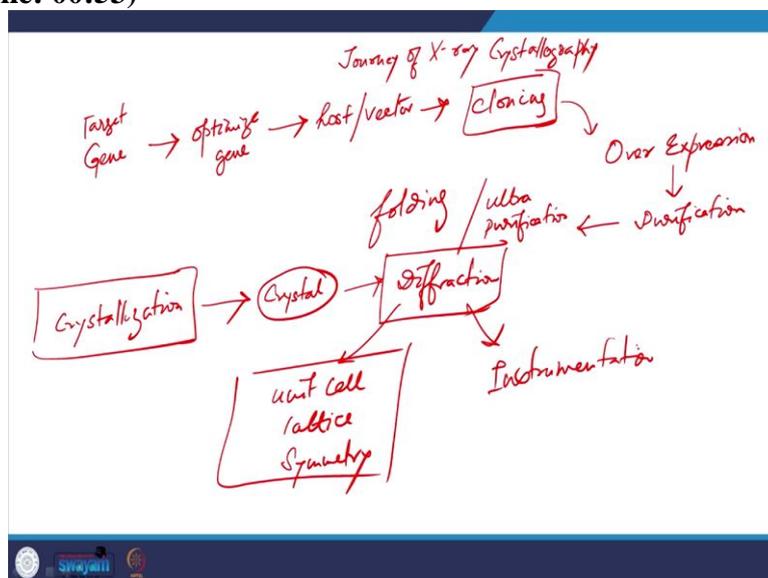


Structural biology
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Department of Biotechnology
Indian Institute of Technology – Roorkee

Lecture – 20
X-ray crystallography: Data collection and processing

Hi everyone, again, welcome to the course on structural biology. We are continuing with the structural biology techniques. Again in structural biology technique, we are talking about X-ray crystallography. Today is the last class of the module. In this class, we will discuss data collection and processing, but before that, I will talk about the journey so far.

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We start with targeting the gene because proper targeting is very important. After targeting, we optimize the gene with engineering then we find a host system vector system that helped us go for cloning. Once the gene is cloned, we go for overexpression because overexpression is very critical here. We do overexpression, and then we need to go for purification.

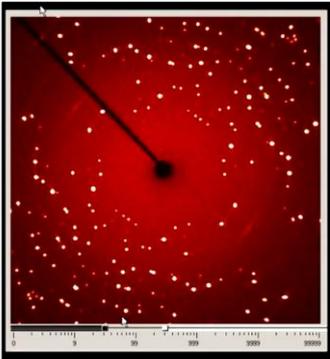
We have discussed that, and then for protein, we have to double-check one ultra-purification, second folding because if the protein is not folded, you will not get a crystal. So, this is the process common to most of the structural technology we will discuss after that. Now, when we have protein, we go for crystallization. As discussed, crystallization is a process that is the check line like if you do everything perfectly still, you have a problem it is the crystallization. And we spend a lot of time looking at how to crystallize the factors affecting crystallization. Though we also admitted that it is not even considered a science, it is considered art like we do the process. It is rather the maintaining of the process and depending on luck. So, but we

did whatever. So, we also look at the science, what the phase transition is happening on how the phase transition could be affected, how our process could work on that, and all.

And also discuss some modern methods by which you could improve. One very important is using microfluidics in a small lab in Chicago, and then from crystallization, we got the crystal. So, we looked at the crystal, and we checked the crystal was protein crystal or not, and all we went for diffraction. We stopped and then looked at the unit cell, lattice, symmetry, and instrumentation. So, this is our journey so far and in this technique.

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X-ray Diffraction Pattern from a Single-crystal Sample:



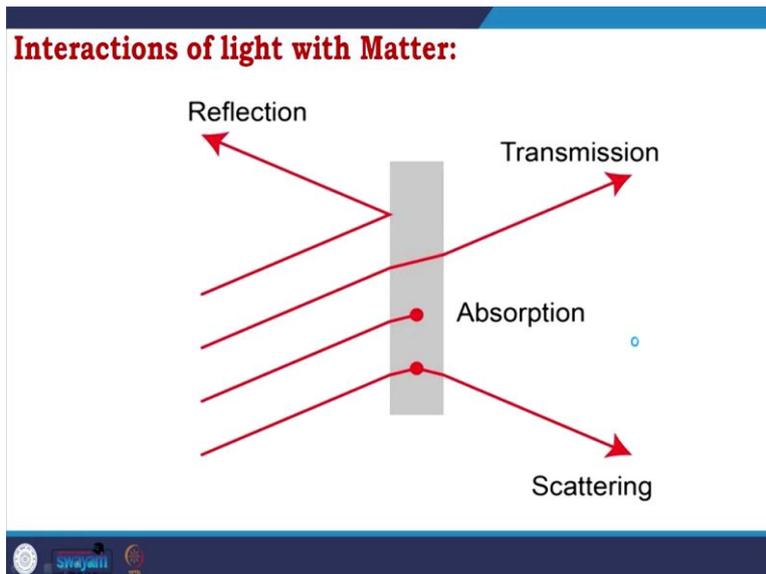
This image happens to be a 360 rotation in **phi**, collected on a CCD detector with all goniometer angles set to zero.

Note that the X-ray diffraction pattern from a single crystal is a set of spots.

Swayam

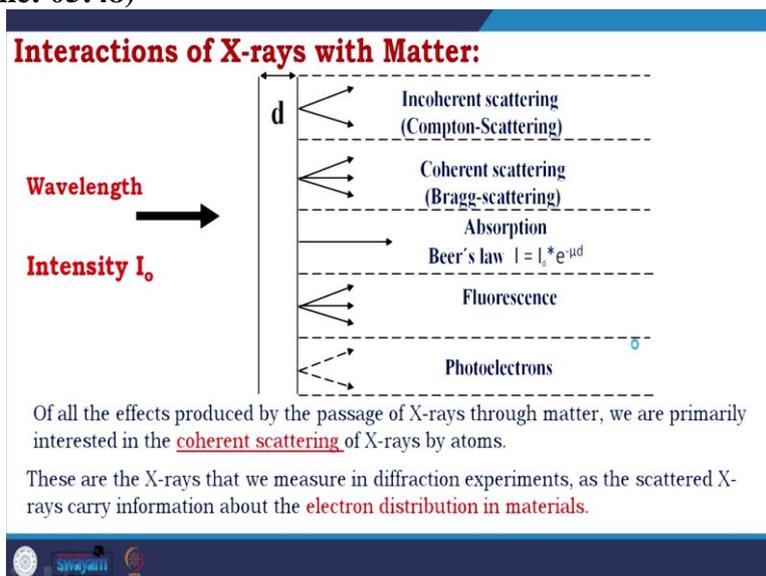
Today, we will look at the diffraction pattern how it is coming. This image happens to be a 360-degree rotation in ϕ , collected on a CCD detector with all goniometer angles set to 0. We talked about changing the angles to make them fixed and align them because alignment is critical. If you do not align it, then with the rotation, the crystal will lose the main contract of the beam, and you will not get data. So, we did the alignment, and if you look at the X-ray diffraction pattern from a single crystal, which is a protein crystal, you will get a set of spots, and we will look at their relation.

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So, before going to that, we will look at how the thing is happening and the science behind it. So, it is very analogous with light, and if you look at the interaction of light, it interacts with the matter, and then several things happen, it could reflect it could transmit, it could absorb, it could scatter.

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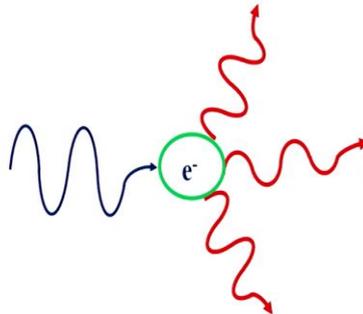


Similarly, here in X-ray, we will look at how its interaction affects matter. So, d is that space, and there are several phenomena like incoherent scattering, which is also known as Compton scattering, coherent scattering, which is Bragg scattering, which we are interested in absorption which follows Lambert Beer's law, fluorescence, photoelectrons, and of all the effects produced by the passage of X-ray through matter.

We are primarily interested in coherent scattering. As I told you, which maintains Bragg's law, we measure these X-rays in diffraction experiments as the scattered X-rays carry electron distribution in materials.

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Coherent Scattering:



Incoming X-rays are electromagnetic waves that exert a force on atomic electrons.

The electrons will begin to oscillate at the same frequency and emit radiation in all directions.

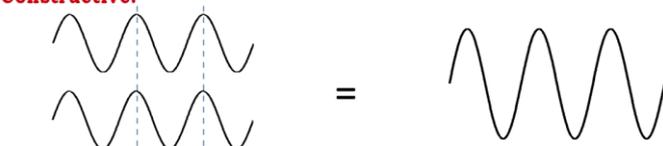
swayam

So, let us look at coherent scattering. The ray comes and hits the electron, and the electron goes in different directions. So, incoming X-rays are electromagnetic waves that force the atomic electrons. And that is why you get the molecular imprint you hit a carbon, and you hit nitrogen, you hit oxygen; you see different effects. And based on that effect, what is coming and detecting, we could go back and calculate which atom it is. We could make that model. We will see that the electrons will begin to oscillate at the same frequency and emit radiation in all directions.

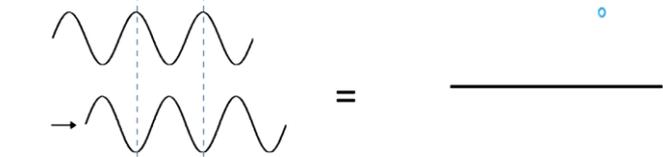
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Constructive and Destructive Interference:

Constructive:



Destructive:



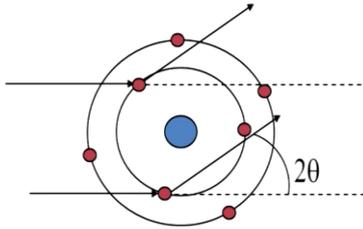
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So, in coherent scattering, there are constructive and destructive interference, which is you all know the property of any wave when they are like having in the same direction, the same phase they are constructive. When they are in the opposite phase, they are destructive.

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Coherent Scattering by an Atom:

Coherent scattering by an atom is the sum of this scattering by all of the electrons.



Electrons are at different positions in space, so coherent scattering from each generally has different phase relationships.

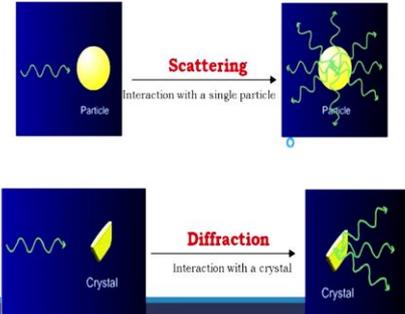
At higher scattering angles, the sum of the coherent scattering is less.

So, coherent scattering by an atom is the sum of the electrons' scattering. Electrons are at a different position in space, so coherent scattering from each generally has a different phase relationship. At higher scattering angles, the sum of the coherent scattering is less.

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Diffraction:

A diffracted beam may be defined as a beam composed of a large number of scattered rays mutually reinforcing each other



So, coming to diffraction, because it is the diffraction that is important for us among the coherence. A diffracted beam may be defined as a beam composed of many scattered rays mutually reinforcing each other.

If you see this is scattering when the X-ray hits a particle and goes in a different direction. In contrast, when it hit a crystal, its interaction with the crystal is called diffraction that is the difference.

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Diffraction:

Random arrangement of atoms in space gives rise to scattering in all directions: weak effect and intensity effect

By atoms arranged periodically in space

In a few specific directions satisfying Bragg's law: strong intensities of the scattered beam :Diffraction

No scattering along directions not satisfying Bragg's law.

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So, the random arrangement of atom in space gives rise to scattering in all direction, which have weak effect and affect the intensity. But when atoms are arranged periodically in space, which is a crystal, in a few specific directions, which satisfy Bragg's law, there are strong intensities in the scattered beam called diffraction. No scattering along the direction, not satisfying Bragg's law. Before going into data collection, let us look at the history. Let us look at where it starts.

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Short History of Data Collection:

❖ 1912 – von Laue et al. Experiment – X-ray Film

❖ 1913 – Bragg – Diffractometer + Detector

❖ ~ 1965 Improved Film (Weissenberg, etc.)

❖ ~1960-1995 – Serial Detector Diffractometer

~ 50 datasets/year

❖ **1995 – present – Image plate/CCD Detectors**

a few hours collection time for a routine structure

❖ ~2007 – Digital Detectors (AXIOM, Pilatus etc.)

❖ new options: shutter less, low noise etc.

It started in 1912, and Von Laue experimented with copper sulfate. In 1913, Bragg started they are father and son as we talked about the work for a long time, developed diffractometer, developed detectors, and could be considered the father of X-ray crystallography in that way contributed a lot. In 1965, an improved film mostly contributed through Weissenberg, but many other contributors. 1960 to 1995, its serial detected diffractometer. So, they have to collect one by one. So, the amount of data set collected is less because it takes a long time to collect as the statistics told it is 50 data set a year, think about you could only have 50 crystal structures. So, if you go back to the protein databank, I will talk about that in detail. A protein databank is a database where all the experimentally solved structures have deposited the coordinates. After 1995, the image plate came, CCD detectors arrived, and it reduced the data collection to a few hours of collection time for a routine structure. Then after 2007, there are digital detectors currently using AXIOM, Pilatus, and all, and new options are coming up.

When we think about data collection, we always talk about the opening and closing of the shutter. Still, shutterless options are coming, the noise is reduced, and the intensity is enhanced. So, with crystals, which we have never thought data could be collected enough to solve a structure now, that data like that crystals are also being able to resolve, and we are getting structure out of that especially called about twinning.

Twin data were like when I started protein crystallography. We used to get twin data like twin crystals. We generally throw the crystals and try to get new crystals sometimes, we solve, but now they are regularly solving. So, these are the improvement.

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Data Collection:

After alignment, the detector distance is set, the Phi axis is locked, the enclosure doors are closed, and the shutter switch is moved to the External position

The crystal is then ready to collect a few initial "oscillation" images, one at 0° and a second at 90°

During collection of the oscillation images, the crystal is rotated about the Phi axis for about 1° as the crystal is exposed, usually for 2-5 minutes per image

The initial images are inspected for crystal quality, and the software then automatically indexes the crystal

Coming to data collection, the source of X-rays is often a home source or synchrotron. For our consideration for further processing, let us assume that we are collecting data from a home source. The typical size for data collection may be 0.3 to 0.3 into 0.1 to 0.5 millimeters, or that is a big crystal. If you have smaller crystals, you do not like to collect data from a home source. Or even if you collect, you do not get the actual resolution you want to solve in the molecule level atomic level. So, it would be best if you had bigger crystals, but again, with the improvement of instrumentation, we are also getting diffraction patterns of smaller crystals in homesource. Generally, a crystal is mounted on the phi axis or spindle, which leaves the crystal upside down or reverse upside down depending on the instrument's setup.

The crystal can either be mounted in a capillary tube, room temperature data collection, or typically in a mounted cryoloop. Let us talk about the advantages when you want to collect the data at room temperature. When you cannot take a temperature of -180 degrees with nitrogen gas or liquid nitrogen, you cannot do freezing that time you try with these capillary tubes. After the crystal is mounted on the instrument, the video screen aligns the crystal to the presumed center position or the X-ray beam. So, if you do not do the perfect alignment with rotation, it would lose the main center and focus of the X-ray beam. After alignment, the detector distance is set, you have to set the detector distance, the phi axis is locked, enclosure doors are closed, and the shutter switch moves to the external position. The crystal is then ready to collect a few initial oscillation images, one at 0 degrees and the second at 90 degrees. I did not talk about these. So, when we started data collection, we did not just go and do the data collection, it generally takes 2, 3 days. So, before you know, you spend 2, 3 days and that much of X-ray, power, consumption, and all you have to make sure that the crystal you are diffracting will give you good data.

So how do you ensure that? We collect 0-degree, 90 degrees, and that also helps us how much exposure time you give. Think about if you want to collect 360-degree images, so 360 per degree 360 images. So, if you choose 1 minute, that is 6 hours, if you choose 10 minutes, that is 60 hours. So, there is a difference of 6 hours to 60 hours if you change the exposure time to 1 minute to 10 minutes.

That is why it is critical to do as much optimization as initially. So, we collect one at 0 degrees and a second at 90 degrees. During the collection of the oscillation image, the crystal is rotated about the phi axis for about 1 degree. This will also depend on you; you could make it 1 degree 0.5 degrees 0.25 degrees depending on you, as the crystal is exposed as an average of 2 to 5 minutes per image we do, but that also depends on how your crystal is diffracting.

So, this part, you need expertise, you collect a few I talked about to 0 degrees and 90 degrees, but you collect few. And then what you have to do is call indexing. I am coming there. The initial images are inspected for crystal quality, and the software then automatically indexes the crystal.

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Data Collection:

- Further, the collected data would be combined computationally with complementary chemical information to produce and refine a model of the arrangement of atoms within the crystal
- The final, refined model of the atomic arrangement-now called a crystal structure is usually stored in a public database
- After the diffraction pattern is obtained, the data is then processed by a computer and the structure of the atom or molecule is deduced and visualized

The slide features a blue header and footer. The footer contains logos for Swayam and other educational institutions.

Further, the collected data would be combined computationally with complementary chemical information to produce and refine a model of the arrangement of atoms within the crystal. The final refined model of the atomic arrangement, now called a crystal structure, is usually stored in public database. After the diffraction pattern is obtained, the data is then

processed by a computer, and the structure of the atom or molecule is deduced and visualized.

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Indexing:

Two separate pieces of information can be found in the reflections of the diffraction images.

The first comes from the geometrical arrangement of the reflections, which gives all the information about the crystal lattice and the symmetry of the crystal.

The second comes from the intensity of the reflection that gives part of the information about the content of the lattice.

Unfortunately the second kind, which is the one that we are actually interested in, is only partial - we lack the phases.

Handwritten notes: "Cubic" with an arrow pointing to a 3D cubic lattice diagram. A circled "A" with an arrow pointing to "Intensity". A circled "B" with an arrow pointing to "Lower Symmetry".

Let us concentrate on indexing now. Because as a new crystallographer, indexing is very critical for you. So here we have to talk or think about two separate things. Two separate pieces of information can be found in the reflection of the diffraction image. The first comes from the reflection's geometrical arrangement, which gives all the information about the crystal lattice and the crystal's symmetry. Now you realize why I have spent two classes explaining the crystal lattice, the unit cell, and the symmetries.

They are important because they will now help you to understand the indexing. The second comes from the intensity of the reflection that gives part of the information about the content of the lattice. So, the first choice of the unit cell and is critical, the choice of a unit cell depends on your choice of model.

Unfortunately, the second kind is the one that we are interested in. Here we have a thing that we lack phase. So, first, we are doing the indexing means we are finding the unit cell then we are going to get the actual coordinate, actual intensity, the electron density, for that we lack phases, which is called the famous phase problem, we will talk in details about that, what is the phase problem and how in protein crystallography people have solved phase problem.

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Indexing & further data collection:

The indexing procedure determines the unit cell dimensions and lattice type of the crystal, as well as its orientation relative to the x-ray beam

Software Used: HKL, Mosfilm

✓ After indexing, the software determines the best starting and ending rotation angles for the data collection

Set up the time of image collection and number of oscillation images to be collected, which usually happened overnight

~~10°~~ ~~4°~~ ~~4°~~
160°

✓ 10° 90°/180°/360° 4°
✓ 1°/0.5°/0.25°
Exposure
Rotation angle
range

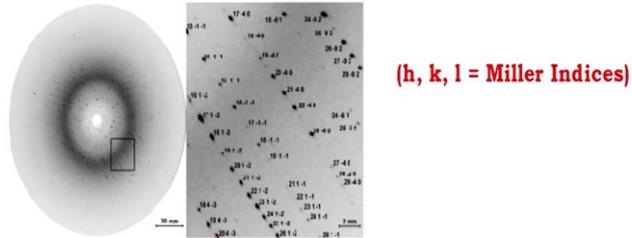
The indexing procedure determines the crystal's unit cell dimension and lattice type and its orientation relative to the X-ray beam. We could use software like HKL, Mosfilm. After indexing, the software determines the best starting and ending rotation angle for the data collection. The initial images would be utilized first, how much exposure you will give, and then what would be your starting and ending rotation angle. So, rotation angle range and third, the degree of the interval you will give 1 degree, 0.5 degrees, 0.25 degree. These three things are practically very critical for getting good data in less time.

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Indexing:

During indexing the spots have to be found, named with integer numbers and the crystal geometry has to be determined accurately

This results the intensities to be integrated accurately and that the space that has to be modelled later on, is defined accurately



During indexing, the spots have to be found, named with integer numbers, and the crystal geometry must be determined accurately. This results the intensities being integrated accurately and that the space that has to be modeled later on is defined accurately. So, if you look at, this is the diffraction pattern we have shown before, and remember I talked for getting good diffraction your crystal ordering would be perfect, there might be a rotational disorder, there might be a translational disorder.

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Relation of Lattice and Spots:

o/y

The length of the reciprocal lattice vectors is of the order $1/a$

Small lattice (like in elemental solids, big reciprocal lattice, points well separated)

Big lattice (like protein crystal) spots VERY close to each other

We need a **VERY collimated monochromatic beam**

The length of the reciprocal lattice vector is of the order $1/a$. If a is the base vector, this is $1/a$. Small lattice like in elemental solids, big reciprocal lattice, points well separated). For example, if many small-molecule synthetic chemists solve the structure of their small-molecule through X-ray crystallography, that is not a problem.

But it is a problem for proteins that form big lattices. The bigger the lattice, the smaller the spot because this is inversely proportional. So, for the protein crystals, there are big lattices. So, the spots are closer, and so, now, because it is closer, it should not be mixed up. So, the beam should be a very collimated monochromatic beam.

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Bragg's Law:

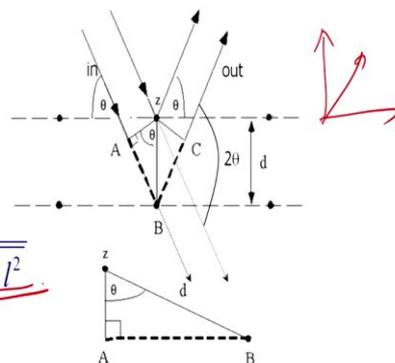
$$n\lambda = 2d \cdot \sin\theta$$

n: Order of reflection

d: Plane spacing

θ : Bragg Angle

$$d = \frac{a}{\sqrt{h^2 + k^2 + l^2}}$$



Path difference must be integral multiples of the wavelength

$$\theta_{in} = \theta_{out}$$

We already talked about sources, but just to remind you, a highly collimated and monochromatic beam is needed for protein crystallography. A synchrotron radiation source only provides it, and when you do not have synchrotron radiation, you have a good X-ray

home source. Now, going towards the further analysis of the data, we will come to Bragg's law, which is

$$n\lambda = 2d\sin\theta.$$

Where n is an integer, and you see that law is applied for the parallel planes where the light rays or any X-rays come in diffraction, the n is the order of deflection, d is the plane of spacing, and θ is the Bragg angle. We will talk about this plane spacing as $a / \sqrt{h^2 + k^2 + l^2}$. Where a is the base vector, we talked about, the a , b , c , and h k l is the reciprocal lattice. Path difference must be integral multiplies on the wavelength, and $\theta_{in} = \theta_{out}$.

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Geometry of Bragg's law:

The incident beam, the normal to the reflection plane, and the diffracted beam are always co-planar

The angle between the diffracted beam and the transmitted beam is always 2θ (usually measured)

$\sin \theta$ cannot be more than unity; this requires $n\lambda < 2d$, for $n=1$, $\lambda < 2d$

λ should be less than twice the d spacing we want to study

Handwritten notes: Cu, 1.54, λ , θ

The incident beam, the normal to the reflection plane, and the diffracted beam are always co-planar. They have to maintain the same plane. The angle between the diffracted beam and the transmitted beam is always 2θ . $\sin\theta$ cannot be more than unity. So, it requires $n\lambda < 2d$ for $n=1$, $\lambda < 2d$. So, the wavelength should be less than twice the spacing we want to study. So, this is a condition which will help you to choose your matter, which metal you are using here. So, we are using copper with $K\alpha$ 1.54.

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Order of reflection:

Rewrite Bragg's law $\lambda = 2d \sin\theta$

A reflection of any order as a first order reflection from planes, real or fictitious, spaced at a distance $1/n$ of the previous spacing

Set $d' = d/n$

$$\lambda = 2d' \sin\theta$$

An n^{th} order reflection from (hkl) planes of spacing d may be considered as a first order reflection from the $(nh \ nk \ nl)$ plane of spacing $d' = d/n$

*The term reflection is only notional due to symmetry between incoming and outgoing beam w.r.t. plane normal, otherwise we are only talking of diffraction.

So, we are rewriting Bragg's law as

$$\lambda = 2 \sin\theta d / n$$

d is the spacing and n is the periodicity a reflection of any order as a first-order reflection from planes, real or fictitious, spaced at a distance $1/n$ of the previous spacing, if we consider that as a

$d' = d/n$, so,

$$\lambda = 2d' \sin\theta$$

An n^{th} order reflection from hkl planes of spacing d may be considered as a first-order reflection from the nh, nk, nl plane of spacing $d' = d/n$.

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Reciprocal lattice vectors:

Used to describe Fourier analysis of electron concentration of the diffracted pattern

Every crystal has associated with it a crystal lattice and a reciprocal lattice

A diffraction pattern of a crystal is the map of reciprocal lattice of the crystal

a₁ b₁ c₁

So, coming to what I was talking about, reciprocal lattice vectors used to describe Fourier analysis of electron concentration of the diffracted pattern, we will talk about the Fourier analysis later every crystal is associated with it a crystal lattice and a reciprocal lattice. A diffraction pattern of a crystal is the map of the crystal's reciprocal lattice.

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Reciprocal Space:

The immediate result of the X-ray diffraction experiment is a list of X-ray reflections ***hkl*** and their intensities ***I***.

We can arrange the reflections on a 3D grid based on their ***h, k*** and ***l*** values. The smallest repeat unit of this **reciprocal lattice** is known as the **reciprocal unit cell**; the lengths of the edges of this cell are inversely related to the dimensions of the real-space unit cell.

This concept is known as **reciprocal space**; it emphasizes the inverse relationship between the diffracted intensities and **real space**.

So, what is reciprocal space? The immediate result of the X-ray diffraction experiment is a list of X-ray reflection hkl and their intensities I . We can arrange the reflections on a 3D grid, based on their h and k and l values. The smallest repeat unit of this reciprocal lattice is known as the reciprocal unit cell; the length of the edges of the cell are inversely related to the dimensions of the real-space unit cell.

So, in previous classes, I talked about real unit cells and the real value of the space. Now, a reciprocal is just inversely proportional. That is what makes the calculation much easier and provable. This concept is known as reciprocal space; it emphasizes the inverse relationship between the diffracted intensities and the real space. You will understand even more when you go to real.

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Real Space and Reciprocal Space:	
<u>Real Space</u>	<u>Reciprocal Space</u>
Unit Cell (a, b, c, α , β , γ)	Diffraction Pattern
Electron Density, $\rho(x, y, z)$	Reflections
Atomic Coordinates – x, y, z	Integrated Intensities – $I(h, k, l)$
Thermal Parameters – B_{ii}	Structure Factors – $F(h, k, l)$
Bond Lengths (Å)	Phase – $\alpha(h, k, l)$
Bond Angles (°)	
Crystal Faces	

So, real space and reciprocal space, you have a crystal structure, crystal lattice, unit cells document when it comes to that diffraction pattern, you get a reciprocal lattice, which helps you get the structure factor for further solution. So, this is the real space, real space lattice, and reciprocal lattice. So, in real space, what information you get, you get the unit cell a, b, c alpha, beta, gamma, the base vector their value and their angles, electron density the rho, because I will show you how to perform the Fourier. And how we come to electron density atomic coordinates, thermal parameters, which is B factor we will talk about that, bond length, bond angle, the bond length would be an angstrom, the bond angle would be in degrees and the faces of the crystal. In the reciprocal space, we get the diffraction pattern. We get the reflections. We get integrated intensities, which is $I(h, k, l)$, and structure factor, which is $F(h, k, l)$, phase which is $\alpha(h, k, l)$.

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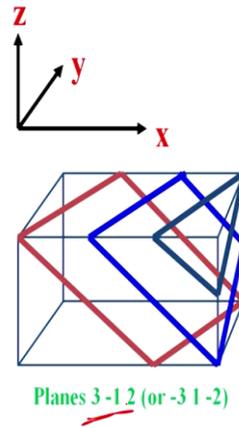
Reflection Indices:

These planes must intersect the cell edges **rationally**,

otherwise the diffraction from the different unit cells would **interfere destructively**.

We can index them by the number of times ***h, k and l*** that they cut each edge.

The same ***h, k and l*** values are used to index the **X-ray reflections** from the planes.



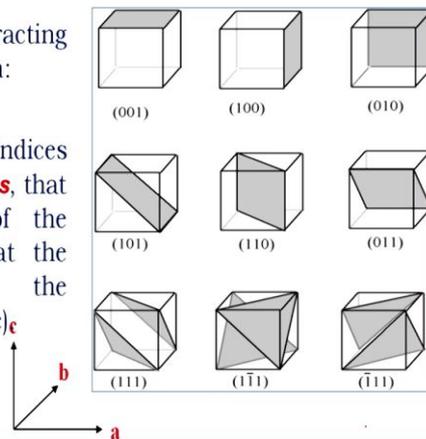
So, if you see, these planes must intersect the cell edge rationally. Otherwise, the diffraction from the different unit cells would interfere destructively. We can index them by the number of times h k l that cut each edge. The same h , k , and l values are used to index the X-ray reflection from the plane. So, if you see here, it is 3-1 and 2 or with the inverse operation -3 1 and -2.

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Examples of Diffracting Planes and their Miller Indices:

Method for identifying diffracting planes in a crystal system:

A plane is identified by indices (hkl) called **Miller indices**, that are the **reciprocals** of the fractional intercepts that the plane makes with the crystallographic axes $(abc)_c$



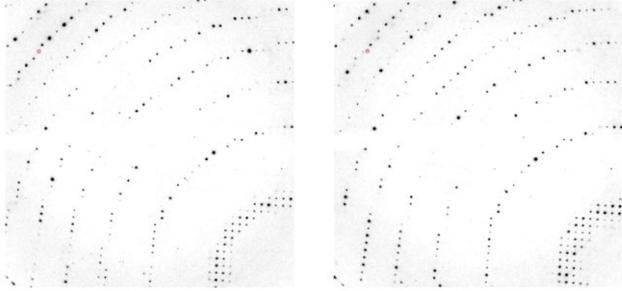
So, examples of the diffracting plane and their miller indices: It is a method of identifying diffracting plane in the crystal system, a plane is identified by indices hkl , they are called as miller indices that are the reciprocals of the fractional intercepts that the plane makes with the crystallographic axis or the real axis. So, they are inversely proportional. So, you have a , b and c .

And if you see, in the case of miller indices, you get all those in integer number 001,100,010,101,110,011,111,1 bar 1 bar 1, 1 bar 11. So, they are all integers. That is the biggest advantage here: you get an integer or 0, whereas, for a real axis, you get a fraction or infinity.

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Diffraction Patterns:

Two successive CCD detector images with a crystal rotation of one degree per image:



For each X-ray reflection (black dot), indices h,k,l can be assigned and an intensity $I = F^2$ measured

So, we are talking about the diffraction pattern, two successive CCD detector images with a crystal rotation of 1 degree per image, for each X-ray deflection, which is the black dots you would see, indices h, k, l can be assigned. An intensity $I = F^2$ is measured.

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Diffraction Patterns:

When Bragg's Law is satisfied through the appropriate combinations of crystal orientation angles with respect to the incident X-ray beam, reflections appear on the detector.

Previous example shows two successive CCD detector images with a crystal rotation of one degree per image.

A complete dataset consists of the measurement of all independent intensities in the diffraction pattern out to a specified resolution limit.

90°
180°
360°
720°

Bragg's law is satisfied by the appropriate combination of crystal orientation angles regarding the incident X-ray beam and reflection appearing on the detector. The previous example talked about two successive CCD detector images with a crystal rotation of 1-degree. A complete dataset consists of the measurement of all independent intensities in the diffraction

pattern out to a specified resolution limit, which means if you have to collect 90 or 180 or 360 or 720 images, each of them has one intensity. Then you have independent intensities, and you get the summation of that to get the total number.

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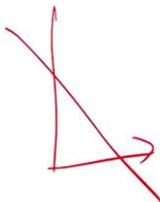
Miller Index:

Miller indices form a notation system in crystallography for planes in crystal (Bravais) lattices.

Characteristics:

Set of three integers (hkl)

Inversely proportional to where face or crystallographic plane (e.g. cleavage) intercepts axes




Coming to the concept of miller index. Miller indices form a notation system in crystallography for planes in Bravais lattices. They are set of three integers, h, k, and l, inversely proportional to face or crystallographic plane intercept axes.

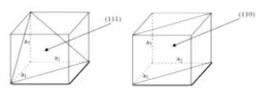
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Miller Index:

The crystal lattice may be regarded as made up of an infinite set of parallel equidistant planes passing through the lattice points which are known as lattice planes.

In simple terms, the planes passing through lattice points are called 'lattice planes'.

For a given lattice, the lattice planes can be chosen in a different number of ways.

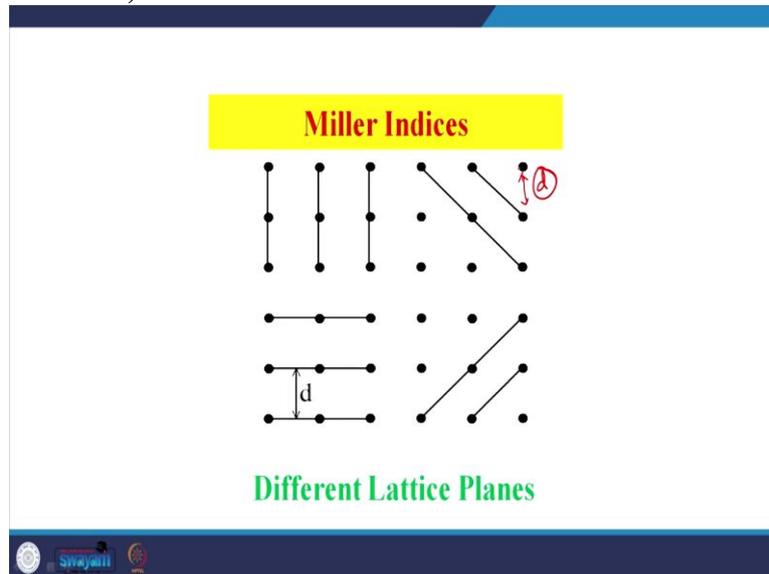




A crystal lattice may be regarded as an infinite set of parallel equidistant planes passing through the lattice points known as the lattice plane. If you remember when we made our journey from 1d to 2d to 3d, consider the 1d lattices as equidistance.

Here in the miller indices, the crystal lattices may be regarded as made up of an infinite set of parallel equidistant planes passing through the lattice point. So, now, in simple terms, the

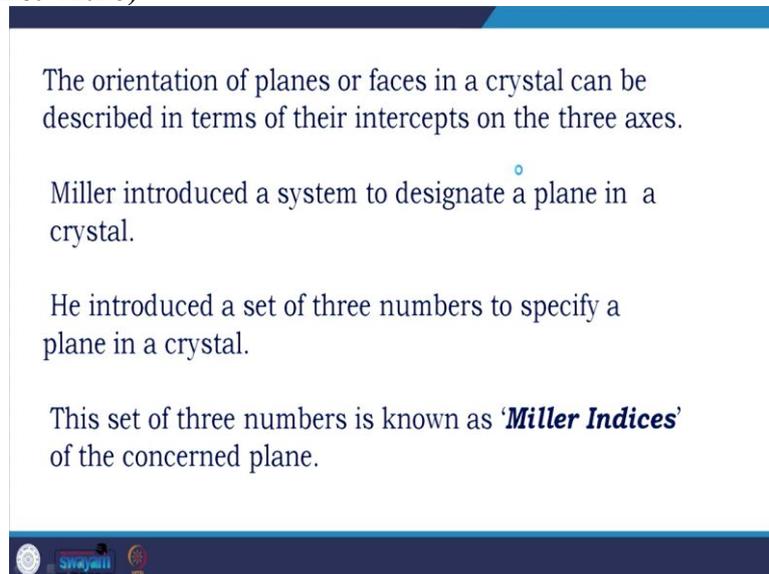
planes passing through lattice points are called lattice planes for a given lattice. The lattice planes can be chosen in a different number of ways.

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So, as you see here, these are Miller indices of different lattice spaces where all the points have the same spacing, which is d , but you have to get the relations where they intersect from the parallel lines.

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The orientation of planes or faces in a crystal can be described in terms of their intercepts on the three axes. Miller introduced this system to designate a plane in a crystal. He introduced a set of 3 numbers, which we call h, k, l , Miller indices, to specify a plane in the crystal. This set of 3 numbers is known as Miller indices of the concerned plane.

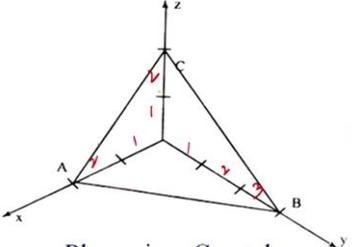
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Miller indices is defined as the **reciprocals** of the **intercepts** made by the plane on the **three axes**.

So, Miller indices are defined as the reciprocal of the intercept made by the plane of the three axes.

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Step 4: The result is written in paranthesis. This is called the 'Miller Indices' of the plane in the form $(h\ k\ l)$.



Plane ABC has intercepts of

- ✓ 2 units along X-axis,
- ✓ 3 units along Y-axis

and,

- 2 units along Z-axis.

Planes in a Crystal

Let us see how you could get or find a Miller index to determine the intercept of the plane along the axis x y. So, if you have these, x, y, z in that way, in terms of the lattice constants, a, b and c, determine the reciprocal of these numbers, find the least common denominator and multiply each by this the least common denominator, so, get the integer, the result is written in parentheses. This is called Miller indices of the plane in the form h k l. So, if you see here, you get the planes, which is 123. This is 1 2, and this is 1 1. So, in x-axis around this is two units, three units along y-axis and two units along z-axis.

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Determination of Miller Indices:

Step 1: The intercepts are 2, 3 and 2 on the three axes.

Step 2: The reciprocals are $1/2$, $1/3$ and $1/2$.

Step 3: The least common denominator is '6'.
Multiplying each reciprocal by lcd,
we get, 3, 2 and 3.

Step 4: Hence Miller indices for the plane ABC is
(3 2 3)

So, in that way, you could determine, see, look at these. The intercepts are 2, 3, 2 on the three axes, the reciprocals are then. So, it is $1/3$, it is $1/2$, and this is $1/2$. The least common denominator is six, multiplying each reciprocal by the LCD (least common denominator). So, we get 3, 2, and 3. Hence, Miller indices of the plane are 3 2 3.

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X-ray Diffraction Data Processing:

From data collection we have found number of oscillation images.

In data processing, the set of oscillation images is converted into a file that contains the intensities (I) and sigmas (σ) of all of the symmetrically unique hkl reflections that were measured.

There are essentially four steps involved in data processing

- (1) collection of initial images, crystal evaluation and indexing
- (2) collection of the full set of oscillation images
- (3) integration of reflection intensities, and
- (4) Scaling, merging, and averaging of reflection intensity data

What is unique hkl reflections? What is intensities (I) and sigmas (σ)?

So, coming to the X-ray diffraction data processing, which we will discuss in the next module. We have found the number of oscillation images in the data processing from data collection. A set of oscillation images is converted into a file containing the intensities I and the sigma with all of the symmetrically unique hkl reflection measured. There were essentially four steps involved in data processing, collection of initial images, crystal evaluation, and indexing.

We will talk about collecting the full set of oscillation images, integrating reflection intensities and scaling, merging, and averaging up reflection intensity data. We talked about what is unique hkl reflection, and we will talk about intensity and sigma. So, then we will proceed with the solution. Thank you very much for listening to me send the questions. Please continue listening, and please continue learning. I will try my best to develop much better things in the following classes. Thank you very much.