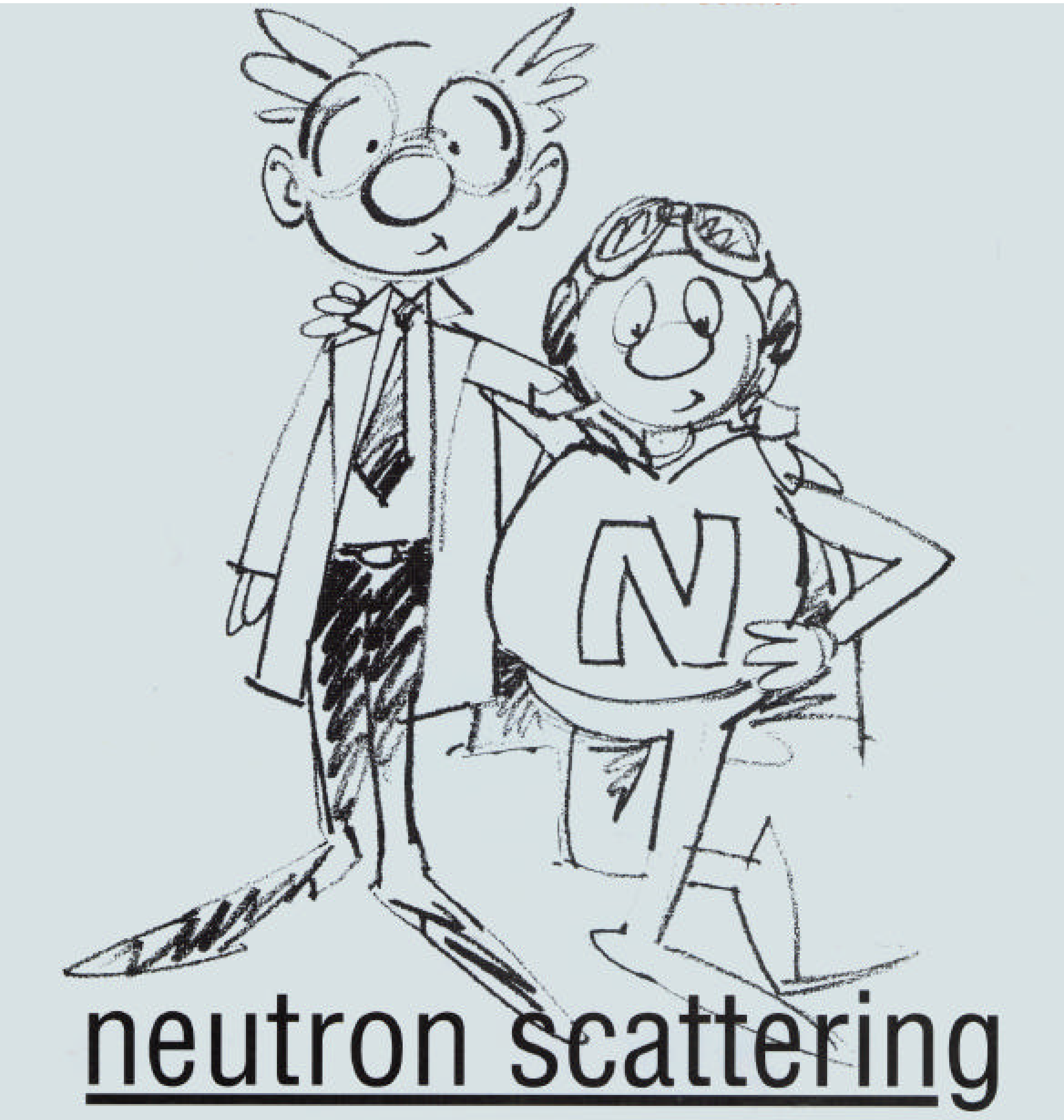

LECTURES 3 & 4: Diffraction

by

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This Lecture

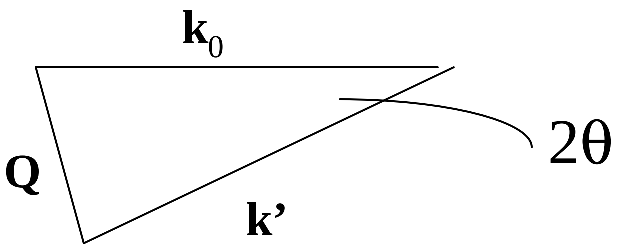
- Elastic coherent scattering from a periodic array of nuclei
- Direct and reciprocal lattices
- Effect of thermal atomic vibrations on diffracted intensity
- Formal expression for Bragg diffraction
- The structure factor – phase and amplitude
- Single crystal versus powder diffraction
- Diffractometers for single crystal & powder diffraction
- The Rietveld method
- Comparison of x-ray and neuron crystallography
- The Laue method
- Examples of the Laue method applied to biomolecular crystals
- The Pair Distribution Function (pdf) method

From Lecture 2:

$$\frac{ds}{d\Omega} = \frac{\text{number of neutrons scattered through angle } 2q \text{ per second into d}\Omega}{\text{number of incident neutrons per square cm per second x d}\Omega}$$

$$\left(\frac{ds}{d\Omega}\right)_{coh} = \sum_{i,j} b_i^{coh} b_j^{coh} e^{i(\vec{k}_0 - \vec{k}').(\vec{R}_i - \vec{R}_j)} = \sum_{i,j} b_i^{coh} b_j^{coh} e^{-i\vec{Q}.(\vec{R}_i - \vec{R}_j)}$$

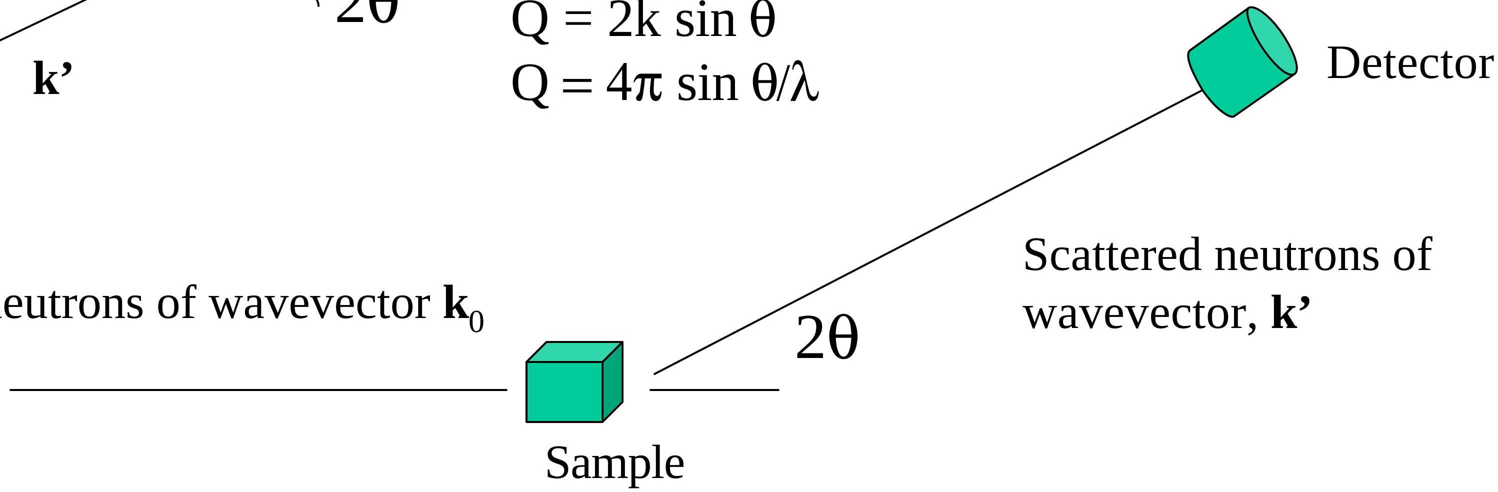
where the wavevecto r transfer $\vec{Q}$ is defined by $\vec{Q} = \vec{k}' - \vec{k}_0$



For elastic scattering k_0=k’=k:

Q = 2k sin θ

Q = 4π sin θ/λ



Detector

Scattered neutrons of wavevector, **k’**

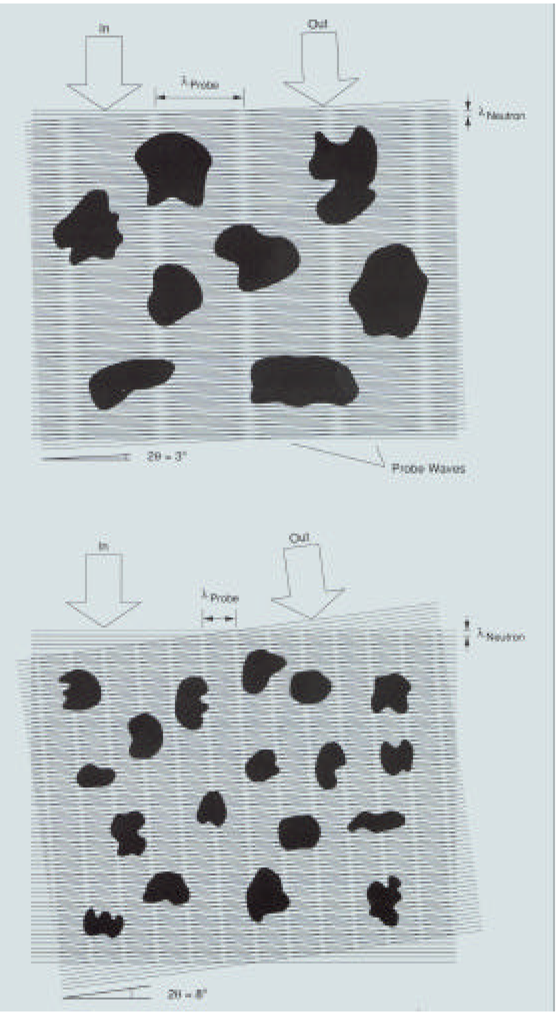
Incident neutrons of wavevector $\mathbf{k}_0$

2θ

Sample

Neutron Diffraction

- Neutron diffraction is used to measure the differential cross section, dσ/dΩ and hence the static structure of materials
  - Crystalline solids
  - Liquids and amorphous materials
  - Large scale structures



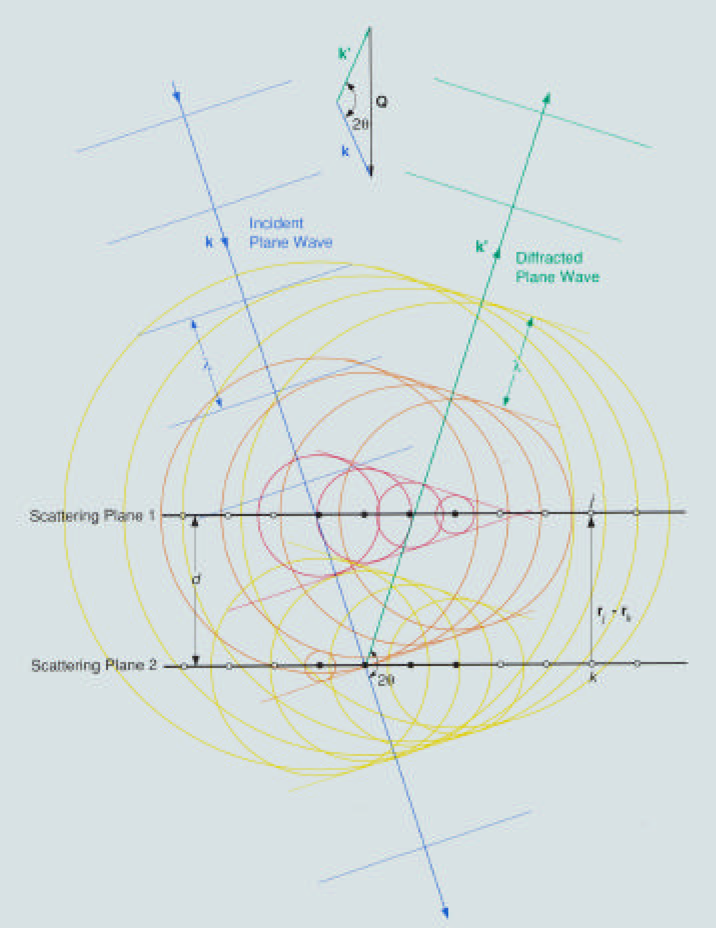
- Depending on the scattering angle, structure on different length scales, d, is measured:

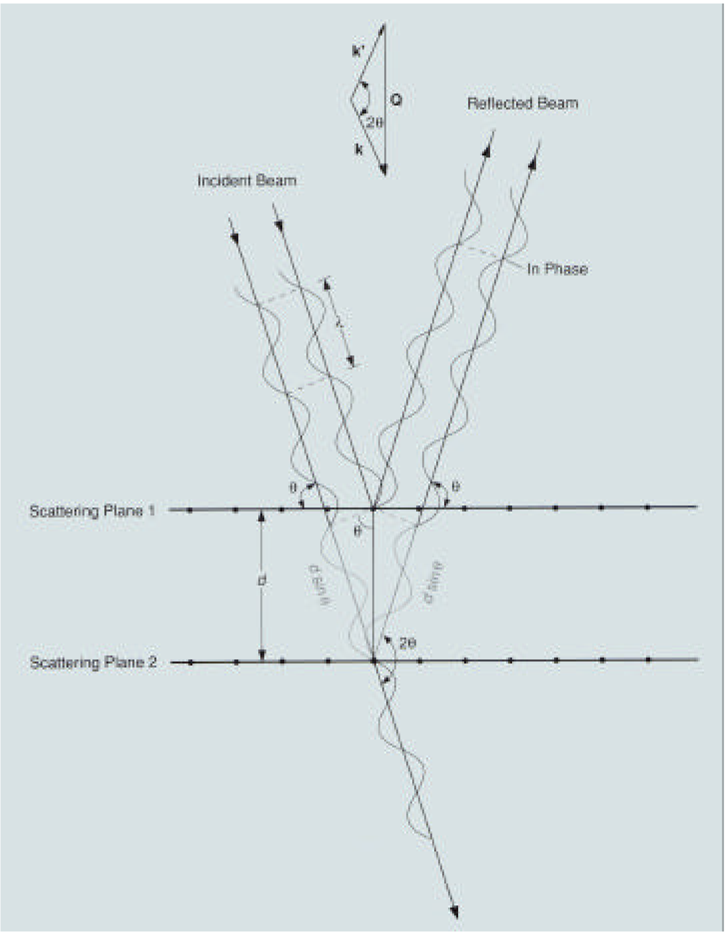
$$2\pi / Q = d = \lambda / 2\sin(\theta)$$

- For crystalline solids & liquids, use wide angle diffraction. For large structures, e.g. polymers, colloids, micelles, etc. use small-angle neutron scattering

For Periodic Arrays of Nuclei, Coherent Scattering Is Reinforced Only in Specific Directions Corresponding to the Bragg Condition:

$$\lambda = 2\, d\, \sin(\theta)$$

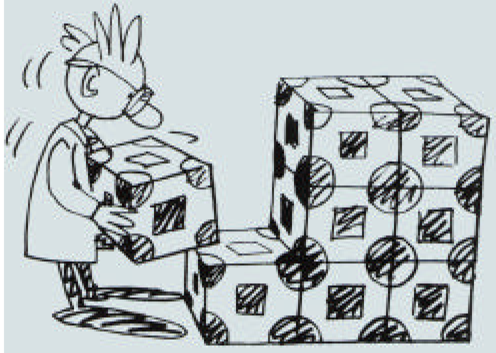




Path difference between waves scattered from planes 1 and 2 is 2 d sinθ. Only constructive Interference when $n\lambda = 2\, d \sin\theta$

Diffraction by a Lattice of Atoms

- Remember that the scattering is given by where the $\vec{R}_i$ are the atomic positions $S(\vec{Q}) = \frac{1}{N}\left\langle \sum_{i,j} e^{-i\vec{Q}.(\vec{R}_i - \vec{R}_j)} \right\rangle$
- The sum is over all interatomic separations and will only be non-zero if $\vec{Q}.(\vec{R}_i - \vec{R}_j) = 2Mp$ for all atom pairs
- This will only happen for specific values of $\vec{Q}$: those that correspond to the Bragg condition on the previous slide
- To find these values we look at the definition of the atomic positions
  - We'll do the easy case first, with only one atom per unit cell



unit cell

The repeating unit of a crystal.

Direct and Reciprocal Lattices

In a Bravais lattice, we can write $\vec{R}_i = m_{1i}\vec{a}_1 + m_{2i}\vec{a}_2 + m_{3i}\vec{a}_3$ where $\vec{a}_1, \vec{a}_2, \vec{a}_3$ are the primitive translation vectors of the unit cell (see previous viewgraph).

Let's define $\vec{a}_1^* = \frac{2p}{V_0}\vec{a}_2 \times \vec{a}_3; \quad \vec{a}_2^* = \frac{2p}{V_0}\vec{a}_3 \times \vec{a}_1; \quad \vec{a}_3^* = \frac{2p}{V_0}\vec{a}_1 \times \vec{a}_2$

where $V_0 = \mathrm{a}_1.(\vec{a}_2 \times \vec{a}_3)$ = the volume of the unit cell.

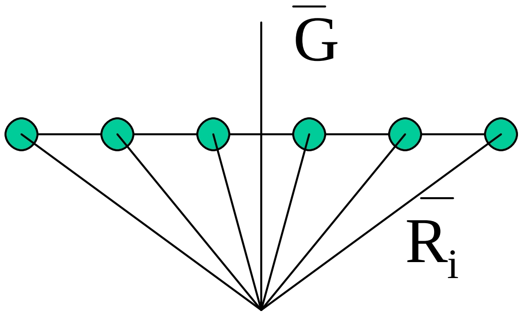
The $\vec{a}_i^*$ have the dimensions of (length) $^{-1}$ and the property that $\vec{a}_i^*.\vec{a}_j = 2pd_{ij}$, i.e. $\vec{a}_1^*$ is perpendicular to the plane defined by $\vec{a}_2$ and $\vec{a}_3$ etc.

If we choose a vector $\vec{G}_{hkl}$ defined by $\vec{G}_{hkl} = h\vec{a}_1^* + k\vec{a}_2^* + l\vec{a}_3^*$ then $\vec{G}_{hkl}.(\vec{R}_i - \vec{R}_j) = 2Mp$.

i.e. $\vec{G}_{hkl}$ is normal to sets of planes of atoms spaced $2p / G_{hkl}$ apart

Scattering from a lattice of atoms occurs only when $\vec{Q} = \vec{G}_{hkl}$

The vectors $\vec{G}_{hkl}$ define a lattice of points called the reciprocal lattice



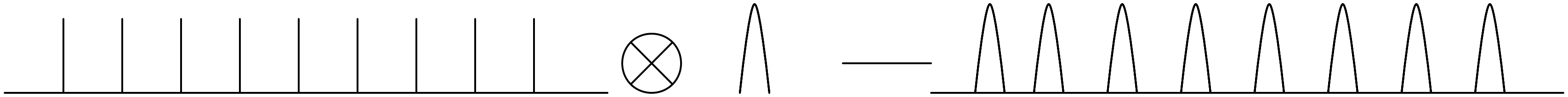
Homework: verify that Bragg's (λ = 2 d sinθ) follows from the above

Notation

- $\overline{G}_{hkl}$ is called a reciprocal lattice vector (node denoted hkl)
- h, k and l are called Miller indices
- (hkl) describes a set of planes perpendicular to $\overline{G}_{hkl}$, separated by $2\pi/G_{hkl}$
- {hkl} represented a set of symmetry-related lattice planes
- [hkl] describes a crystallographic direction
- <hkl> describes a set of symmetry equivalent crystallographic directions

Atomic Vibrations

- The formalism on the previous slide works fine if the atoms are stationary: in reality, they are not
- Remember, from the last lecture that $S(\vec{Q}) = \frac{1}{N}\left\langle \sum_{i,j} e^{-i\vec{Q}.(\vec{R}_i-\vec{R}_j)} \right\rangle_{\text{ensemble}}$
- We average over the (fluctuating) atomic positions by introducing a probability that an atom will be at given position. Instead of the Fourier Transform of d functions, this gives the FT of the d functions *convolved* with a spread function. The result is that S(Q) is *multiplied* by the FT of the spread function i.e. by $\exp - Q^2\langle u^2\rangle/3$ if we use a Gaussian spread function



- Atomic vibrations cause a decrease in the intensity of Bragg scattering. The “missing” scattering appears between Bragg peaks and results in inelastic scattering

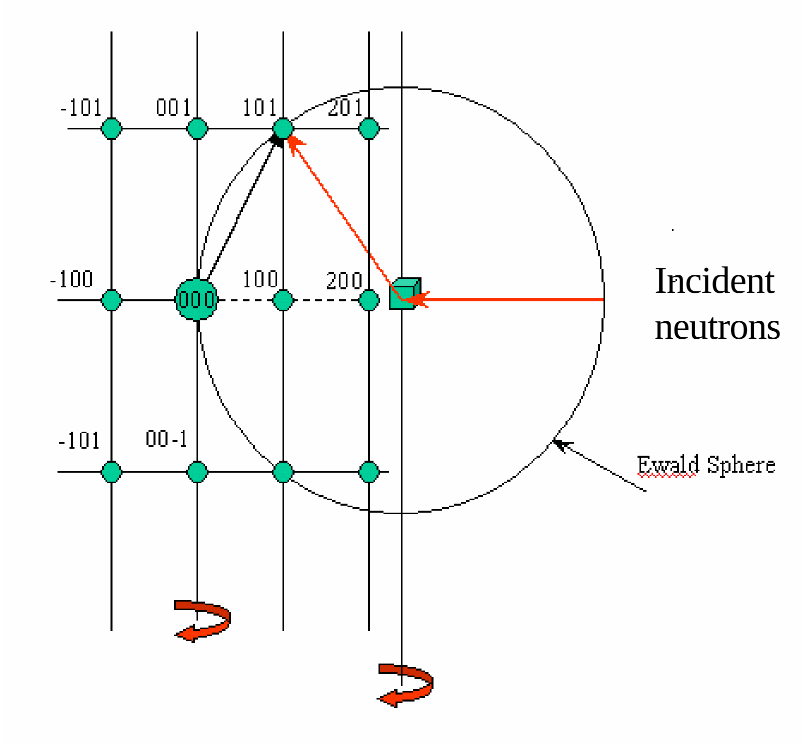
Key Points about Diffraction

- A monochromatic (single λ) neutron beam is diffracted by a single crystal only if specific geometrical conditions are fulfilled

- These conditions can be expressed in several ways:
  - Laue's conditions: $\vec{Q}.\vec{a}_1 = h; \quad \vec{Q}.\vec{a}_2 = k; \quad \vec{Q}.\vec{a}_3 = l$ with h, k, and l as integers
  - Bragg's Law: $2d_{hkl} \sin\theta = \lambda$
  - Ewald's construction

    see http://www.matter.org.uk/diffraction/geometry/default.htm

- Diffraction tells us about:
  - The dimensions of the unit cell
  - The symmetry of the crystal
  - The positions of atoms within the unit cell
  - The extent of thermal vibrations of atoms in various directions



Bragg Scattering from Crystals

Working through the math (see, for example, Squires' book), we find :

$$\left(\frac{ds}{d\Omega}\right)_{Bragg} = N\frac{(2p)^3}{V_0}\sum_{hkl} d(\vec{Q}-\vec{G}_{hkl})\left|F_{hkl}(\vec{Q})\right|^2$$

where the unit - cell structure factor is given by

$$F_{hkl}(\vec{Q}) = \sum_{d} \bar{b}_d e^{i\vec{Q}.\vec{d}} e^{-W_d}.$$ d labels the various atoms in the unit cell

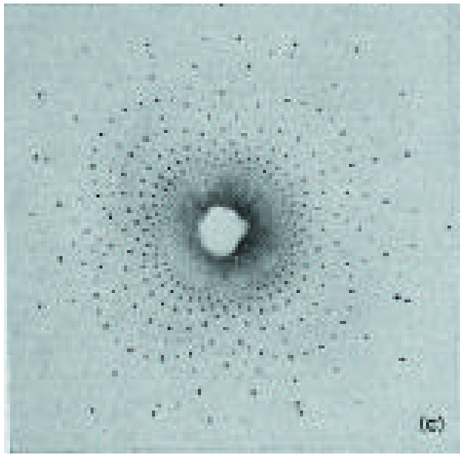
and W_d is the Debye - Waller factor that accounts for thermal motions of atoms

- Using either single crystals or powders, neutron diffraction can be used to measure F^2 (which is proportional to the intensity of a Bragg peak) for various values of (hkl).
- Direct Fourier inversion of diffraction data to yield crystal structures is not possible because we only measure the magnitude of F, and not its phase => models must be fit to the data
- Neutron powder diffraction has been particularly successful at determining structures of new materials, e.g. high T_c materials

The Structure Factor

- The intensity of scattering at reciprocal lattice points is given by the square of the structure factor $F_{hkl}(\vec{Q}) = \sum_d \bar{b}_d e^{i\vec{Q}.\vec{d}} e^{-W_d}$

- Crystallography attempts to deduce atomic positions and thermal motions from measurements of a large number of such “reflections”
  - (Reciprocal) distance between diffraction “spots” => size of unit cell
  - Systematic absences and symmetry of reciprocal lattices => crystal symmetry (e.g. bcc h+k+l=2n)
  - Intensities of “spots” => atomic positions and thermal motions



Laue diffraction pattern
showing crystal symmetry

We would be better off if diffraction measured phase of scattering rather than amplitude! Unfortunately, nature did not oblige us.

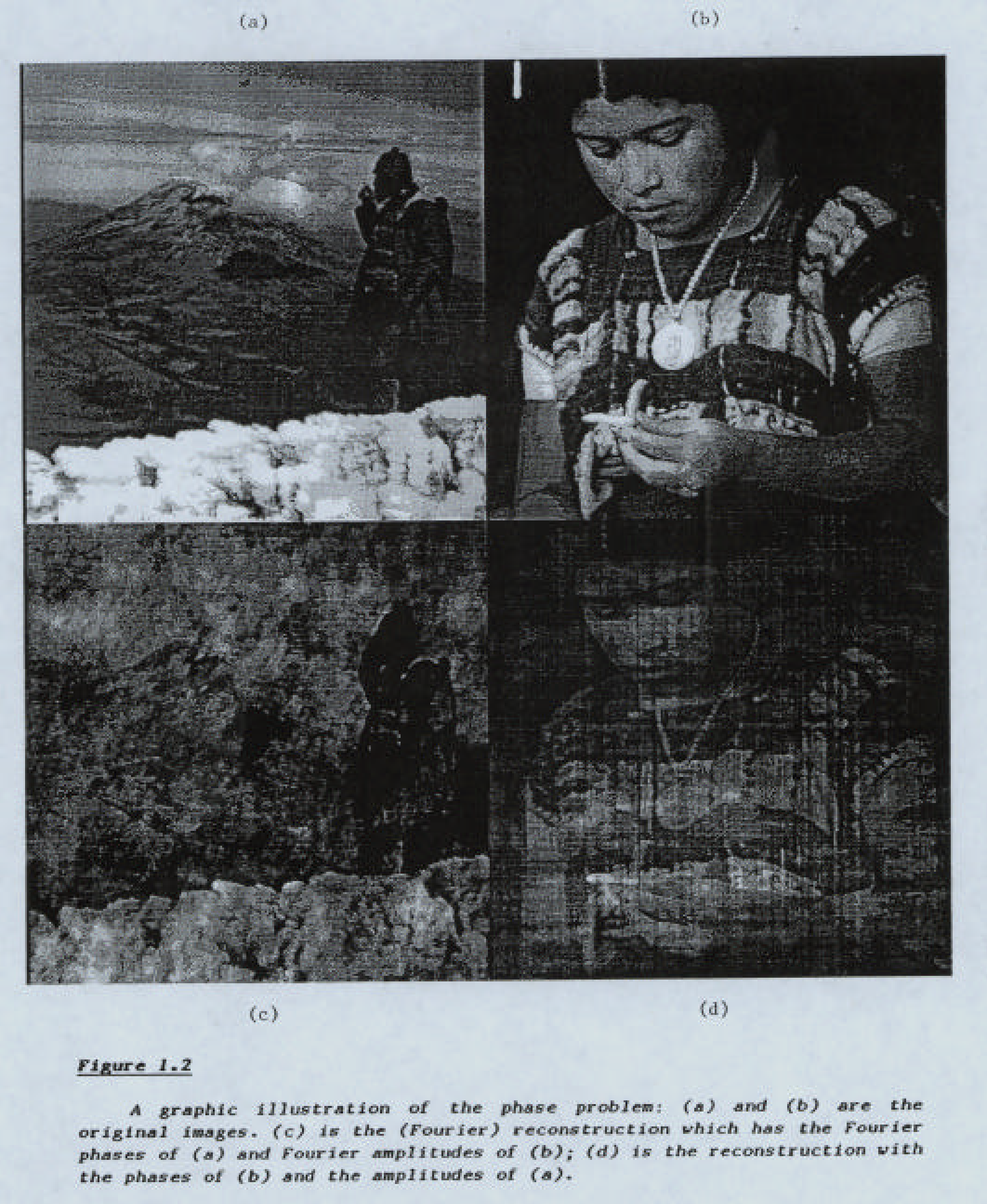


Figure 1.2

A graphic illustration of the phase problem: (a) and (b) are the original images. (c) is the (Fourier) reconstruction which has the Fourier phases of (a) and Fourier amplitudes of (b); (d) is the reconstruction with the phases of (b) and the amplitudes of (a).

Picture by courtesy of D. Sivia

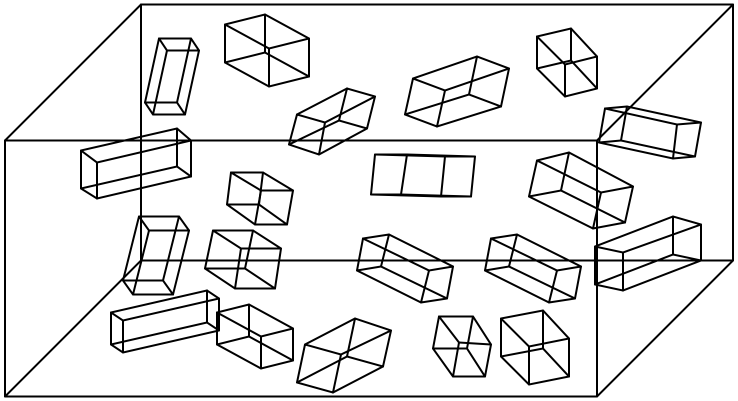
Homework

- The following sites provide tutorials on diffraction. Please go through them and try the examples. Bring questions to the next class

- http://www.matter.org.uk/diffraction/introduction/default.htm

- http://www.uni-wuerzburg.de/mineralogie/crystal/teaching/teaching.html

Powder – A Polycrystalline Mass



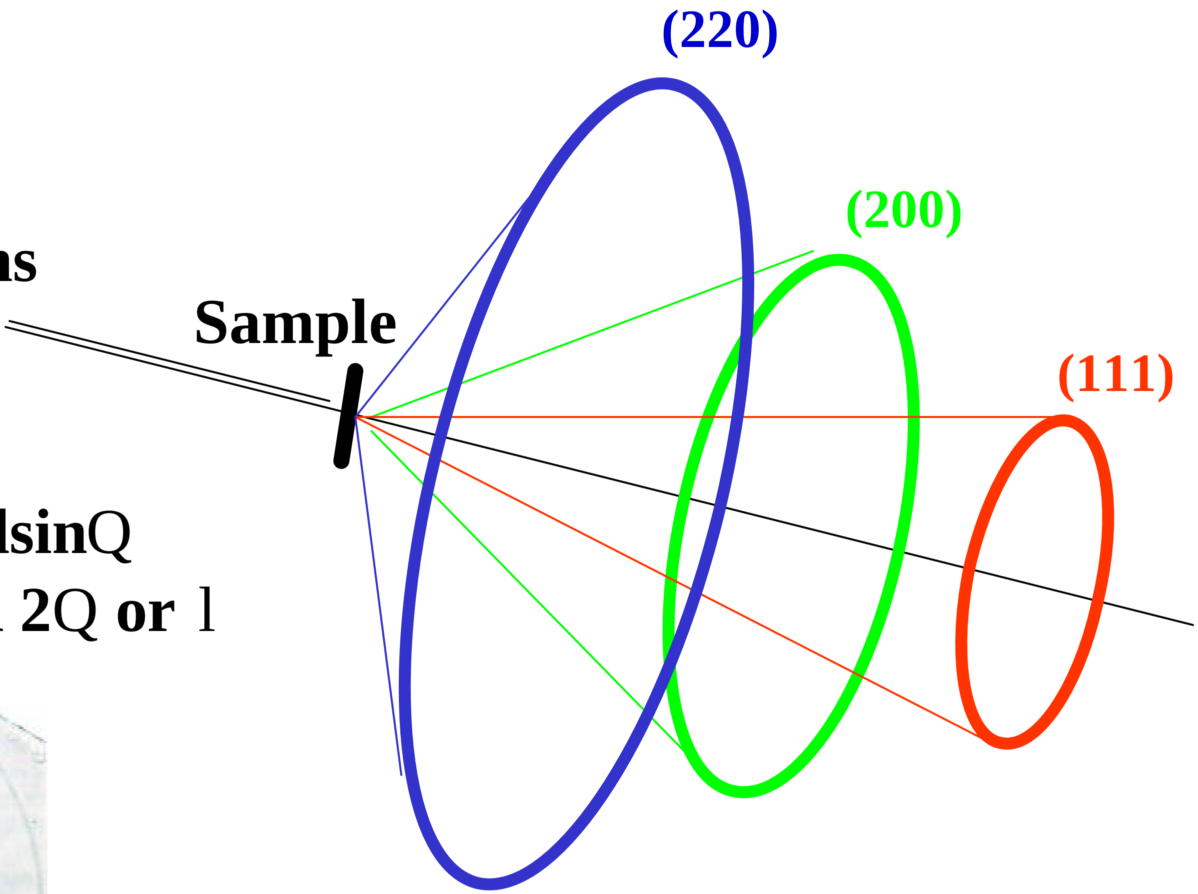
All orientations of crystallites possible

Typical Sample: 1cc powder of 10μm crystallites - 10^9 particles if 1μm crystallites - 10^{12} particles

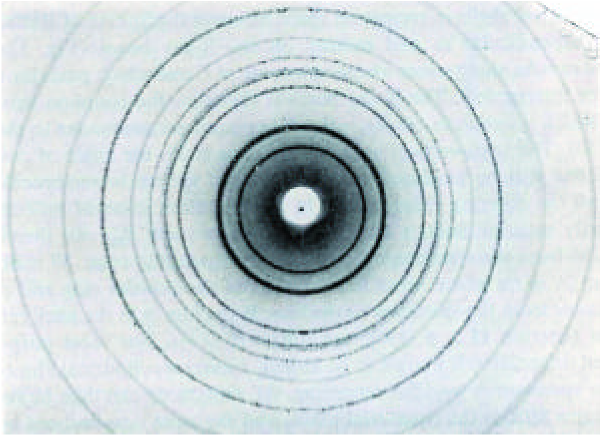
Single crystal reciprocal lattice - smeared into spherical shells

Powder Diffraction gives Scattering on Debye-Scherrer Cones

Incident beam
x-rays or neutrons

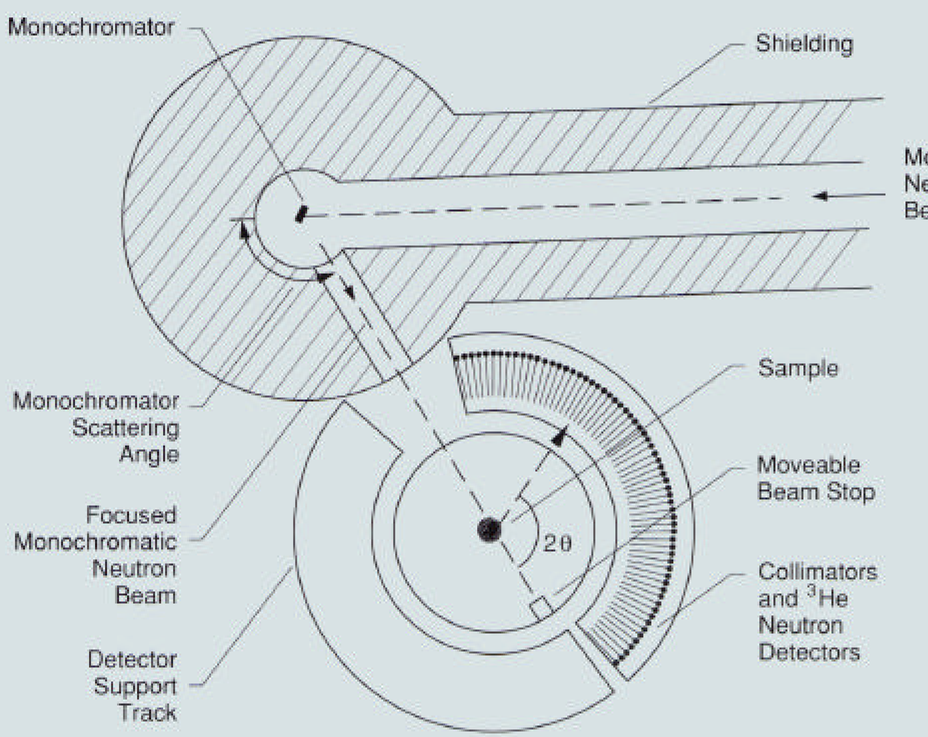


Bragg's Law   l = 2dsinQ
Powder pattern – scan 2Q or l



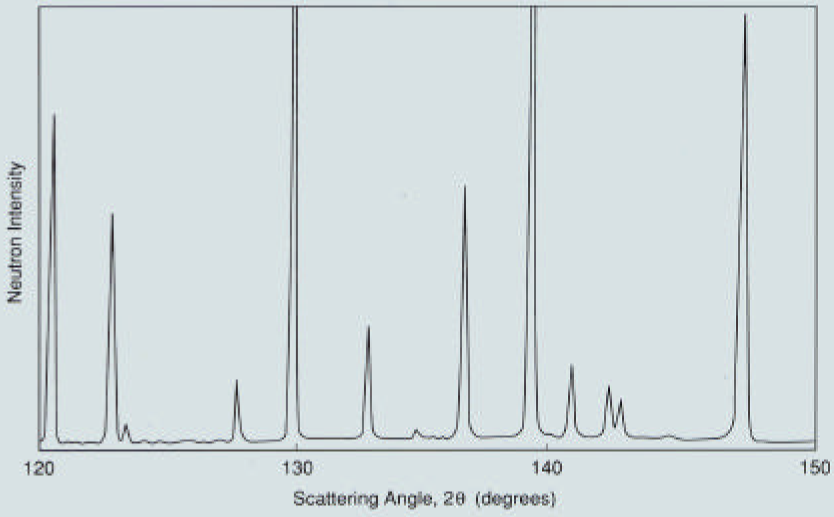
A typical powder diffraction pattern

Measuring Neutron Diffraction Patterns with a Monochromatic Neutron Beam



POWDER DIFFRACTION AT A REACTOR SOURCE

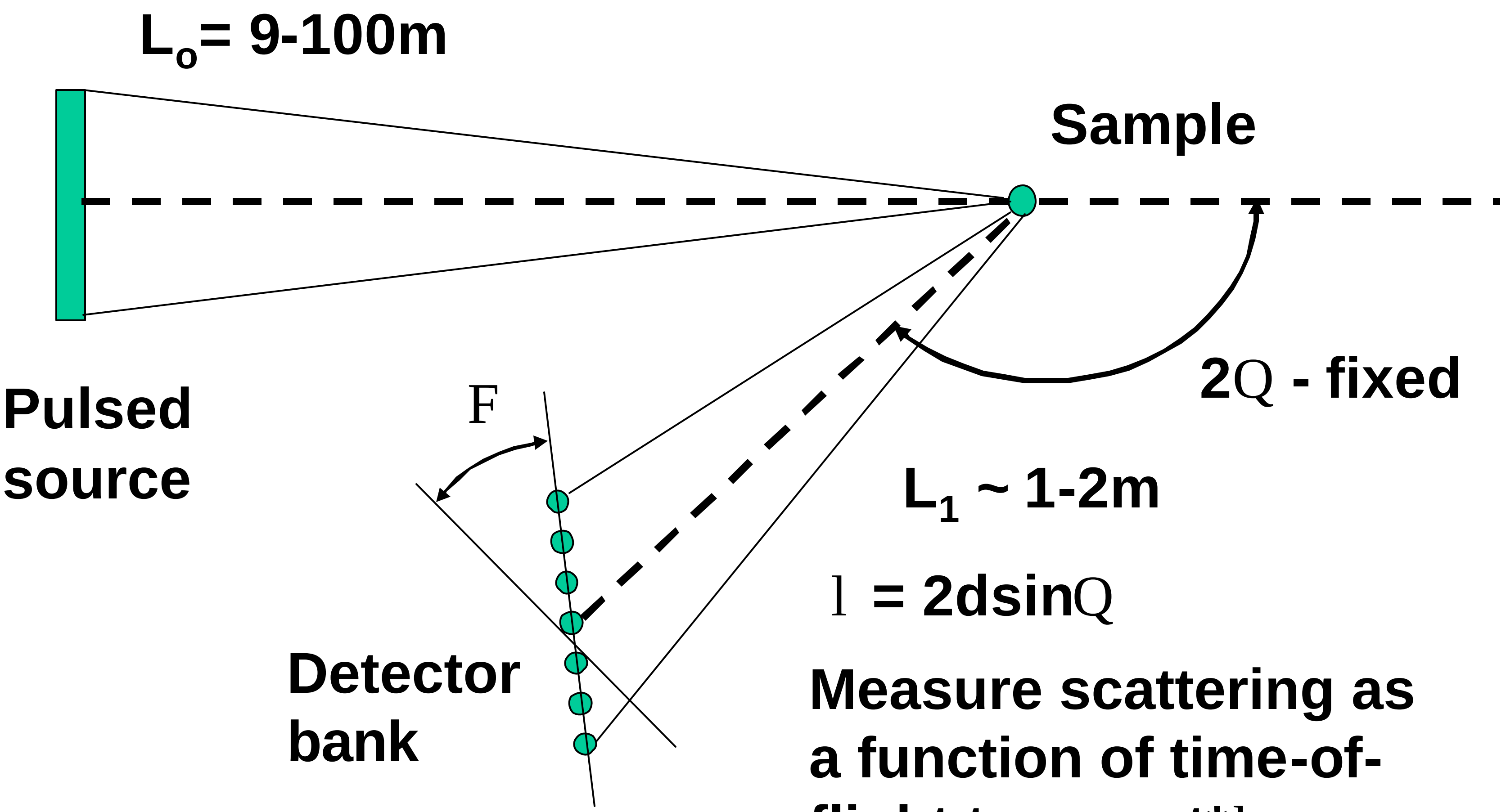
Use a continuous beam of mono-energetic neutrons.



A POWDER DIFFRACTION PATTERN RECORDED AT A REACTOR

Since we know the neutron wavevector, k, the scattering angle gives G_{hkl} directly:
$G_{hkl} = 2\,k\sin\theta$

Neutron Powder Diffraction using Time-of-Flight



L_o= 9-100m

Sample

Pulsed source

F

2Q - fixed

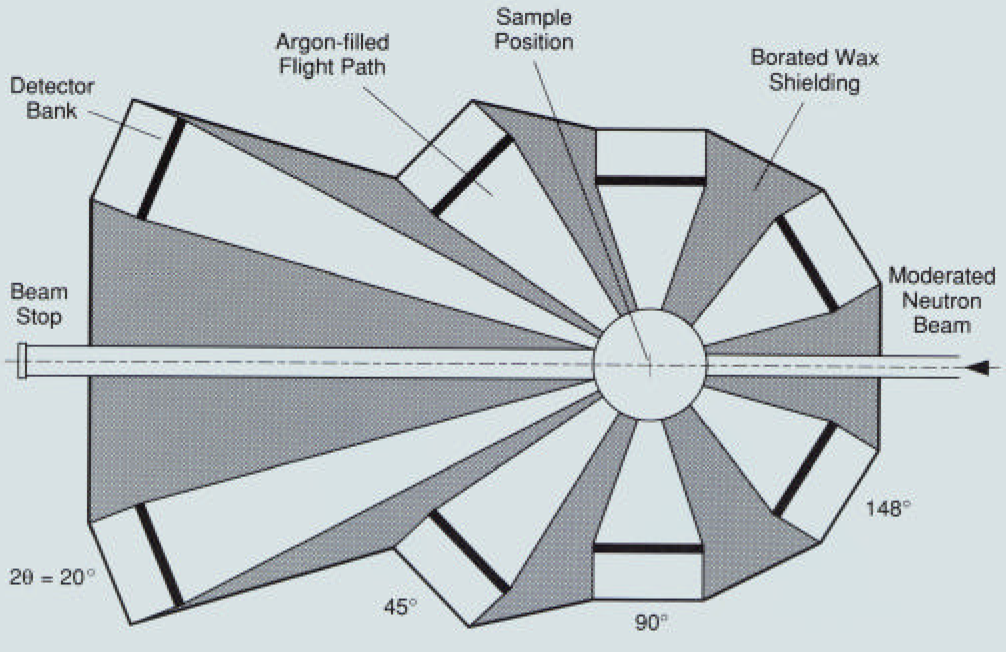
L_1 ~ 1-2m

Detector bank

l = 2dsinQ

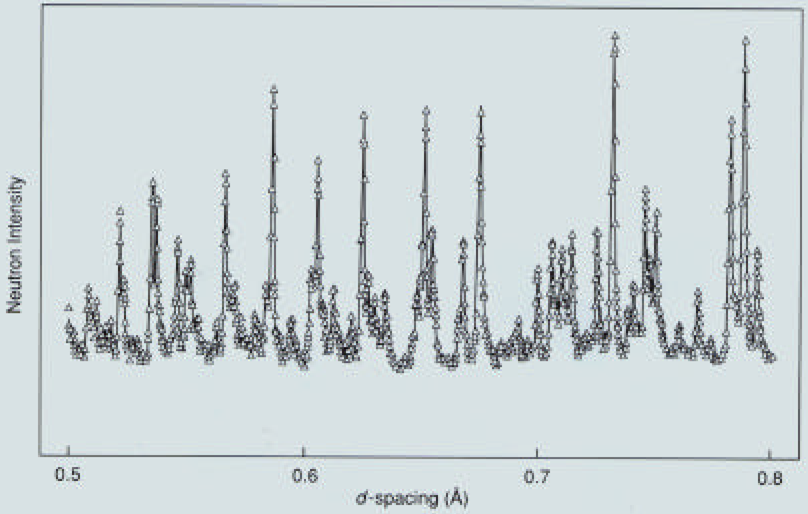
Measure scattering as a function of time-of-flight t = const*l

Time-of-Flight Powder Diffraction



POWDER DIFFRACTION AT A SPALLATION SOURCE

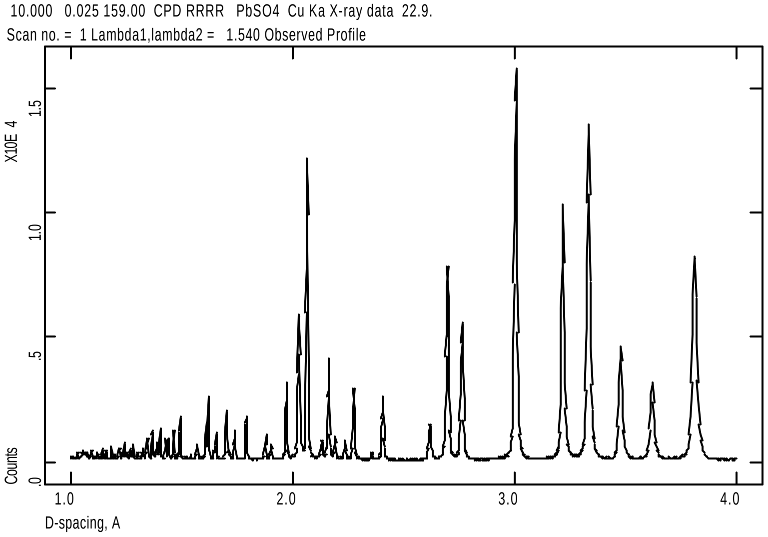
Use a pulsed beam with a broad spectrum of neutron energies and separate different energies (velocities) by time of flight.

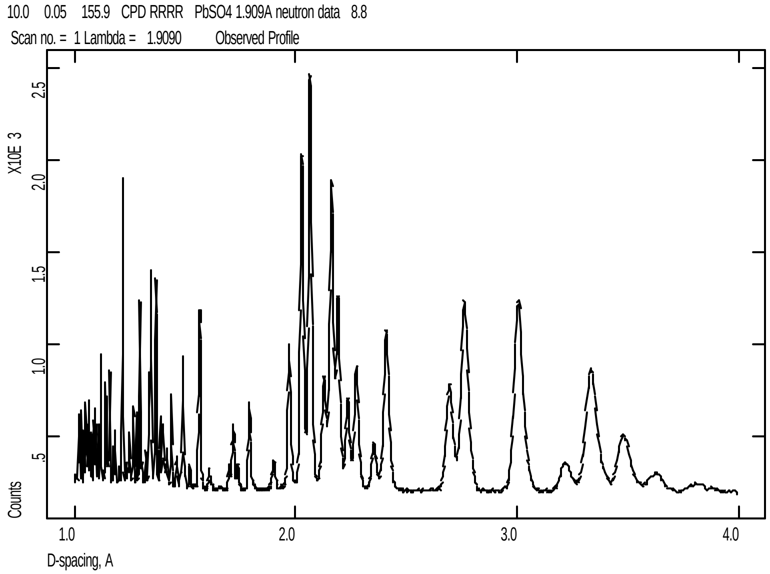


A POWDER DIFFRACTION PATTERN RECORDED AT A SPALLATION SOURCE

Compare X-ray & Neutron Powder Patterns

X-ray Diffraction - CuKa
Phillips PW1710
- **Higher resolution**
- **Intensity fall-off at small d spacings**
- **Better at resolving small lattice distortions**



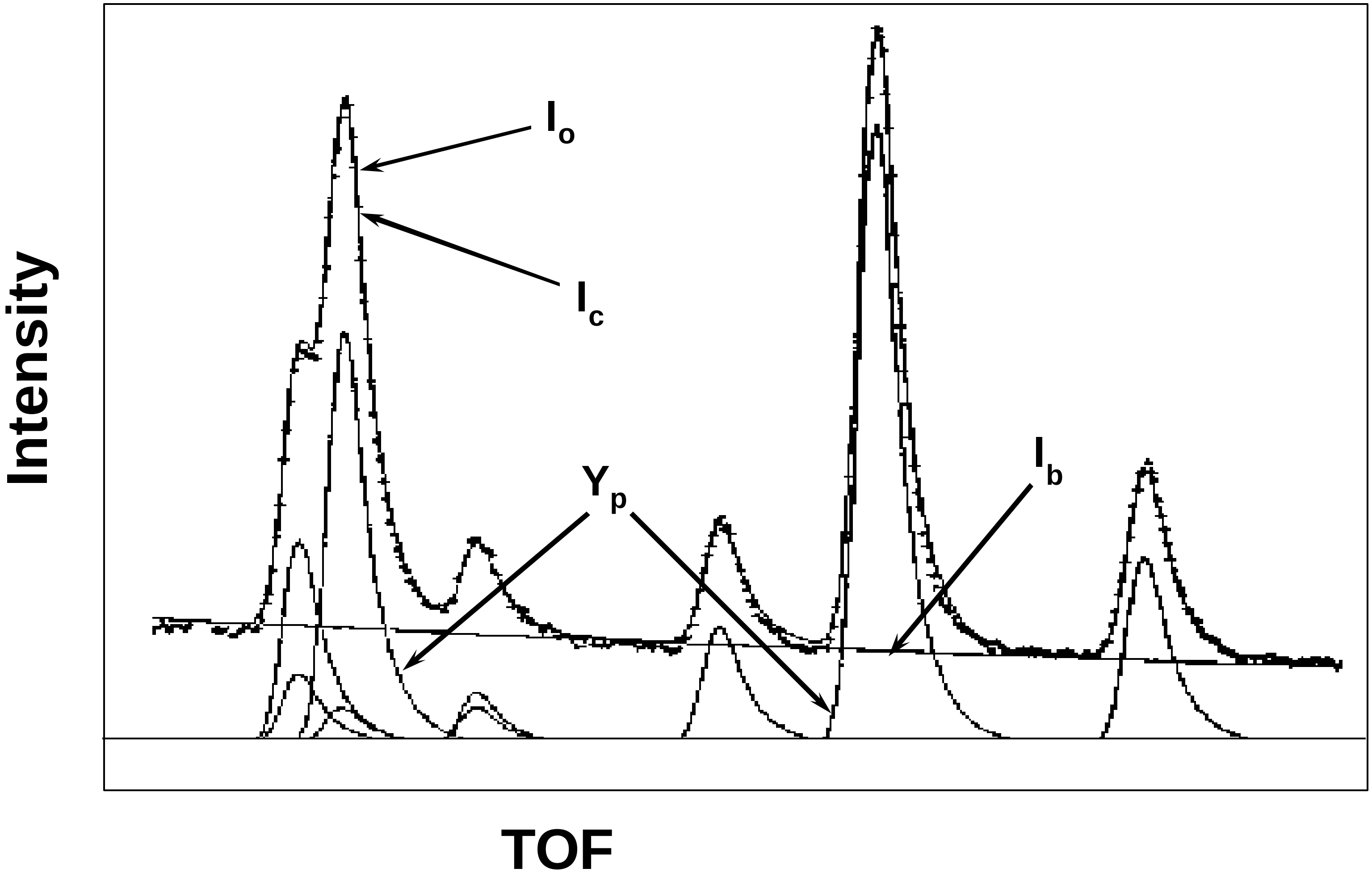


Neutron Diffraction - D1a, ILL
λ=1.909 Å
- **Lower resolution**
- **Much higher intensity at small d-spacings**
- **Better atomic positions/thermal parameters**

X-ray powder diffraction is beginning to be used to obtain structures of biomolecules

There’s more than meets the eye in a powder pattern*

Rietveld Model $\mathbf{I}_c = \mathbf{I}_b + S\mathbf{Y}_p$



*Discussion of Rietveld method adapted from viewgraphs by R. Vondreele (LANSCE)

The Rietveld Model for Refining Powder Patterns

$$I_c = I_o\{S k_h F^2_h m_h L_h P(D_h) + I_b\}$$

I_o - incident intensity - variable for fixed 2Q

k_h - scale factor for particular phase

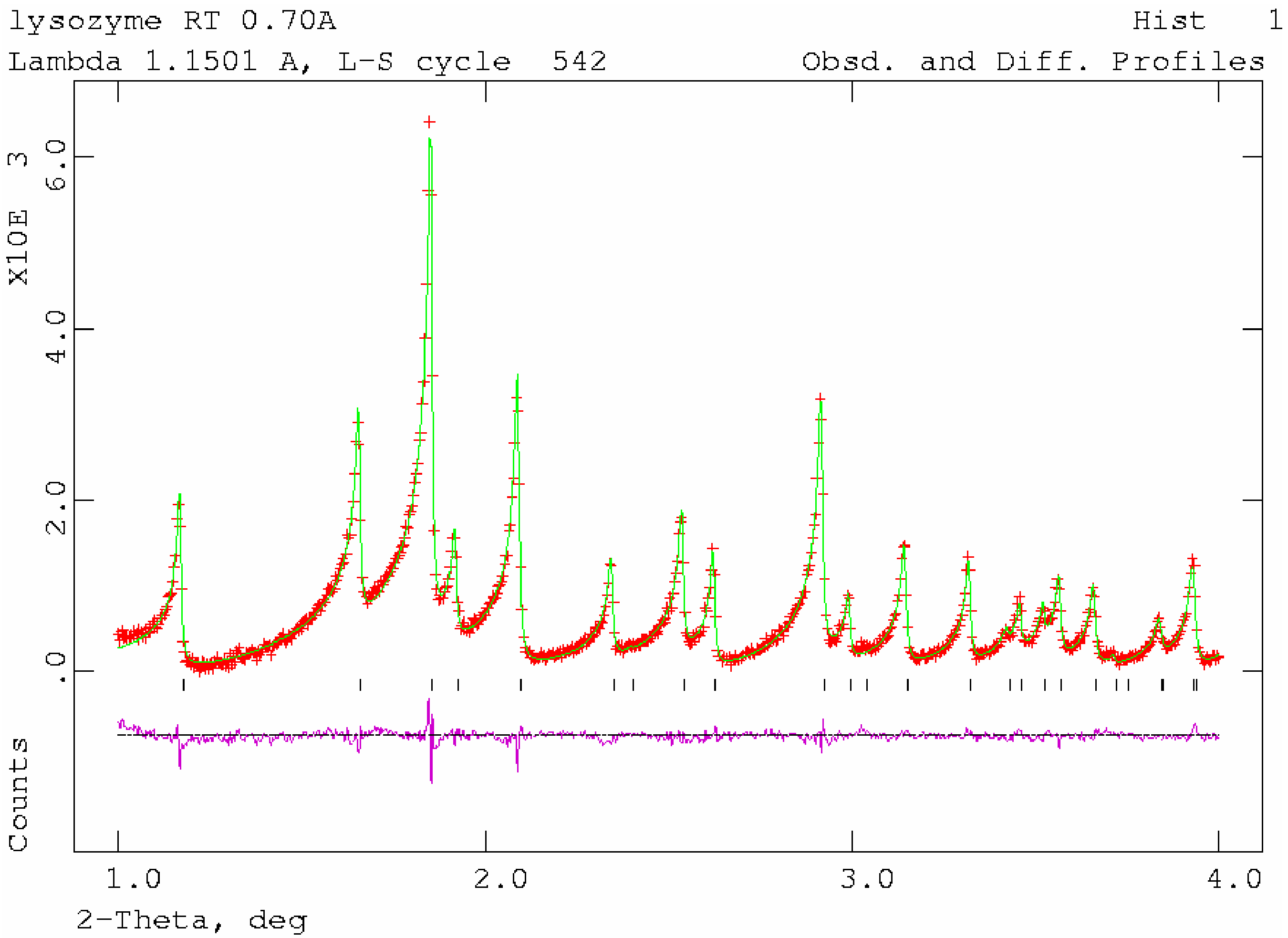
F^2_h - structure factor for particular reflection

m_h - reflection multiplicity

L_h - correction factors on intensity - texture, etc.

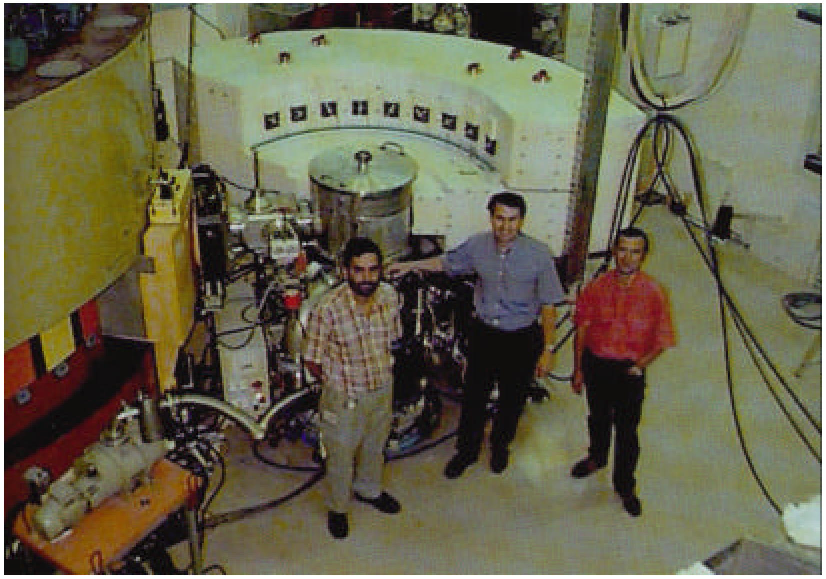
$P(D_h)$ - peak shape function – includes instrumental resolution, crystallite size, microstrain, etc.

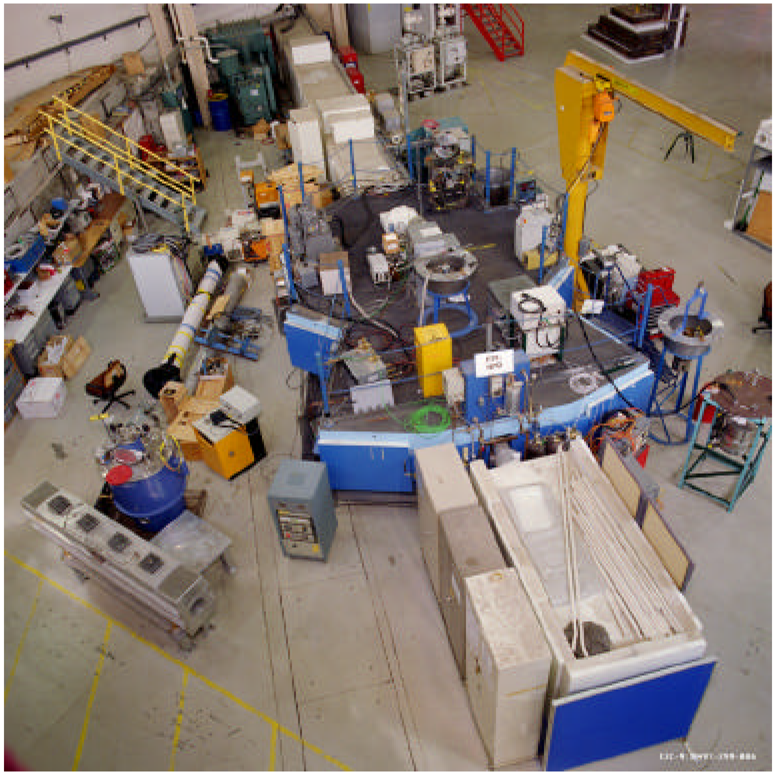
How good is this function?



Protein Rietveld refinement - Very low angle fit
1.0-4.0° peaks - strong asymmetry
“perfect” fit to shape

What do Neutron Powder Diffractometers look like?



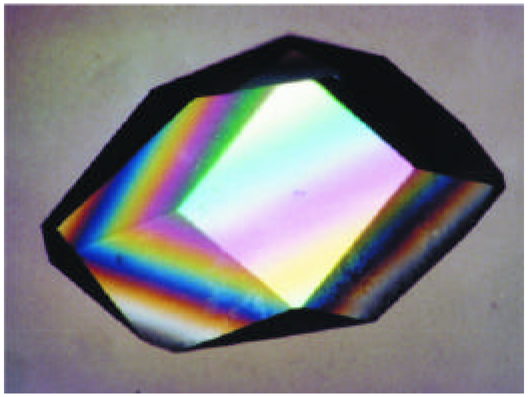


Note: relatively massive shielding; long flight paths for time-of-flight spectrometers; many or multi-detectors on modern instruments

Macromolecular Crystallography using Synchrotron Radiation provides Detailed Molecular Structures

- The principle steps are:
  - Isolation, purification
  - Cloning and expression (several mg are required)
  - Crystallization
  - Preliminary x-ray survey – cell dimensions, space group, quality of crystal, sensitivity to radiation damage
  - Data collection (perhaps including MAD) – 1 Å resolution usually requires measurement of several x 100,000 unique Bragg reflections
  - Phase determinations
  - Electron density maps
  - Structure refinement

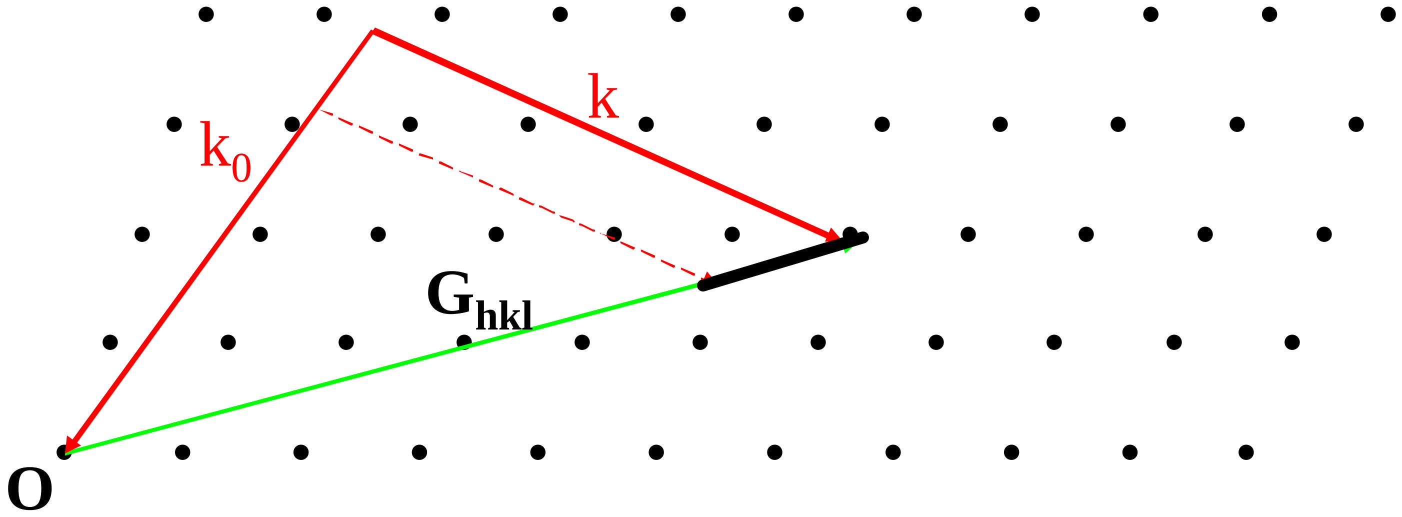
- For neutrons we must add:
  - Producing even bigger crystals (several mm^3)
  - Deuteration (may reduce crystal size needed)
  - Largest MW is less than x-rays
    - Current neutron record same as synchrotron record in 1990



Large lysozyme crystal grown on the space shuttle

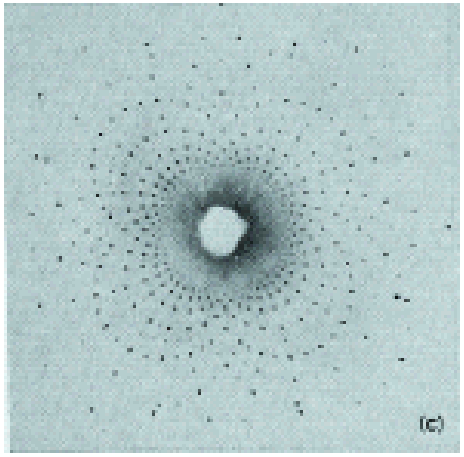
The Reciprocal Lattice for Multiple Wavelengths

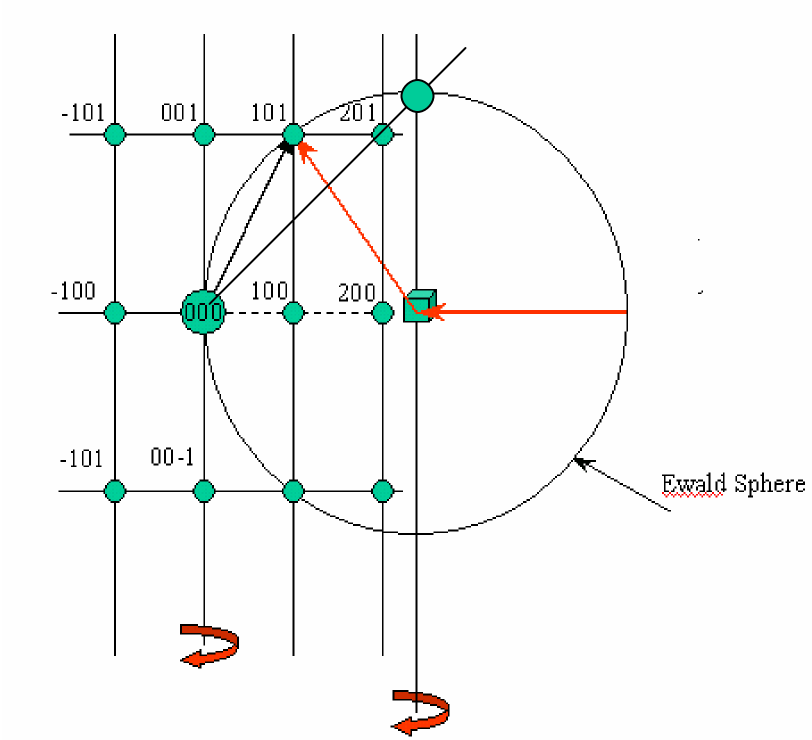
- With a continuous band of wavelengths, each reciprocal lattice point becomes a line pointing towards the origin



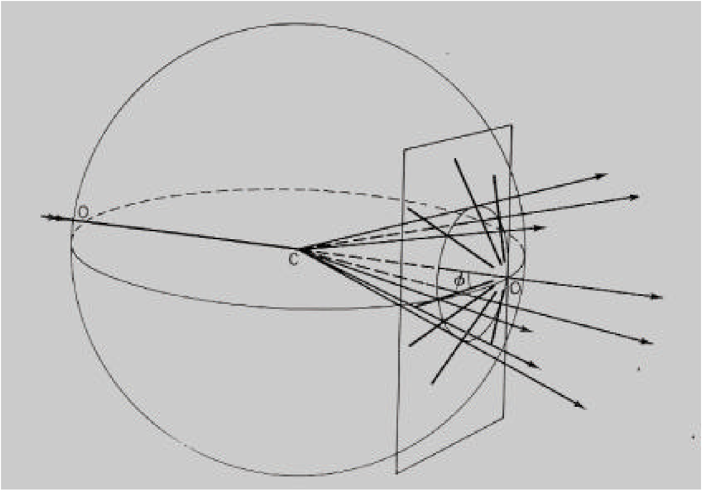
What does the Scattering Pattern look like for Multiple Wavelengths?

- Many of the radial rods intersect the Ewald sphere and given rise to Bragg reflections
- The Laue pattern reflects the crystal symmetry





Note that diffraction orders overlap for a broad wavelength band



What is the Role for Neutron Scattering in Protein Crystallography?

- Even the highest resolution synchrotron x-ray studies have trouble locating protons in protein crystals
  - Especially true if there is a close-by metal atom (e.g. in enzymes)
  - X-rays measure electron density – if a proton and its electrons are displaced x-rays will give a false impression of proton location

- Neutron diffraction can locate protons even in moderate resolution studies (~2 Å)
  - Neutrons locate nuclei not (generally) electrons
  - Either H or D scatter comparably with other nuclei

- There are many cases were H plays a vital role in proteins
  - Primary motive power for many enzymatic reactions
  - Hydrogen bonding and hydration contribute to structural stability

The Laue Method is a Powerful Tool for Neutron Protein Crystallography

- “Quasi-Laue” technique implemented at continuous neutron sources
  - “quasi” because it uses restricted wavelength band (1.5 to 2 Å typically) to avoid overlapping Bragg peaks
  - Use image-plate detectors that measure over a wide range of scattering angles but with relatively low efficiency
  - Typically requires crystals of several mm^3 and MW less than ~50 kDa

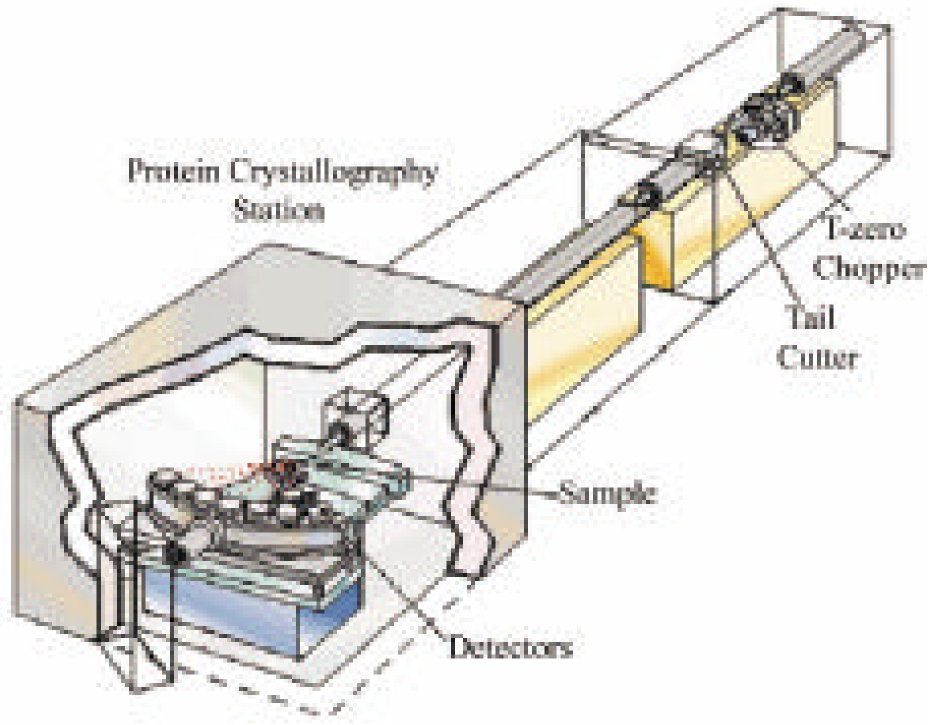
- Full Laue method implemented at LANSCE*
  - Uses wavelengths from ~0.6Å to ~6.5Å, separated by TOF
  - Peak-to-background ratio is excellent because bgr is spread over many TOF channels
  - Has recently solved structure with MW ~ 160 kDa

*Langan et al J. App. Cryst., <u>37</u>, 24 (2004)

The Protein Crystallography Station (PCS) at Los Alamos

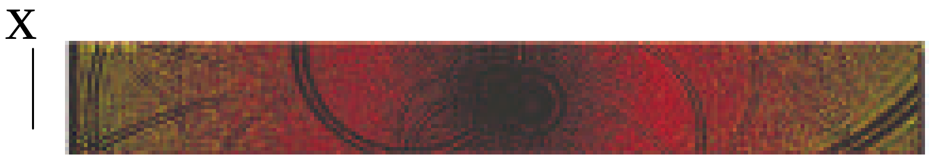
- The PCS sees a broad wavelength neutron beam, pulsed at 20 Hz
- The time-of-flight of a neutron from source to detector determines

  λ (Å) ~ 4 t(ms)/L(m)

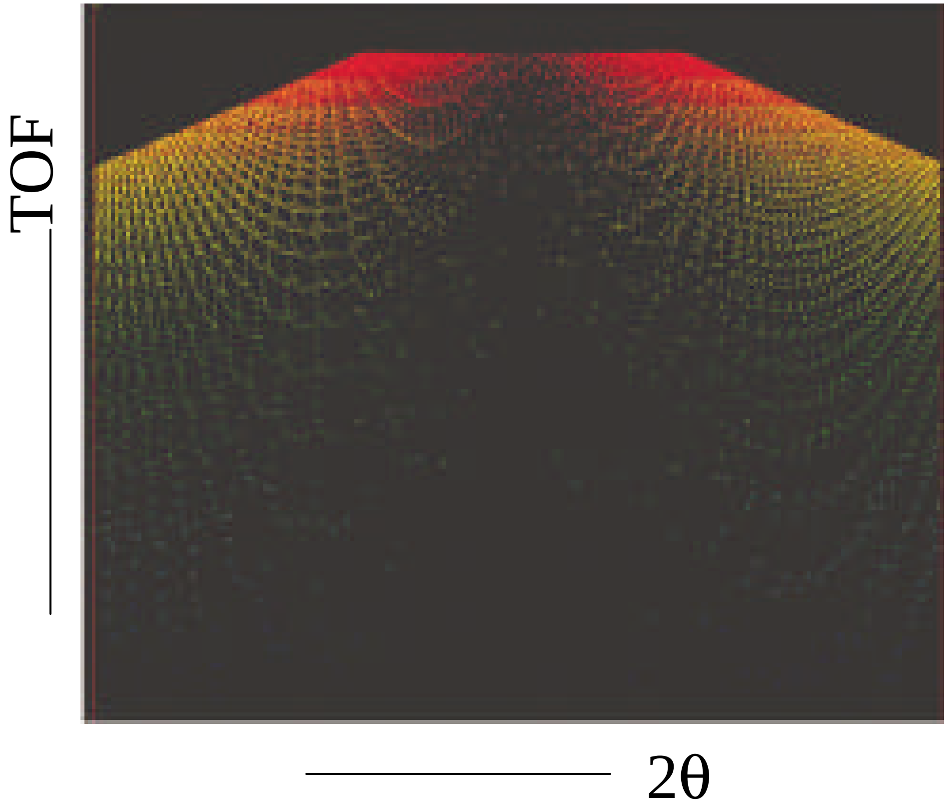


The heart of the PCS is an advanced neutron detector that subtends 16^{o} x 120^{o} at the sample position ($0.3\ m^2$ active area with a spatial resolution of ~1.5mm)

Neutron TOF Laue Patterns for Cubic Insulin*



Detector image integrated over λ – equivalent to the pattern obtained at a reactor



The same data, integrated over x, showing how the reflections are separated in TOF i.e. in λ.

The data are color coded with the shortest wavelength (hot) neutrons in red and the longest wavelength (cold) neutrons in blue

*Langan and Greene, J. Appl. Cryst., 37, 253 (2004)

Preparing a Neutron Protein Crystallography Experiment

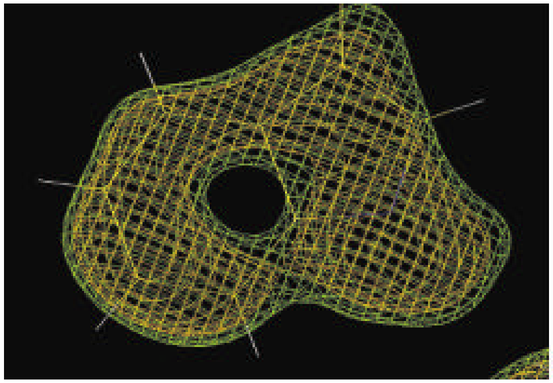
- Need to know x-ray structure and have a good scientific case for needing to know H positions
- X-ray crystals are ~0.1 mm^3; neutrons need 1 mm^3 or larger for hydrogenated samples
- Scattering power increases by ~10x if crystal is deuterated
- Typically, insert gene that encodes protein in *E Coli* and grow up using D_2O and deuterated nutrient – probably need about 5 L of final medium for neutron experiment (this can be done at LANSCE)
- Extract protein and grow deuterated crystal – crystal mosaic needs to be 0.2^o to 0.3^o
- MW limit is currently about 150 kDa for good quality crystals

The Process of Obtaining Neutron Laue Patterns

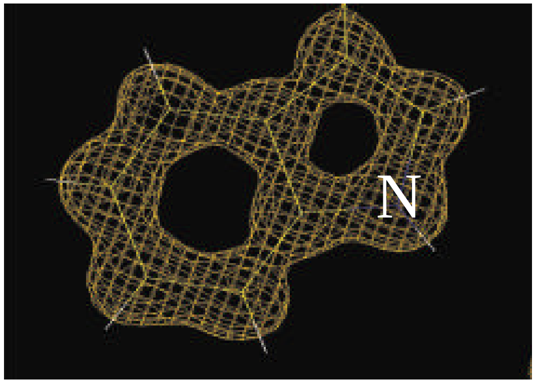
- Detector response correction – use incoherent scatterer
- Check crystal centering – telescope
- Collect data (~12 hours per crystal orientation)
- Check diffraction quality – intensity & standard deviation
- Locate diffraction spots in x, y and λ
- Check no spurious peaks
- Index reflections & determine crystal orientation
- Predict reflection positions & overlay on observations
- Integrate reflections
- Perform wavelength normalization ($I \sim \lambda^2$)
- Average intensities of equivalent reflections
- Refine crystal structure (use x-ray structure)

A Recent Example: Preliminary Measurements on D-xylose Isomerase*

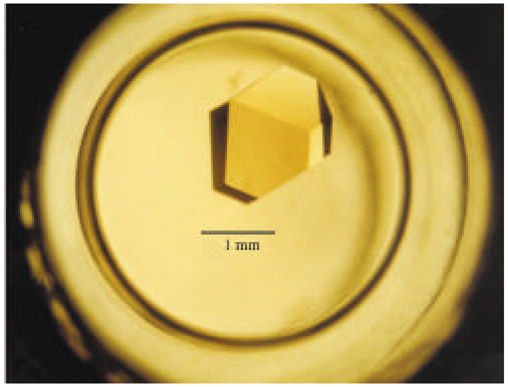
- X-ray structure known with very high resolution (<1 Å) (MW=160kDa)
- Unable to decide between postulated catalytic mechanisms
  - Enzyme action transfers an H from one C atom of substrate to an adjacent C
  - Three different mechanisms proposed – each involves differences in H-atom orientations at active sites



Neutron



X-ray



Crystal used in experiment

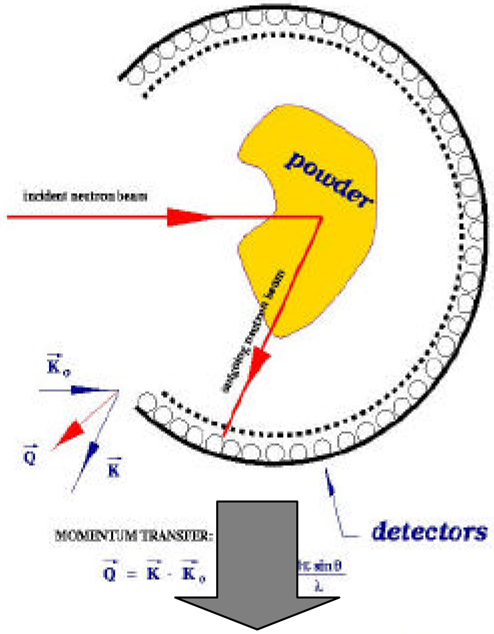
Neutron data show that the ring N atom on Trp137 is deuterated: cannot see this in electron density deduced from x-ray data

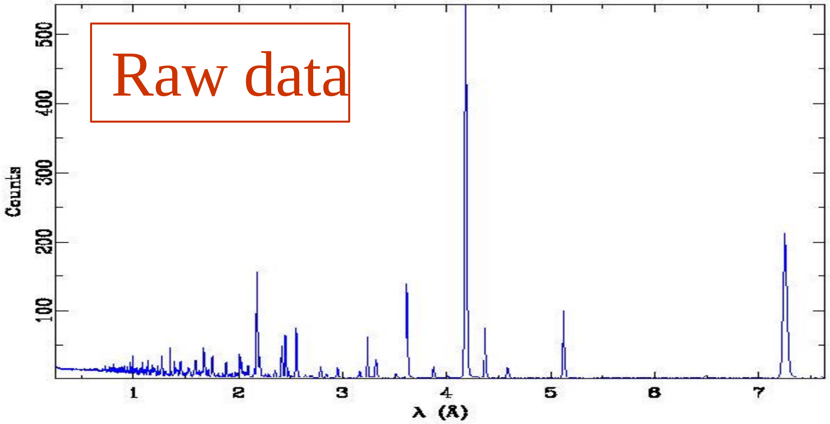
Hanson et al., Acta Cryst., D60, 241 (2004)

Pair Distribution Functions

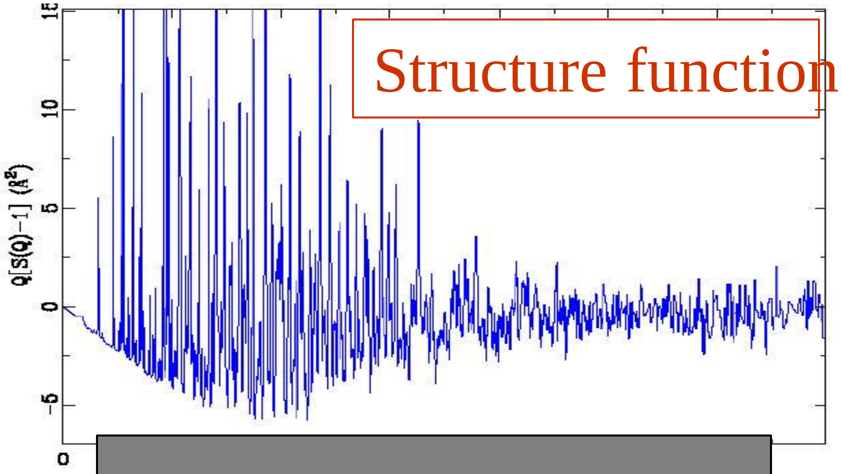
- Modern materials are often disordered.
- Standard crystallographic methods lose the aperiodic (disorder) information.
- We would like to be able to sit on an atom and look at our neighborhood.
- The PDF method allows us to do that (see next slide):
  - First we do a neutron or x-ray diffraction experiment
  - Then we correct the data for experimental effects
  - Then we Fourier transform the data to real-space

Obtaining the Pair Distribution Function*



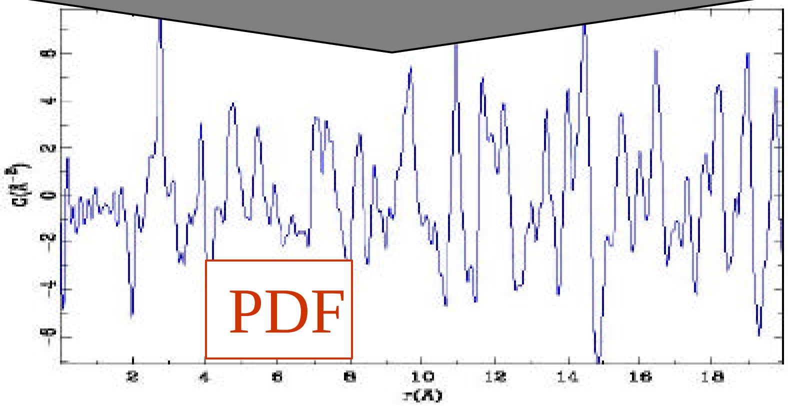


Raw data



Structure function

$$G(r) = \frac{2}{p}\int_0^\infty Q[S(Q)-1]\sin Qr\,dQ$$

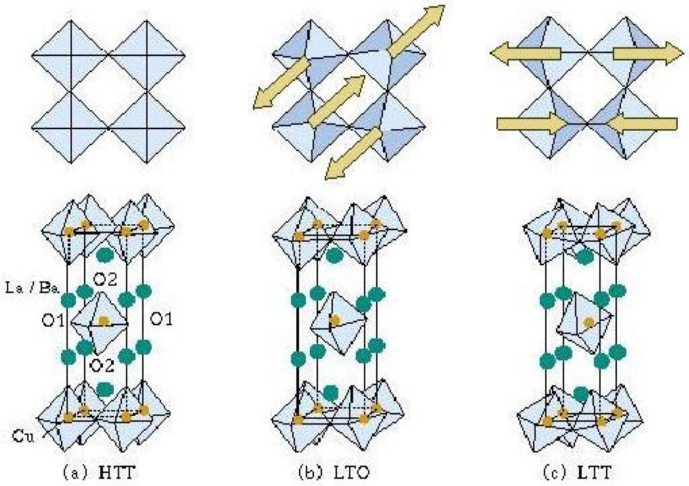


PDF

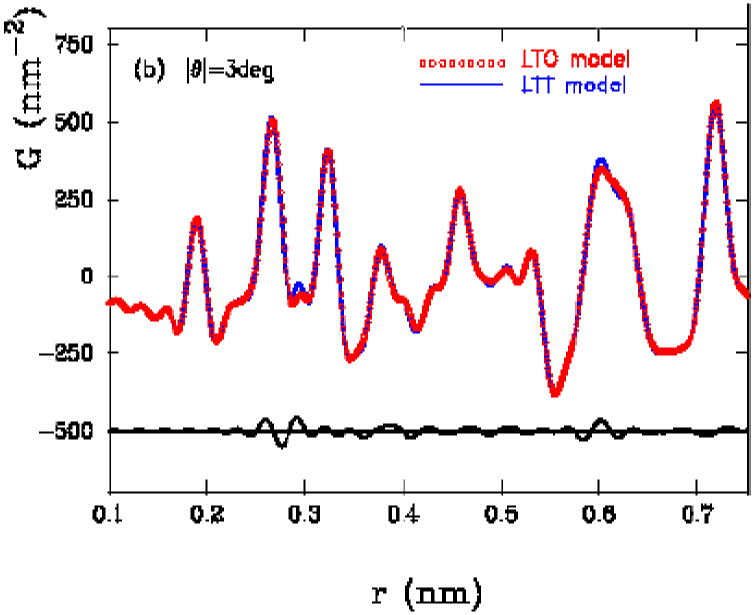
* See http://www.pa.msu.edu/cmp/billinge-group/

Structure and PDF of a High Temperature Superconductor

The structure of $La_{2-x}Sr_xCuO_4$ looks like this: (copper [orange] sits in the middle of octahedra of oxygen ions [shown shaded with pale blue].)



(a) HTT (b) LTO (c) LTT

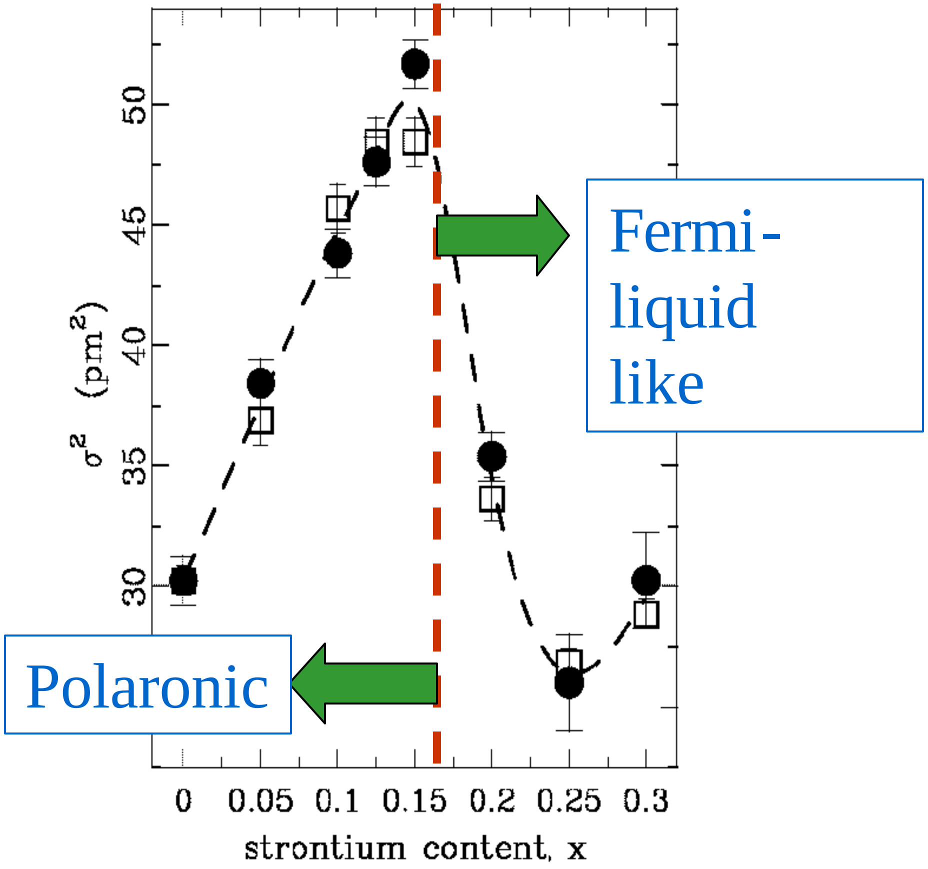


The resulting PDFs look like this. The peak at 1.9A is the Cu-O bond.

So what can we learn about from the PDF?

Effect of Doping on the Octahedra

- Doping holes (positive charges) by adding Sr shortens Cu-O bonds
- Localized holes in stripes implies a coexistence of short and long Cu-O in-plane bonds => increase in Cu-O bond distribution width with doping.
- We see this in the PDF: σ^2 is the width of the CuO bond distribution which increases with doping then decreases beyond optimal doping



Fermi-liquid like

Polaronic

Bozin *et al*. Phys. Rev. Lett. Submitted; cond-mat/9907017

Writing Proposals for Neutron Beam Time

- What’s the problem?
  - Describe the scientific problem you want to solve in a manner that would be clear to a scientist who works in the general area of your proposed experiment, but who may be unfamiliar with the details.

- Why do we care?
  - Include a well-developed motivation for the experiment including objectives, relation to theory, scientific or programmatic merit, and relevant references.

- What do we do about it?
  - Explain how the experiment you propose can answer the scientific question you have described.
  - The proposal should include a description of the intended data analysis procedure. This will be especially important for experiments that may require the development of novel data analysis techniques

- What have others done?
  - Summarize the current state of knowledge relevant to your proposed experiment, including prior work and preliminary data, e.g., x-rays, light scattering, structure determination, NMR, modelling.

Writing Proposals *(cont'd)*

- Why will neutron scattering help?
  - What information can you get from neutrons that you can't get from another technique?
  - Give any arguments that support the feasibility of your experiment and the requested measuring time, including count rates, backgrounds, resolution, detector systems, and ability of the research team to carry out the project.
  - What complementary experiments will be done to fully develop the science?
  - How was your beam time request calculated?

- Dos and Don'ts
  - Do not assume Program Advisory Committee members will read references you cite. Include relevant arguments from these references in the proposal.
  - If your proposal is part of a continuing research program, describe the overall goal of the program. It is also essential that you submit Experimental Reports from earlier experiments.
  - The Committee may not receive more than a certain number of figures or color copies – check before you write the proposal
  - Check facility web site for spectrometer details
  - Talk to the Instrument Scientist about your experiment, especially if you need non-standard instrument parameters or sample environment

Homework

- Explore the following sites for information about the proposal process and bring questions to the next class

www.ncnr.nist.gov
www.lansce.lanl.gov
http://neutrons.ornl.gov/
www.pns.anl.gov/