X-ray Diffraction
Waves: constructive and destructive interference

Light shines from the left on a 1-d set of evenly spaced holes, and angles where diffraction occurs (constructive interference) and where it does not (destructive interference) are shown.

(a-d) **constructive**
Interference:

Note: the figure does not show the waves are added together increasing the amplitude of the resultant wave

(e) **destructive** interference
no diffraction.
Reminder of differences and similarities between X-ray and EM

There is no lens that can focus X-rays. Thus, we must use the Fourier transform to understand X-ray diffraction and structure determination.
Set of points ~ atoms

Point 1. the position of the spots in D are ONLY dependent on the lattice.

Point 2. if we superimposed D on B the intensity at each point in d corresponds to the intensity in the continuous transform shown in b. Thus, intensity is dependent on atoms/posit

Point 3. The real (red) and reciprocal (yellow) lattices are shown (reciprocal)
A diffraction pattern is a Fourier Transform

The diffraction pattern of the cosine grating consists of only three spots, referred to as the +1, −1, and zeroth orders. (“Orders” is a historical usage associated with gratings; the word reflection is used in most contexts.)

1. Diffraction pattern is centrosymmetric.
2. Reciprocal nature of the diffraction pattern: As d gets smaller (period) 1/d gets larger.

**Real space**, Lattice represented by cos wave

**Reciprocal space**, diffraction pattern
Representing a square grating with cosine waves

\begin{align*}
\gamma_s & \quad d_1 \\
q_1 & = \frac{1}{d_1} \\
\text{Zero term} & \\
1 & \\
0 & \quad x \\
\text{1st term:} & \quad \frac{2}{\pi} \cos 2\pi q_1 x \\
\text{3rd term:} & \quad \frac{2}{3\pi} \cos (2\pi \cdot 3q_1 x)
\end{align*}
Diffraction pattern of a square grating

Diffraction pattern of a square wave grating consists of 7 spots.

The square wave grating can be represented by 3 cosine waves with different orders 1, 2, 3. Note the period of each wave is getting shorter, or frequency greater.

This figure shows the contribution of each Cos wave to the diffraction pattern

The bottom panel shows the sum of all 3 cos gratings, which accounts for the diffraction pattern

Note: the higher frequency wave results in a reflection further out in the diffraction pattern. This is the concept of resolution

Sum of 3 cos waves

\[1+2+3\]
Families of planes that make rational intercepts with the unit cell edges are defined by 3 integers $h$, $k$, $l$. The integers divide $a$, $b$, $c$ axes into $h$, $k$ and $l$ parts. E.g. the 0 0 2 is a plane outlined by $a$ and $b$ and cuts the $c$ axis in 2 parts.

The spacing between each $h$, $k$, $l$ plane is called $d_{hkl}$.

For example, for unit cell $a=50\text{Å}$, $b=100\text{Å}$, $c=80\text{Å}$, $\alpha=90$, $\beta=90$, $\gamma=90$

The 002 $d_{hkl}$ spacing is 40Å.

Figure 3.1. Bragg planes in a crystal unit cell. Eight different families of planes are shown. Each family $h$, $k$, $l$ divides the $x$-axis into $h$ parts, divides the $y$-axis into $k$ parts, and divides the $z$-axis into $l$ parts. Each of these families of planes gives rise to a distinct X-ray reflection. The values $h$, $k$, $l$ are called Miller indices. Note that some of the indices are negative; the signs of the indices specify the direction of the tilt for that family of planes. Adapted from McPherson: *Preparation and Analysis of Protein Crystals*.
What are the Miller indices for these planes on this 2d point lattice?

One is given:

For you-
Bragg’s law – condition for diffraction
An X-ray diffraction experiment may be described as waves being reflected off of planes defined by miller indices.

1) Defines when diffraction will occur for given $\lambda$.
2) With closer spaced planes, $\theta$ increases.
3) Calculate resolution of a reflection, set the edge of detector for an experiment.
4) Resolution dependent on $\lambda$ used in the experiment

$$n\lambda = 2d_{hkl} \sin \theta$$

$$\frac{n\lambda}{2d_{hkl}} = \sin \theta$$
Bragg’s law, diffraction geometry

\[ d_{hkl} = \frac{\lambda}{\sin \theta} \]

A = crystal-to-film distance
R = distance from the center of the detector (beam spot) to the reflection furthest from the center, which can be identified on the film. Both values usually in mm.
1. Origin of the reciprocal lattice can be anywhere relative to crystal origin, choose (O).

2. Euclid, states QOP is a right triangle. OP perpendicular to QP, which is parallel to hkl planes.

3. CP is a diffracted ray and the $s$ vector (OP) has magnitude $1/d_{hkl}$ ($d_{hkl}^*$).

4. Take home: diffraction occurs when reciprocal lattice point is on the surface of the sphere.
a* and c* are **reciprocal axes** with directions:
a* perpendicular to the bc plane.
c* perpendicular to the ab plane.

The length of $a^* = 1/a$  $c^* = 1/c$ for orthogonal axes. Otherwise, must correct for the angle (in this case $\beta$). $1/d_{100}$ (e.g. plane spacing) assumes this correction.

e.g. For monoclinic cell, $a^*$ and $c^*$ lengths are: $a^* = 1/a \sin \beta$,  $c^* = 1/c \sin \beta$

Note the directions of $a^*$ and $c^*$ are perpendicular to the planes defined by the miller indices. For example, $a^*$ perpendicular to 100 planes and $c^*$ is perpendicular to the 001 planes.

$a^*$ and $c^*$ correspond to $d^*_{hkl}$ for the 100 and 001 miller indices. However the “s” vector (see previous slide) increases in length as integrals of $a^*$ and $c^*$ with increasing hkl values. Eg. The 500 (e.g. divides a by 5) will have an s vector 5 x a*
Relationship between real space and reciprocal space (diffraction space)
From the diffraction pattern:
We can derive a, b, c, and alpha, beta, and gamma. For a given unitcell (grating), the positions of the intensities will be the same.

In the orientation shown, reflections off the a (100) and b (010) bragg planes result to diffraction vectors with length $1/d_{hkl}$ ($d^{*}hkl$), with hkl index 100 and 010.

Note: Every diffraction pattern is centrosymmetric. (inversion).

How do we rotate the crystal to observe c* or “l” reflections?
This is only a single 2D image. How do we get a 3D data set? Ewald sphere, or sphere of reflection
What happens to the Ewald Sphere and the diffraction image when:

1. $\lambda$ gets bigger or smaller?
2. When white radiation is used?
3. When the $d_{hkl}$ spacing is large/small?
4. Large, or small crystal rotation around axis perpendicular to page at the crystal origin – (C)

Ewald Sphere used to explain/calculate diffraction images

1. When / Where will diffraction will occur?
2. X-ray beam properties, crystal mosaicity (crystal parameter), Rocking curve.
3. Dataset completeness.
4. Strategy for collecting data most efficiently.
Ewald Sphere of reflection, reciprocal lattice

Monochromatic x-rays are used: thus, the sphere size is fixed for a give exp.

The crystal is rotated through the reciprocal lattice in small oscillations (~1 deg) bringing each h k l in the reciprocal lattice (fixed by unit cell/space group) into diffracting conditions.

A complete dataset might be composed of 180 1 oscillation frames

Fig from Crystallography 101 website
Ewald Sphere of reflection

1. Crystal lattices are three dimensional.
2. Thus, reciprocal lattices are three dimensional.
3. Data collection $\lambda$ is fixed when using monochromatic radiation.
4. Thus, one rotation image in a x-ray diffraction experiment samples only a small slice of the reciprocal lattice.
5. A complete dataset is collected by rotating the crystal to bring all reciprocal lattice points into diffraction position.
6. Number of Bragg reflections, corresponding to a complete dataset can be calculated where $V = \text{volume of the unit cell in } \text{Å}^3$ and $d_{\text{min}}$ is the minimum interplanar spacing in $\text{Å}$ (resolution), $n = 1$ for primitive, 2 for face centered lattice. Alternatively, as a function of $\lambda$.

$$N = \frac{4\pi}{3} \frac{V}{n} \left(\frac{1}{d_{\text{min}}}\right)^3$$

$$N = \frac{4\pi}{3} \frac{V}{n} \left(\frac{8}{\lambda^3}\right)$$
**Symmetry, Ewald sphere, of reflection**

**Space group P21**

Symmetry equivalent posit.

1) \(x, y, z\)

2) \(-x, y+1/2, -z\)

**Diffraction pattern symmetry (laue symmetry)**

1. Remove translations
2. Add center of symmetry

\(h, k, l\) \(-h, -k, -l\)

\(-h, k, -l\) \(h, -k, l\)

If SG p2\(_1\), need to collect \(1/4\) of the sphere to measure all unique data once.

Redundancy: Measure intensities 4 x by collecting entire sphere.

**Friedel pairs** - reflections related by a center of symm. (e.g. collect a reflections on each side of hkl planes)

**Bijvoet pairs** - symmetry-related Friedel pairs.
Protein crystal mounted in a glass capillary with mother liquor (crystal stabilizing solution)

Ends of the capillary are sealed with wax

Now, crystals are frozen in a nitrogen gas stream at ~170°C for data collection
Crystals being mounted in nylon loops for low temperature data collection

Crystals being mounted

Mounted “frozen” Crystal
When does diffraction occur?
What is the relationship between the crystal and diffraction pattern
Evacuated chamber

Common Laboratory X-ray source: rotating anode

Rotating anode

Characteristic wavelengths (Cu)

- $K_\alpha = 1.3922$
- $K_\beta = 1.5405$
- $K_\gamma = 1.5443$
- $K_\delta = 1.5418$

Wavelength (Å)
Synchrotron sources

SSRL

APS
FIG. 2.39  Synchrotron radiation source. A storage ring has high-energy electrons held in orbit by bending magnets (A). As the electrons accelerate around the curve they emit synchrotron radiation (B). Because the beam is so intense, all experiments are done in shielded hutchies that are interlocked so that personnel cannot be inside while the shutters are open. A wiggler (C) is a method of increasing the brilliance of the X-rays by combining several beams from local excursions of the electron path.
High Intensity, tunable, synchrotron radiation allows data collection on small weak diffracting crystals.

3rd Generation Synchrotron.

Brilliance $10^{17}$ to $10^{20}$

Brilliance = $I / \text{mm}^2$

Rotating anode

Brilliance $10^9$ to $10^{11}$

Synchrotrons are tunable
X-ray Sources

Home:
1. Fixed wavelength
   a. Cukα – 1.54Å
   b. Cr 2.26Å
   c. Mo 0.7Å

Synchrotron:
1. Brilliant (photons/mm²)
2. Tunable
3. Automated mounting capabilities
4. Rostering/crystal imaging
5. State-of-the-art equipment / detectors

Monochromatic x-rays
2.2 5,635 eV
Cukα 1.54Å 8,050 eV
1.0 12,398 eV
0.7 17,712 eV

White radiation
• Select crystal
  Collect a few images to judge quality

• Decide strategy and collect all images

• Integration
  • Index
    choose lattice
  • Refine unit cell
  • Integrate

• Choose Laue group (point group)

• Scale & merge

• Convert I to F

Decisions

Is this your best crystal?
Mosaicity, resolution, size, ice

Total rotation, rotation/image, exposure time, position of detector. Programs: DNA, BEST

Correct lattice
Integration parameters: box size, overlap check

How good is the dataset?
Any bad bits?

Is the crystal twinned?
From an initial diffraction Image (NOT A COMPLETE DATA SET), the unit cell and possibly the space-group can be determined.

Determine the reciprocal Lattice...
1. Spacings between spots
2. Angle between spots
3. 3\textsuperscript{rd} dimension requires a computational algorithm, but can be obtained from 1 image.
Based on this crude 2D Indexing, what can you conclude about the spacegroup?
Consider every possible direction in turn as a possible **real-space** axis, i.e. perpendicular to a reciprocal lattice plane. Project all observed vectors on to this axis.

Lattice plane normal to lattice plane: vectors cluster at lengths which are multiples of the lattice spacing. Fourier transform shows sharp peaks.

Non-lattice direction, random length. No peaks in Fourier transform.
An indexed x-ray diffraction pattern
Back-project each spot on to Ewald sphere, then rotate back into zero-φ frame

An integration box is placed around each diffraction spot on the detector. The spot is Integrated (summed), resulting in a list of H K L indexes, I, σI for all reflections on the frame.

Full and partial reflections for each frame Are summed
A final 3-dimensional data set is obtained.
Fully recorded and partially recorded reflections

A fully-recorded spot is entirely recorded on one image

Partials are recorded on two or more images

“Fine-sliced” data has spots sampled in 3-dimensions

Illustrations from Elspeth Garman
User defined variables required for X-ray data collection:

- **Wavelength**
  *Tuneable: Only at a Synchrotron Facility. 0.6-2.5Å generally described in KeV (~5-15KeV).

  *fixed at 1.54178Å on a rotating anode generator (lab source)

Take a single image, index and use a prediction/strategy program to determine:

- **Oscillation range per frame** (no overlaps, suff. # of spots)

- **Total Oscillation range for the dataset** (req. for complete data)

- **Exposure Length for each oscillation** (radiation damage)

- **Crystal-to-Detector Distance** (spot separation, low background use the entire detector surface)
Data processing and structure factor files in practice

1. 2D Frames of ~1 degree oscillations of full and partial reflections. Diffraction exp.

2. Frames are indexed – Unit cell parameters determined. (HKL2000)

3. Based on the indexing, 2D frames are integrated to give intensities and error values for each h, k, l. The unit cell and intensities define the spacegroup.

4. Intensities are converted to structure factor amplitudes (F) and an error estimate. (σF) This file is deposited with the PDB file when the structure is published.

5. Statistics of the processing are provided in table format in all crystal structure pubs. NOW this has been largely moved to the supplemental data file in major journals.

The structure factor file is the experimental DATA for a crystal structure.

The crystal structure (PDB file) is the interpretation of this diffraction data Usually, a very good interpretation.
Crystal

Indexing / reflection prediction
Data collection Strategy
Data Collection

Images

Integration

h k l \(\sigma(l)\)

Scaling & merging (data reduction)

h k l F \(\sigma(F)\)

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Space group determination
Quality assessment