Macro-Molecular Fiber Diffraction

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Why Fiber Diffraction ?

- Atomic level structures from crystallography or NMR = "gold standard" for structural inferences

- But there is a large class of "fibrous proteins"

  e.g: actin, myosin, intermediate filaments, microtubules, bacterial flagella, filamentous viruses, amyloid, collagenous connective tissue

- Will not crystallize but can be induced to form oriented assemblies

- Some systems *naturally* form ordered systems

Dimensional hierarchy of Biophysical (X-ray) techniques

1D   Low angle solution or powder diffraction

large macromolecular assembles

2-3D   Fiber diffraction

fiber forming arrays – muscle, collagen, DNA, amyloids, various carbohydrates, often *super-macromolecular scale*
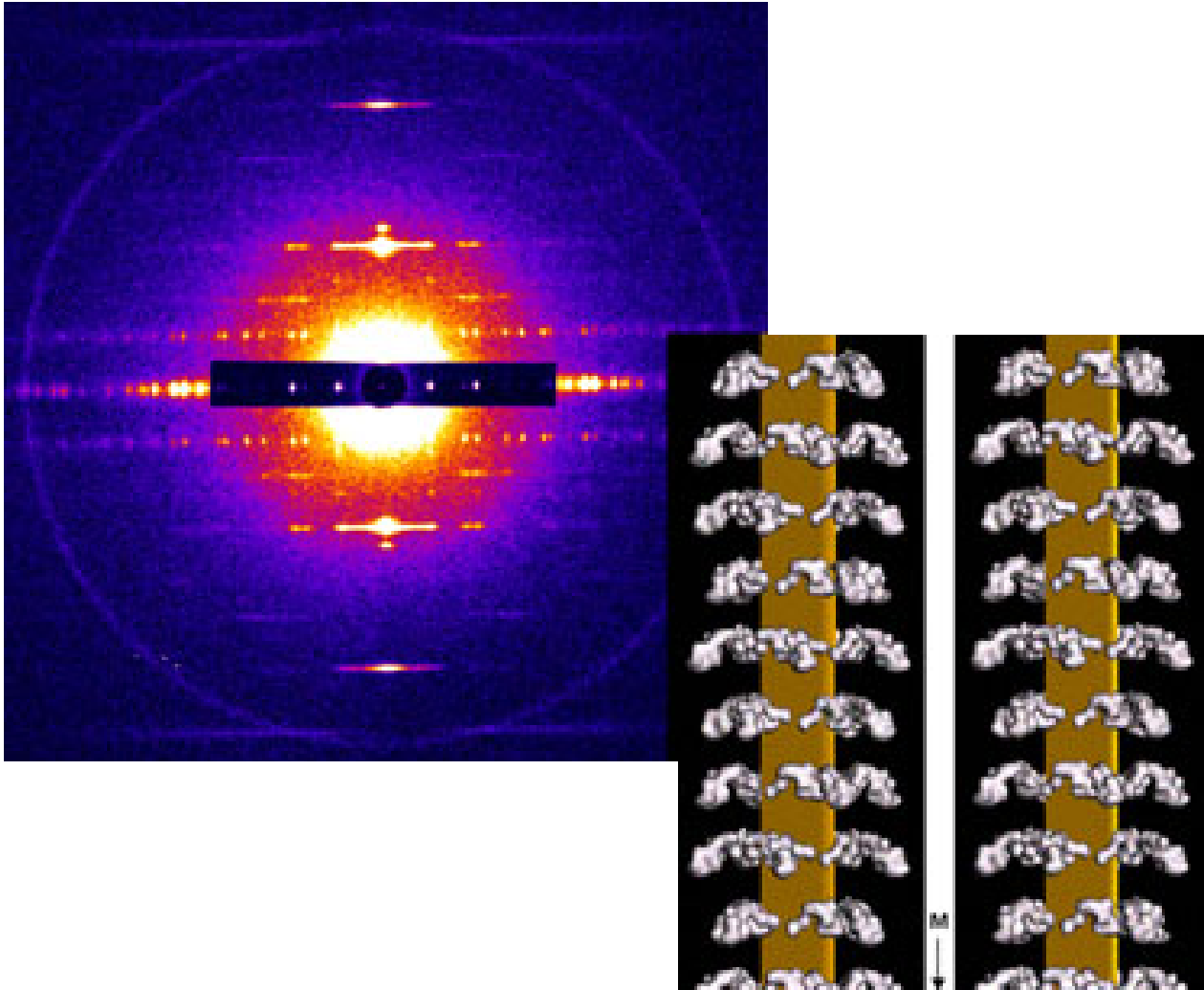
3D   Single crystal X-ray diffraction

anything that can crystallize, must be (initially) soluble, usually relatively small in comparison to the above, *molecular to macromolecular scale*
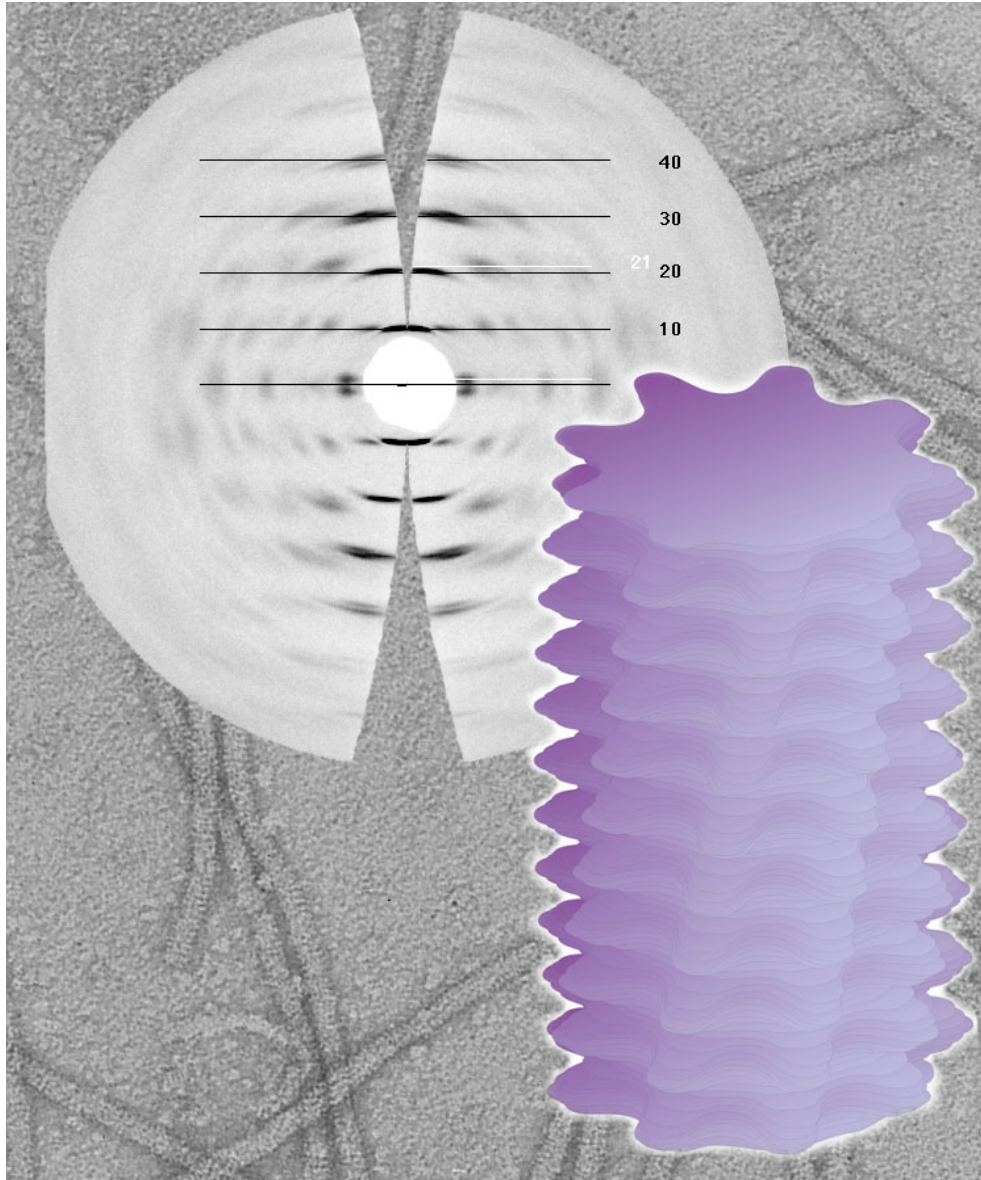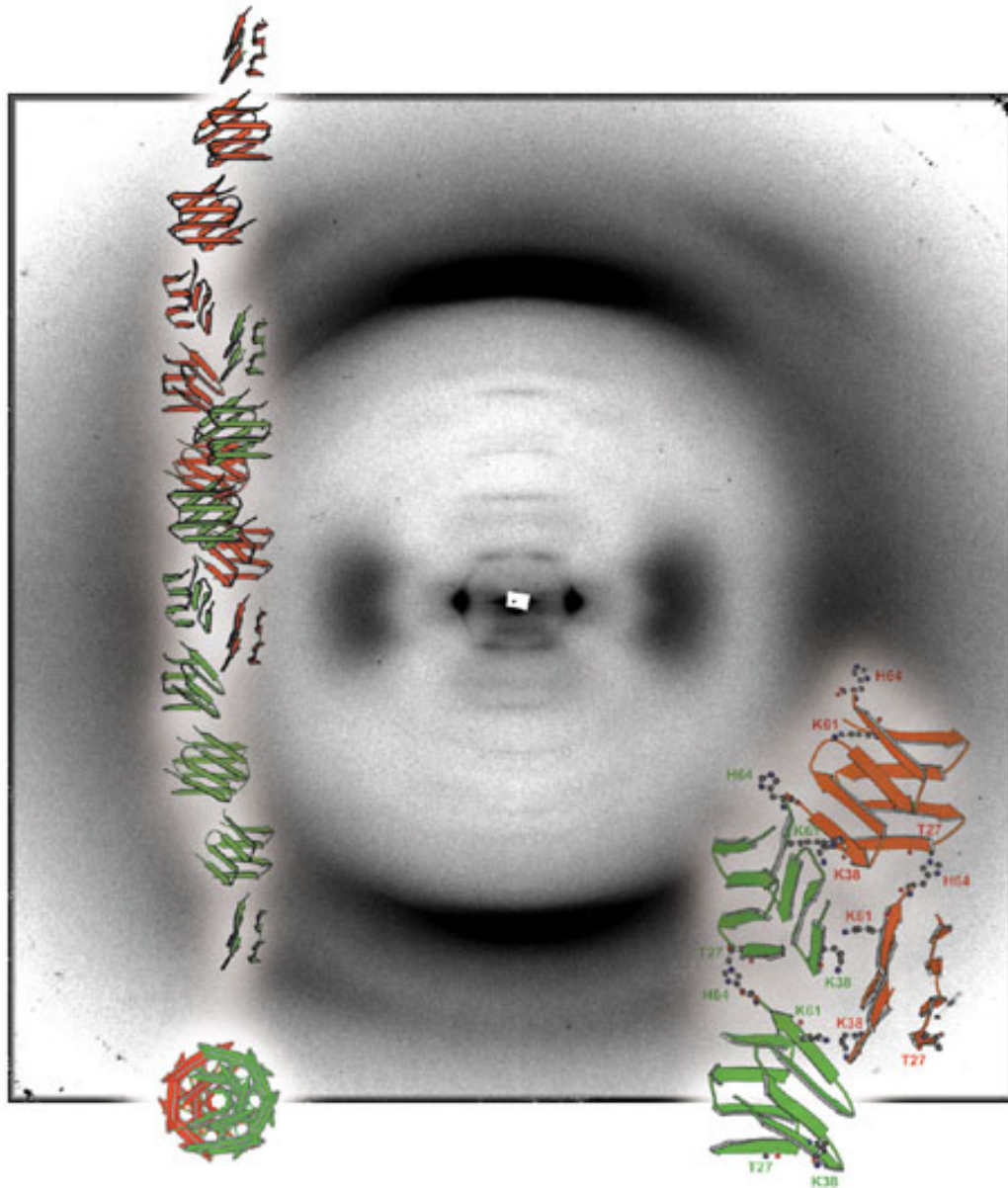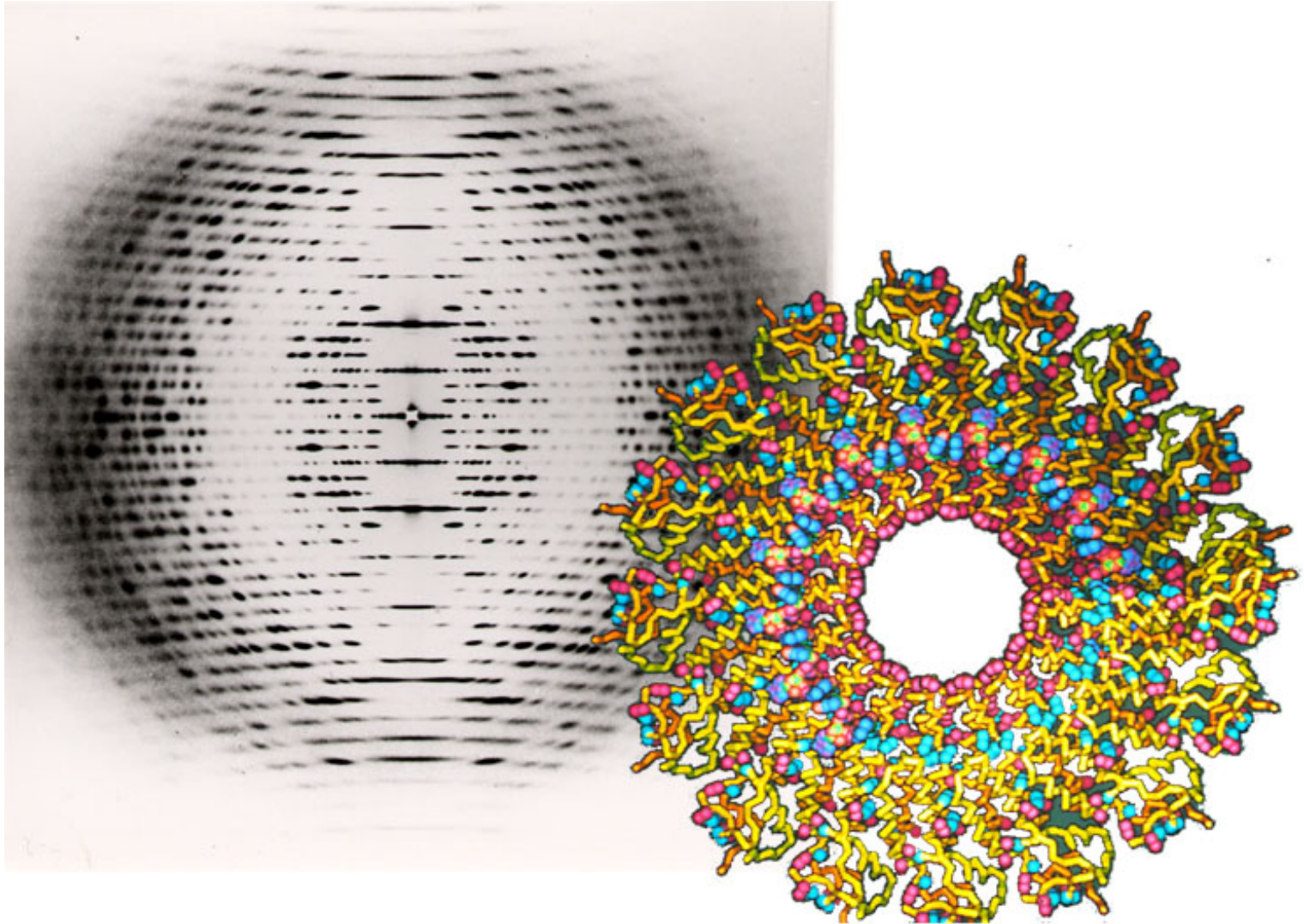
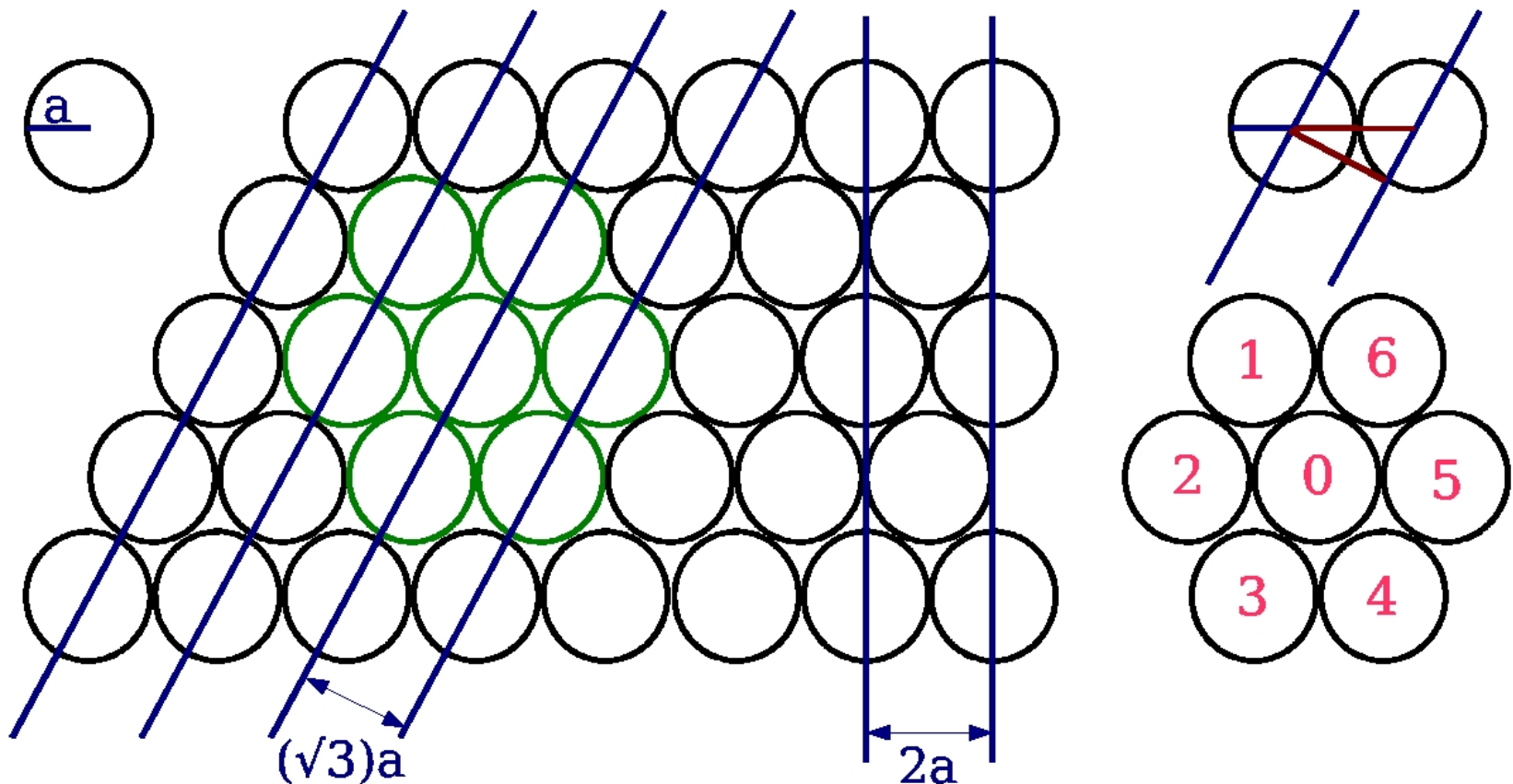Potato virus X

Insect flight-muscle
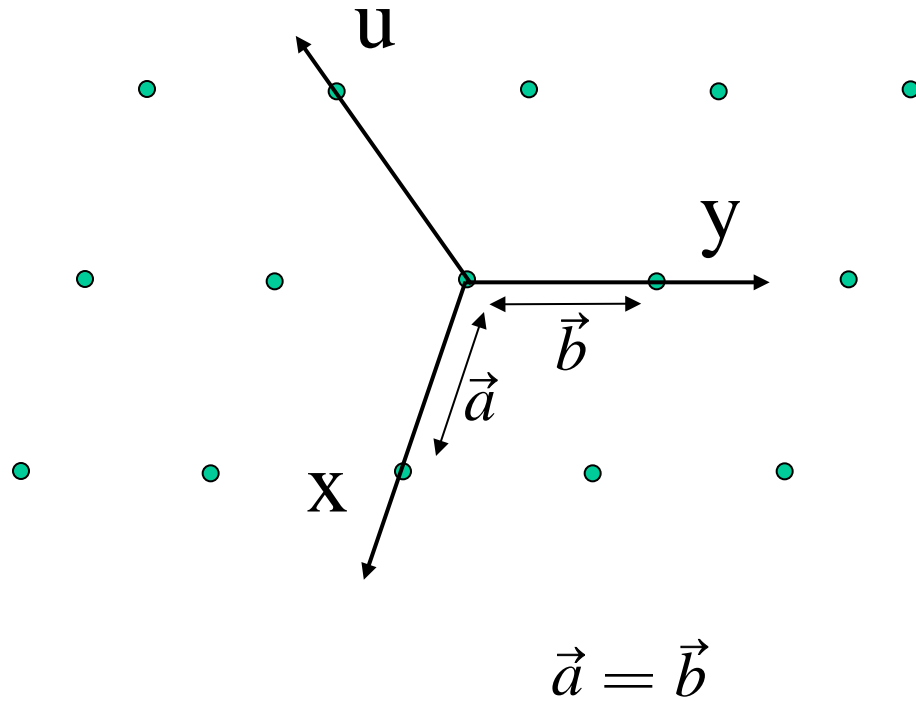
Engineered amyloid fiber

Tobacco mosaic virus

Fiber Diffraction Theory
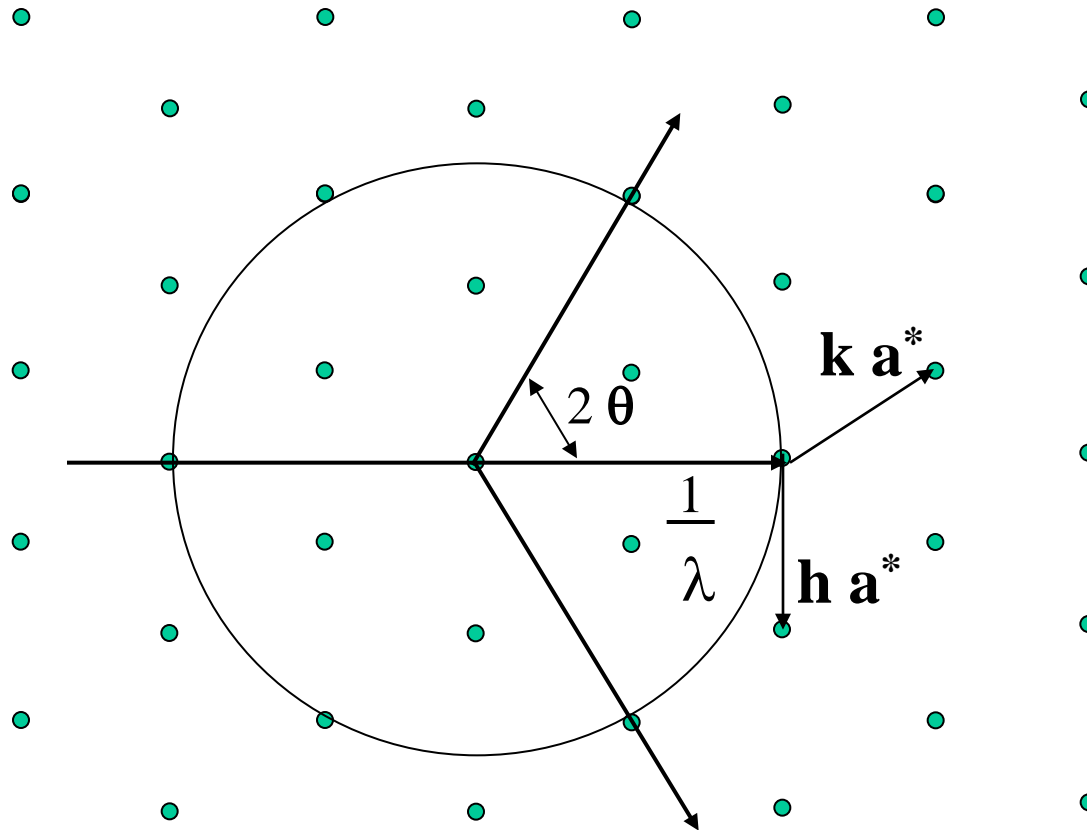## Fibers (essentially rods/cylinders) usually hexagonally packed

Hexagonal Lattice variables
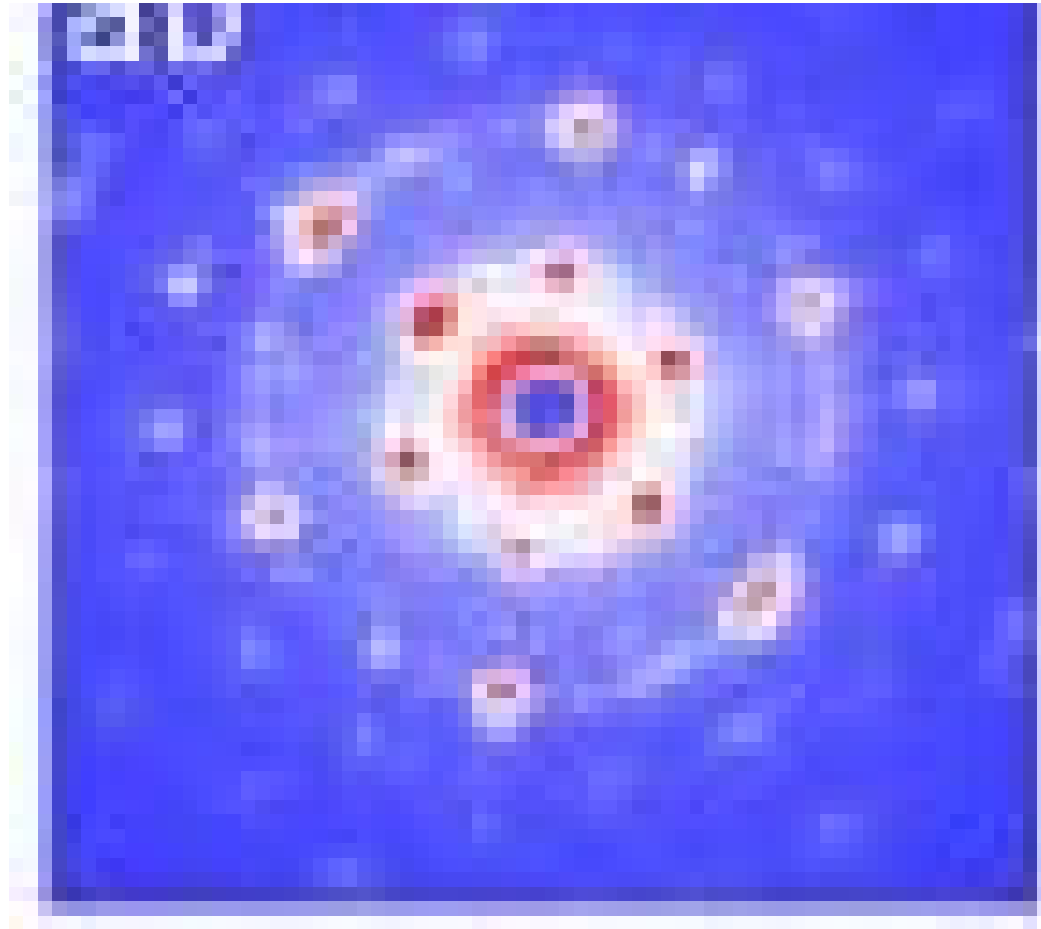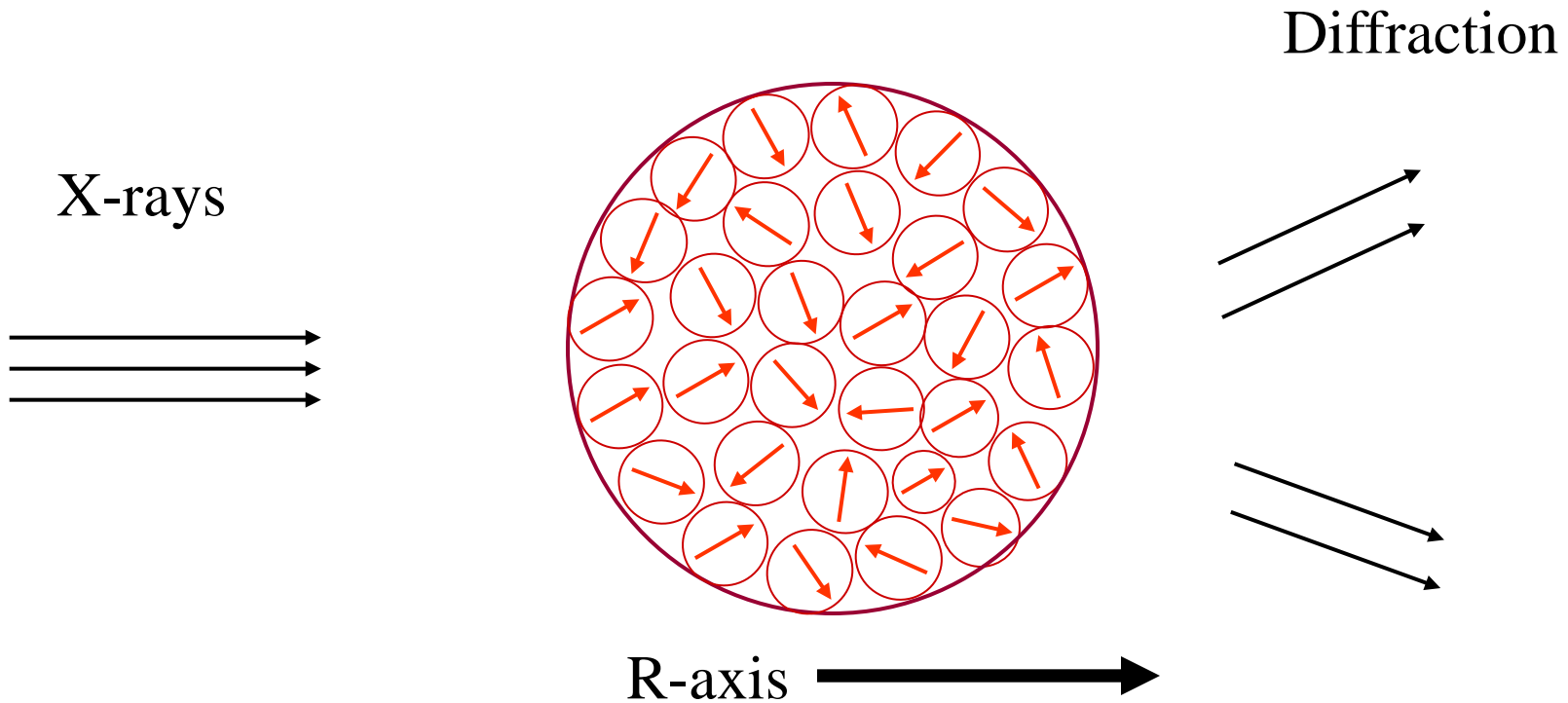

$$\vec{a} = \vec{b}$$

Ewald Sphere

End on view of hexagonal reciprocal lattice

Fiber Cross-section

X-rays

Diffraction

R-axis
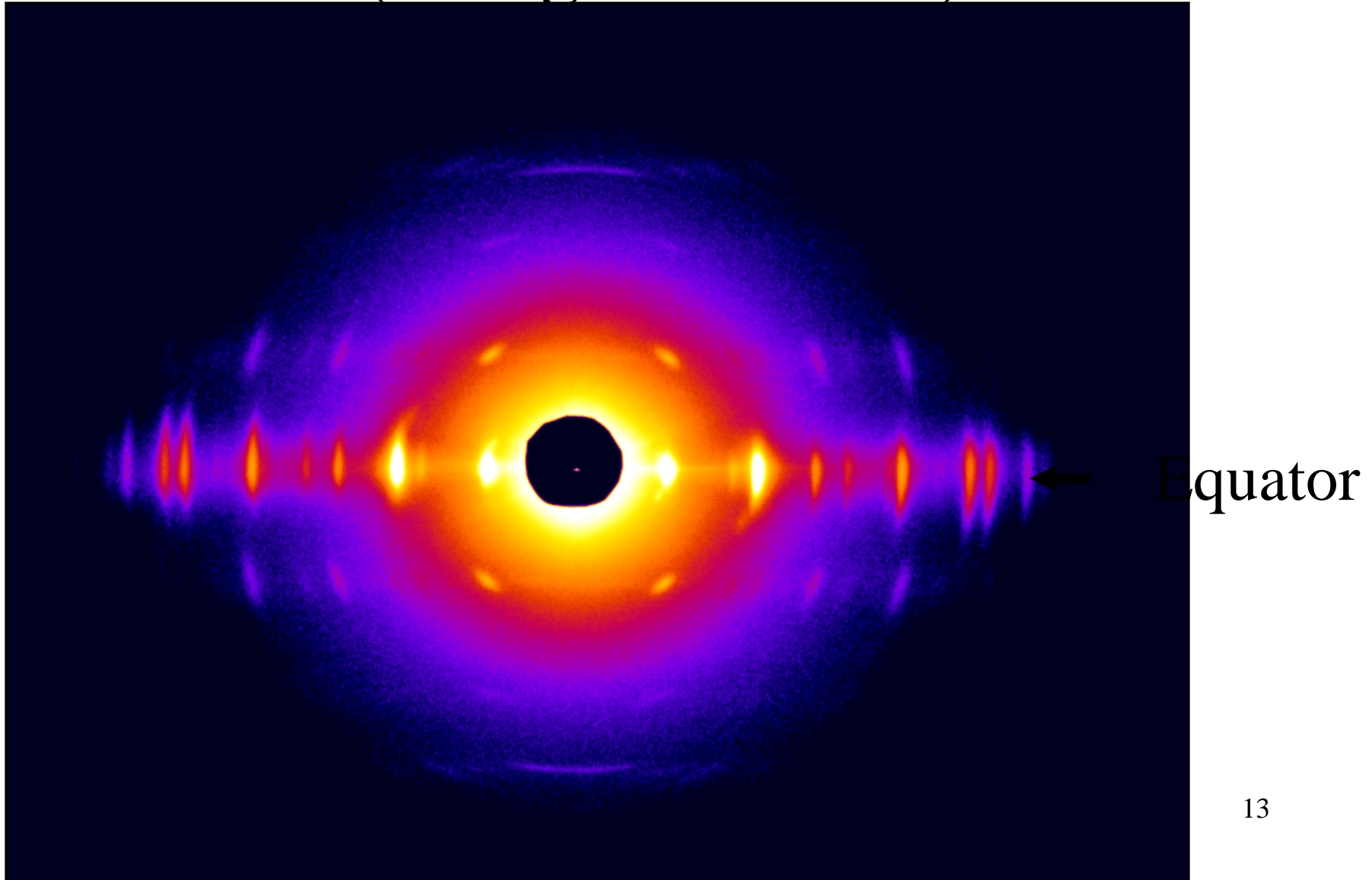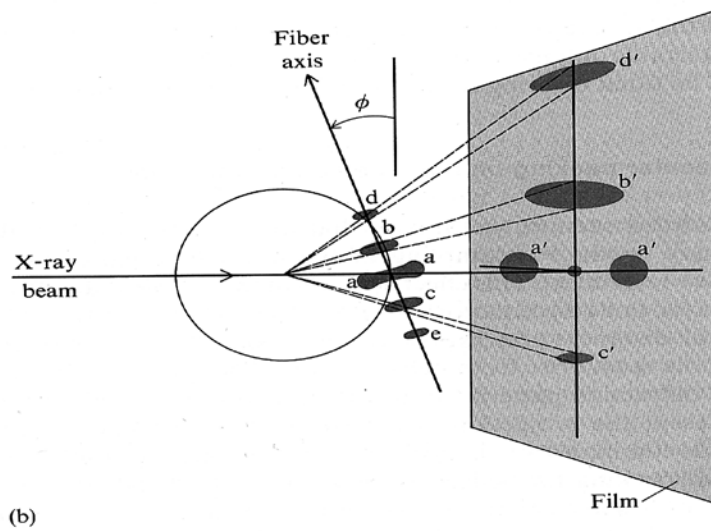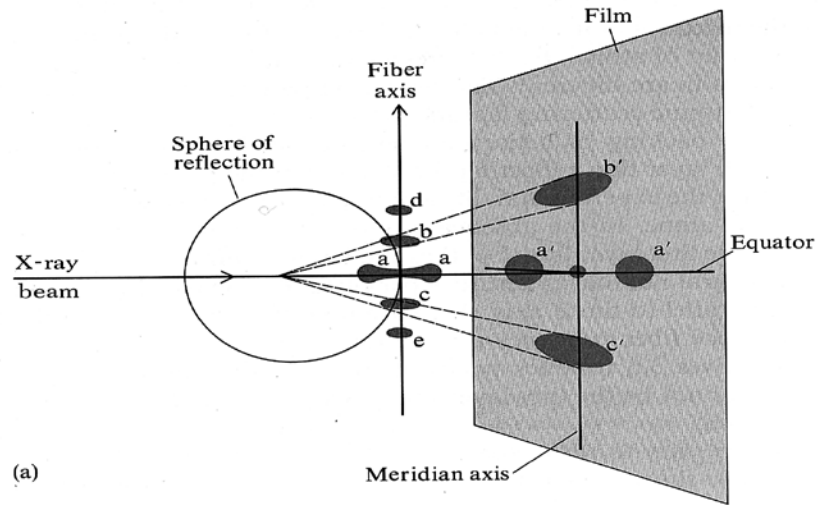
Crystallites randomly orientated around the axis
perpendicular to the fiber axis (the '**R**' – axis: Sum of
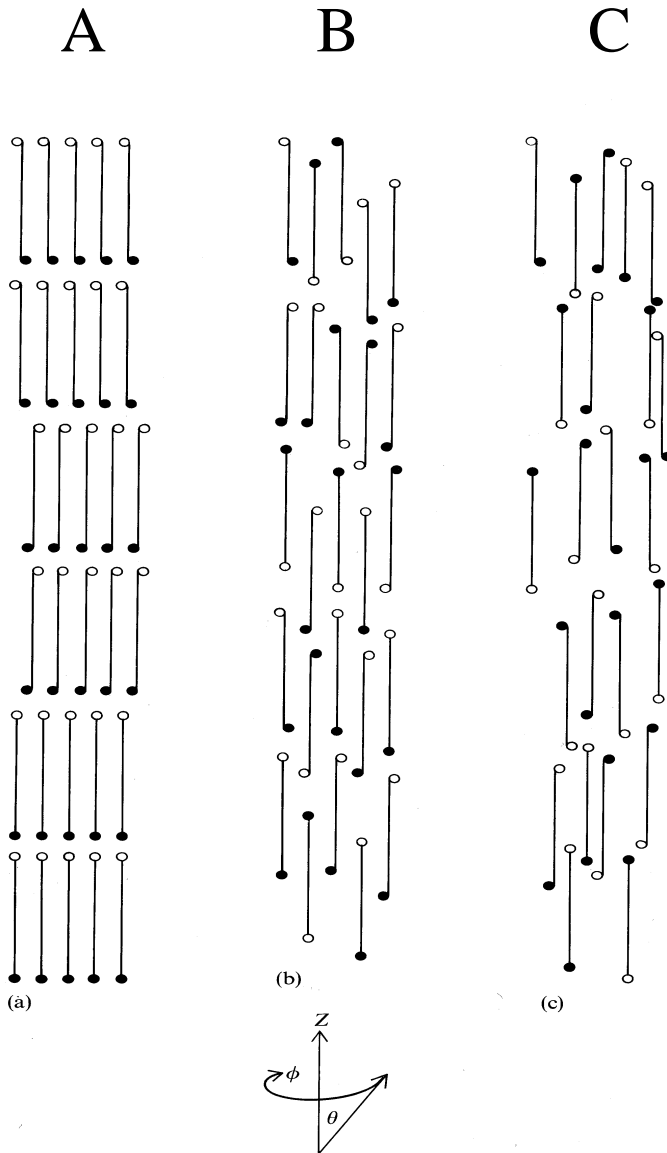Rotation of crystallites assumed = 360 degrees)

12

Fiber diagram - Insect Muscle (hexagonal lattice)

Equator

Geometry of Fiber Patterns

14

A B C
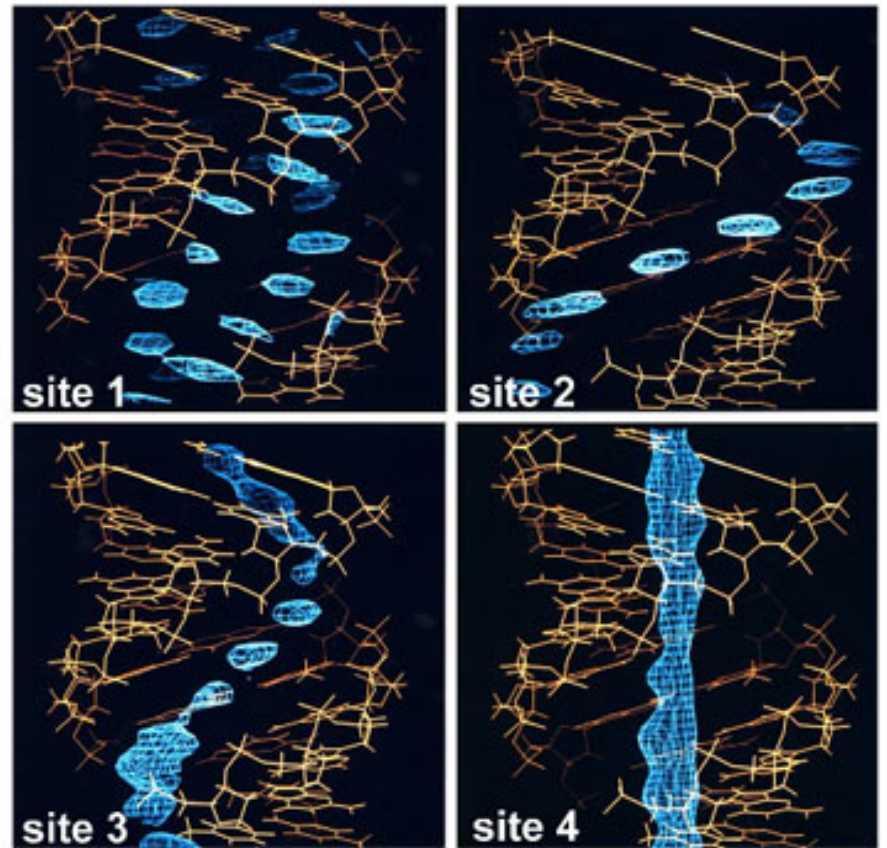
Ordering in Fibers:

A - Crystalline fiber

B Semicrystalline Fiber

C Non-crystalline fiber

(a) (b) (c)

14-1

$$\langle I(s)\rangle = \langle |F_m(S)F_L(S)|^2\rangle$$

Average over all molecular and lattice orientations

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B-form DNA - Non-crystalline

site 1

site 2

site 3

site 4
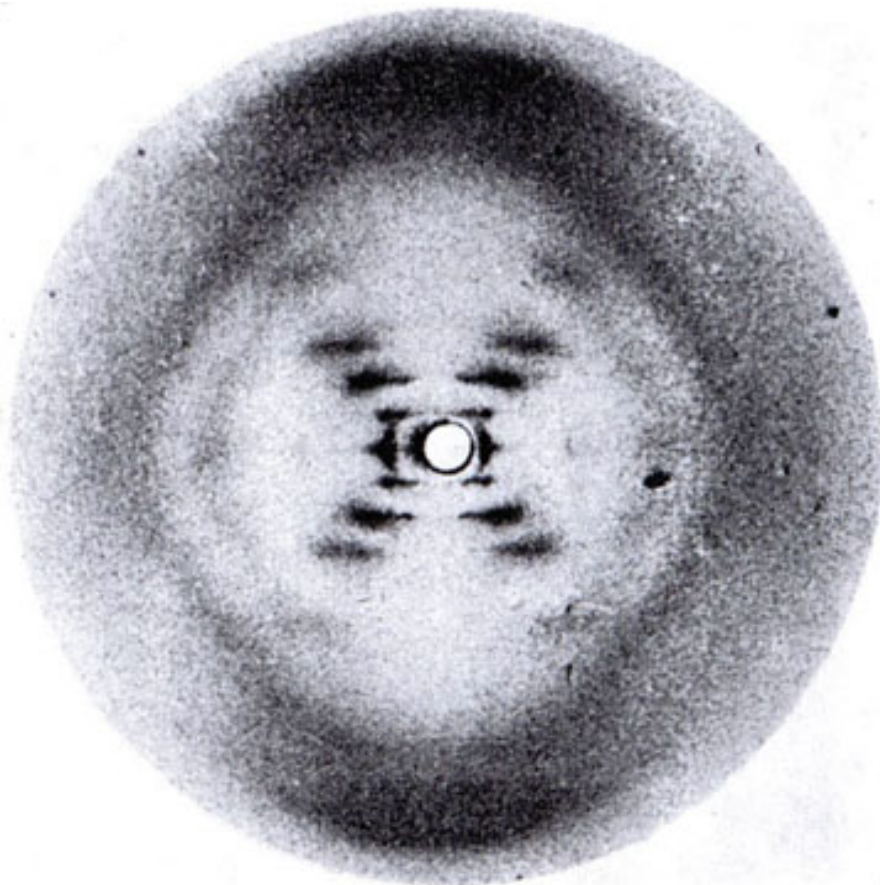
Insect flight-muscle - crystalline
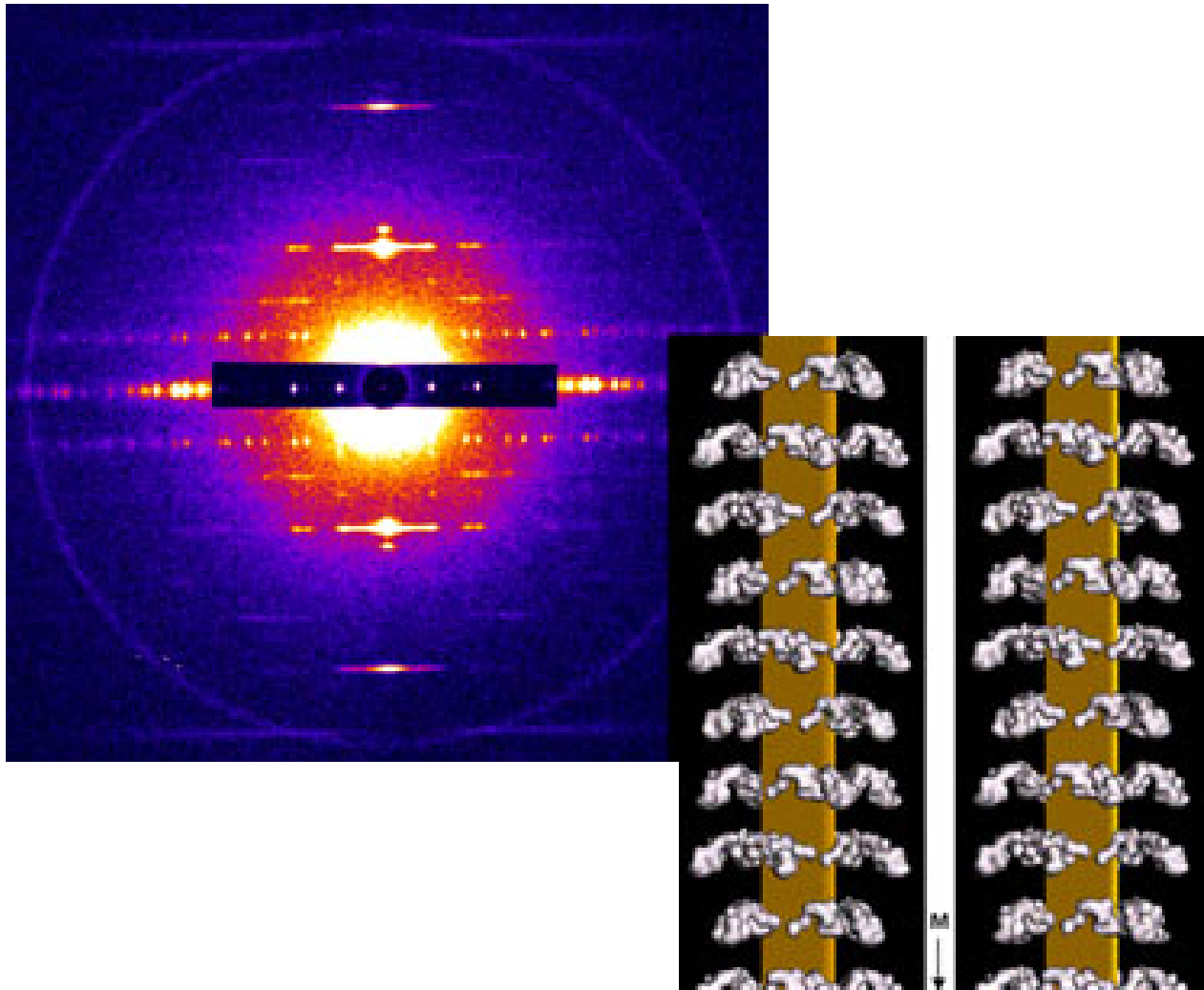
Fibrous Proteins Usually Show Helical Symmetry

axis
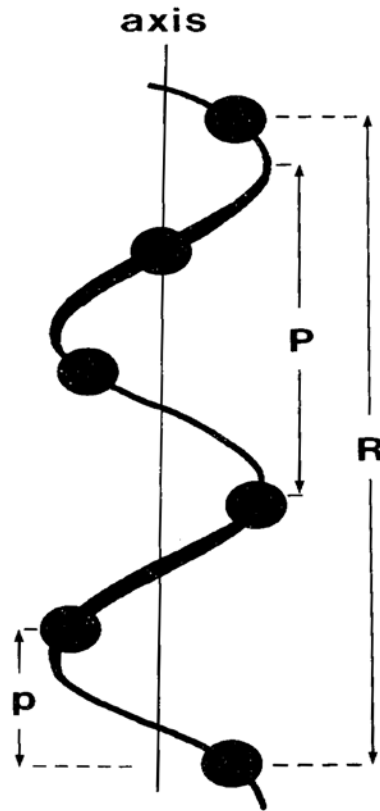
P = pitch

p = subunit axial translation distance

R = true repeat distance

P

R

p

a   b   c

d   e   f

$$I = |F_M F_L|^2$$

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Diffraction from a continuous Helix

Bessel Functions and Layer lines
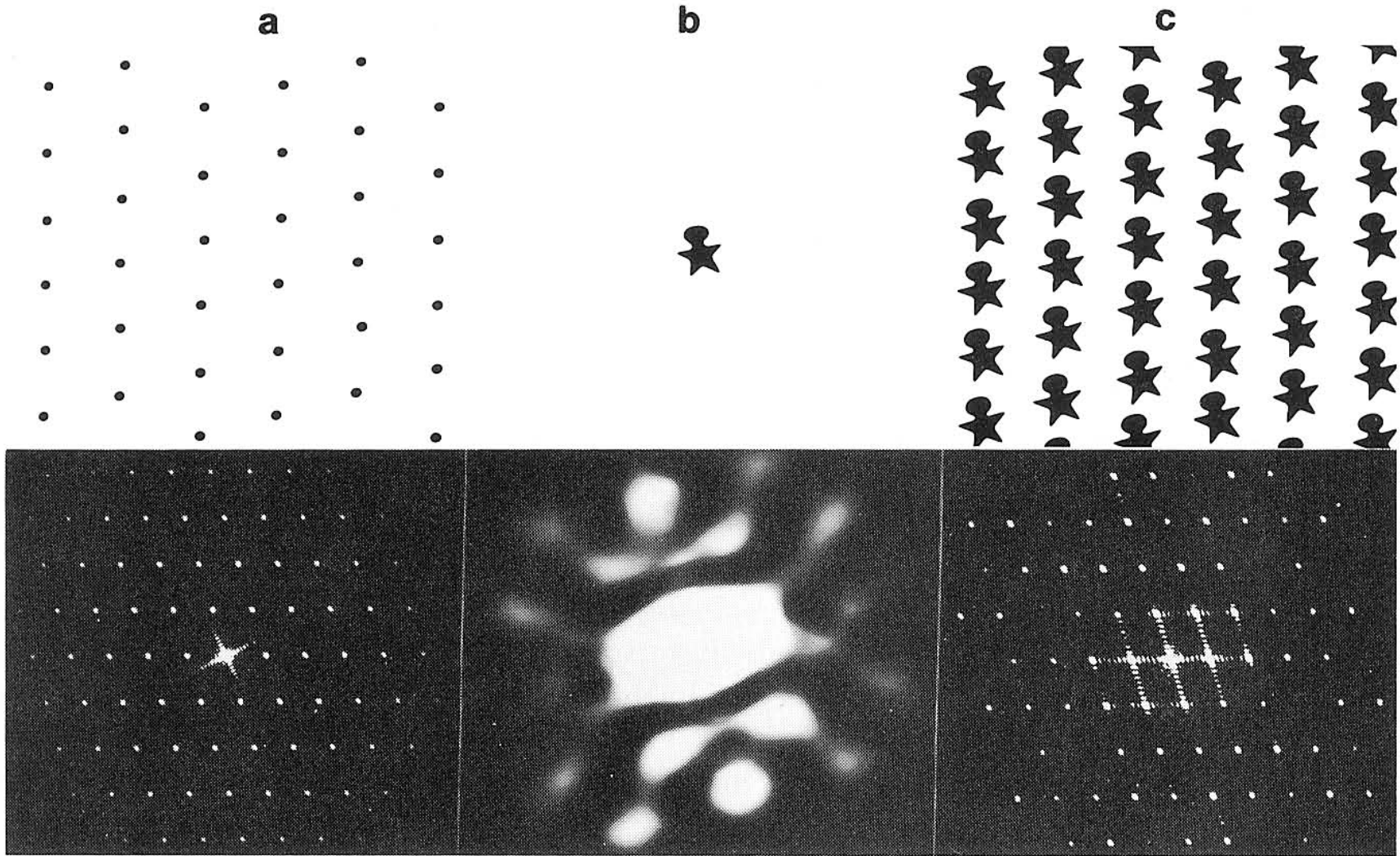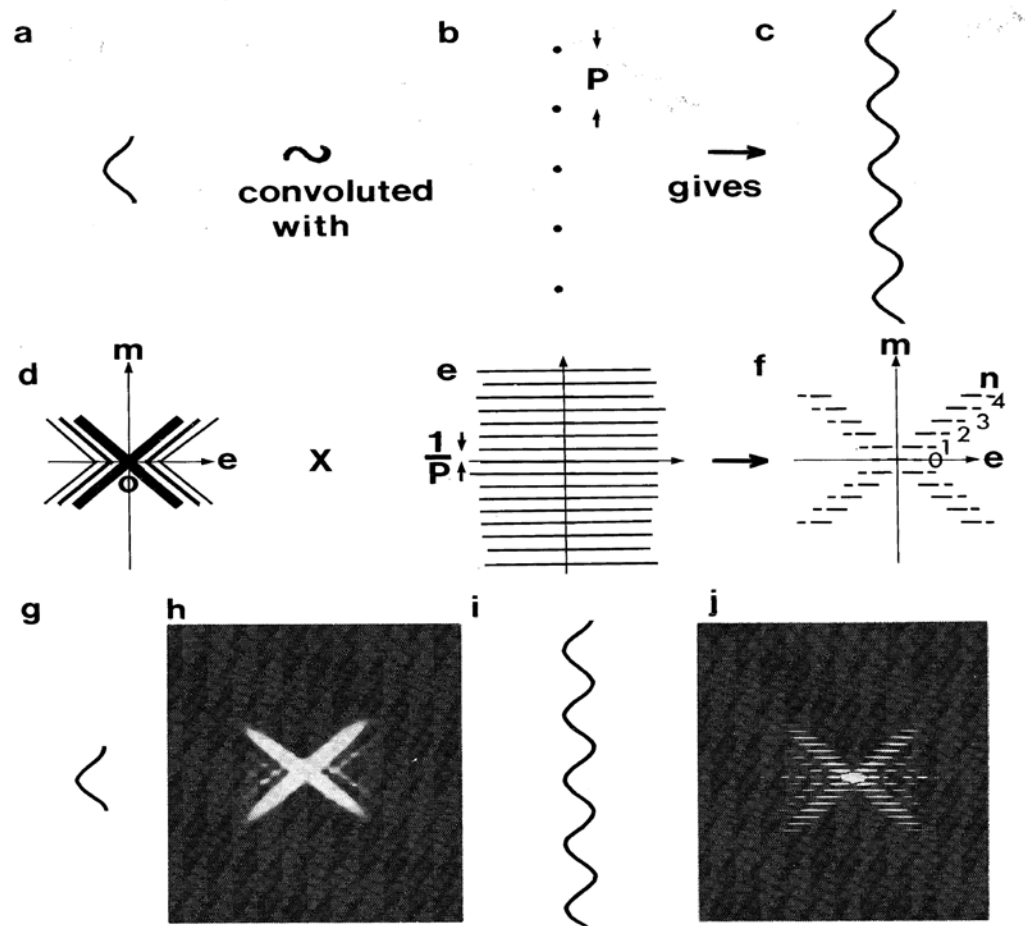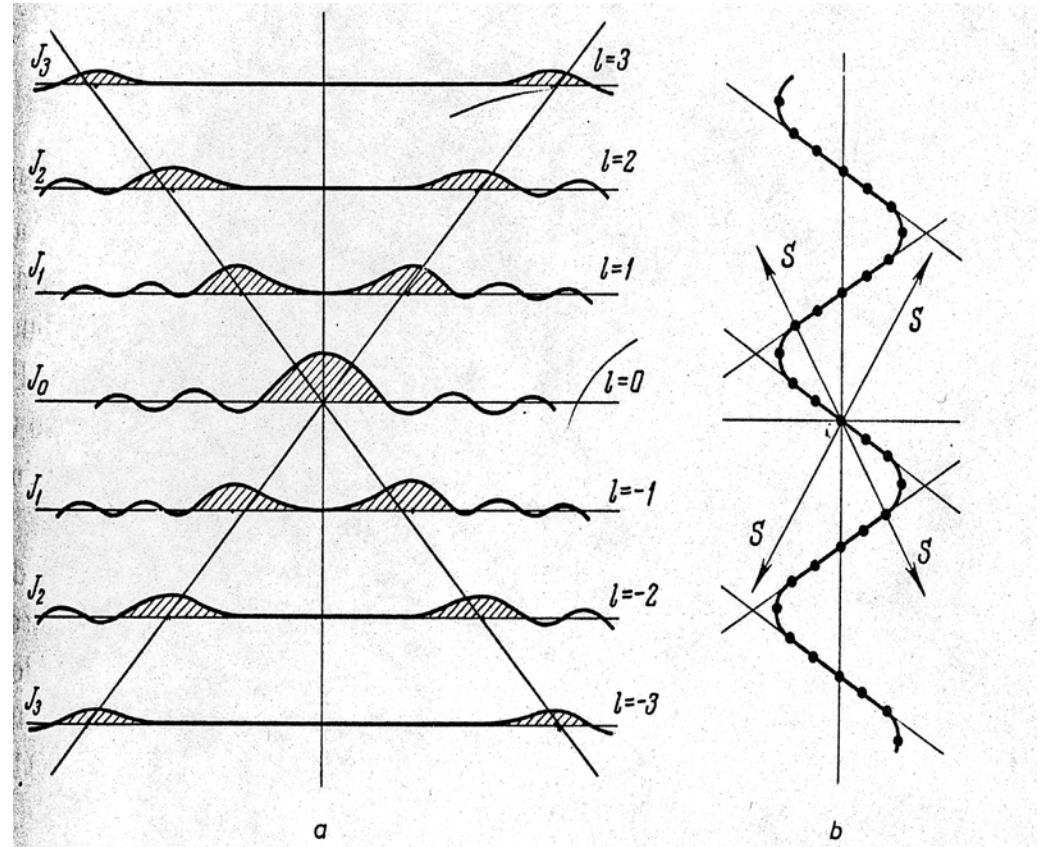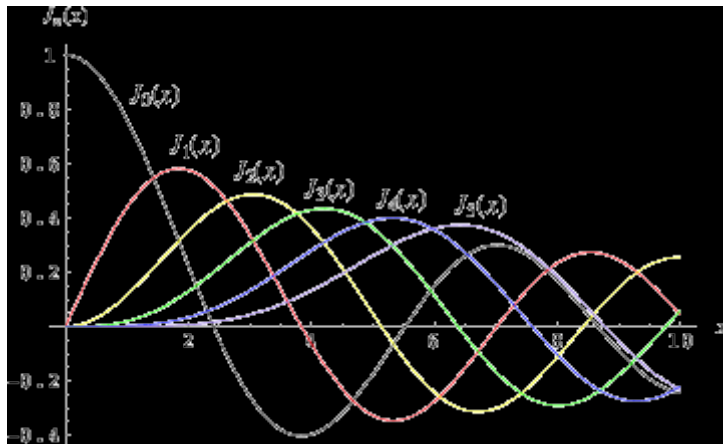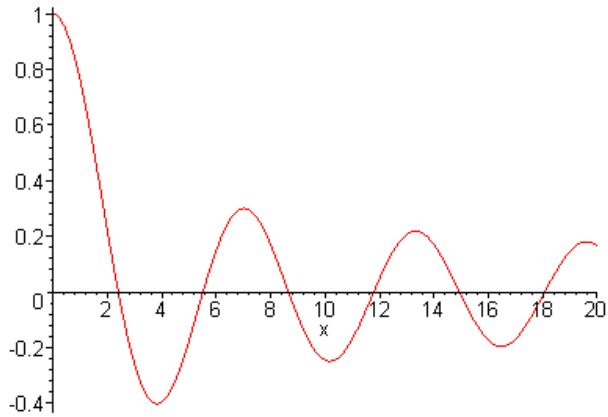

Transform of a cylinder

Diffraction From a Discontinuous Helix

A set of points that are regularly spaced along a helical path

Figure 3.17 Generation of the form of the diffraction pattern from an 18/5 α-helix. Such a helix (c) can be considered as the product of a continuous helix of pitch 5.4 Å (a) and a set of density planes spaced 1.5 Å apart (b). The helix cross diffraction pattern (d) from (a) needs to be convoluted with the diffraction pattern from (b), a set of meridional points (e), to give the diffraction pattern (f) from the α-helix. Strong reflections occur on layer lines for which l is 5, 13, and 18.

Diffraction From a Discontinuous Helix

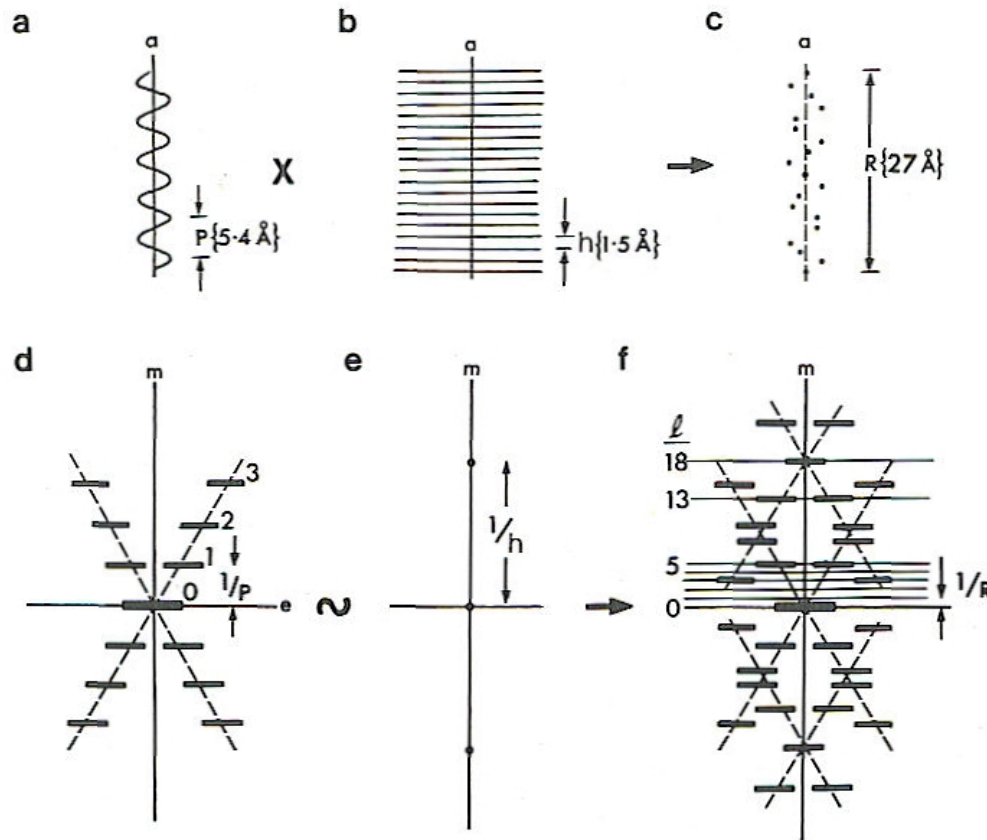A set of points that are regularly spaced along a helical path

Figure 3.17 Generation of the form of the diffraction pattern from an 18/5 α-helix. Such a helix (c) can be considered as the product of a continuous helix of pitch 5.4 Å (a) and a set of density planes spaced 1.5 Å apart (b). The helix cross diffraction pattern (d) from (a) needs to be convoluted with the diffraction pattern from (b), a set of meridional points (e), to give the diffraction pattern (f) from the α-helix. Strong reflections occur on layer lines for which l is 5, 13, and 18.
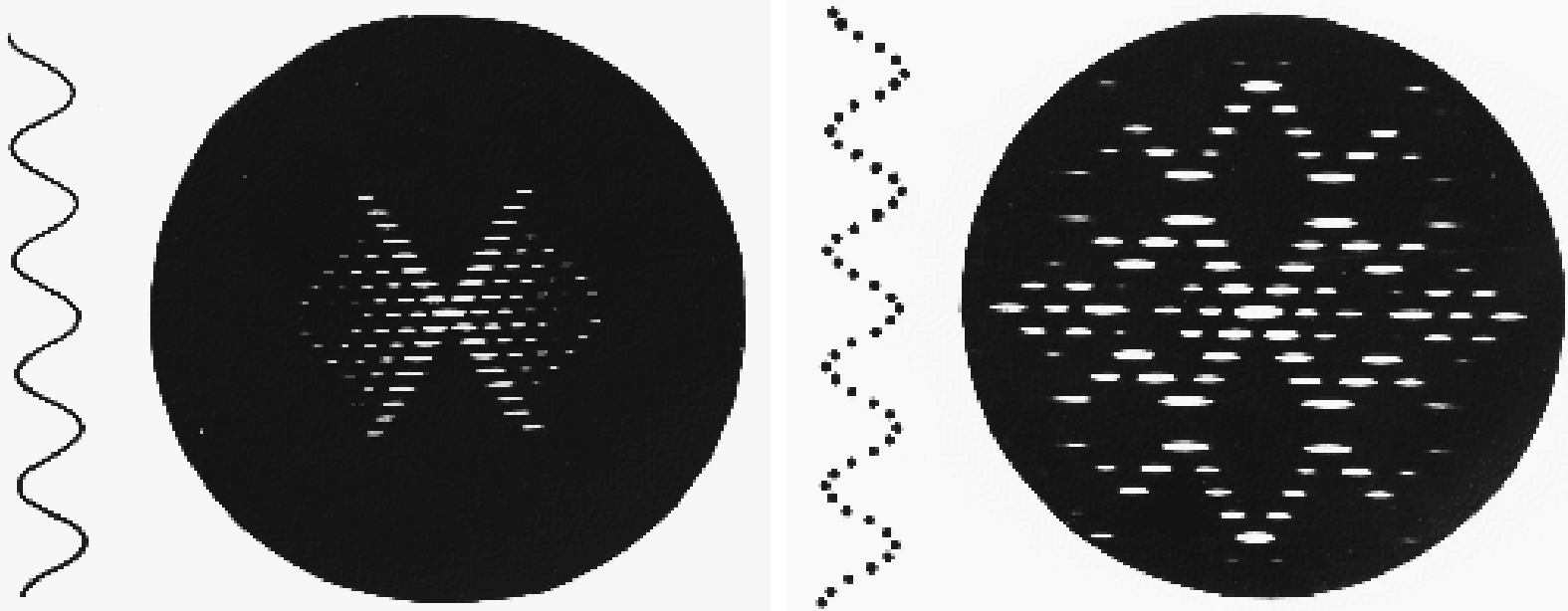
Diffraction from a helix: comparison

The main effect of shifting from a continuous to a discontinuous helix is to introduce new helix crosses with their origins displaced up and down the meridian by a distance $1/p$
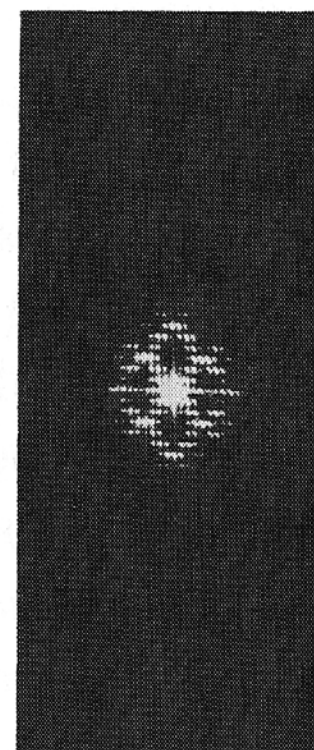
Helical Selection Rule

Which Bessel function order will turn up on what layer line for a more complicated helix?

For a non-integral helix (repeats after two or more turns), with **u** subunits in **t** turns, allowed Bessel functions on layer line *l* are:

$$l = \mathbf{m.u} \ + \ \mathbf{n.t}$$

m is an integer indicating translational periodicity index of helix lattice

Crystals of Helical Molecules

a   b   c   d   e
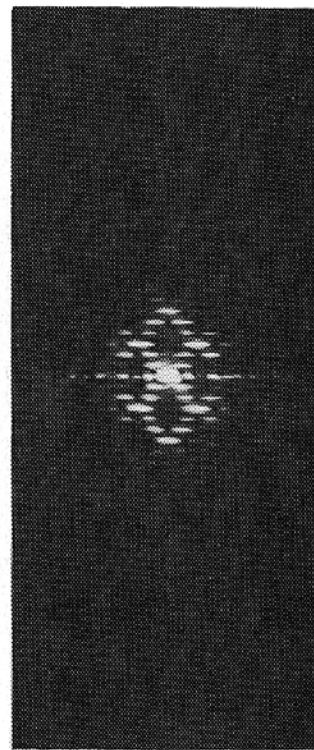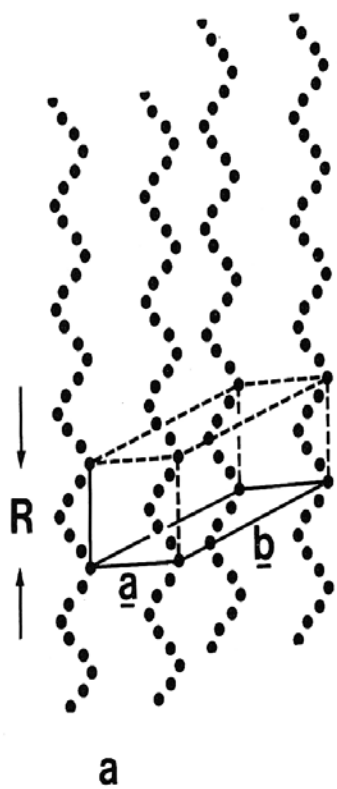
Multi-Stranded (coiled coil) Helices

a   b   c   d

If N strands
Only every
Nth Layer -
line allowed

Fiber Diffraction Often Just Used to Find Gross Molecular Parameters
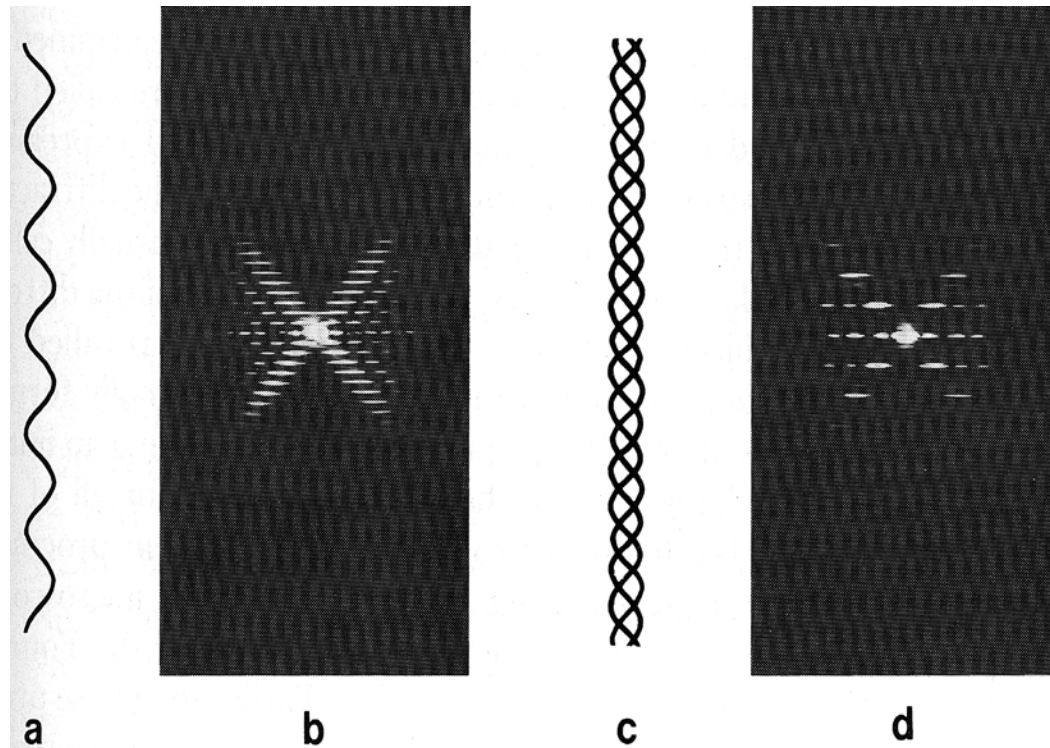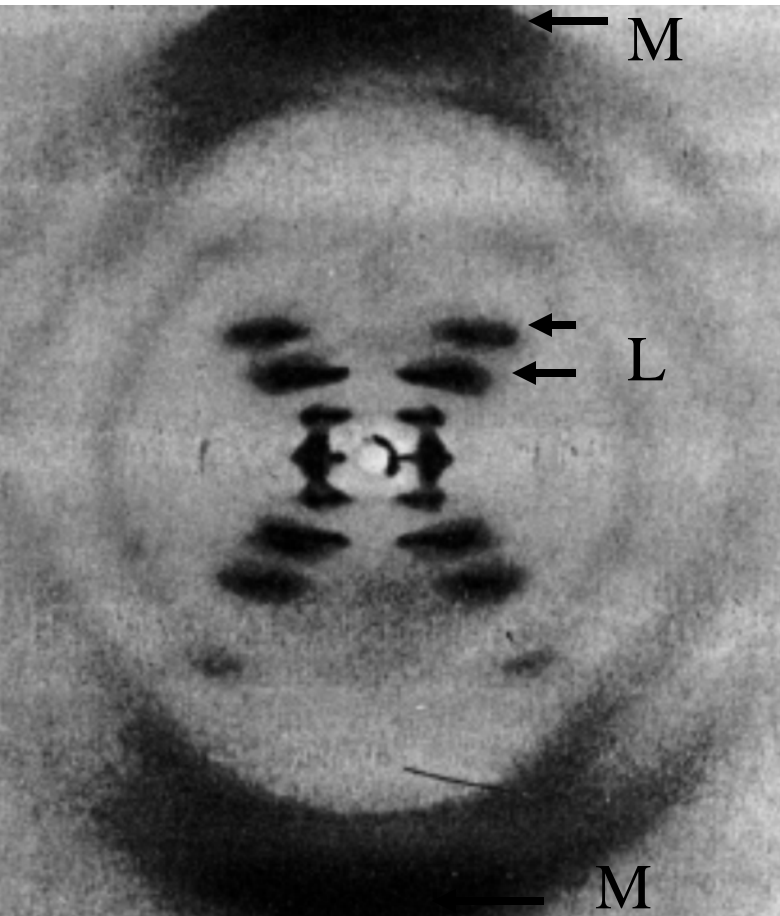
- In many cases one can make structural inferences without a full-blown structure solution

- Helical parameters in Polyamino-acids and nucleic acids

- Topology of viruses and other large molecular complexes

- Test hypotheses concerning influence of inter-filament lattice spacing

Rosalind Franklin's Pattern from B-DNA

Layer lines (L) separated by 34 Å nm

Meridional (M) reflection at 3.4 Å

=> 10 residues/turn

Diffraction from Poly L-Alanine -α-helix

(a)

(b)
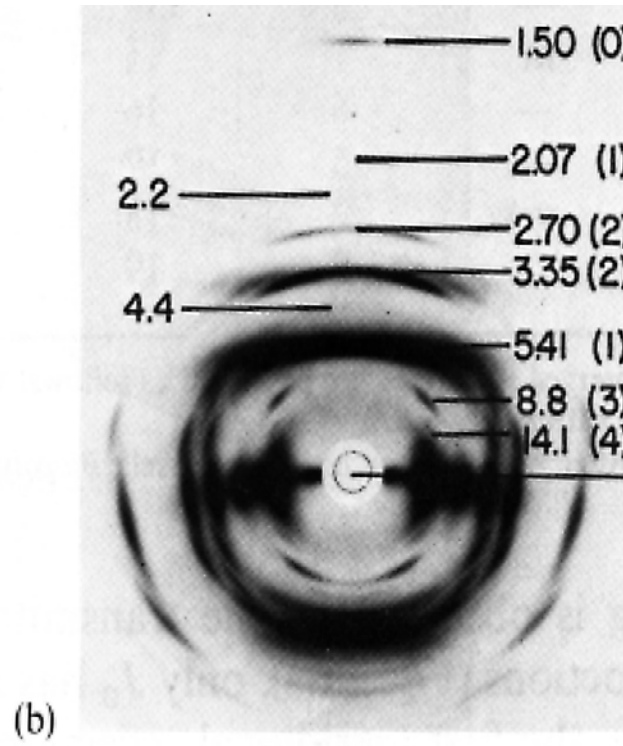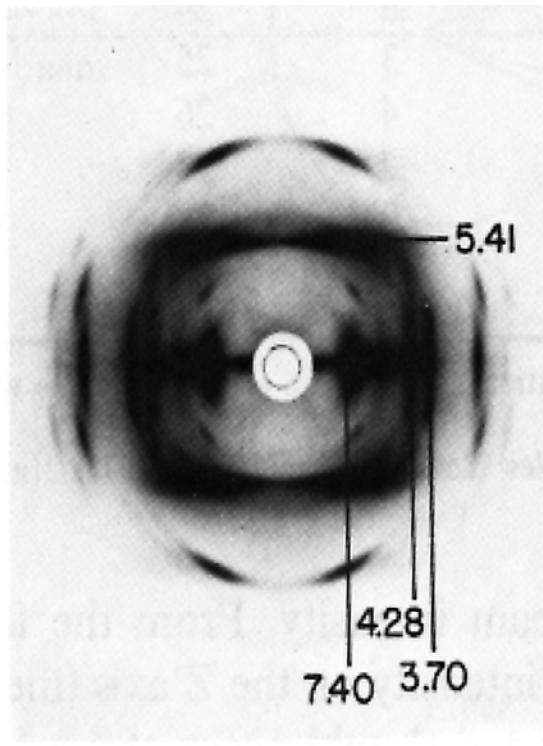
1.5 Å/residue

Pitch 5.4 Å

18 residues/5 turns

Layer lines every $(5.4 * l/5)^{-1}$

$\boldsymbol{l = m.u \; + \; n.t}$

l=18m+5n

X-ray reflections: direct test of hypothesis

1,1   1,0   1,0   1,1

X-ray $\Rightarrow$ LS
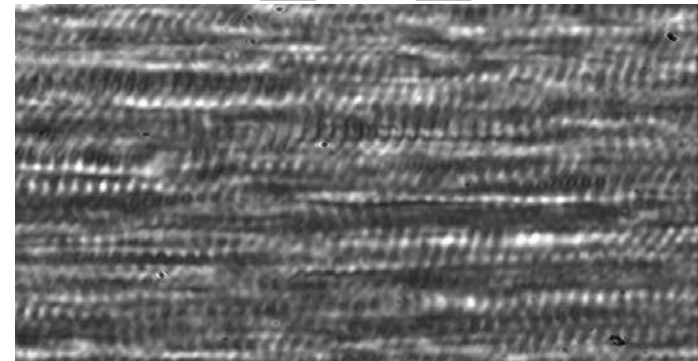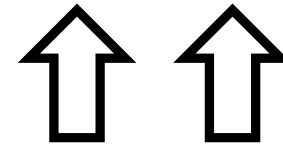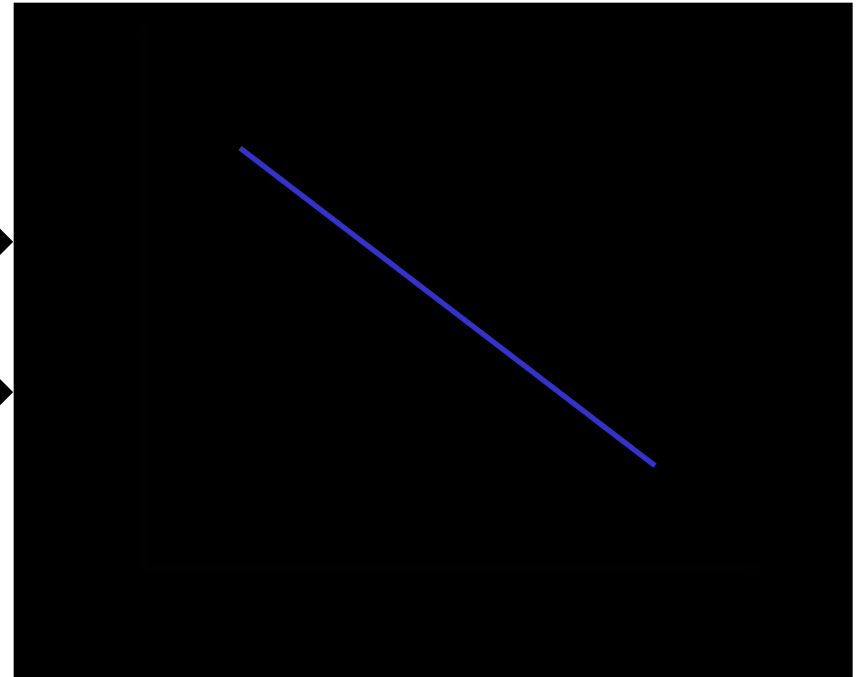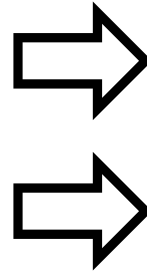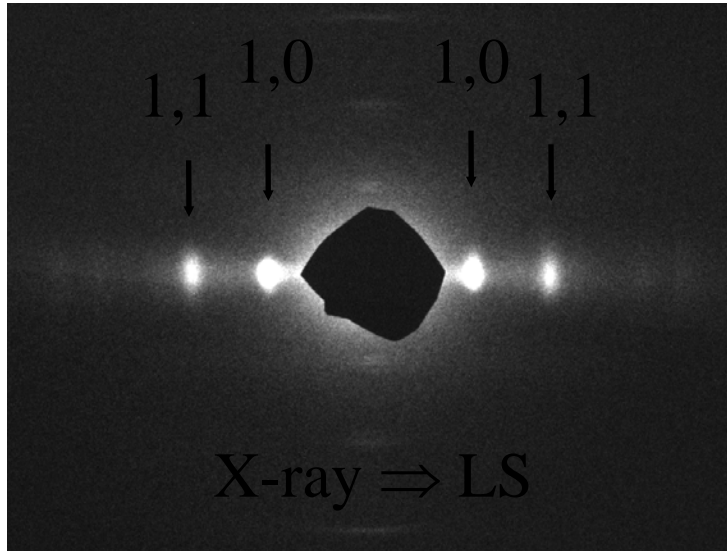
Irving, Konhilas, Perry, Fischetti, and de Tombe. *AJP* 279: H2568-H2573, 2000.

Video $\Rightarrow$ SL

Lattice spacing in skinned rat trabeculae shrinks with increasing sarcomere length

Hypothesis

SHORT SL   LONG SL
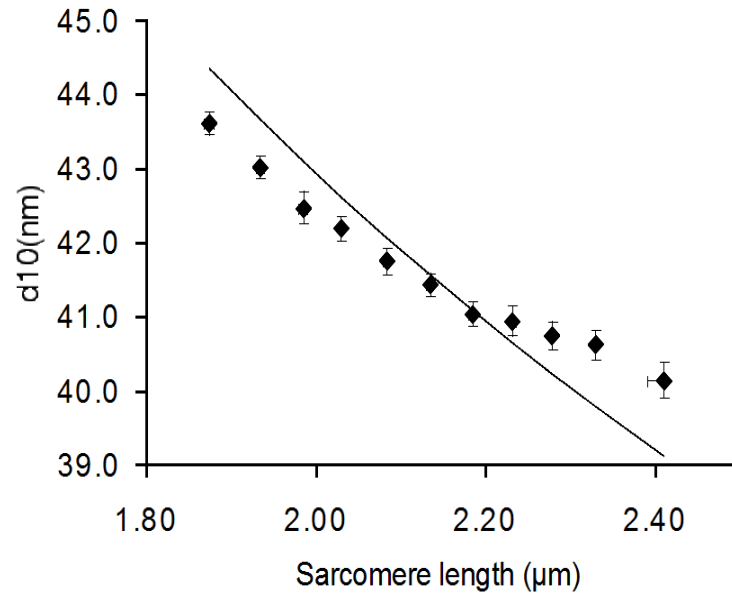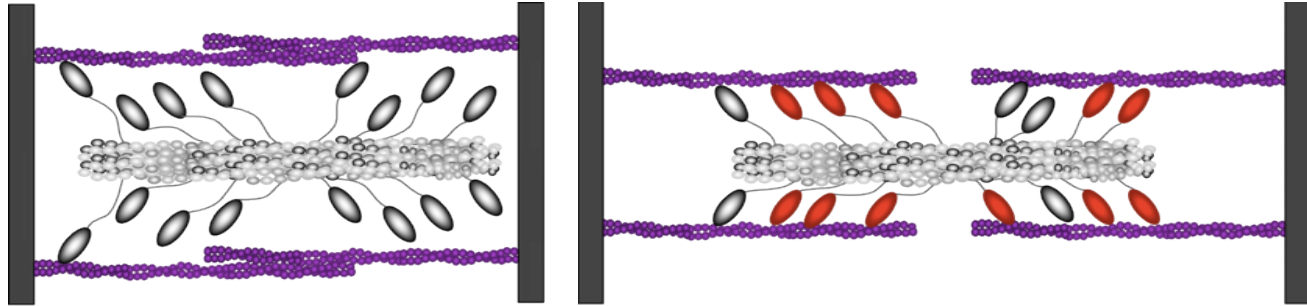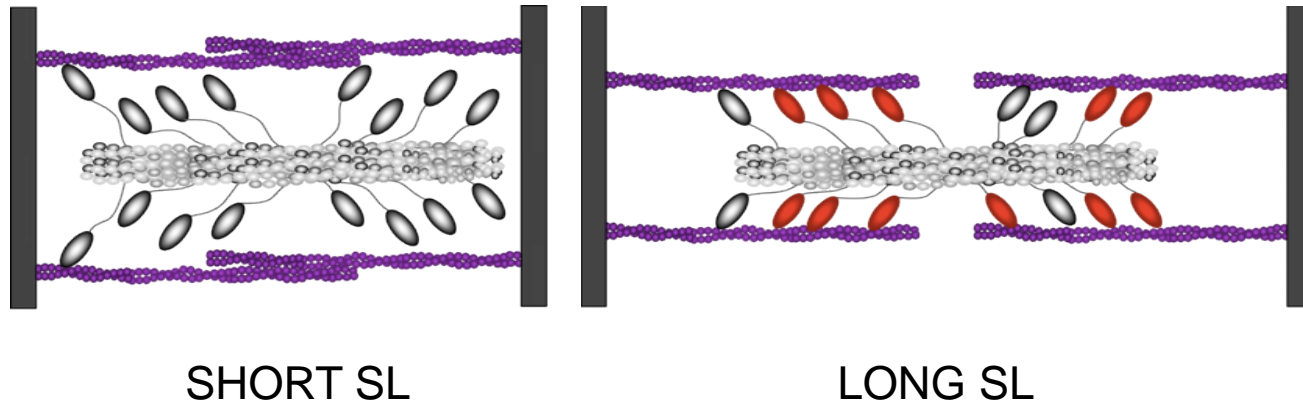
Myosin heads on the thick filaments move inwards or outwards when muscle changes length

Layer Lines Allow Direct Measurement Radius of Heads
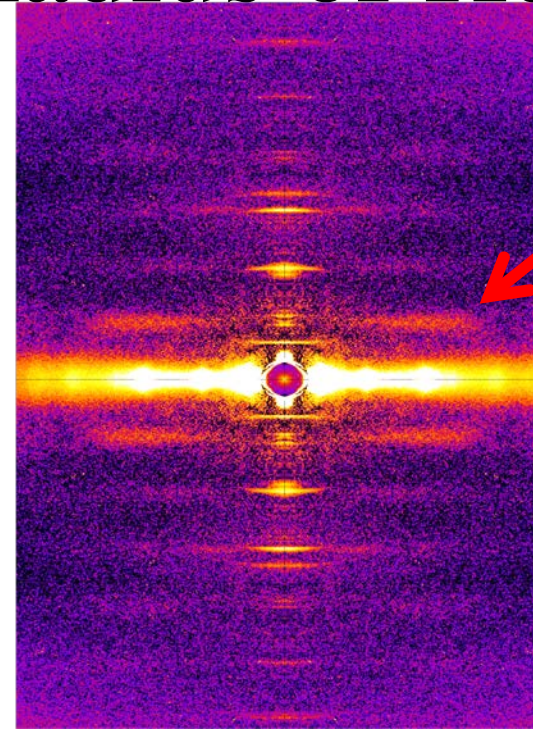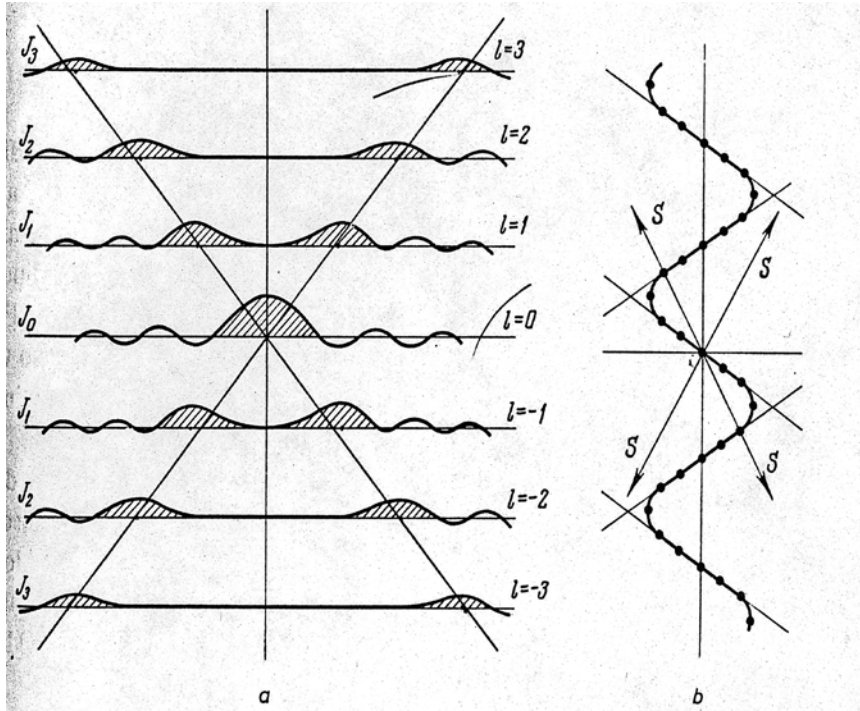
First myosin layer line (M1)

Position of maxima on layer lines inversely proportional to radius to the center of mass of myosin heads

Myosin layer lines show inward radial motion of myosin heads with stretch
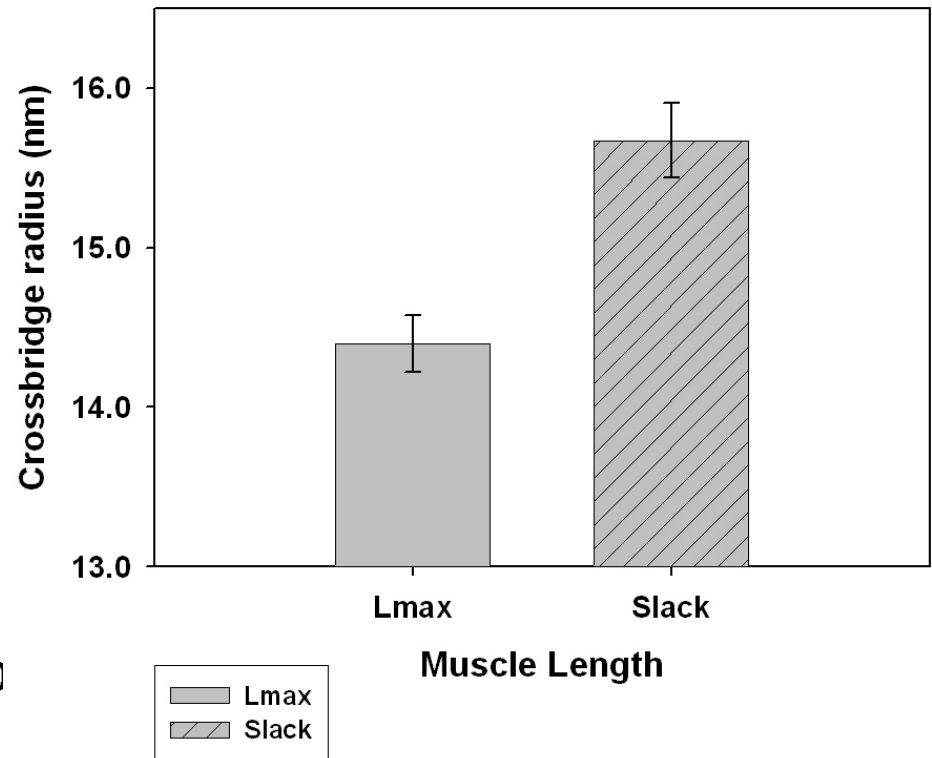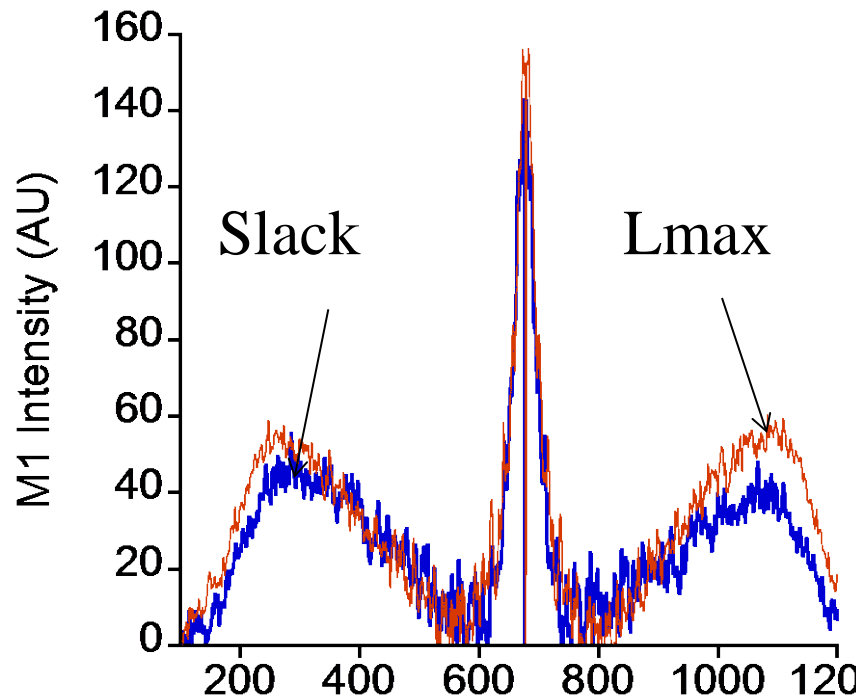
Summary
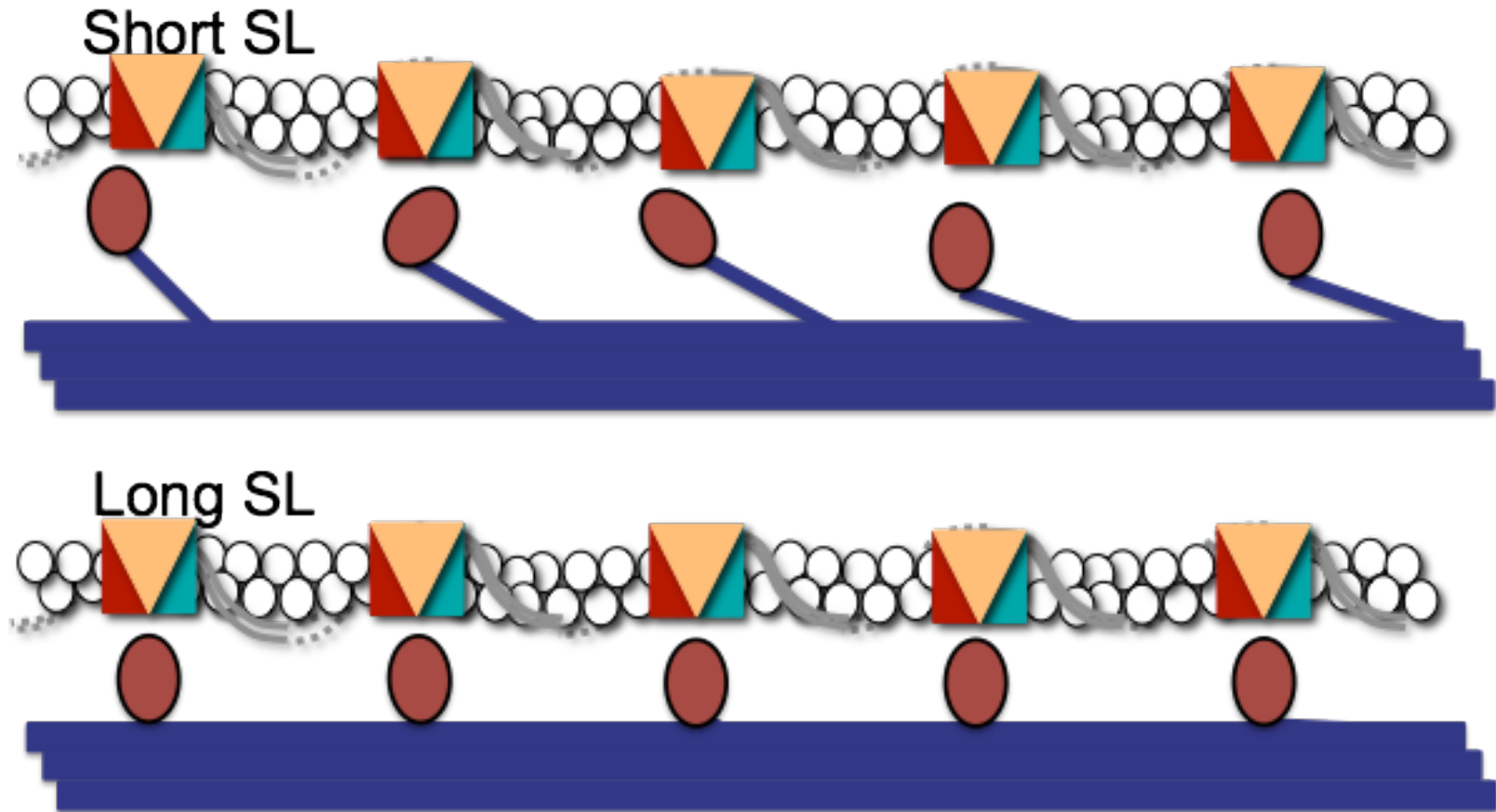
Increased calcium sensitivity at long SL is due to a more perpendicular orientation of the myosin heads

Meridionals Allow Measurement of Strain in Fibers
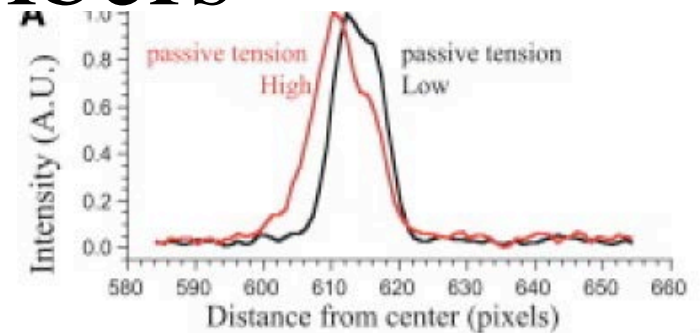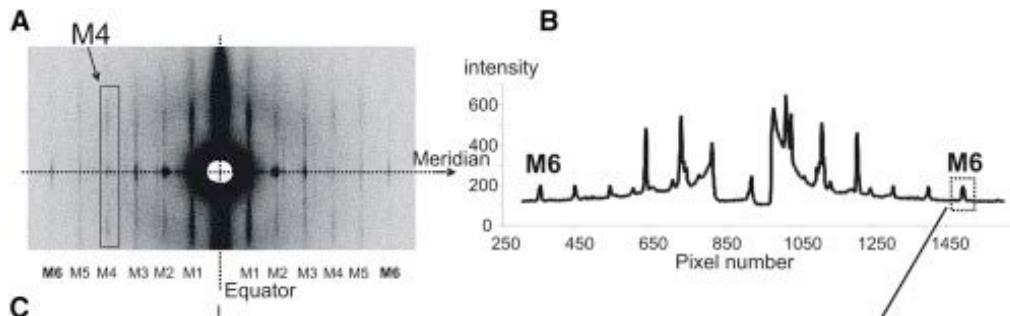
In this example the 6th order meridional reflection from myosin in skeletal muscle was used to measure elastic strain in the myosin containing thick filament
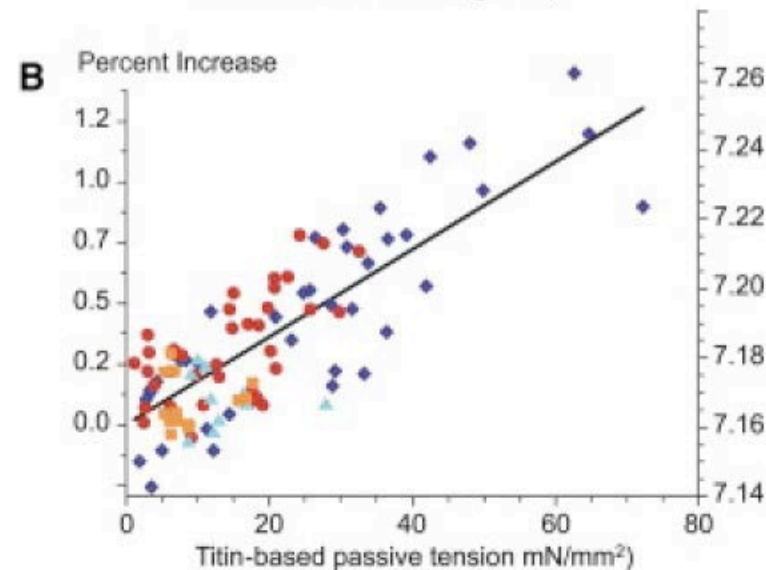
Fiber Crystallography

- Most fiber "structures" result of model building studies
- There have been a small number of Fiber "structure solutions".
- High resolution structure by Keichi Namba on bacterial flagella (Yamashita et al., 1998 Nature SB) aligned by high magnetic fields
- Orgel et al. (2001 Structure) published first MIR structure of a natural fiber - Type I Collagen from rat tail collagen

Collagen type I sub-fibrillar structure

7
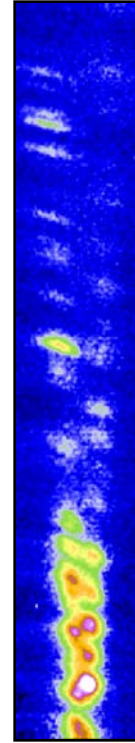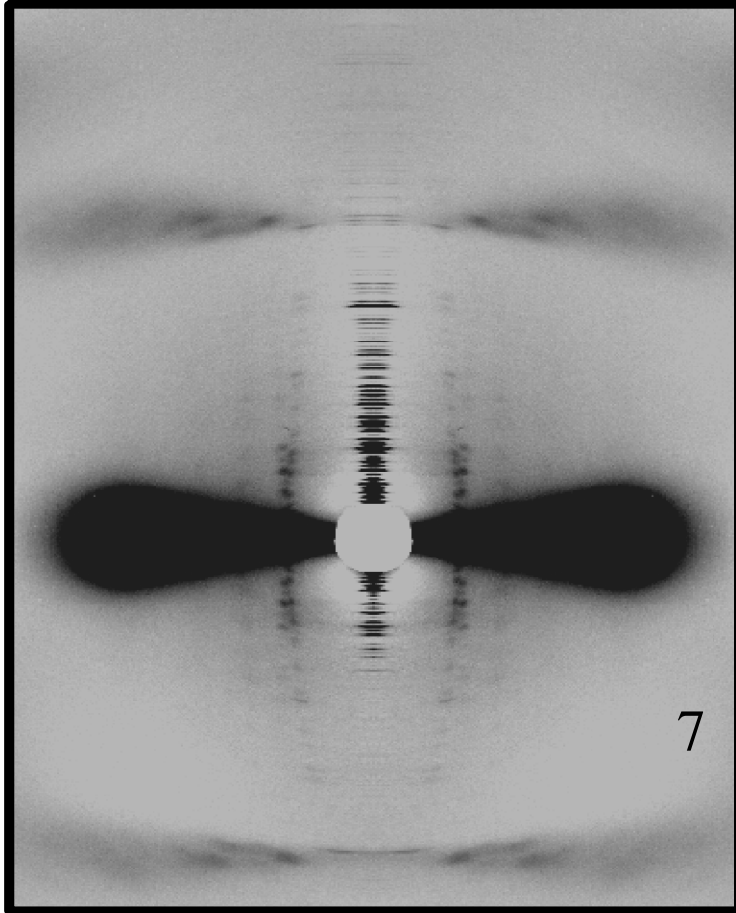
C
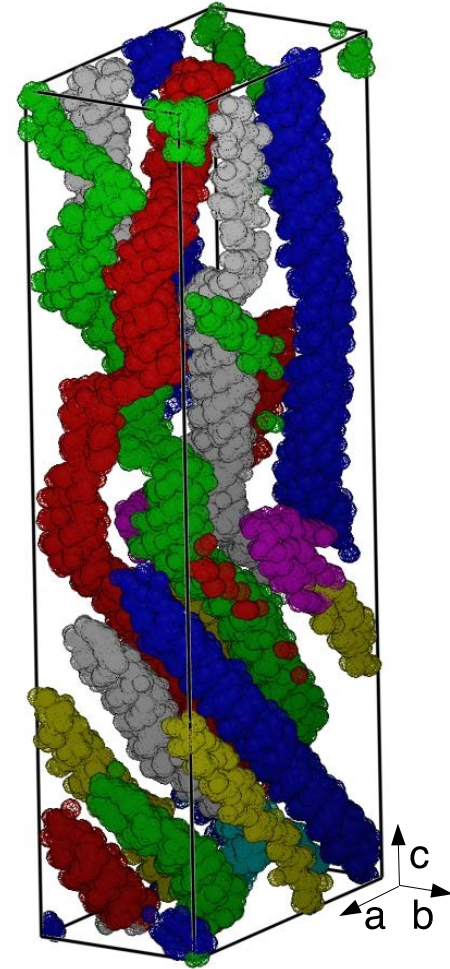
Orgel et al., 2006, Proc Natl Acad Sci. 103: 9001–9005.

Resolution ~ 11 /5.16 Å
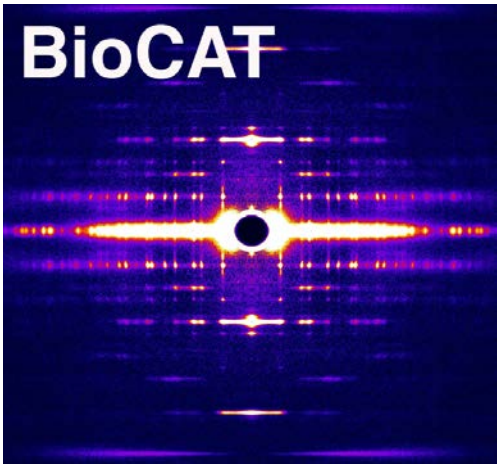
Data Analysis for Fiber Diffraction

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Existing Tools for Fiber Diffraction Analysis

- Tradition of "rolling your own" software
- Community small so relatively difficult to get support behind new large-scaledevelopments
- There are several packages useful for fiber diffraction
- Difficulty of obsolescence of existing packages
- Badly need new developments!

Useful packages

- Fit2D
  - Pros:
    - generally useful and very powerful package for analysis of general diffraction programs
    - Supports many detector formats
    - Supports macros
    - Runs on Windows and Linux (Mac's sort of)
  - Cons
    - Hasn't been recompiled in 8 years
    - Documentation inconsistent – most powerful features least well documented
    - Not supported, future uncertain

Fit2D

- Things to watch for:
  - Intensity projections plot AVERAGE values not SUMMED
  - The dreaded "X-pixel size bug"

Fibrefix

- Outgrowth of CCP13 project
- Pros:
  - Comprehensive package for analysis of crystalline fibre diffraction patterns
  - Unique background subtraction tools
- Cons
  - Less useful for continuous diffraction data
  - Relatively difficult to use
  - Not amenable to automation
  - No longer maintained
  - Needs .NET framework 1.1 – risk of obsolescence

Background Subtraction with Fibrefix
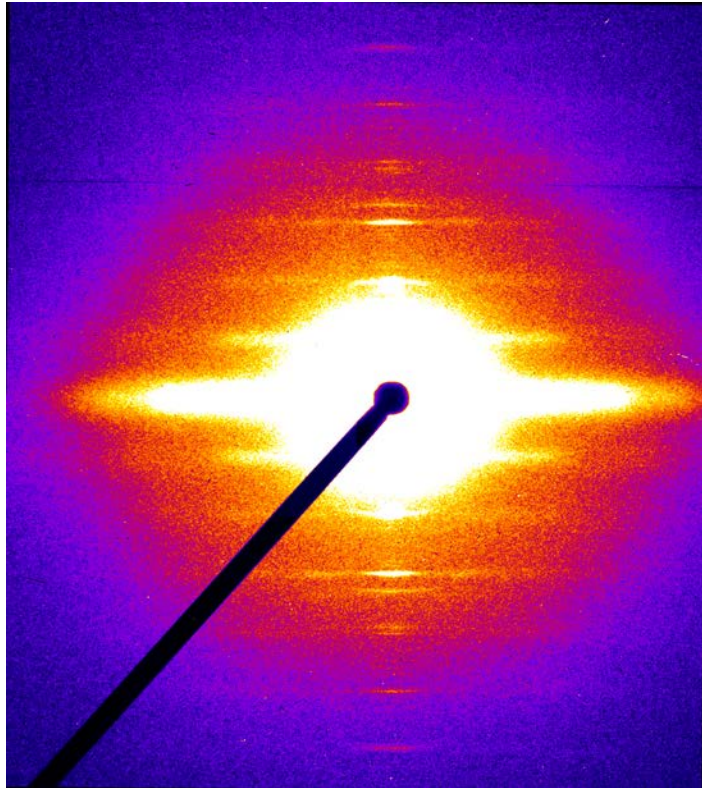
Raw pattern
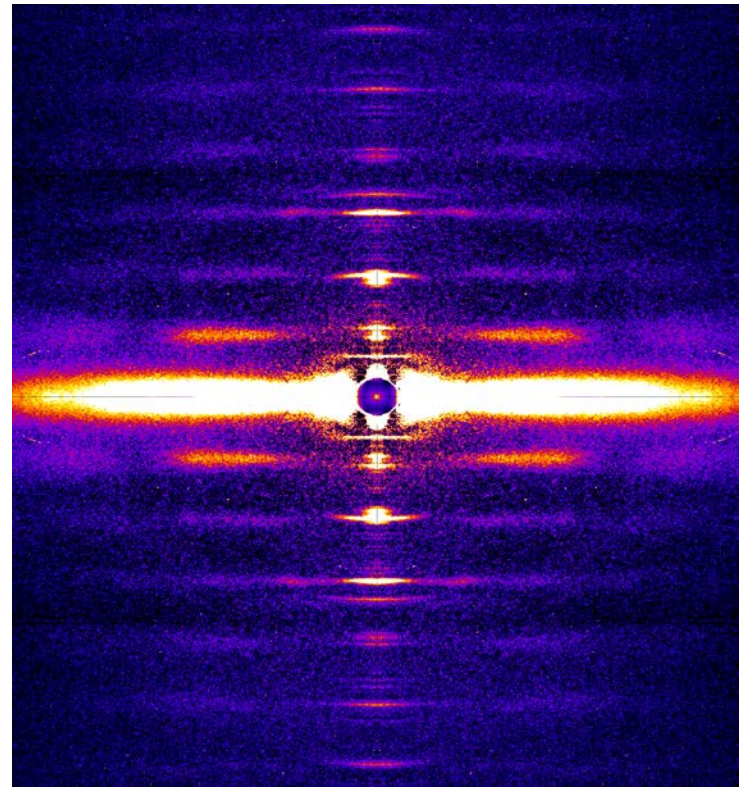
Quadrant folded and Background subtracted

WCEN

- Developed Gerald Stubbs group, Vanderbilt university
- Pros:
  - Nice data visualization tools
  - Cool "Healing" tool for detector defects
  - Good for continuous fiber diffraction
  - Still being actively maintained
  - Works Macs and Linux
- Cons:
  - Not widely used yet so very small user base

ImageJ

- Powerful general purpose image processing package
- Pros:
  - Written in Java – easily extensible
  - Well supported at NIH and Community
  - Nice image rotation tools, lookup tables
- Cons
  - None really, no diffraction specific tools

Fityk

- http://www.unipress.waw.pl/fityk/
- Pros:
  - Powerful curve fitting package with many built in functions
  - User defined functions
  - Functionality available as a python library
- Cons:
  - Needs more flexible input/output

Workflow in Irving Lab

- Background subtraction in Fibrefix (optional)
- Distance measurements and 1D traces in Fit2D
- Pattern splitting, background subtraction Chiplot.Analyze.py
- Curve fitting in FITYK
- Data extraction into .csv files from Fit2d and Fityk log files using custom perl scripts
- Use ImageJ for image rotations and preparing figures for publication

The Future

- New tools incorporated in GDA
- Integrated workflows at the beamline to yield partially or reduced data
- Suggestion has been made to develop some tools in the context of ImageJ plugins
- GDA is an inherently JAVA based environment
- ???

References

- **Basics**:

  C. Cantor and P. Schimmel "Biophysical Chemistry part II: Techniques for the study of Biological Structure and Function" Chapter 14. Freeman, 1980

- **A terrific introduction**:

  John Squire "The Structural Basis of Muscular Contraction" Plenum, 1981

- **Definitive Reference**:

  B.K. Vainshtein "Diffraction of X-rays by Chain Molecules" Elsevier, 1966.

More references:

Good introduction to "Fiber crystallography":

Chandrasekaran, R. and Stubbs, G. (2001). Fiber diffraction. in *International Tables for Crystallography, Vol. F: Crystallography of Biological Macromolecules* (Rossman, M.G. and Arnold, E., eds.), Kluwer Academic Publishers, The Netherlands, 444-450.