Introduction

- Earlier in the semester we developed a crude model for macromolecules using sedimentation analysis.
  - Prolate and oblate ellipsoids of revolution

- Since the late 1950's we have had models with atomic resolution.
Protein Data Bank

Electromagnetic Radiation

Snell’s Law

\[ n_1 \sin(\theta_1) = n_2 \sin(\theta_2) \]
To use electromagnetic radiation to create images of objects requires electromagnetic radiation having wavelengths that are equal to or smaller than the objects being imaged.

In 1924, Louis de Broglie proposed that just as light has a particle-like nature, matter also has a wave-like nature.

\[ E = h \nu \quad \text{Planck} \]

\[ \lambda = \frac{h}{p} \quad \text{de Broglie} \]

\[ \frac{h}{mv} \]

\[ E = \frac{1}{2}mv^2 \]

\[ E = mc^2 \]
High Energy Electron Beam

- For a 100 kV beam, in which an electron is accelerated through a 100,000 V potential field.

\[ \lambda = \frac{h}{mv} \]
\[ = 0.004 \text{ nm} \]
\[ = 0.04 \text{ Å} \]

Microscopy

Comparing light microscopy to electron microscopy

X-ray Crystallography

The Crystal Lattice

X-ray Crystallography

The Unit Cell
X-ray Crystallography

The Bravais Lattice Types

X-ray Crystallography

Protein Crystals

X-ray Crystallography

Protein Crystals

X-ray Crystallography

Growing Crystals
In light microscopy, lenses are used to collect the light scattered from an object and use it to form an image.

There are no X-ray lenses
• Computers are used to simulate a lens
• To understand how this works, we need to understand the elements of a diffraction pattern

Constructive and Destructive Interference

Bragg's Law describes the conditions required for obtaining a spot.

• Constructive interference occurs when
  \[ 2d \sin(\theta) = n\lambda \]  
  (Bragg's Law)

- William Henry Bragg and his son William Lawrence Bragg shared the Nobel Prize in Physics in 1915.
The Diffraction Pattern

Crystal Diffraction
- The spots arise from reflections off of the crystal planes

The spots in the diffraction pattern arise from constructive and destructive interference between x-rays scattered from the different planes in the unit cell.

The spacings and angles depend on the unit cell dimensions and angles \((a, b, c, \alpha, \beta, \gamma)\).

Demos
- **XRayView 3.0**
  - Simulates diffraction from a crystal
- **Optical Diffraction**
  - Diffraction patterns are reciprocally related to the object responsible for the diffraction

...
The Diffraction Pattern

The diffraction pattern is also referred to as the reciprocal lattice.

The Fourier Series

Fourier Series
- Sine and Cosine waves have
  - Amplitude, $F$
  - Frequency, $h$
  - Phase, $a$

$$f(x) = F_h \cos(2\pi hx + a)|$$

or

$$f(x) = F_h \sin(2\pi hx + a)|$$

Fourier Synthesis
- Approximating a square wave by a Fourier Series

$$f(x) = F_h \cos(2\pi [hx + a_1]) + F_h \cos(2\pi [hx + a_2]) + F_h \cos(2\pi [hx + a_3]) + \ldots + F_h \cos(2\pi [hx + a_n])$$

$$= \sum_{n=0}^{\infty} F_n \cos(2\pi [hx + a_n])$$

Fourier Series

An alternative to designating a phase angle, $a$, is to combine cosine and sine waves
- The sine is equal to a cosine which has been shifted by an angle of $\pi/2$ (90°)

$$\sin(x) = \cos(x + \pi/2)$$

- This allows us to replace

$$f(x) = \sum_{n=0}^{\infty} F_n \cos(2\pi [hx + a_n])$$

with

$$f(x) = \sum_{n=0}^{\infty} (F_{n,\cos} \cos(2\pi hx) + F_{n,\sin} \sin(2\pi hx))$$
Fourier Series

It is also mathematically convenient to represent this relationship using complex numbers.

\( f(x) = \sum_{n=0}^{\infty} F_n \cos(2\pi nx) + i \sin(2\pi nx) \)

where the amplitude, \( F_n \), is also a complex number

- The cosine portion represents the Real part, while the sine portion represents the Imaginary part, of the complex number

Another mathematical convenience comes from making use of Euler’s formula:

\( \cos(x) + i \sin(x) = e^{ix} \)

- This allows us to cast the Fourier series into a nice, compact, complex exponential form:

\( f(x) = \sum_{n=0}^{\infty} F_n e^{i2\pi nx} \)

The complex polar coordinate system

Applying the Fourier Series to Diffraction

- The diffraction pattern also called the reciprocal lattice.
- Each spot in the diffraction pattern is called a structure factor
  - The structure factors are represented in three dimensions by the symbol
  \( F_{h,k,l} \)
  - Where \( h, k, \) and \( l \) are the Miller indices, which describe the sets of planes that can be used to divide up the unit cell in the crystal lattice.
Applying the Fourier Series to Diffraction

- The diffraction pattern also called the **reciprocal lattice**.
- Each spot in the diffraction pattern is called a **structure factor**.
  - The structure factors are represented in three dimensions by the symbol $F_{h,k,l}$.
  - Where $h$, $k$, and $l$ are the Miller indices, which describe the sets of planes that can be used to divide up the unit cell in the crystal lattice.
  - Remember, according to Bragg’s Law, it is the reflections from these planes that give rise to the diffraction pattern.

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The Interactive Structure Factor Tutorial

Welcome to the Interactive Structure Factor Tutorial! The aim of this tutorial is to help understand structure factors, plane waves, and the relationship between the structure factor and the electron density. This is achieved through the use of interactive windows, which allow you to visualize reflections and their values by moving through the unit cell. Structure factors also play a crucial role in determining the electron density in the crystal lattice, which dictates the electrons' position. Together, this tutorial aims to help you understand the role of reflection in the unit cell, developing an intuitive understanding of electron density.
Applying the Fourier Series to Diffraction

The structure factors (spots), $F_{h,k,l}$

$$F_{hkl} = f_A + f_B + ... + f_A' + f_B' + ... + f_F'$$

Sum over atoms

The structure factors (spots), $F_{h,k,l}$

$$F_{hkl} = f_1 + f_2 + ... + f_m + ... + f_n$$

Sum over volume elements

The structure factor can be related to the electron density using a complex exponential Fourier Series

- Each spot in the diffraction pattern is associated with a particular structure factor, $F(hkl)$

$$F(hkl) = \sum \sum \sum \rho(xyz) e^{2\pi i (hx + ky + lz)}$$

- Every location in the unit cell contributes to each structure factor
  - If the electron density fluctuates with position at a frequency corresponding to $h$, $k$ and $l$, then this will produce a strong intensity for this particular structure factor, $F(hkl)$

Fourier Transforms

When one function represents the amplitude for the Fourier series of a second function then the dependent variables for the two functions are reciprocally related.

- In X-ray diffraction, the dependent variables for the electron density function, $x$, $y$ and $z$, have dimensions of distance, where as the dependent variables for the structure factor, $h$, $k$ and $l$, have dimensions of frequency, or one over distance.

- When this situation exists, there exists a mathematical operation called the Fourier Transform, can be used to convert between the two functions
Fourier Transforms

For example, for a one dimensional, discrete function, \( F(h) \), which is represented as the Fourier series of a second function, \( f(x) \):

\[
F(h) = \sum_{k=0}^{n} f(x) e^{2\pi i k x}
\]

- The Fourier transform is given as:

\[
f(x) = \sum_{k=0}^{n} F(h) e^{-2\pi i k x}
\]

When this is extended to 3-dimensions:

\[
F(hk \ell) = \sum_{x} \sum_{y} \sum_{z} f(xyz) e^{2\pi i (hx + ky + lz)}
\]

- The Fourier transform is given as:

\[
f(xyz) = \sum_{x} \sum_{y} \sum_{z} F(hk \ell) e^{-2\pi i (hx + ky + lz)}
\]

Substituting \( \rho(xyz) \) for \( f(xyz) \) we get:

\[
F(hk \ell) = \sum_{x} \sum_{y} \sum_{z} \rho(xyz) e^{2\pi i (hx + ky + lz)}
\]

- The Fourier transform is given as:

\[
\rho(xyz) = \sum_{x} \sum_{y} \sum_{z} F(hk \ell) e^{-2\pi i (hx + ky + lz)}
\]

This provides us with a method for determining the electron density function, \( \rho(xyz) \), from the structure factors, \( F(hk \ell) \).

Solving for the Electron Density Functions

\[
\rho(xyz) = \sum_{x} \sum_{y} \sum_{z} F(hk \ell) e^{-2\pi i (hx + ky + lz)}
\]

As indicated earlier, each structure factor \( F(hk \ell) \) is itself a complex number that is given by an amplitude, phase and frequency

- The Interactive Structure Factor tutorial demonstrates this.
Solving for the Electron Density Functions

- The frequency is determined by the Miller indices \((h,k,l)\), which determine the frequency of the planes cutting through the unit cell.

- The amplitude of each structure factor \(|F(hkl)|\), can be determined from the intensity of each spot \(I(hkl)\):
  \[
  |F(hkl)| = \sqrt{I(hkl)}
  \]

- What’s missing is the phase information
  - We have essentially taken a black-and-white photo instead of a colored one

Solving the Phase Problem

For unit cells with small numbers of atoms, a Patterson Map can be used to determine the distances and directions between the atoms in the unit cell.

- A Patterson Map is constructed by assuming the phases are all zero

  \[
  P(xyz) = \sum_k \sum_l |F(hkl)| e^{-i2\pi(hx+ky+lz)}
  \]

- The Interactive Structure Factor tutorial demonstrates this.

Solving the Phase Problem

- Patterson Map

Solving the Phase Problem

For protein crystals, can approximate this situation by using isomorphic replacement

- A small number of metal ions are introduced into the crystal.

Another method used to solve for the structure factor phases is Molecular Replacement

- In this method a homologous protein with a known structure is packed into the cell and used to determine the phases using an Inverse Fourier Transform

  \[
  F(hkl)_{calc} = \sum_x \sum_y \sum_z \rho(xyz) e^{i2\pi(hx+ky+lz)}
  \]
Model Building

A model is fit to the electron density:

The model can be used to calculate a new set of phases:

\[ F(hkl)_{\text{calc}} = \sum_{\text{xyz}} \rho_{\text{xyz}} e^{i2\pi(hx+ky+lz)} \]

- And this is used to determine a calculated structure factor that can be compared to the observed structure factor:

\[ R = \frac{\sum |F(hkl)_{\text{calc}}|}{\sum |F(hkl)_{\text{obs}}|} \]

This is an iterative process.