Diffraction – Theory & Data Collection

Lewis & Clark Workshop
Macromolecular Crystallography
© Michael S. Chapman
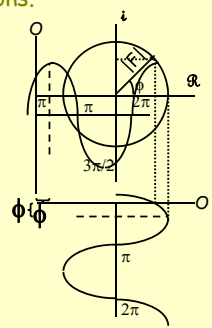
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Part 1:

X-RAYS

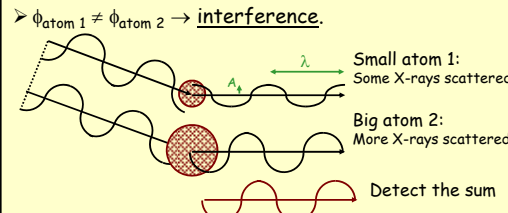10/16/2009   Michael S. Chapman (Oregon Health & Science University)   2

Definitions.

- Electromagnetic waves.
- Amplitude (A) varies as cosine of distance from origin (O). |F|
- Wavelength = λ, split into 360° or 2π radians.
- Phase (φ or α)
  - Measured:
    - origin → +ve peak
    - angle from ℜ-axis (anticlockwise)

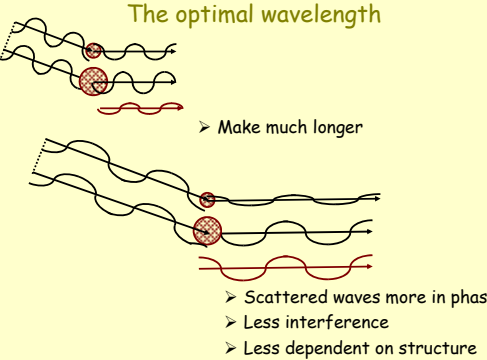10/16/2009   Michael S. Chapman (Oregon Health & Science University)   3

Why X-rays?

- Atoms scatter X-rays in all directions.
- Phase change is exactly π rad.
- Measure sum of scattering.
- Path length through 2nd atom differs.
- $\phi_{atom\ 1} \neq \phi_{atom\ 2} \rightarrow$ interference.

Small atom 1:
Some X-rays scattered

Big atom 2:
More X-rays scattered

Detect the sum

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The optimal wavelength

- Make much longer

- Scattered waves more in phase
- Less interference
- Less dependent on structure

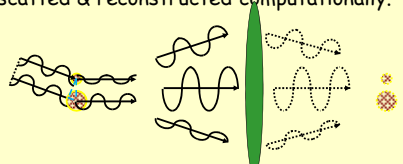10/16/2009   Michael S. Chapman (Oregon Health & Science University)   5

X-rays have wavelengths close to optimal

- Max. interference: path length difference ≈ λ.
- λ ~ interatomic distances.
- Choose 0.7 Å ≤ λ ≤ 1.8 Å
- Commonly λ ~ 1.5 Å ~ C--C bond.

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Why Diffraction? – Crystallography in a nutshell
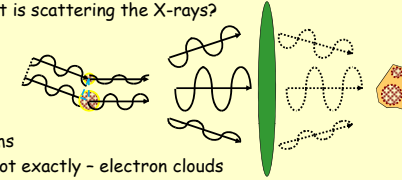
➤ X-ray refractive indices close to 1.0.
➤ ~No refraction or reflection → No lenses.
➤ Uncharged →No electrostatic lenses like E.M..
➤ Left with diffraction!
➤ X-rays scatted & reconstructed computationally.

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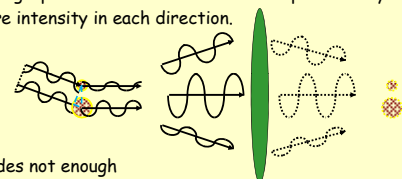
Atoms or Electron Density

➤ What is scattering the X-rays?

➤ Atoms
  ▪ Not exactly – electron clouds
➤ Image is electron density
  ▪ Atomic Structure is an interpretation…
    ▪ Nuclear positions consistent with electron density.
  ▪ Exptl error in density can → difficult interpretation.

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Crystallography in a nutshell

➤ Microscopy without the lens…
➤ Lens would physically "sum" scattered waves.
➤ Crystallographers have to "sum" waves computationally.
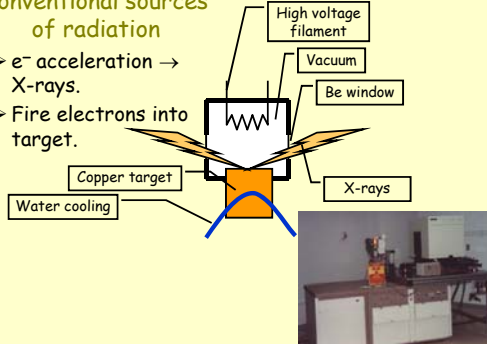➤ Measure intensity in each direction.

➤ Amplitudes not enough
➤ Phases – synchronization of waves
  ▪ How they line up, how far peaks lag behind each other.
  ▪ Can't be measured directly – "*Phase Problem*" Challenge.

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Conventional sources of radiation

High voltage filament
Vacuum
Be window

➤ e⁻ acceleration → X-rays.
➤ Fire electrons into target.

Copper target
Water cooling
X-rays

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Synchrotrons

➤ e⁻ accelerated in circular path
  ▪ Relativistic speeds
➤ Energy (X-rays) tangential
➤ High intensity
  ▪ More data before damage spreads
➤ Tunable wavelength
  ▪ Phasing
➤ Shared multi-user facilities

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Preparation of X-rays

➤ Fine parallel beam
  ▪ Collimator
  ▪ Focusing mirrors
➤ Monochromatic (single wavelength)
    ▪ 1.51 Å or 0.91 Å or adjusted 0.7 – 2.0 Å
  ▪ Monochromator
  ▪ Focusing mirrors

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Interaction of X-rays with samples
- Absorption (fluorescence)
  - Mostly minor annoyances
- Scattering
  - Important – because no lenses for imaging
  - Need understanding of how atomic structure affects the scattering of materials
- Elastic (Coherent aka Thompson) scattering
  - No loss of energy (λ unchanged; $\Delta\varphi = \pi$).
  - Dominates diffraction.
- Inelastic (Incoherent aka Compton) scattering
  - Collision w/ e^- (energy transfer)...
  - Background scattering

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Electromagnetic waves.
$$E_i = E_{Oi}[\cos 2\pi\nu(t - x/c) + i\sin 2\pi\nu(t - x/c)]$$
- E_i is field @ time t, position, x from origin O.
- $i = \sqrt{-1}$; E_i represented as complex vector.
  - Only amplitude observed, but
    - complex form allows addition of waves.
- By de Moivre's theorem ($e^{ix} = \cos x + i\sin x$):
- $E_i = E_{Oi}\exp 2\pi i\dot\nu(t - x/c)$
- Field accelerates a charged particle with frequency ν.
  - Max. acceleration as particle passes node @ max E_i.
  - Thus (electron) particle displacement $\pi/2$ from E_i.
- The accelerating orbital electron initiates a second electromagnetic wave with a 2nd phase change of $\pi/2$.

E_{Oi} E_i

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Part 2

DIFFRACTION BY FOURIER TRANSFORM

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Interference of Scattered X-rays
- Scatter from O and O' related by vector $\mathbf{r}$.
- Path-difference:
  - $|OA| + |OB| = |\mathbf{r}|\cos A\hat{O}O' + |\mathbf{r}|\cos B\hat{O}O'$
    $= \mathbf{r} \cdot (\mathbf{s} - \mathbf{s_o})$
    - $\mathbf{s_o}$ unit vector along incident direction.
    - $\mathbf{s}$ unit vector along scattered direction.
- $\Delta\phi = (2\pi/\lambda)\mathbf{r} \cdot (\mathbf{s} - \mathbf{s_o})$

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Interference of Scattered X-rays (2)
- $\Delta\phi = 2\pi\mathbf{r}\cdot\mathbf{r^*}$
- where $\mathbf{r^*} = (\mathbf{s} - \mathbf{s_o})/\lambda$
- $\mathbf{r^*}$
  - units: reciprocal distance;
  - vector in *reciprocal space*.
- Consider plane/line $OQ \perp \mathbf{r^*}$:
- $|\mathbf{r^*}| = r^* = 2\sin\theta/\lambda$
- Angles of incident and scattered beams to OQ are both θ.
  - Imagine beam reflected from plane $OQ \perp \mathbf{r^*}$.

Direction of r* comes from eqn 2 of this page

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William & Lawrence Bragg
- Max von Laue demonstrated wave character of x-rays
  - Diffraction by crystals
- Braggs – father & son
  - 1913 - 1914
  - Realized that x-ray scattering could be imagined as reflection from planes
  - Solved NaCl.
  - Nobel prize 1915

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Atomic Structure Factor eqn.

- $_{ABS}E_d = E_{Od} \exp 2\pi i \nu(t-r/c)-i\pi$
  - From last lecture
- $_{REL}E_{Od} \exp 2\pi i\dot\nu(r/c) = {}_{REL}E_{Od} \exp 2\pi i\dot r/\lambda$
- Substitute $\Delta\phi = r/\lambda$ (= $2\pi r \cdot r^*$)
  - & sum over N scattering points:
- Let r_j be center of jth atom.
- $F(r^*) = \Sigma^N_{j=1} A_j \exp 2\pi i r^* \cdot r_j$
- Consider scattering relative to a free electron.
  - By Convolution Theorem (later):
  - $F(r^*) = \Sigma^N_{j=1} f_{at,j}(Z, r^*, U) \exp 2\pi i r^* \cdot r_j$

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Atomic Structure Factor eqn.(2)

- $F(r^*) = \Sigma^N_{j=1} f_{at,j}(Z, r^*, U) \exp 2\pi i r^* \cdot r_j$
- $f_{at} \equiv$ *atomic scattering factor* $\equiv \mathcal{FT}$ isolated atom (later):
- Depends on # electrons, thermal vibration.
  - Tabulated theoretical or experimental values.
- Can be approximated roughly by spherically symmetric Gaussian.
- Scattering $\equiv \mathcal{FT}$(molecule)

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Scattering by elements of electron density

- $F(r^*) = \Sigma^N_{j=1} A_j \exp 2\pi i r^* \cdot r_j$
- Let r_j be small element of electron density, ρ.
- Consider total scattering:
  - $F(r^*) = \int_V \rho(r) \exp 2\pi i r^* \cdot r$ dr
- Right-hand side $\equiv \mathcal{FT}(\rho)$.
- Structure determination:
  - measure amplitude
  - determine phase throughout (continuous) function, $F(r^*)$
  - compute inverse $\mathcal{FT} \rightarrow$ electron density:
- $\rho(r) = T^{-1}[F(r^*)] = V^* \int_{V^*} F(r^*) \exp -2\pi i r^* \cdot r dr^*$

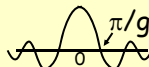10/16/2009   Michael S. Chapman (Oregon Health & Science University)   21

Fourier Transforms (1)

- If $f(x)$ is piecewise integratable, it can be approximated by a Fourier transform (T):

  Do not confuse this f (general function) with f – the atomic scattering factor

- $f(x) = \int_{x^*} F(x^*) \exp -2\pi i x \cdot x^* dx^*$
- $F(x^*)$ is
  - a "Fourier coefficient"
  - given by an inverse Fourier transform (T^{-1}):
- $F(x^*) = \int_x f(x) \exp 2\pi i x \cdot x^* dx$
  - Both F, f may be complex.
  - Crystallographically: $\rho = \Re(f)$.
- Designation of forward, inverse is conventional for crystallography. As both are Fourier transforms, they have corresponding mathematical properties.

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Fourier Transform of a Step Function

- $-g < x < g$: $f(x) = c$; else $f(x) = 0$
- $F(x^*) = \int_{-g}^{g} (c) \cdot \exp 2\pi i x^* x\, dx$
- $F(x^*) = c\int_{-g}^{g} \cos 2\pi x^* x\, dx + i c\int_{-g}^{g} \sin 2\pi x^* x\, dx$
- $f(x)$ is even function, so 2nd integral = 0:
- $F(x^*) = c[(\sin 2\pi x^* x)/2\pi x^*]_{-g}^{g}$
- $= c((\sin 2\pi g x^*)/2\pi x^* -(\sin -2\pi g x^*)/2\pi x^*))$
  $= c(\sin 2\pi g x^*)/\pi x^*$
- Note wider step (large g)
  - --> short period of $F(x^*)$.
- **Generally: large objects in real (direct) space are small in reciprocal (Fourier transform) space** *et vice versa.*

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Infinite 1-D lattice -- Lessons

- $\mathcal{FT}$(lattice) = (lattice), in reciprocal space.
- Spacing of reciprocal lattice inversely proportional to real lattice.
- Diffraction of crystals $\rightarrow$ relatively strong diffraction spots and insignificant intensity between spots.
- Need only use lattice points --> discrete transform.

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Fourier Series

- $\mathcal{FT}$ can approx. any piecewise integratable function.
- Coefficients each have amplitude and phase
- Effect of truncation

Fig. 18. Periodic function.

Fig. 19. Summation of waves approaches the shape of the periodic function.

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Electron Density Equation

- $\rho(\mathbf{x}) = 1/V \, \Sigma_\mathbf{h} F_\mathbf{h} \exp -2\pi i\, \mathbf{h}\cdot\mathbf{x}$
- $\rho(x,y,z) = 1/V \, \Sigma_{h=-\infty,+\infty} \, \Sigma_{k=-\infty,+\infty} \, \Sigma_{l=-\infty,+\infty}$
  $[A_{hkl}\cos\{-2\pi(hx+ky+lz)\} + i\, B_{hkl}\sin\{-2\pi(hx+ky+lz)\}]$
  - Where $\mathbf{F} = A + i\, B$;
    - $\mathbf{h} = (h,k,l)$, "Miller indices";
    - $\mathbf{x} = (x,y,z)$ r.h. unit cell vectors
    - V is unit cell volume
  - $A = |\mathbf{F}| \cos \phi$; $B = |\mathbf{F}| \sin \phi$
    - $|\mathbf{F}|$ is structure factor amplitude (measured experimentally).
    - ϕ difficult to determine → "The phase problem"

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Structure Factor Equation

- $\mathbf{F}_\mathbf{h} = 1/V^* \, \Sigma_\mathbf{x}\rho(\mathbf{x})\exp 2\pi i\, \mathbf{h}\cdot\mathbf{x}^*$
- $\mathbf{F}_{hkl} = V \, \Sigma_{x=0,1} \, \Sigma_{y=0,1} \, \Sigma_{z=0,1}$
  $\rho(x,y,z)[\cos 2\pi \, (hx+ky+lz) + i \sin 2\pi \, (hx+ky+lz)]$
- Where $\mathbf{x} = (x,y,z)$ are
  - right-handed coordinates
  - measured in fractional units
    - fraction of unit cell (a,b,c)
    - $V = \mathbf{a}\cdot\mathbf{b}\wedge\mathbf{c}$.
- Note symmetry w/ electron density equation

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Atomic Structure Factor Equation

- $\mathbf{F}_{hkl} = \Sigma^N_{j=1} \, f_{at,j} \exp 2\pi i\, \mathbf{h}\cdot\mathbf{x}_j$
- $\mathbf{F}_{hkl} = \Sigma^N_{j=1} \, f_{at,j} \, [\cos 2\pi(hx + ky + lz)$
  $+ i \sin 2\pi(hx + ky + lz)]$
- Summing over atoms instead of grid points
  - Computationally less efficient if many atoms
  - Structure factor equation can use FFT

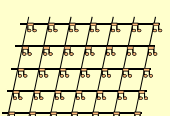10/16/2009   Michael S. Chapman (Oregon Health & Science University)   28

LATTICES

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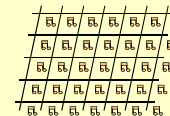
Crystalline Lattices

- Previously learned…
  - Diffraction pattern = Molecular transform sampled at reciprocal lattice points.
  - Reciprocal lattice = FT or diffraction pattern from crystalline lattice
- Why understand real-space lattices?
  - → prediction of location of diffraction spots.
  - → interaction of neighboring molecules in crystal lattice
  - (molecular symmetry)

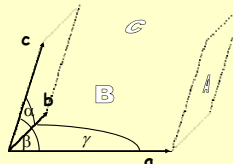10/23/2009   Workshop: Diffraction (c) 2009, M.S.Chapman   30

Lattices

➢ Grid: equally spaced || lines (planes).
➢ Choice of origin is arbitrary.
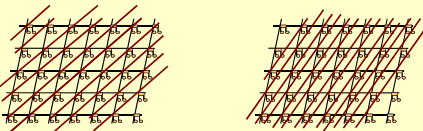
Unit Cells

➢ Axes labeled (a, b, c)
  ▪ by convention.
➢ Right-handed system
➢ Specify 3 vectors
  ▪ **a, b, c**.
➢ or 6 parameters
  ▪ 3 lengths + 3 angles
  ▪ a, b, c   + α, β, γ
  ▪ α = angle between **b** and **c** axes *etc.*.
➢ Faces labeled A,B,C
  ▪ with A intersected by a-axis *etc.*.

Lattice Planes in 2-D

➢ Line can be drawn between any 2 grid points.
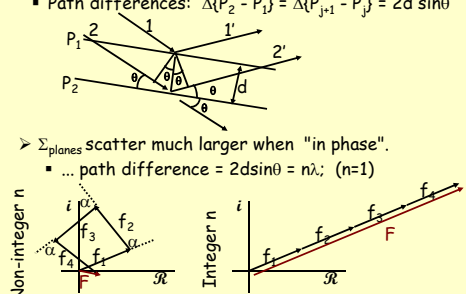➢ Parallel lines pass through every grid point.

Bragg's Law (Real space) 1

➢ Previously showed scattering like reflection from plane through scatterer.
➢ Path length same for all points on plane:
➢ All points scatter in phase.
➢ Planes containing many identical scatterers have strong scattering (Lattice planes).

Bragg's Law -- Real space 2

➢ Consider || planes $P_1, P_2, ... P_j, P_{j+1}, ... P_N$.
  ▪ Path differences: $\Delta\{P_2 - P_1\} = \Delta\{P_{j+1} - P_j\} = 2d \sin\theta$

➢ Σ_{planes} scatter much larger when "in phase".
  ▪ ... path difference = $2d\sin\theta = n\lambda$; (n=1)

Bragg's Law -- Reciprocal lattice

➢ Diffraction only at integral path difference = $2d\sin\theta = n\lambda$
  ▪ Spots spaced regularly
    ▪ Lattice in 3-D
➢ This *reciprocal* lattice has spacing inversely proportional to crystal lattice spacing
  ▪ Directions of axes depend on crystal
    ▪ Let $\tilde{d}_{100}$ be unit vector || d^*_{100}:
    ▪ $\tilde{d}_{100} = \mathbf{b}\wedge\mathbf{c}/(|b||c|\sin\theta)$
    ▪ $\mathbf{d}^*_{100} = |d^*_{100}| \tilde{d}_{100}$
    ▪ $|d_{100}| = V/(|b||c|\sin\theta) = 1/|d^*_{100}|$
    ▪ $\mathbf{d}^*_{100}$ = 1. $\mathbf{b}\wedge\mathbf{c} /(d_{100} |b||c|\sin\theta) = \mathbf{b}\wedge\mathbf{c}/V \equiv \mathbf{a}^*$
    ▪ (Remember that we are using **bold** for vectors)

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Bragg's Law – Graphically -- Implications

- For any point, P, on the the surface of the sphere, $OP = 2\sin\theta/\lambda$.
- $OP = d^*$ is a solution to Bragg's law
- Diffraction only at reciprocal lattice points
  - … when sphere and lattice point superimpose
  - Scattering || CP

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Bragg's Law Graphically – Ewald construction

- For given wavelength and crystal orientation…
  - only those *relps* on surface of the
    - *"sphere of reflection"*
  - → observable reflections.
- To see more spots, rotate crystal:
  - Rotates reciprocal lattice through sphere
  - → reflections pass through sphere

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Resolution

- Let D_{max} be the distance of furthest spot from the direct beam.
- Let d_{min} be its interplanar spacing.
- $d_{min} = \lambda/(2\sin\theta_{max}) = \lambda/2\sin\{\frac{1}{2}\tan^{-1}(D_{max}/\ell)\}$
- d_{min} is *de facto* resolution limit.
- Note d_{min} refection at max, i.e. farthest from beam.

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Topic to be skipped…

SYMMETRY

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Part 4

DATA COLLECTION & PROCESSING

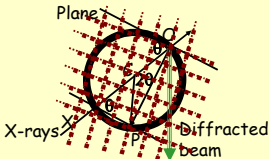10/16/2009   Michael S. Chapman (Oregon Health & Science University)   41

Diffraction Conditions

- Reminder: Bragg's Law shows that diffraction only occurs when a reciprocal lattice point lies on the sphere of reflection.

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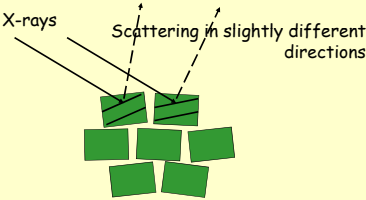
No reflections?

- No reflections on sphere of reflections if
  - Truly monochromatic radiation
  - and infinitely thin reciprocal lattice points
- Why do we see any reflections for still crystal?
  - Filters & Monochromators: $\Delta\lambda \neq 0$.
  - "Mosaic spread" → finitely large reciprocal lattice points.
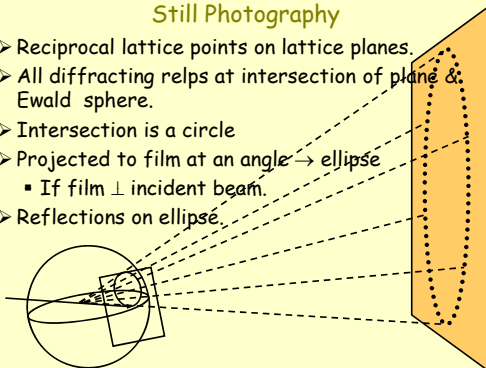
Plane

X-rays

P

Diffracted beam

Mosaic Spread

- Crystal = a mosaic of sub-micron crystalline blocks
  - separated by fault lines or other defects.
  - The variation of orientation 0.15°.

X-rays   Scattering in slightly different directions
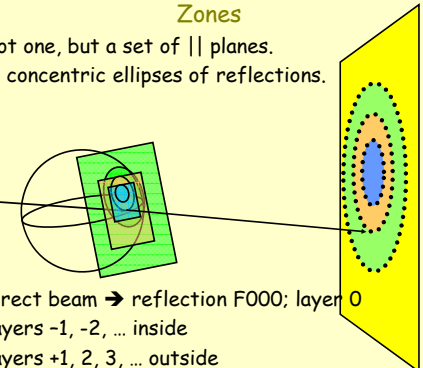
Still Photography

- Reciprocal lattice points on lattice planes.
- All diffracting relps at intersection of plane & Ewald sphere.
- Intersection is a circle
- Projected to film at an angle → ellipse
  - If film ⊥ incident beam.
- Reflections on ellipse.

Zones

- Not one, but a set of || planes.
- → concentric ellipses of reflections.
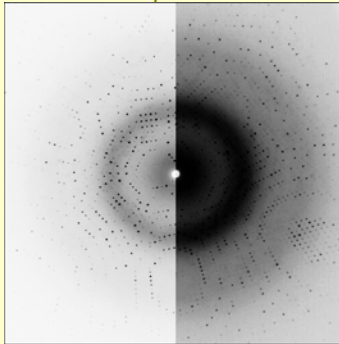
- Direct beam ➔ reflection F000; layer 0
- Layers –1, –2, … inside
- Layers +1, 2, 3, … outside

Zones - example
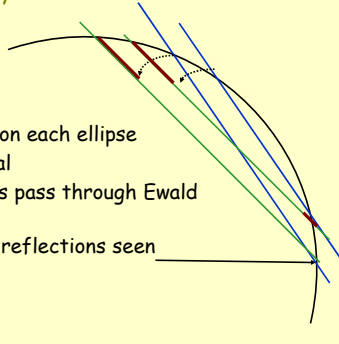
- Crystal rotated
  - (a little)

Moving the crystal

- Still crystal
  → few spots on each ellipse
- Rotate crystal
  - Red regions pass through Ewald sphere
  - Additional reflections seen

Diffraction of moving crystal

➢ All spots between ellipses between
  ▪ Start of rotation
  ▪ End
➢ Regions of reflections = "lunes"

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Lunar or spatial overlap

➢ Wide rotation
  ▪ Fat ellipses
  ▪ Many reflections from each plane
➢ Planes project to same part of detector
  ▪ Lunes overlap
  ▪ Spots may overlap

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Avoiding Overlap

Oscillation method
➢ Small rotations
➢ Overlap more w/ finely spaced planes
  ▪ Large unit cells
  ▪ Rotations < 0.5°
➢ Typical unit cells
  ▪ Rotations: 2.5 to 5°
➢ Also depends on
  ▪ Resolution
  ▪ Mosaic spread

Precession method
➢ Metal screen
  ▪ Allows through xrays of one plane only
  ▪ Circular aperture
➢ Large rotations in all directions
  ▪ ~ All reflections in one plane
➢ Film rotated to be || to crystal plane
  ▪ Undistorted image of lattice
  ▪ "Precession"

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Rotation or Oscillation Photography

➢ Crystal rotated - small angle, $\Delta\phi \perp$ beam; ...
➢ 10 fast oscillations better than 1 slow rotation:
  ▪ Averages crystal decay & beam fluctuations
  ▪ during angular sweep.

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Rotate the Crystal or the Generator?

➢ Most prefer to rotate the crystal
➢ Diagrams easier if rotate the laboratory
  ▪ Equivalent

Ewald sphere at start

End of rotation

Will now focus on parts of planes that have cross the Ewald sphere

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Full and Partial Reflections

➢ Reciprocal lattice points have finite size
  ▪ Mosaic spread
➢ Whole reflections
  ▪ Crossed completely by Ewald sphere w/in $\Delta\phi$.
➢ Partial reflections
  ▪ On sphere at start or end of rotation
  ▪ Only part of reflection recorded
  ▪ Fraction recorded = "partiality"

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Structure amplitudes from partials

- Measured intensity = F^2/partiality
- Partiality depends on experiment, not structure
- Need to correct
  - Make equivalent to full reflection
    - Partiality = 1
- Two approaches
  1. Series of contiguous oscillations
     - Add intensities from successive images
     - Errors – changing beam intensity etc..
  2. Estimate partiality → mathematical correction
     - Errors - Requires accurate understanding of crystal orientation
- Partial reflections less accurate

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Crystal Alignment

- Some orientations → more efficient collection
  - Approximate alignment OK (not like precession)
- Determine orientation then re-orient
  - Center zones from still images
  - Computer-auto-indexing (details later)
    - From 1 to 3 still images
- No longer necessary to *precisely* orient before data collection
- Some use the American Method
  - Shoot first – ask questions later
  - When crystal lifetime in beam is short

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Prolonging Lifetime w/ Cryocrystallography

Radiation damage
- Reduced at 100 K
- Caused by:
  - Crystal heating
  - Ionizing radiation → roaming free radicals
    - Changing covalent structure
    - Abated by reducing diffusion in ice

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Freezing Crystals

- Vitreous ice (glass) OK
- Ice crystals:
  - Destroy protein crystal
  - Add ice diffraction
  - Must be avoided
    - Cryo-protection
      - Antifreeze
      - Glycerol, PEG, MPD…
    - Oil coating
    - Search for one that does no damage
    - Serial transfer
      - Reduce osmotic shock

Flash freezing – 2 ways:
- Plunge into liq N_2, propane
  - Then put on x-ray camera
    - Keeping at 100K
- Place on camera at 20°
  - Quickly start N_2 stream
- Maintain in cold N_2 gas stream

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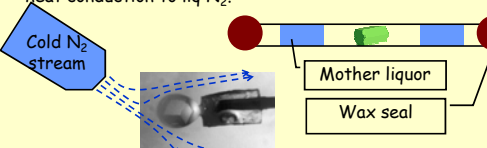
Crystal Mounting

Cryo-data collection
- Drop of frozen mother liquor
- Held in loop of fiber
- Scooped up using surface tension
- Open crystal → better heat conduction to liq N_2.
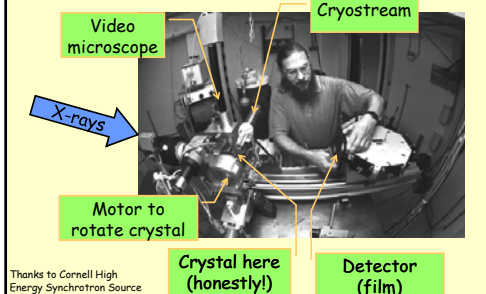
Collection at 4 to 30°C
- In sealed capillary
  - Maintain humidity
  - Stop crystal drying
- Capillary made of quartz
  - Minimize x-ray absorption

Cold N_2 stream

Mother liquor

Wax seal

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Data Collection Instrumentation - Synchrotron

Video microscope

Cryostream

X-rays

Motor to rotate crystal

Crystal here (honestly!)

Detector (film)

Thanks to Cornell High Energy Synchrotron Source

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Data Collection Instrumentation

X-rays

Helium path to minimize air-absorption

Motor to rotate crystal

Detector (image plates + automatic scanner)

Thanks to Soma!

Crystal here (honestly!)

Generator

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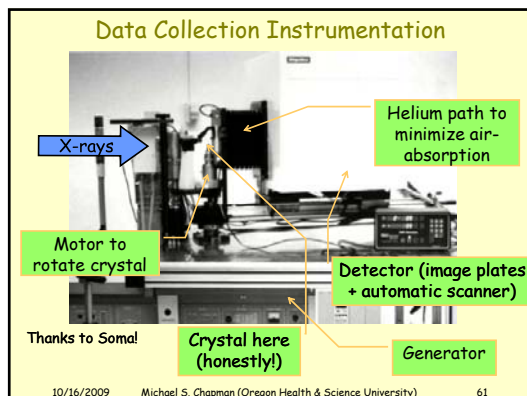
Detectors

Type	Advantages	Disadvantages
Scintillation counter	Most precise	One spot at a time (small unit cells)
Film	Spatial resolution < 50 μm	Background, dynamic range, turnaround time
Multiwire	Precise	Spatial resolution, expense
TV detector		Unstable, calibration, expense
Image plate	Size, Medium precision	Scan-time
CCD's	Precise, dynamic range	Large ones very expensive

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Data Processing - Overview

➢ Determine which region contains each reflection
  ▪ **"Indexing"**
  ▪ **Orientational refinement**
➢ **Integrate** intensity near reflection center
➢ Subtract background
➢ **Scaling**:
  ▪ Correct for factors that don't depend on structure, but
    ▪ Geometry of data collection
    ▪ Shape and absorption of crystal
➢ **Merging** – symmetry equivalent measurements
  ▪ Calculate quality statistics

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Scaling - Introduction

➢ Calculate image scale constant
  ▪ Intensities agree w/ symmetry equivalents
➢ Approx. correction for many factors:
  ▪ Crystal Absorption:
    ▪ Depends on path length
      ▪ Depends on crystal orientation and individual reflection.
  ▪ Other absorption: capillary, solvent -
    ▪ varies slowly, can be minimized.
  ▪ Volume of crystal in beam:
    ▪ depends on ϕ, therefore image #
  ▪ Decay due to radiation damage:
    ▪ depends on resolution and time ($\rightarrow \phi$, image #).

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Quality of Scaling

➢ Quality of data is now assessable with a scaling R-factor. $R_i = \sum_h \sum_i |\langle I_h \rangle - K_{hi} I_{hi}| / h\langle I_h \rangle$
➢ Compare this R-factor to the least-squares residual (or corresponding variance):
  ▪ Both depend on magnitude of difference.
  ▪ Residual is squared, so more sensitive to large differences.
  ▪ R-factor is normalized, and expressed as decimal fraction or %.
➢ R_i (R_{sym}) is calculated from intensities.
  ▪ Most other R-factors are calculated from $|F| = \sqrt{I}$.

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Quality of Data

➢ R_i is used as a measure of data quality:
  ▪ $R_i \leq 3\%$: excellent.
  ▪ $R_i \leq 5\%$: typical average protein.
  ▪ $R_i \leq 9\%$: typical large protein.
  ▪ $R_i \leq 13\%$: typical virus capsid.
➢ Partial reflections are usually excluded
  ▪ $\rightarrow$ underestimate of error, especially for large molecules (small $\Delta\phi$, many partials).
  ▪ Inclusion of partials for virus $\rightarrow R_i = 18\%$.

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