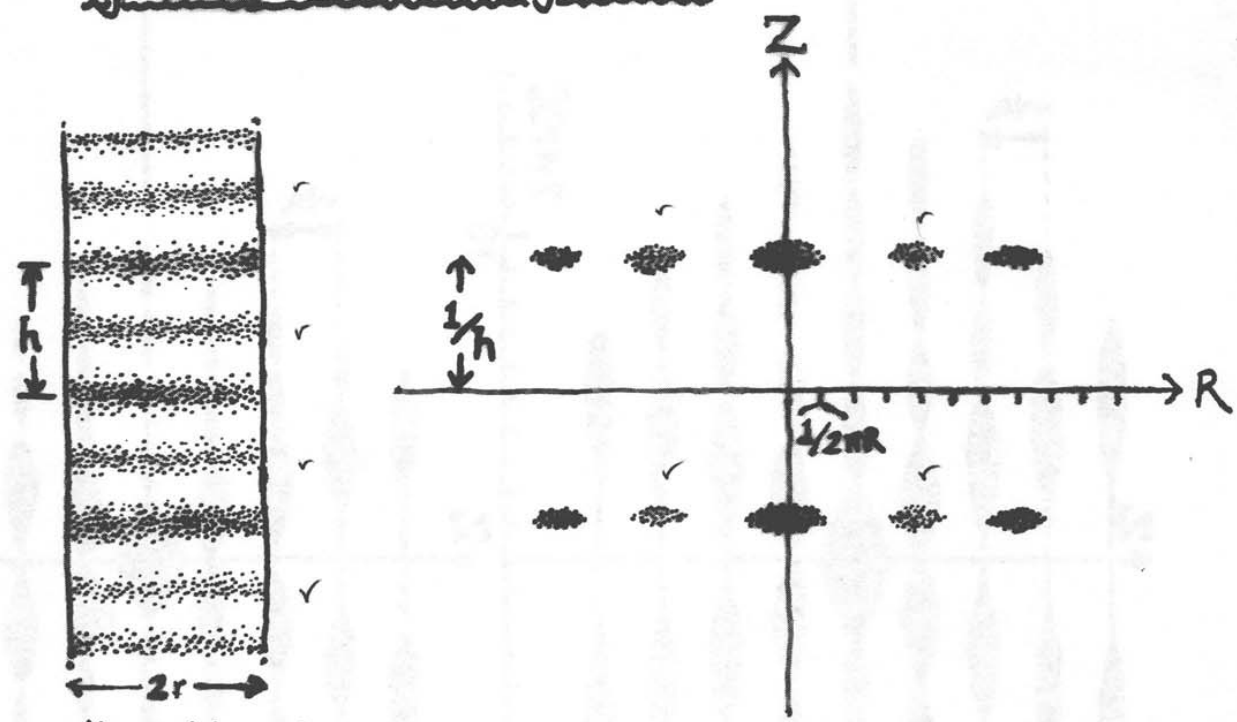




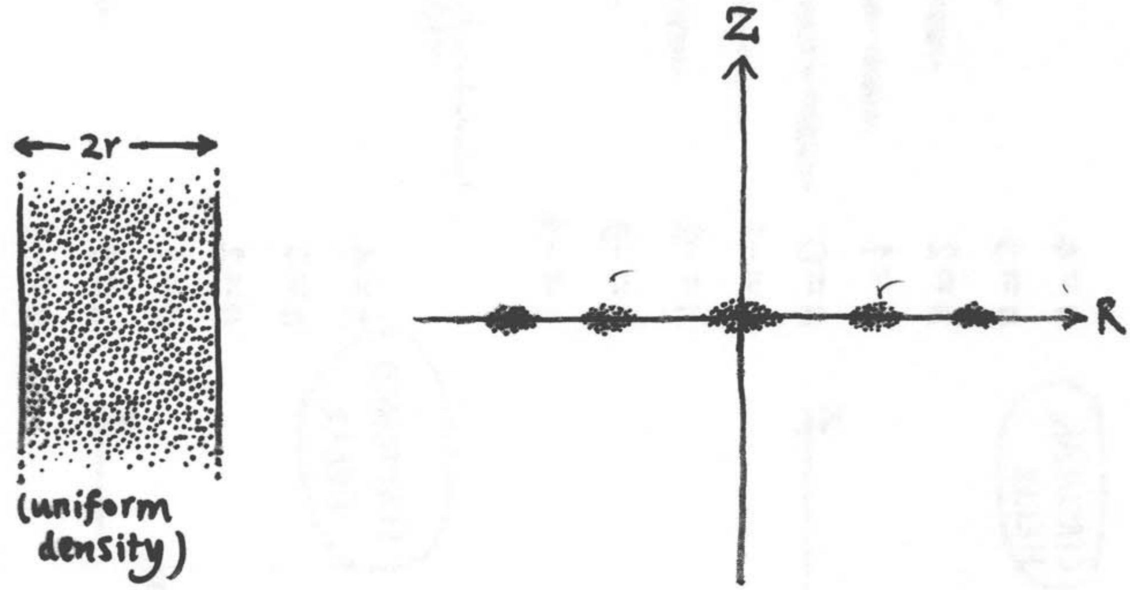


CIRCULAR HELIX
FLATTENED HELIX
z
x
p
2r
πr
n=4
n=3
n=2
n=1
n=0
n=-1
n=-2
n=-3
n=-4
Z
X
1/p
2πrX
-10
10

Cylindrical "Fourier components"



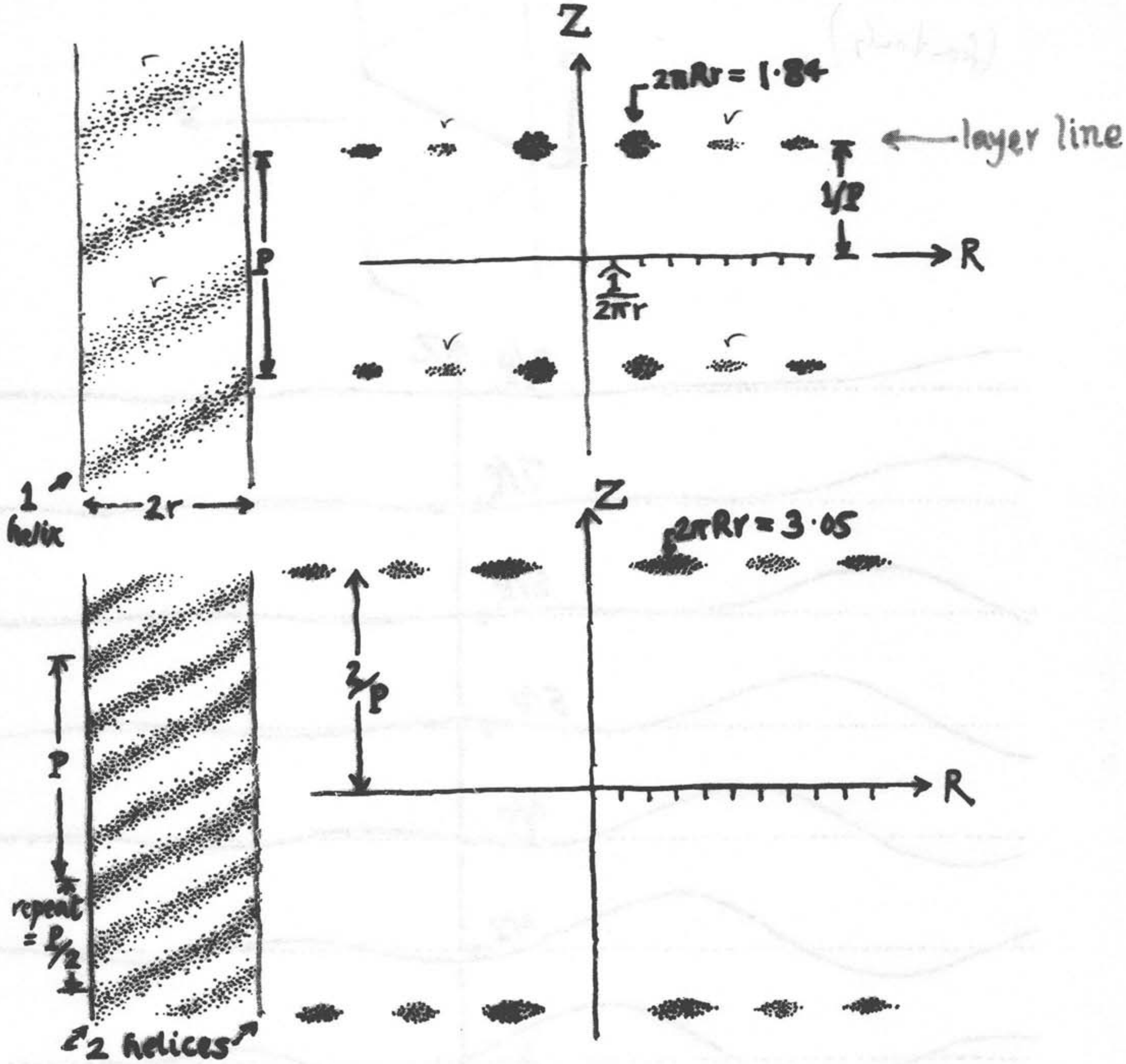
alternating rings of density



(uniform density)

- follows from previous diagramme by applying the scale theorem.

Helical "Fourier components" or Density waves



With n helices, layer lines are at $Z = \pm n/P$.

n density waves ★] P/n = no change

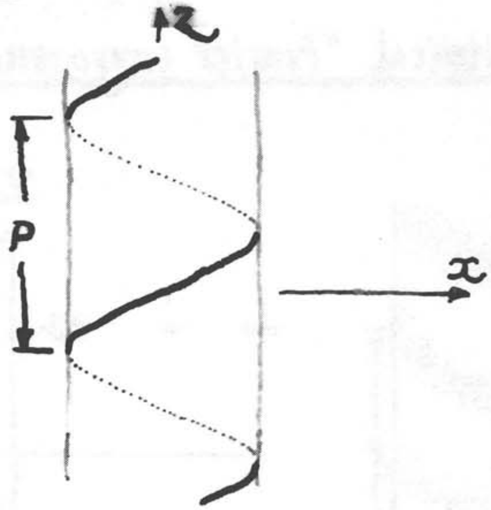
F.T.(n density waves) ×] n/P = no change ; so F.T. on n/P [

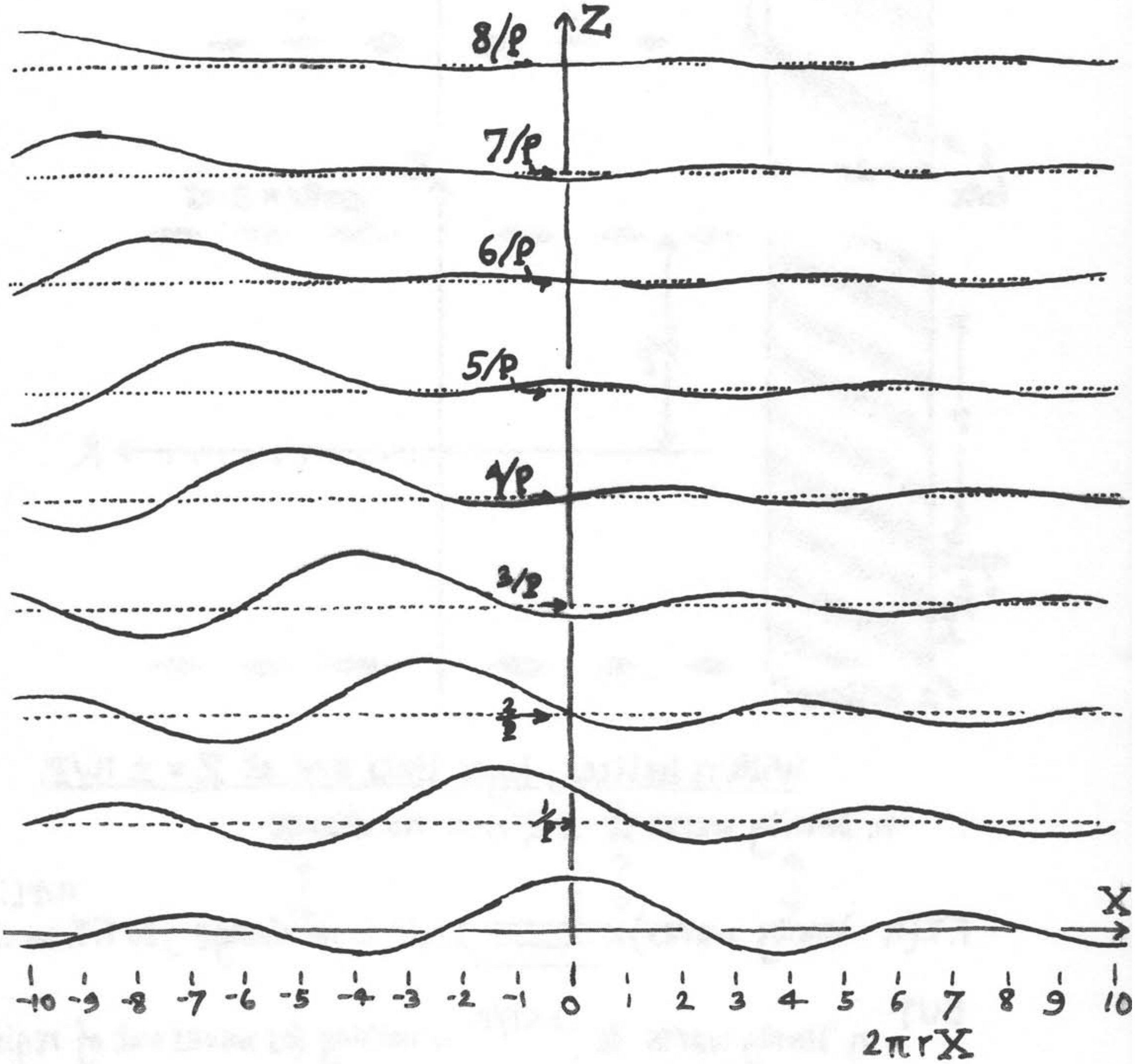
BUT n density waves ★] < P/n = nothing (as waves out of register)

F. T. (n density waves) exists only on $Z = \pm n/P$

F.T. of a half-helix.

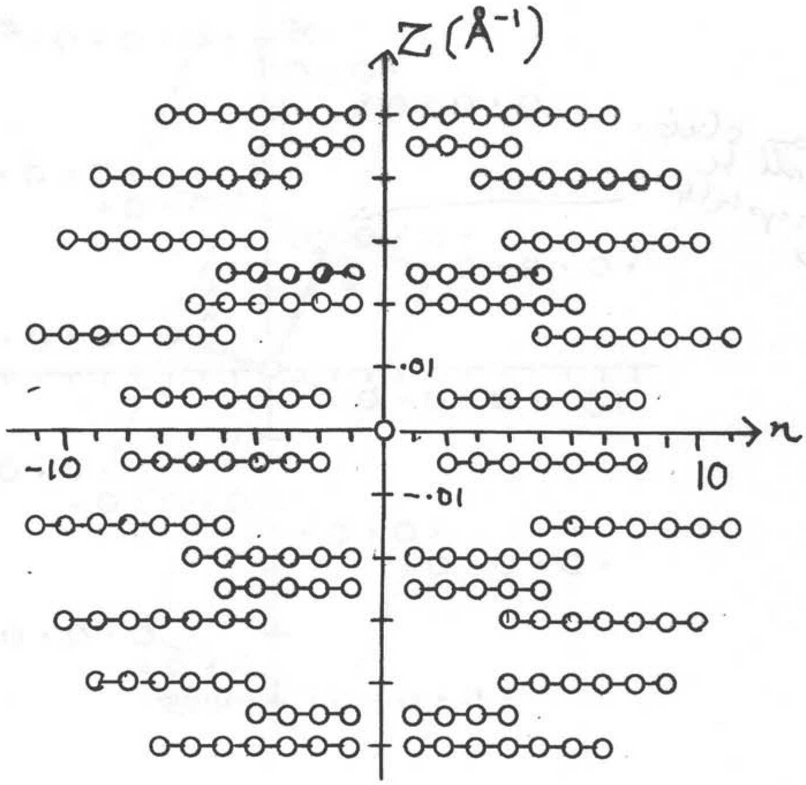
(front only)





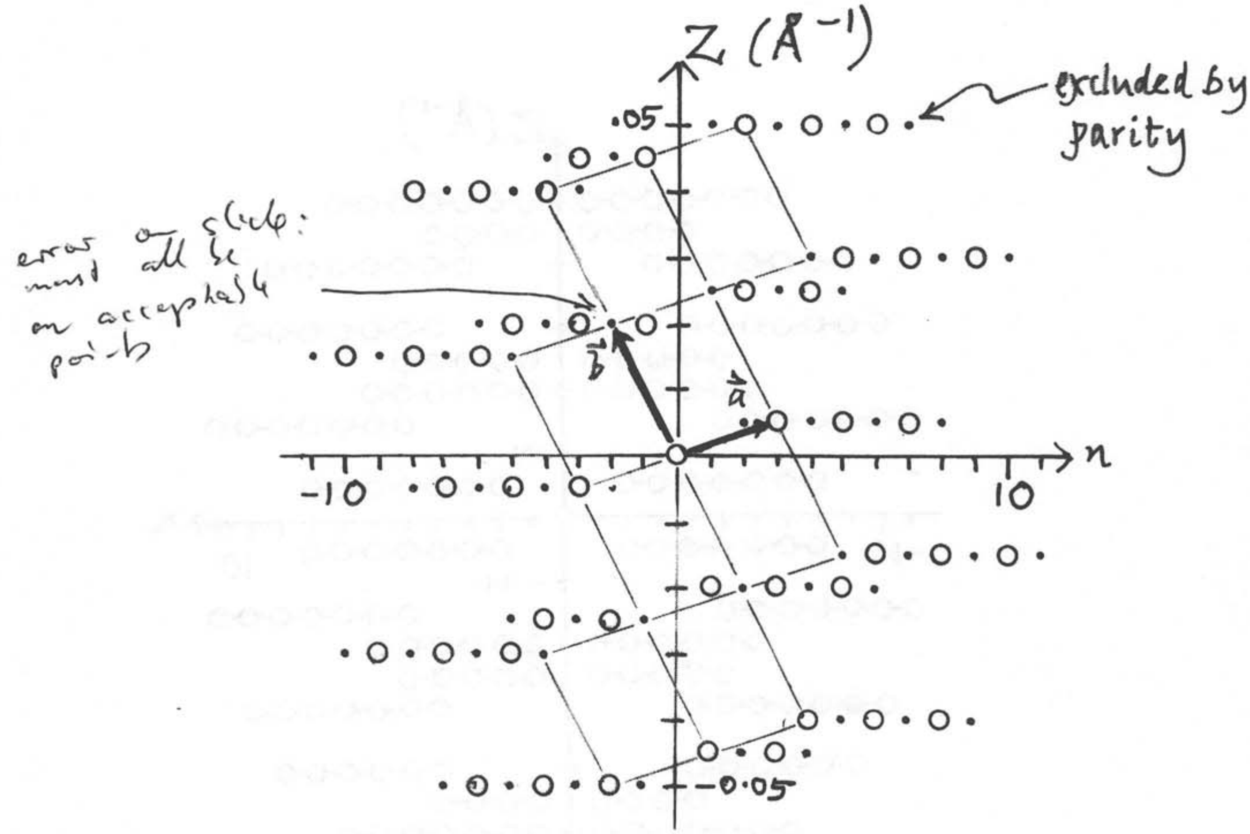
INDEXING HELICES I

Possible n's to fit F.T.



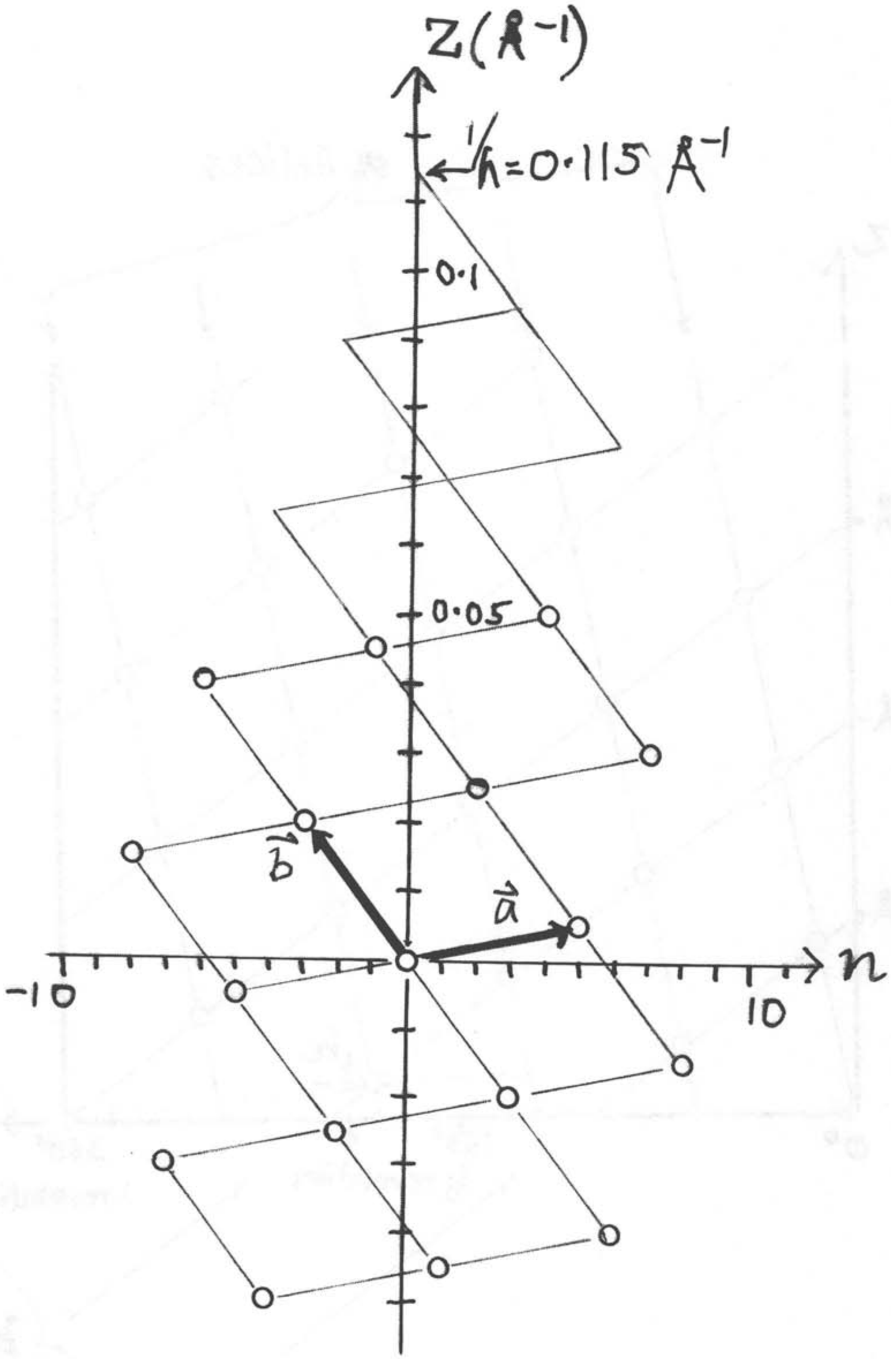
INDEXING HELICES II

Fitting the (n, Z) lattice



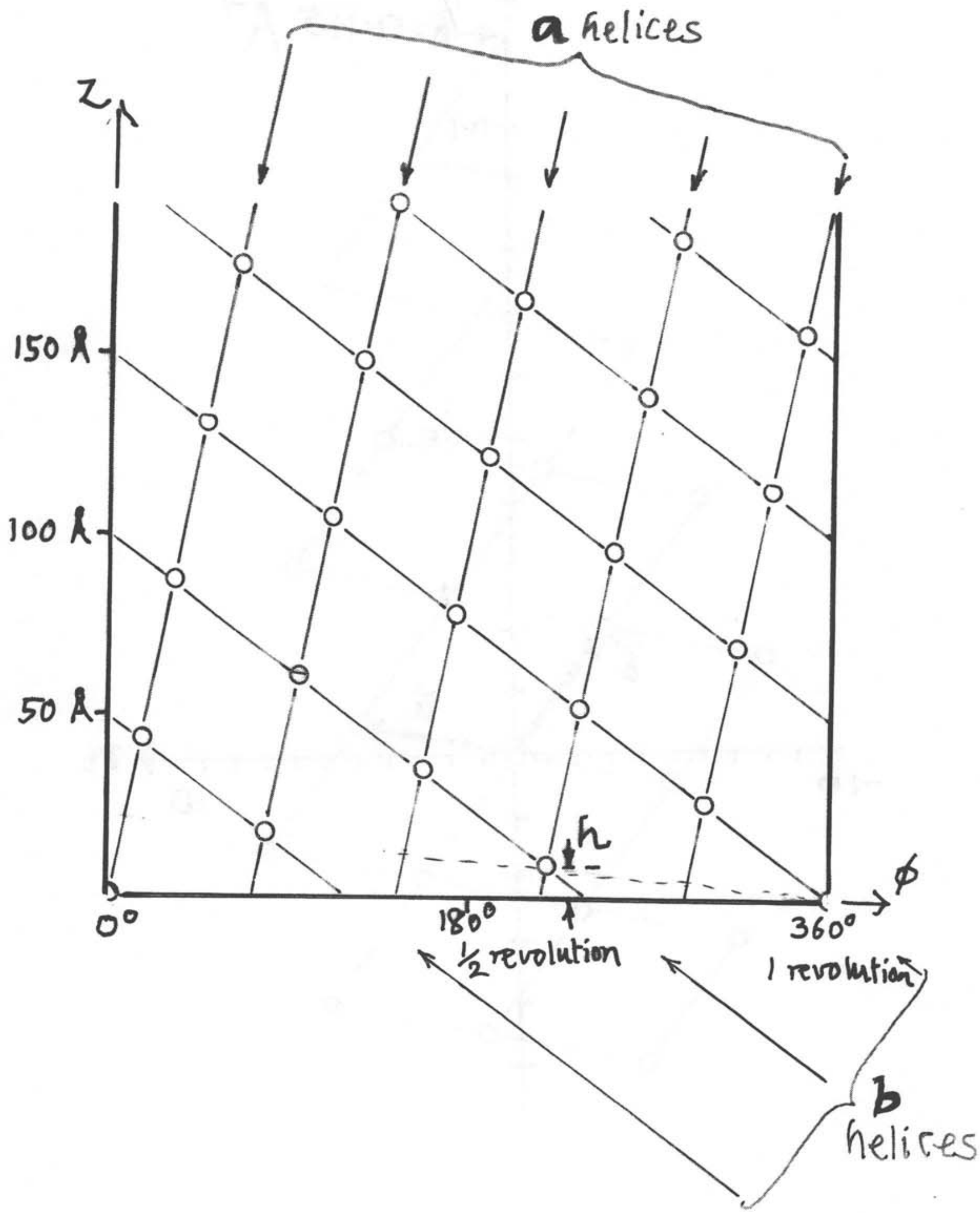
INDEXING HELICES III

Using (n, Z) lattice to find h



INDEXING HELICES IV

Radial projection of the helix



Summary: how to index a helical diffraction pattern

For each layer-line

- measure Z
- estimate n — spacing of peak
  — phase → parity of n

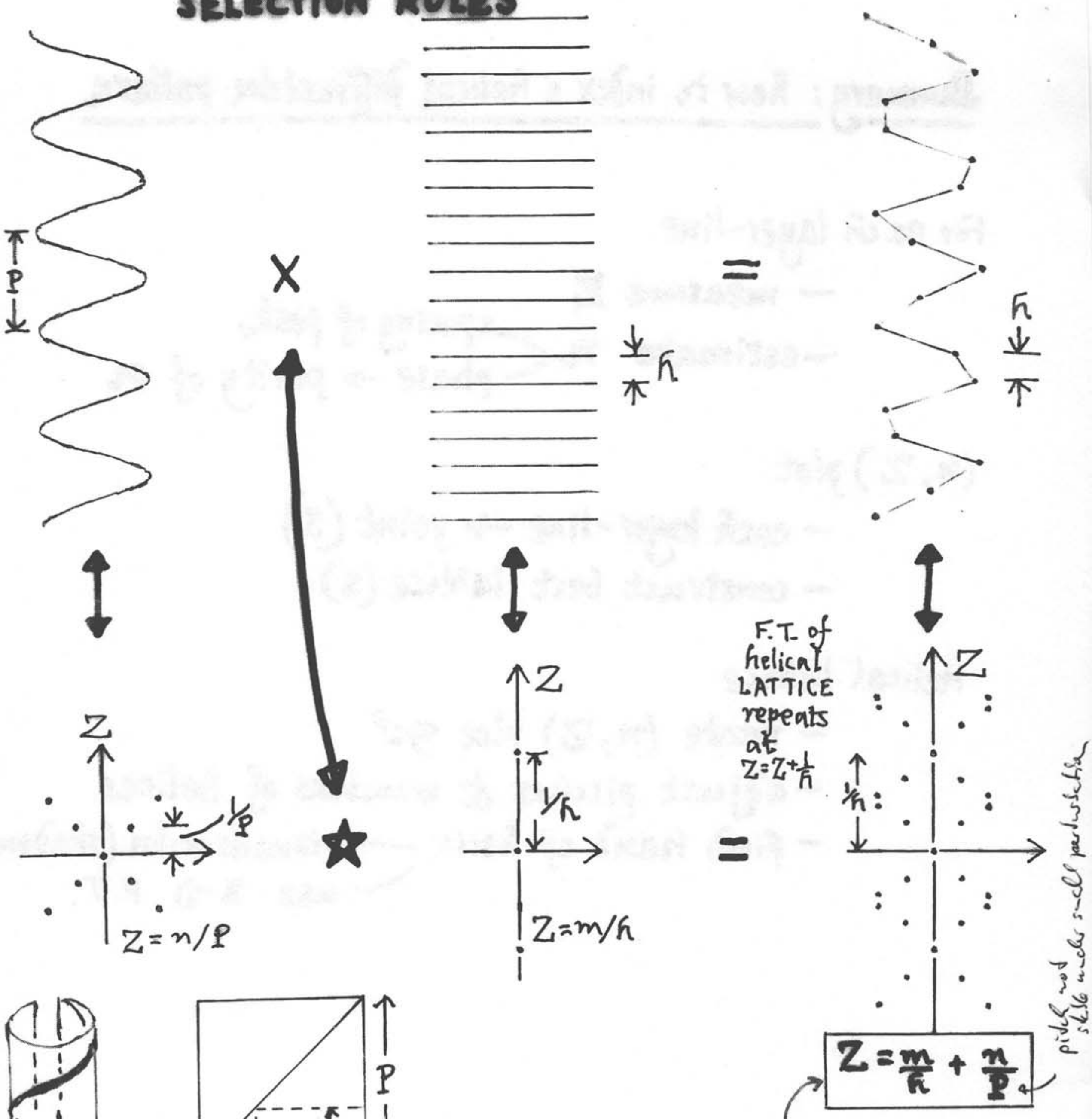
(n, Z) plot

- each layer-line → point(s)
- construct best lattice(s)

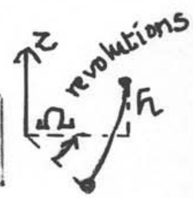
Helical lattice

- rotate (n, Z) plot 90°
- adjust pitches & numbers of helices
- find hand of helix — visualisation (shadowing etc)
  — use 3-D F.T.

SELECTION RULES



$\frac{1}{P} = \frac{\Omega}{h}$, so $Z = \frac{m}{h} + \frac{n\Omega}{h}$, so $\boxed{Zh = m + n\Omega}$



If helix has an exact repeat c, $Z = \frac{l}{c} = \frac{n}{P} + \frac{m}{h}$, so $l = n\left(\frac{c}{P}\right) + m\left(\frac{c}{h}\right)$

If the repeat has t turns and u units, $t = \frac{c}{P}$ and $u = \frac{c}{h}$

not stable under small perturbations → So $\boxed{l = tn + um}$ (also needs c)

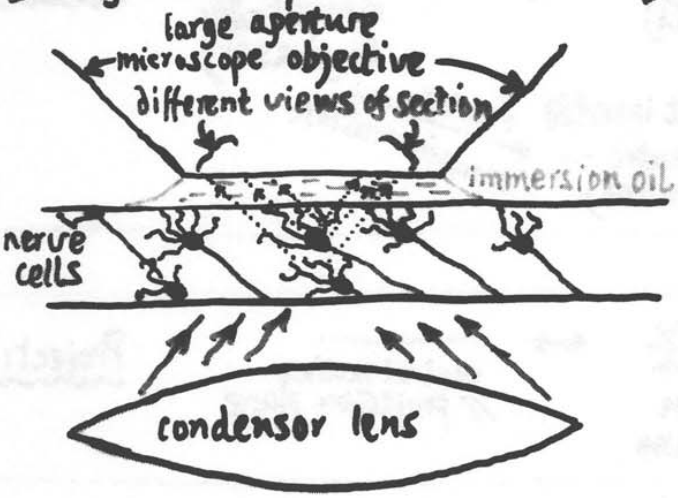
Integ~~ral~~er form (approximations)

& if have exact repeat

3-DIMENSIONAL RECONSTRUCTION

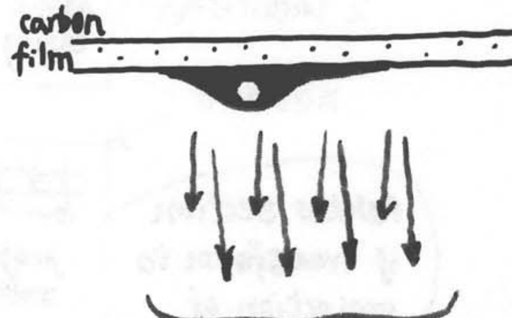
Why does this problem arise only in electron microscopy?

Light microscope:



Many different views
combined by microscope
↓
appearance of depth
(but not really very good)

Electron microscope:



only a very narrow range of angles accepted by <u>electron lens</u> ~ <u>spherical aberration</u>

DIFFERENCE

<u>So ~ only one view of specimen</u>

Different tilts of specimen
↓
Different views

Need to combine these to give 3-dimensional structure

Fundamental problem of 3-D reconstruction

Different mathematical solutions
(e.g. back-projection, matrix inversion, iterative procedures, and Fourier transform method.)

Most widely used in electron microscopy

Fourier Method for 3-D Reconstruction

So, if we can find 3-D F.T., we can find 3-D object

Object (= 3-D array of densities) $\xrightarrow[\text{transformation}]{\text{Fourier}}$ F.T. (= 3-D array of amplitudes & phases) — **Inversion**

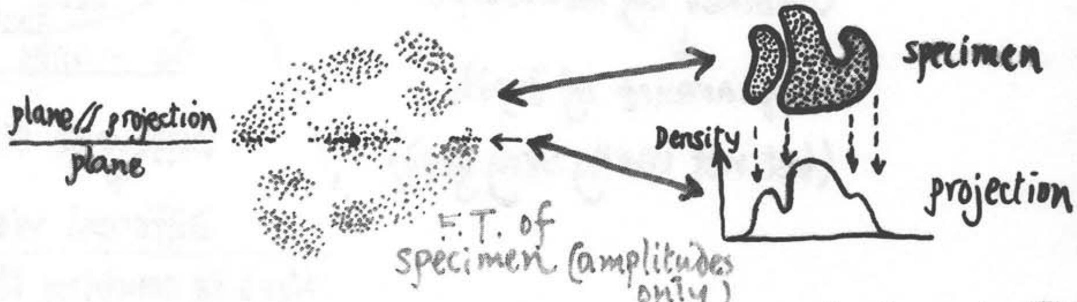
Object (but inverted about its centre — easily corrected) $\xleftarrow[\text{transformation}]{\text{Fourier}}$

2 theorems needed

projection onto plane ↔ central section // projection plane — **Projection**

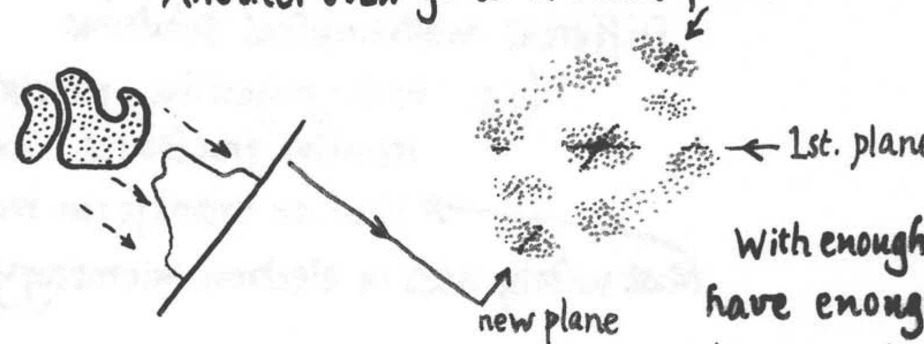
Relates section of transform to projection of object

Micrograph of 1 view of specimen = projection of specimen



So 1 view = 1 projection gives us 1 plane in F.T.

Another view gives a second plane

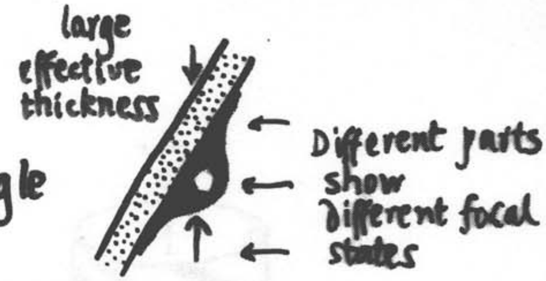


With enough views, we have enough planes to reconstruct the F.T

and, from the F.T., to calculate the specimen

How to record the projections

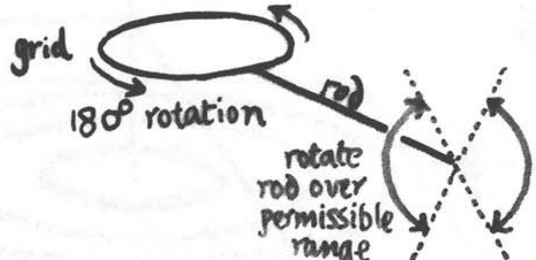
► Tilt the E.M. grid



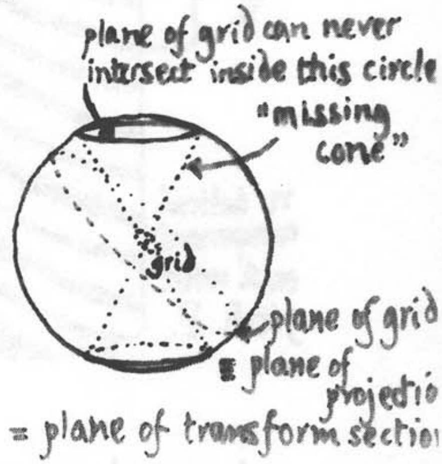
Problem: maximum tilt angle

∴ evenly spaced tilts not possible

Combine maximum tilt range with grid rotation



Hence:

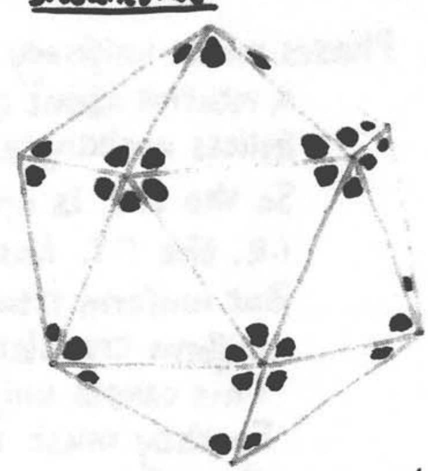


► Use symmetrical objects (require special reconstruction techniques)

Helical:

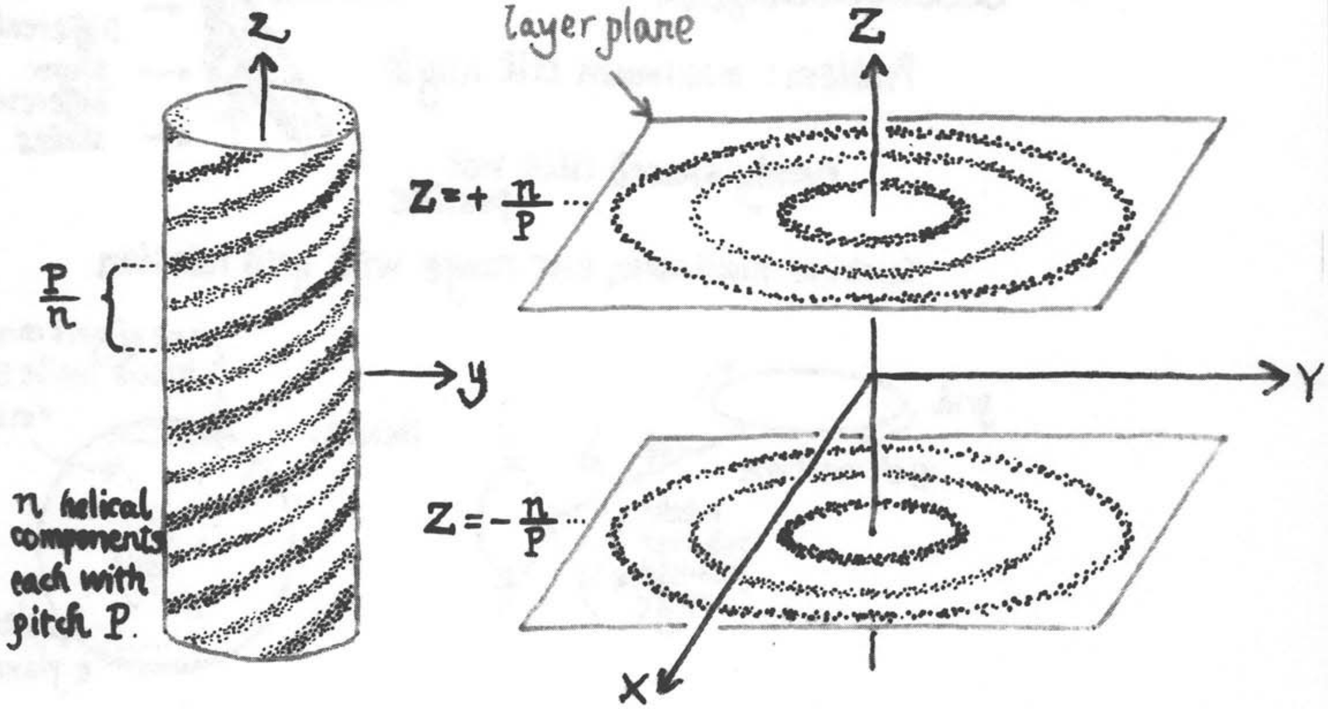
1 projection contains
11 different views
(evenly spaced tilts)

Icosahedral:



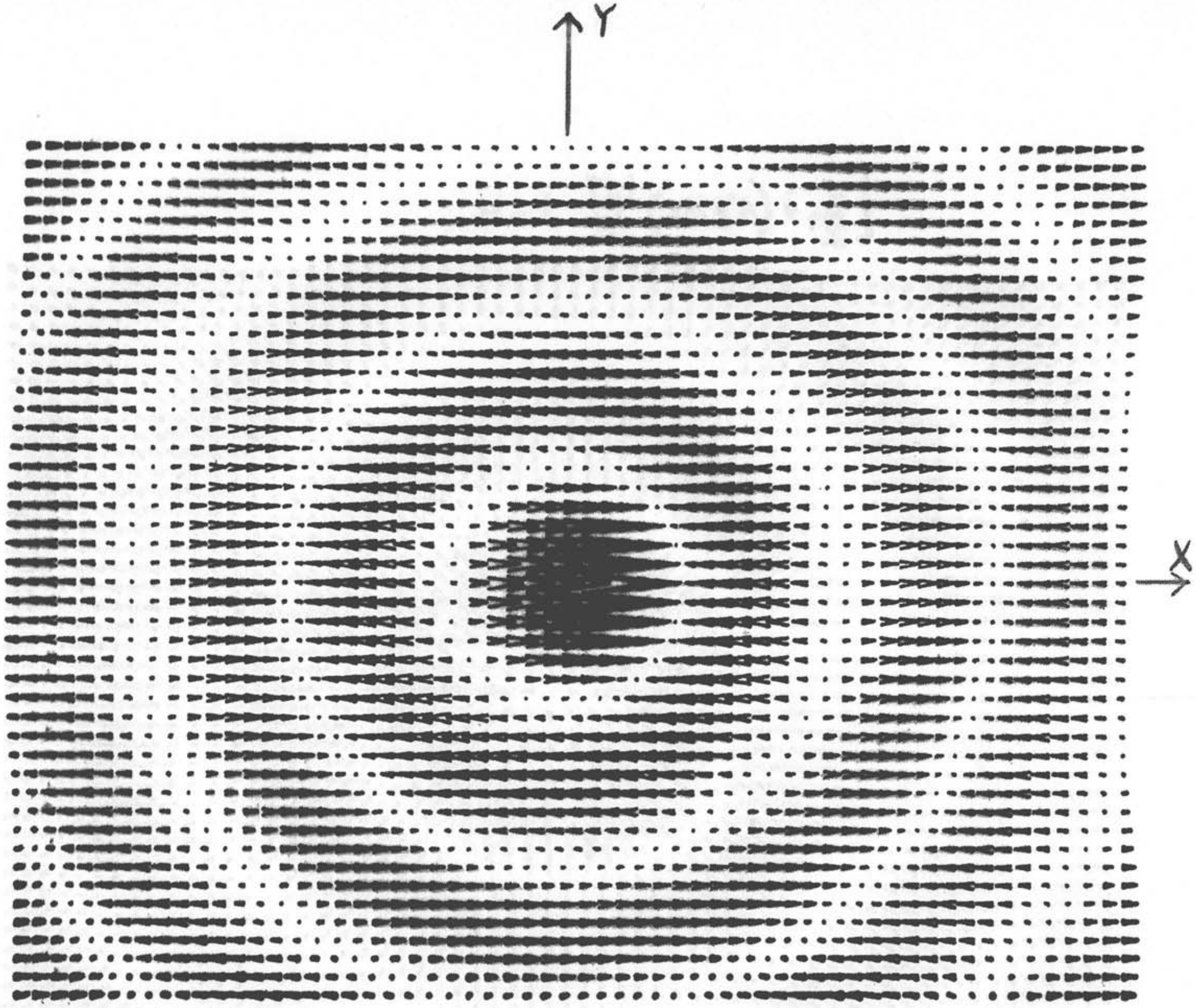
1 projection contains ≲ 60
different views

3-Dimensional F.T. of a set of Helical Components

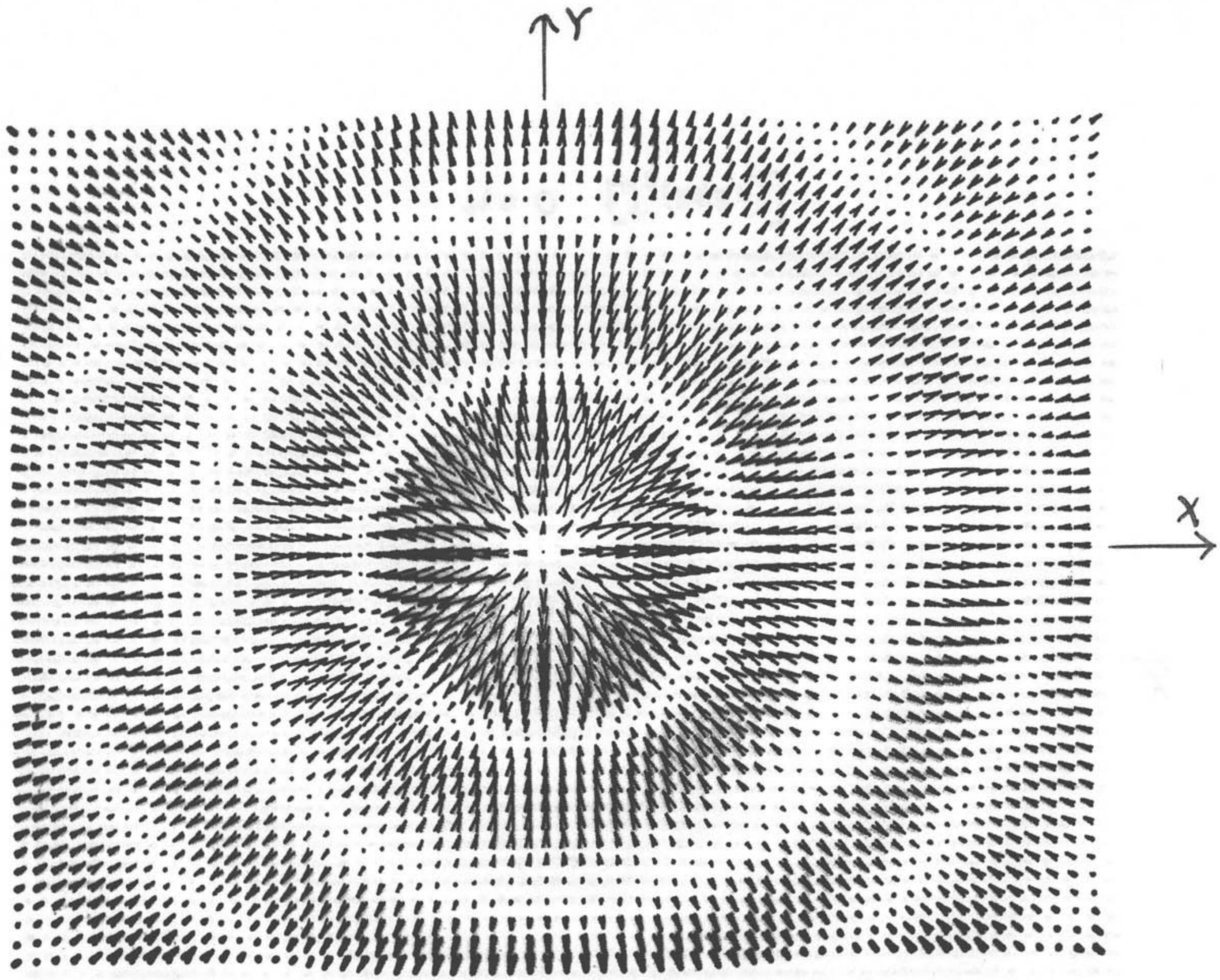


Amplitude consists of rings of density because rotation of helix about z is the same as translation along z, and this changes phases, not amplitudes. So amplitudes are unchanged by rotation about Z, i.e. they consist of rings of density.

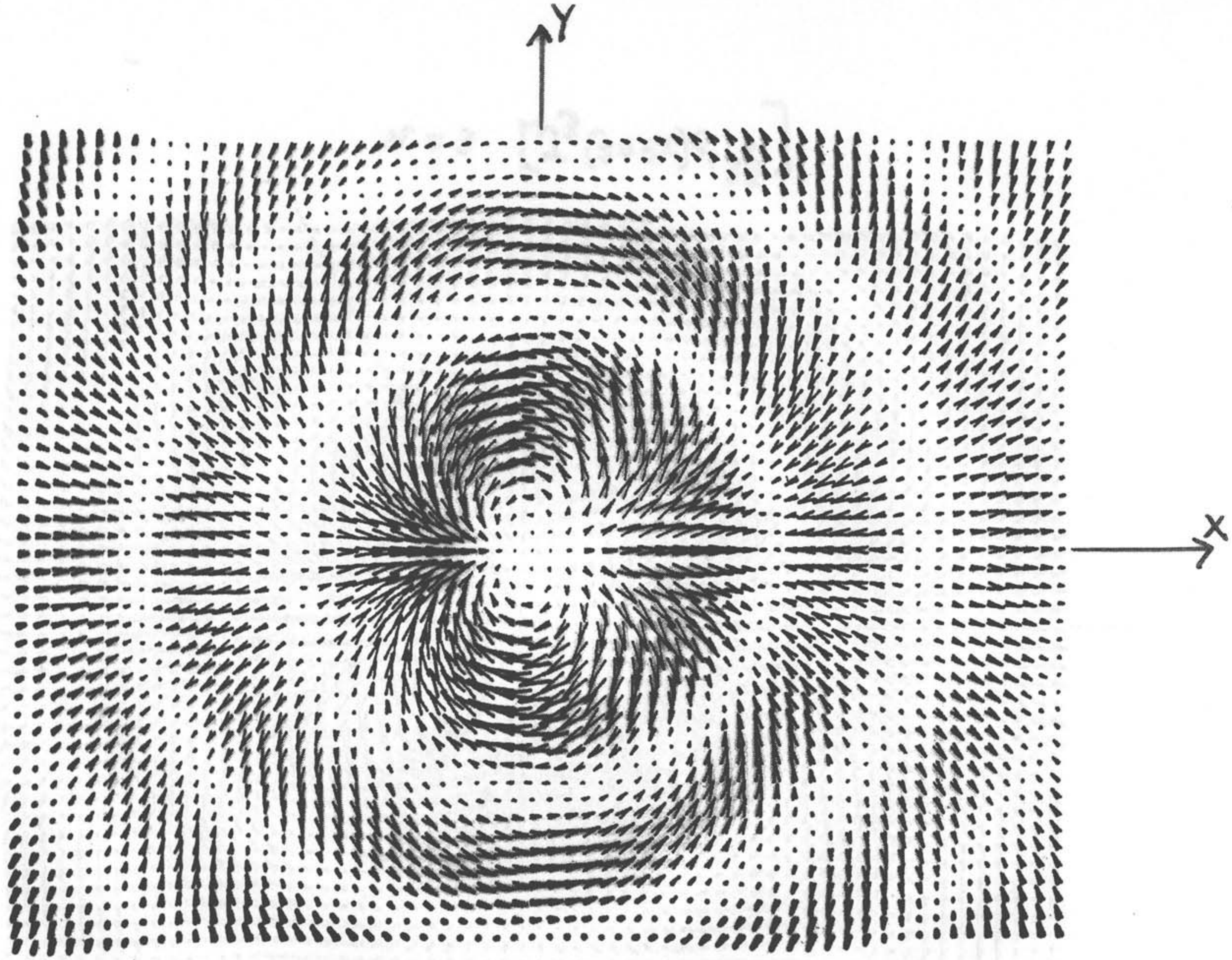
Phases rotate uniformly n times for one revolution around the rings.
A rotation about z by 1/n revolutions leaves the set of n helices unchanged.
So the F.T. is unchanged by the same rotation about Z, i.e. the F.T. has n-fold rotational symmetry.
But uniform rotation of the helix about z is equivalent to uniform translation along z.
This causes uniform rotation of the F.T. phases.
So they must rotate n times for one revolution around the rings.



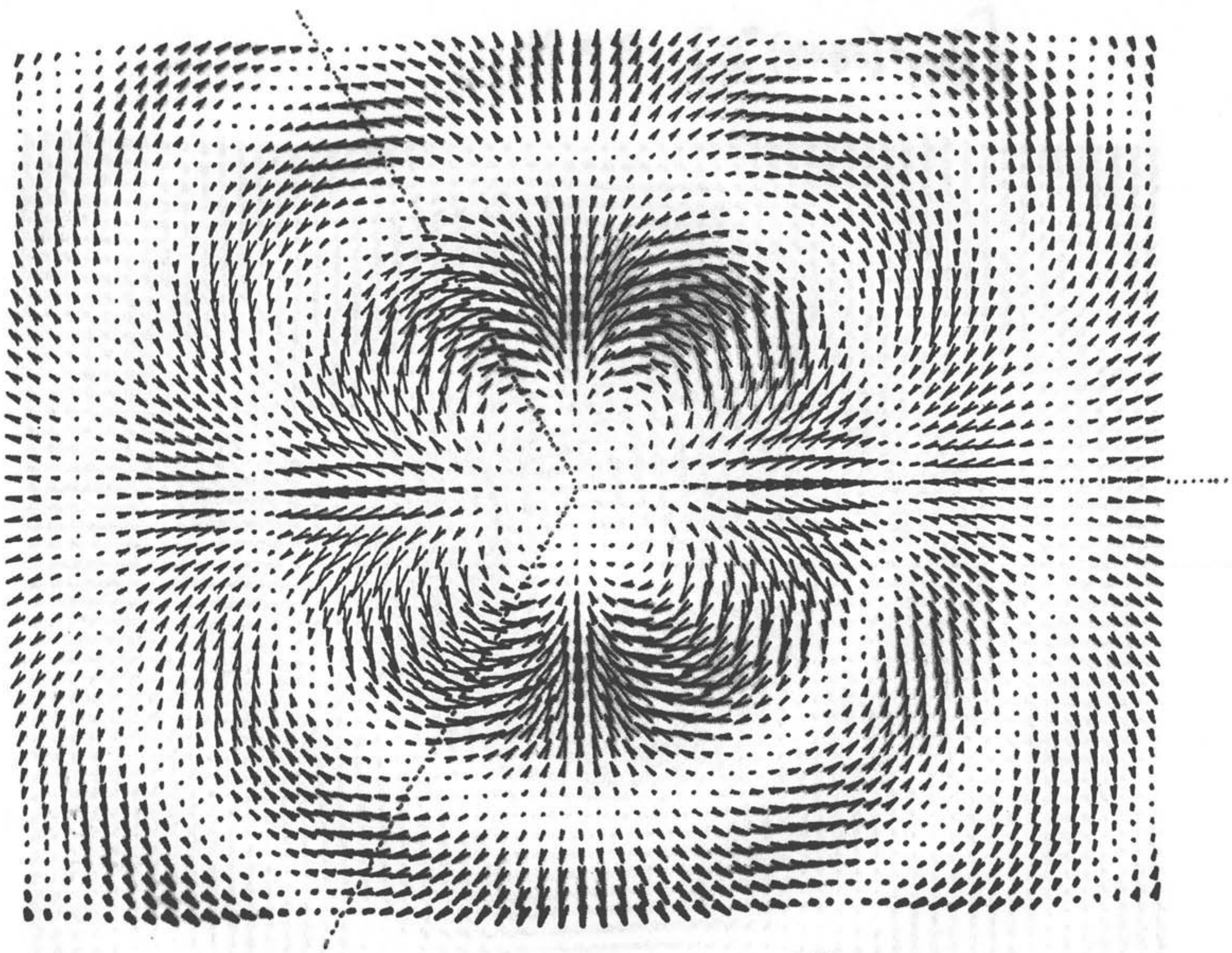
$n = 0 \quad [J_0(2\pi R r)]$



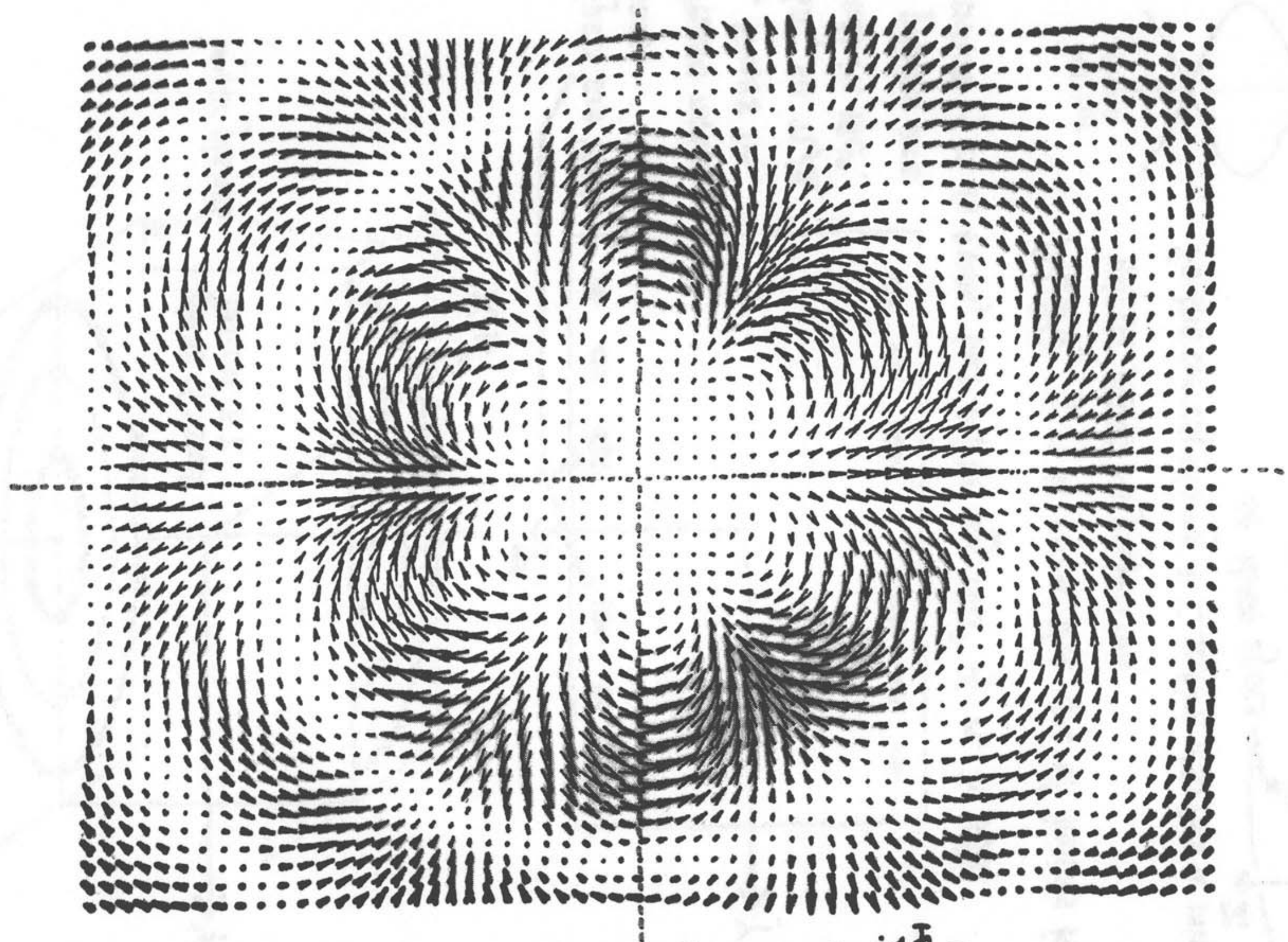
$n = 1 \quad [J_1(2\pi R r)\, e^{i\Phi}]$



$n = 2 \quad \left[J_2(2\pi k r)\, e^{i2\Phi} \right]$



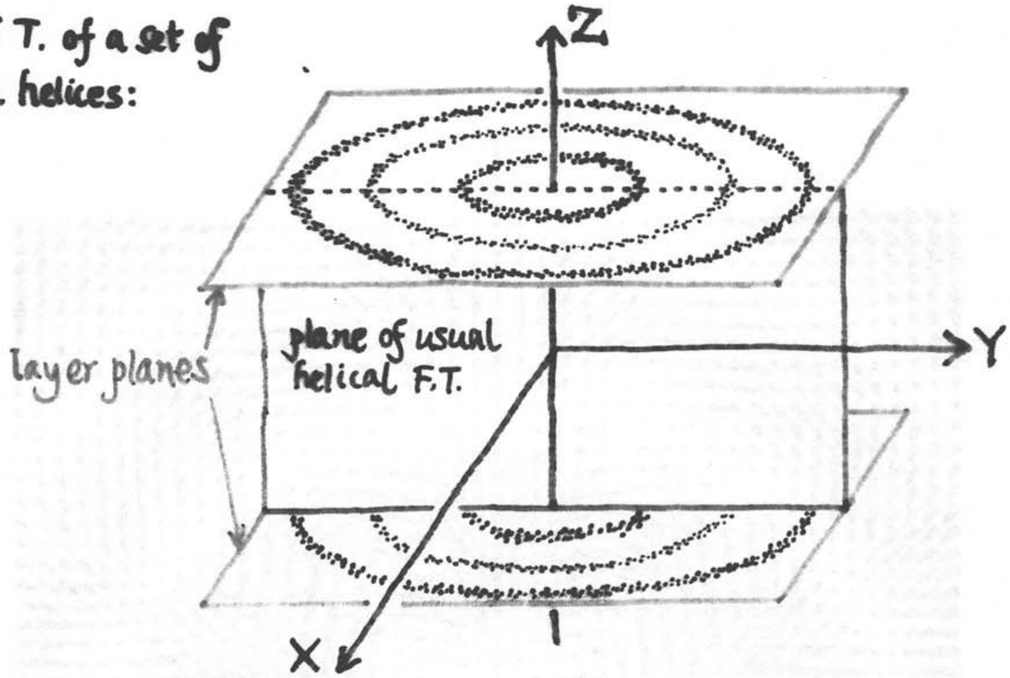
$n = 3 \quad \left[J_3(2\pi R r)e^{i3\Phi}\right]$



$n=4 \quad [J_4(2\pi R r)\, e^{i4\Phi}]$

Parity of a set of helices can be found from phases

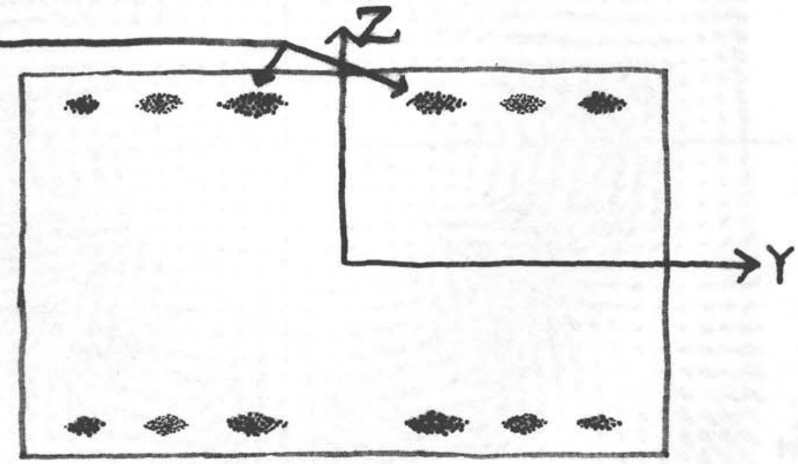
F. T. of a set of n helices:



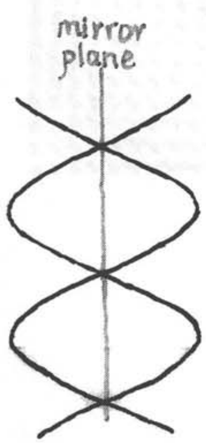
Spots are 180° apart.

Phase rotates n times in 360°, so n/2 times in 180°.

So phases are



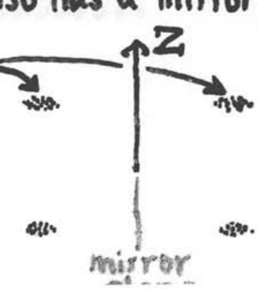
identical if n is even, but differ by 180° if n is odd.



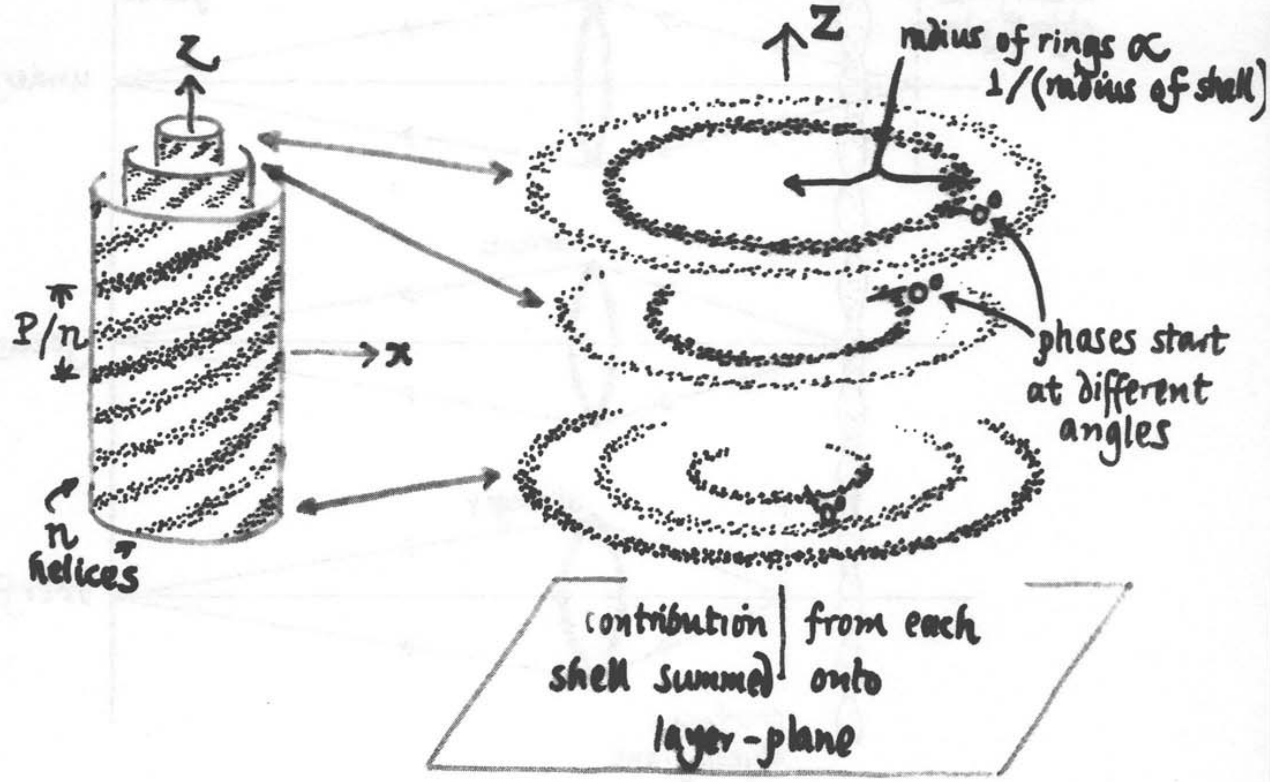
ALSO, if n is even the set of helices has a mirror plane in projection.

So the corresponding F.T. section also has a mirror plane,

so these spots must have the same phase.



Effect of helix thickness on a layer-plane



Resultant layer-plane:

- amplitude is ⊙rly symmetric
- phase rotates n times per revolution
- Z-coordinate is n/P
- can be inverted to give same thick helical waves

So we need to know only

- amplitude distribution
- starting phase

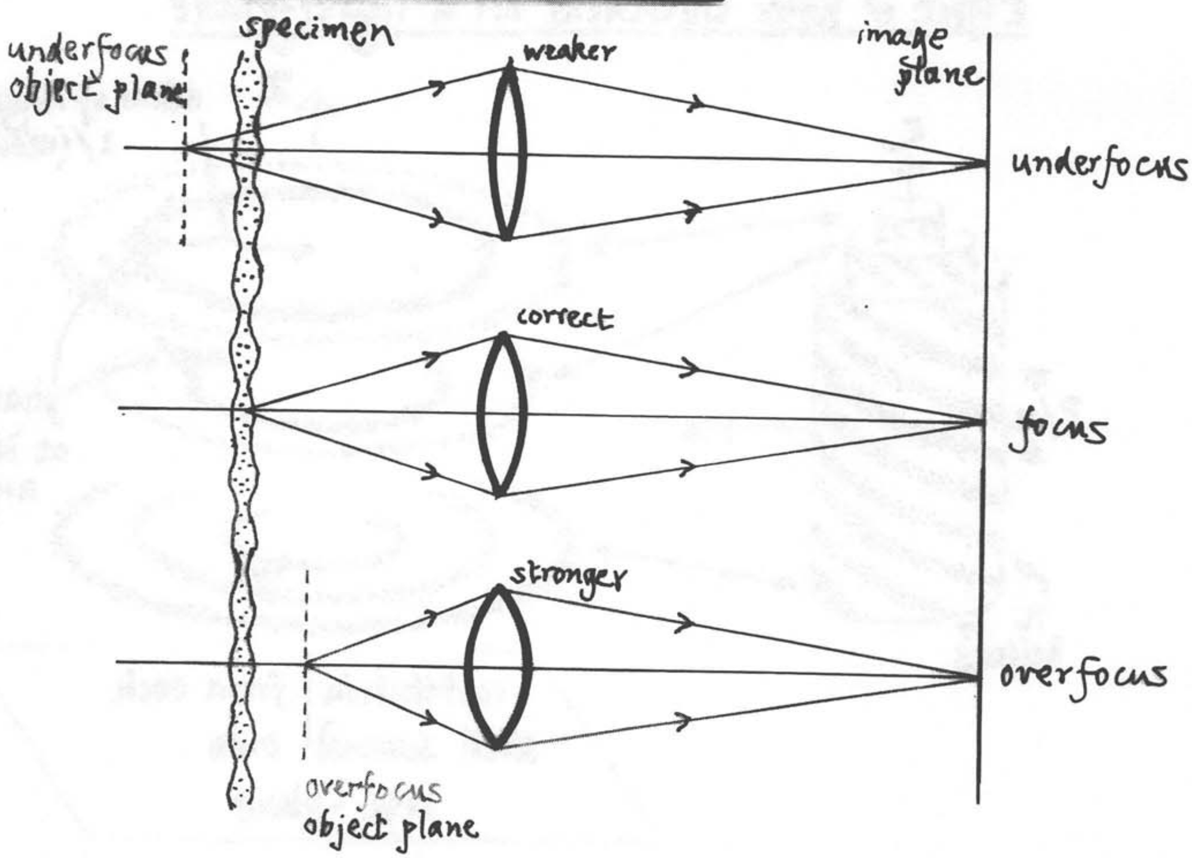
for each layer-plane.

PROVIDED no layer-planes interfere

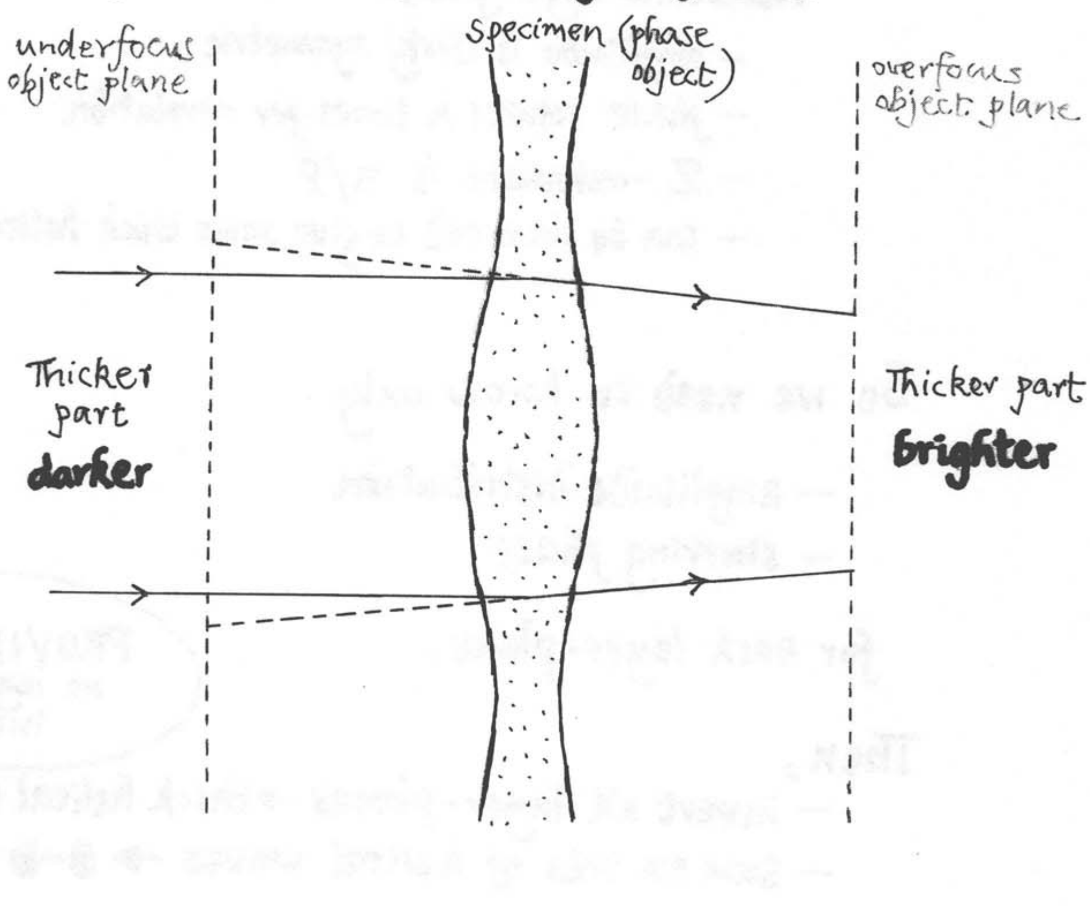
Then,

- invert all layer-planes → thick helical waves
- sum all sets of helical waves → 3-D structure

DEFOCUS IN REAL SPACE

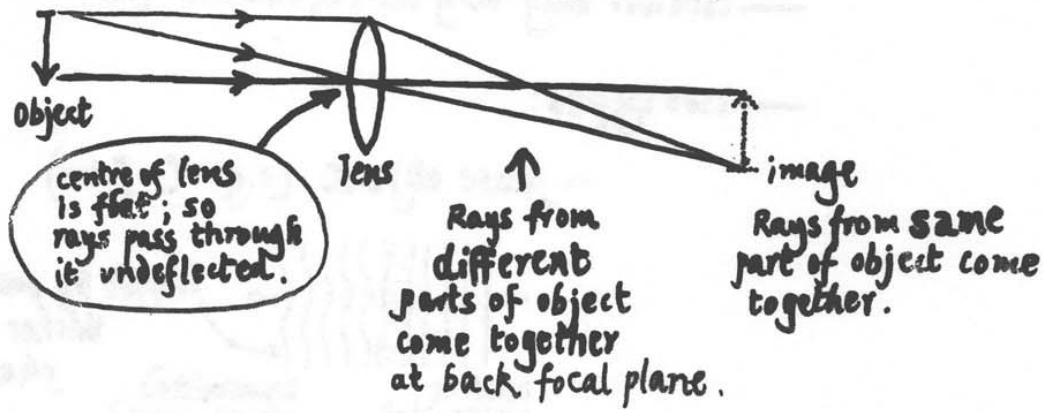


Phase object seen at different object planes.

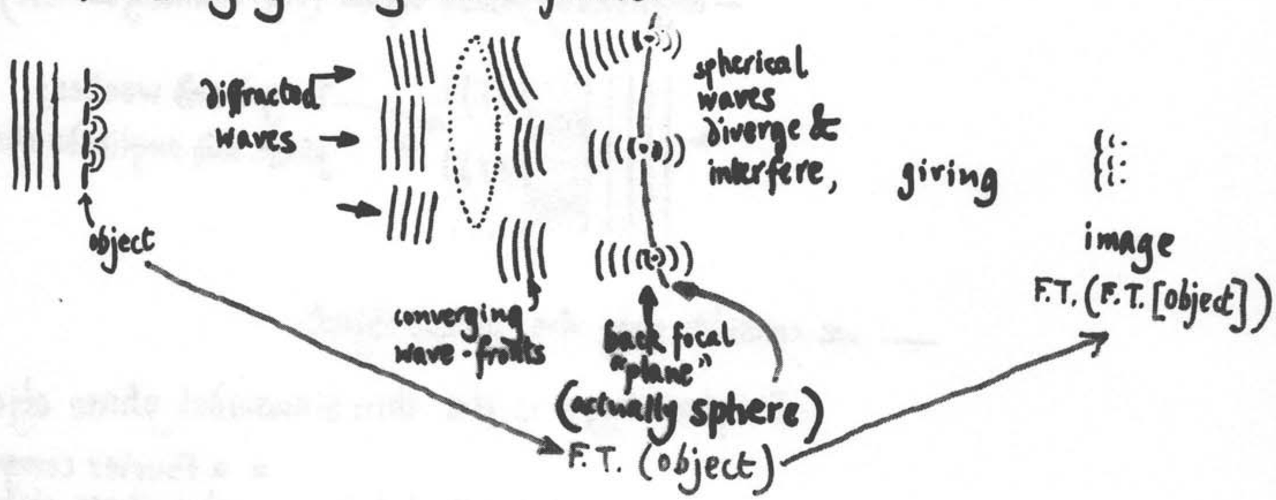


Imaging in the Electron Microscope

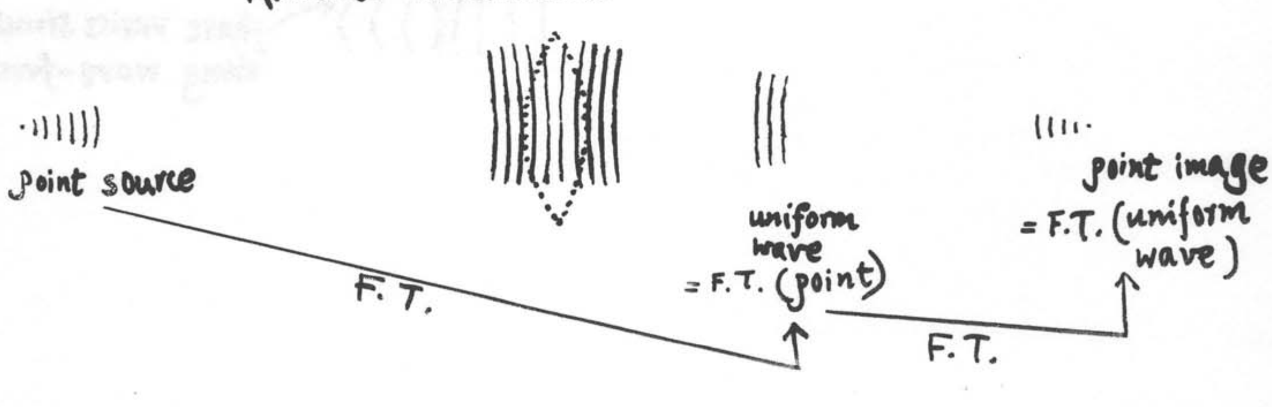
► Reminder of imaging using rays:



► Imaging using wave-fronts:



Another illustration:



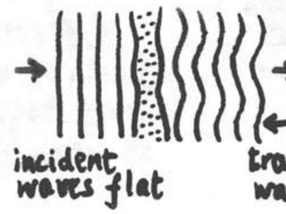
Wave transmitted by object

E.M. specimens

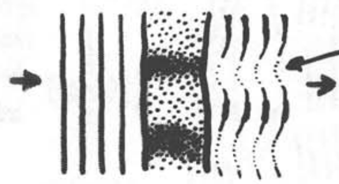
— consider only very thin specimens (electrons scattered once)

— two types:

- phase object (e.g. C-film)



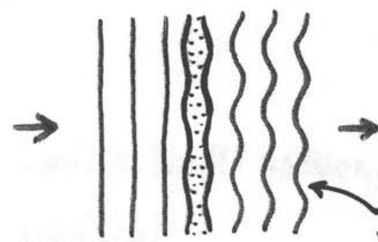
- amplitude/phase object (e.g. stained specimen)



— we consider only the phase object.

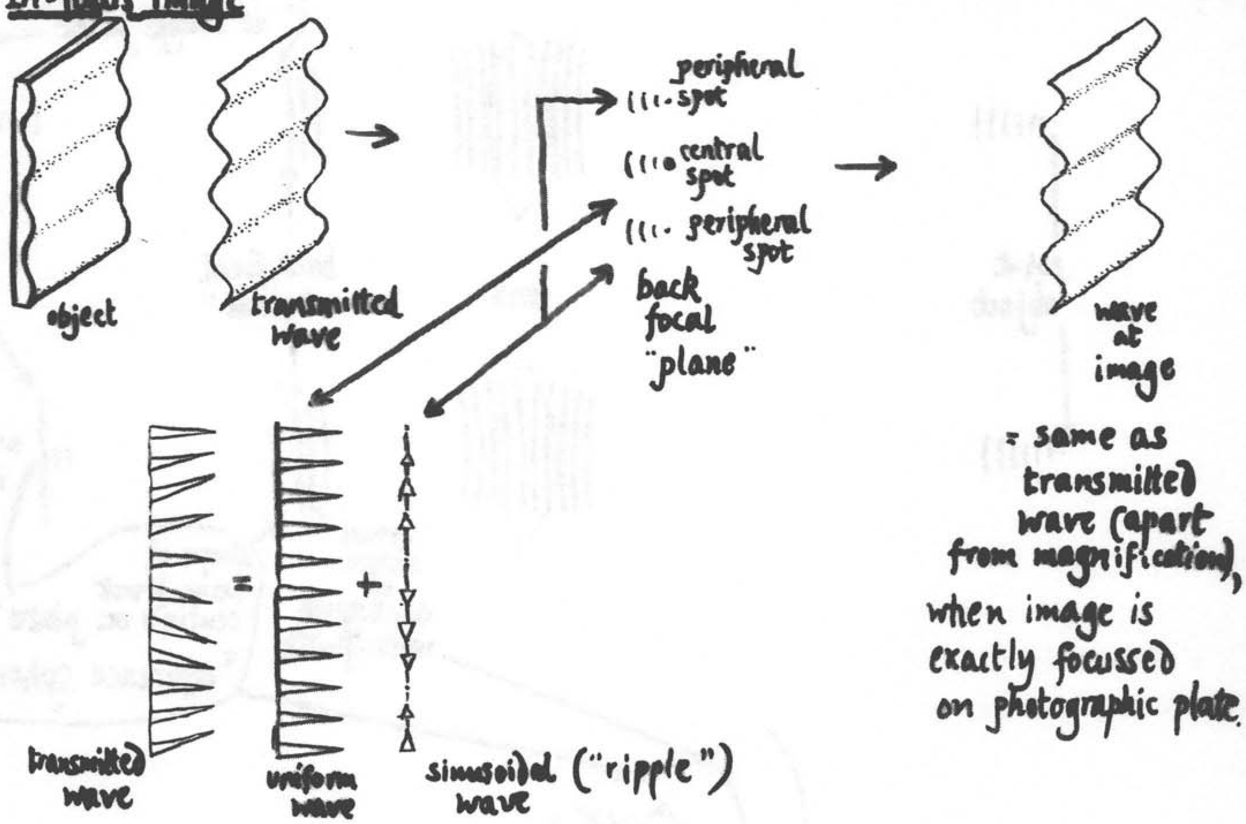
Simplest type is the thin sinusoidal phase object.

= a Fourier component of a phase object.

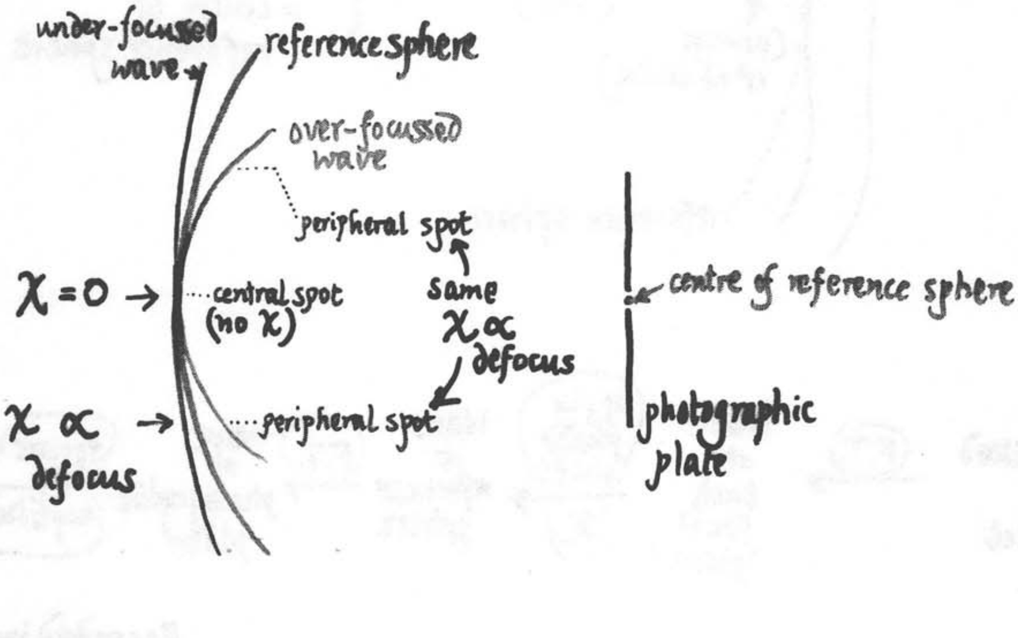


Imaging of a Weak Sinusoidal Phase Object

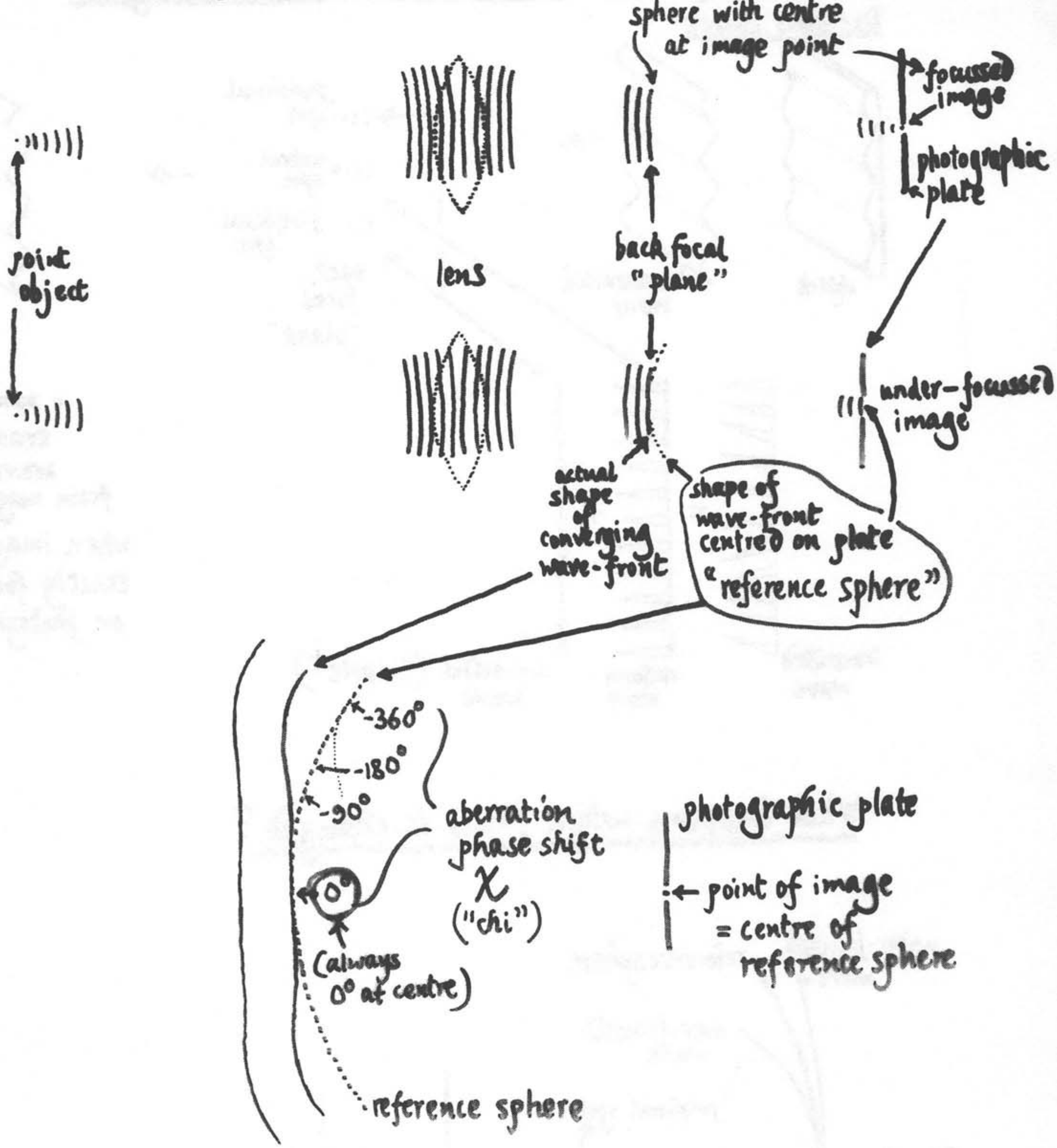
In-focus image



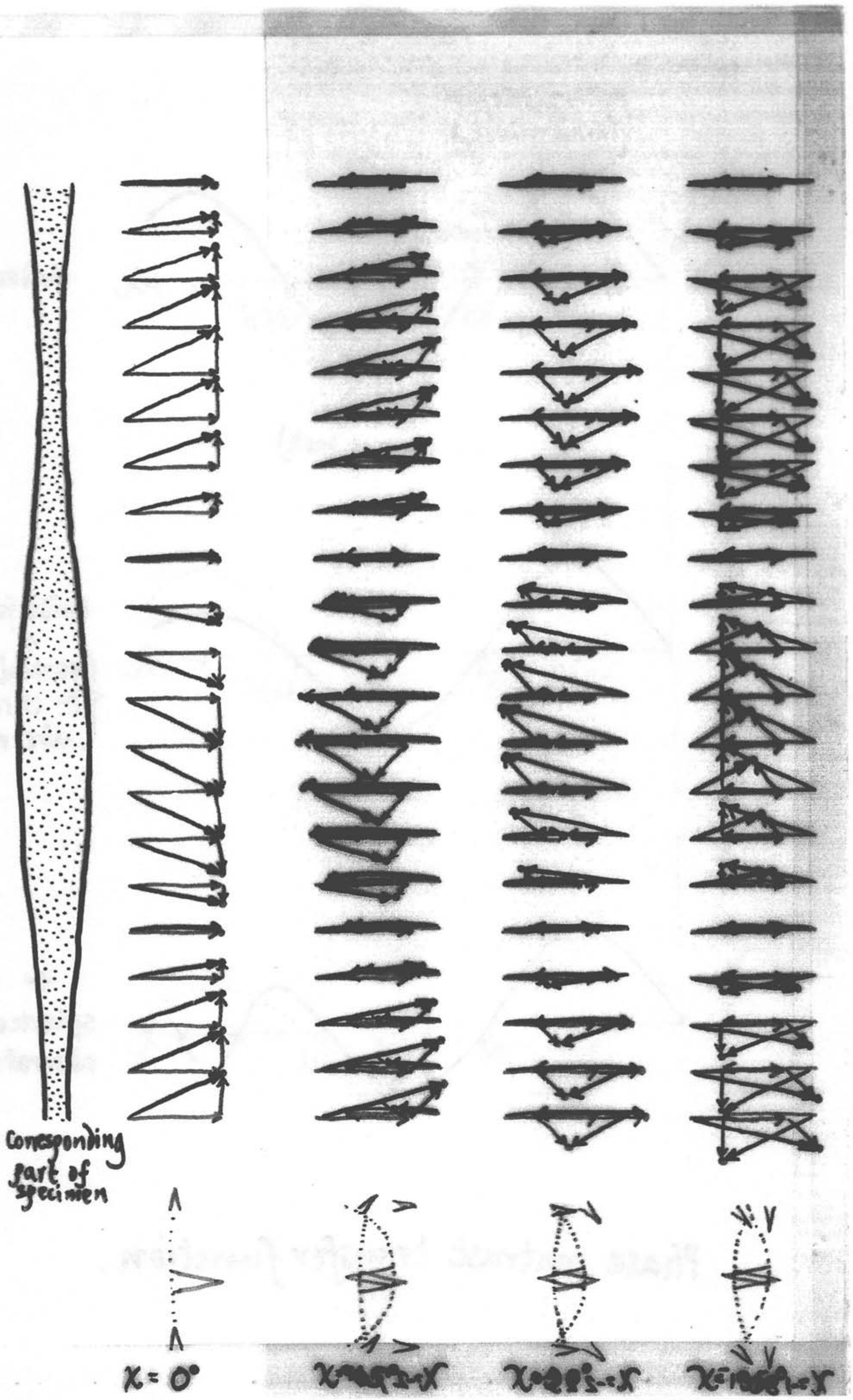
What happens when focus is changed?

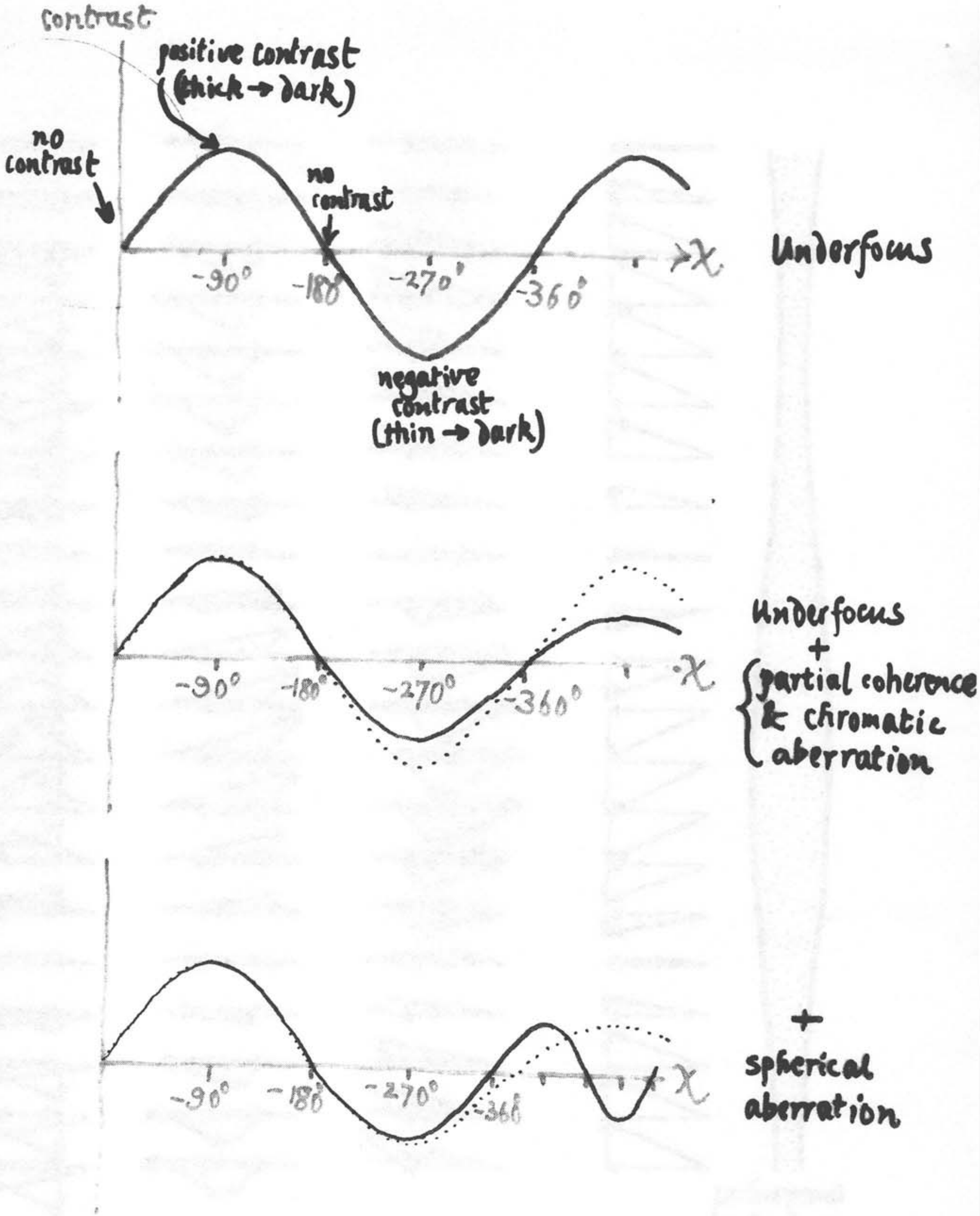


Imaging Aberrations



Wave transmitted by object $\xrightarrow{\text{F.T.}}$ Wave at back focal "plane" $\xrightarrow[\chi]{\text{apply phase shift}}$ Wave at reference sphere $\xrightarrow{\text{F.T.}}$ Wave at photographic plate $\xrightarrow[\text{amplitude}]{\text{square}}$ Recorded image


Corresponding part of specimen
χ = 0°



Phase contrast transfer function.

Effect of under-focus on image of a carbon film.

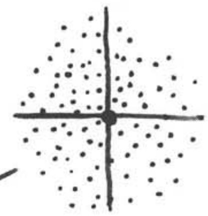
Reminder:

Wave transmitted by object (a) $\xrightarrow{\text{F.T.}}$ Wave at back focal "plane" (b) $\xrightarrow{\chi}$ Wave at reference sphere (c) $\xrightarrow{\text{F.T.}}$ Wave at photographic plate (d) $\xrightarrow[\text{amplitude}]{\text{square}}$ Recorded image (e)

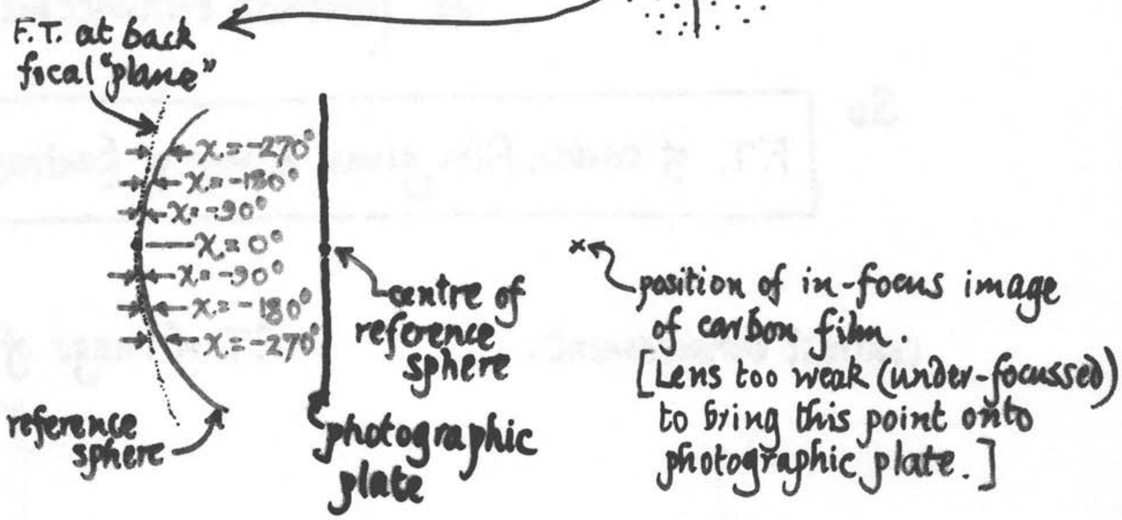
(a) Structure of carbon film is random; so transmitted wave is **noisy**.

(b) F.T. (noise) = noise; so

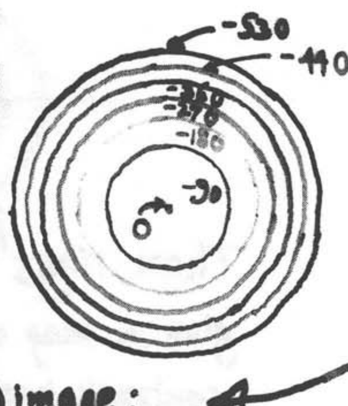
Wave at back focal "plane" =



(c)



Map of reference sphere:



Contrast enhancement to F.T. :

(d) Enlarged image:

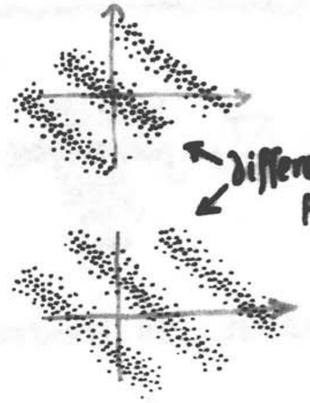
mostly circles whose size is 1/(spatial frequency with maximum enhancement)

⁘ = spatial frequencies with POSITIVE contrast

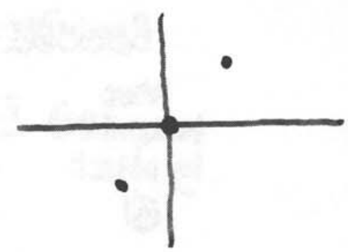
∴ = spatial frequencies with NEGATIVE contrast

F.T. of under-focussed image of carbon film.

+ve contrast



difference is PHASE, so both $\longleftrightarrow$



−ve contrast

Strength of spots

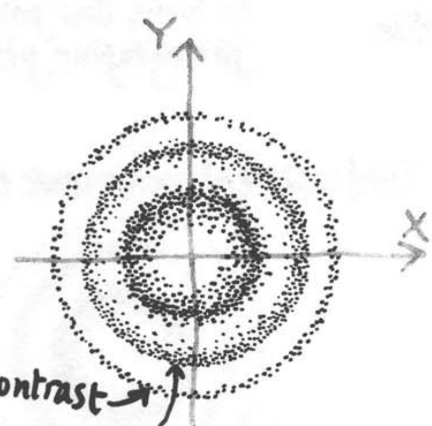
$\propto$ amplitude of spatial frequency

$\propto$ (contrast enhancement$)^2$

So

$\boxed{\text{F.T. of carbon film gives a map of (contrast enhancement)}}$

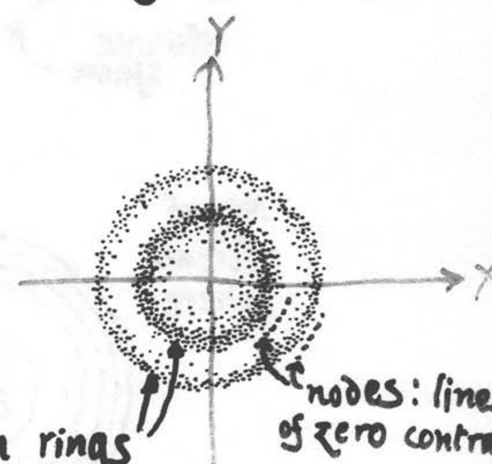
Contrast enhancement:



+ve contrast

−ve contrast

F.T. of image of carbon film:



nodes: lines of zero contrast

Thon rings give a map of the contrast transfer function

Numerical Filtering

Micrograph $\xrightarrow{\text{densitometer}}$ Optical density array

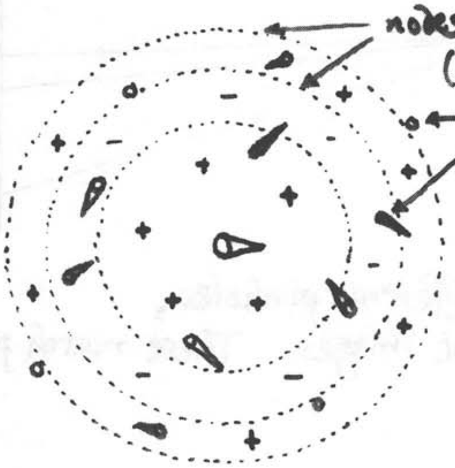
↓ fast F.T. program (FFT)

Fourier transform array – amplitudes / phases

↓ change amplitudes and phases

Filtered image of micrograph $\xleftarrow[\text{2) display}]{\text{1) FFT}}$ Filtered Fourier transform array

▶ Example of use: correction for under-focus



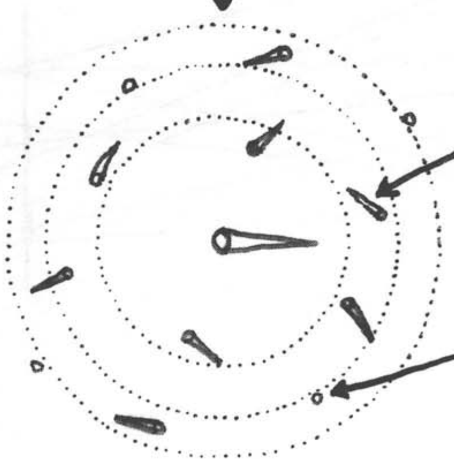
nodes of transfer function (found from Thon rings)

calculated amplitudes & phases at reciprocal lattice points

++ regions of positive contrast

-- regions of negative contrast

↓ divide by transfer function



vectors in middle ring change direction

(These were zero because they lie next to nodes of transfer function)

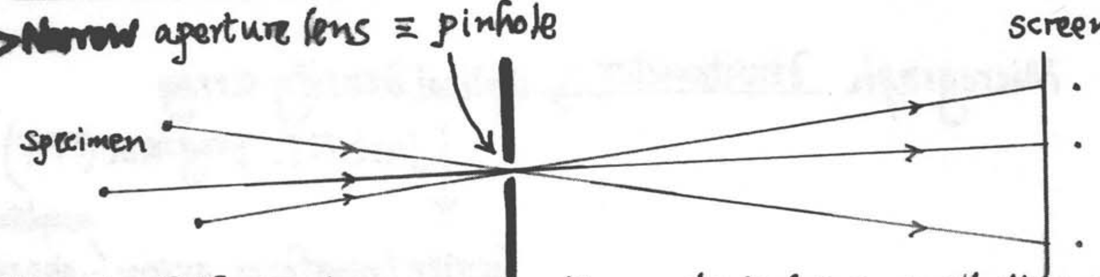
Problem: null vectors; use a different micrograph (taken at a different focal state) to find these.

FIELD-DEPTH

Geometrical Optics

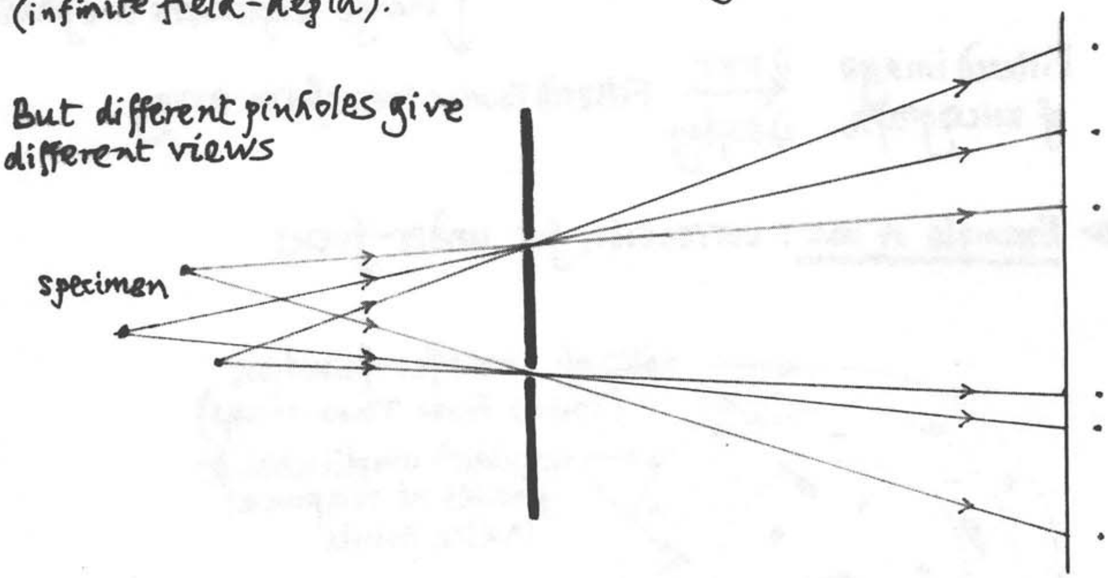
Field-depth depends on lens aperture

▷ **Narrow** aperture lens ≡ pinhole

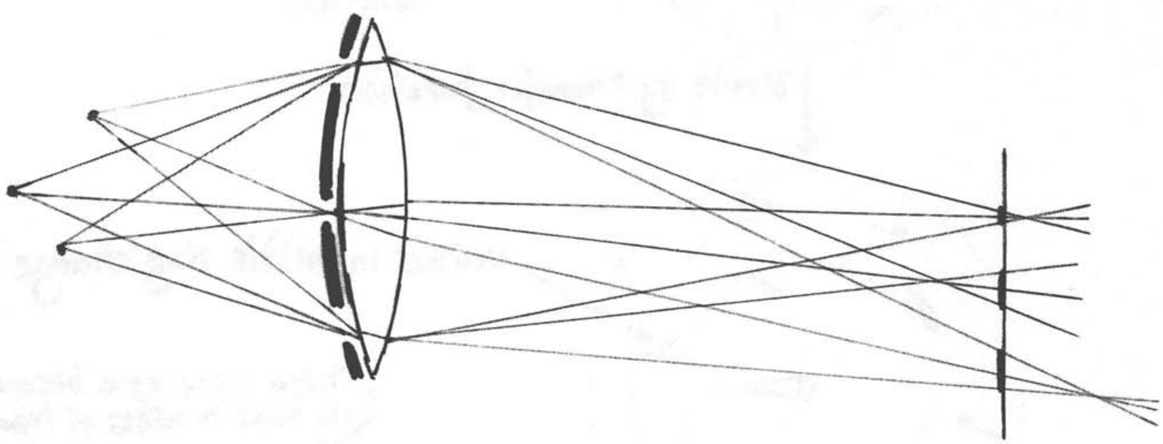


All parts of the specimen are simultaneously in focus at all distances (infinite field-depth).

But different pinholes give different views



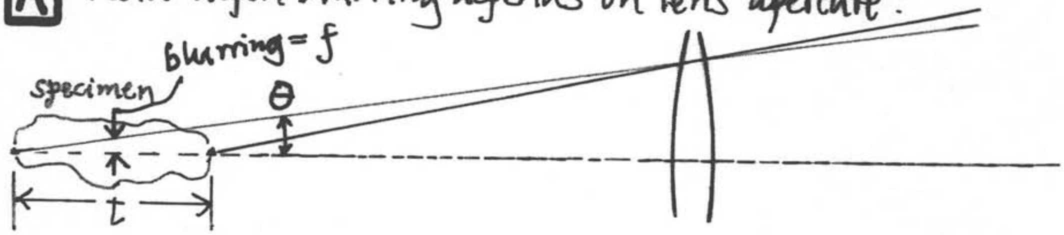
▷ **Wide** aperture lens ≡ many different pinholes, and superposing their different images. These match perfectly

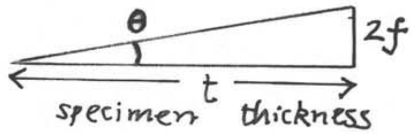


AT WHAT RESOLUTION is FIELD-DEPTH revealed as DEFOCUS?

OR/ when will micrograph no longer show specimen's PROJECTION, needed for conventional 3D reconstruction?

A Field-depth blurring depends on lens aperture:

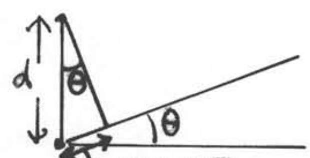




$$\frac{2f}{t} = \theta$$

So the blurring **f** is minimized by making the lens aperture angle **θ** small — but not too small, because:

B Small aperture produces diffraction blurring, resolution = **d**
d = spacing of points whose diffraction pattern just reaches aperture



$$\frac{\lambda}{d} = \theta$$

path difference for first diffracted ray = λ (wavelength)

C Field-depth blurring **f** is just detectable at a resolution **d** when $f = d$. Substituting in the two equations and combining them, $\frac{2d}{t} = \theta = \frac{\lambda}{d}$, or

$$\boxed{t = \frac{2d^2}{\lambda}}$$

simulations show that a better value is 1.4 (DeRosier)

depends on EM voltage: $\frac{1}{25}$ Å at 100 kV
$\frac{1}{50}$ Å at 300 kV

So field-depth defocus occurs at a smaller specimen thickness **t** when resolution **d** is small or wavelength **λ** is big (low voltage).