

## **Experimental Nanobiotechnology**

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### **Lecture 09: X-Ray Diffraction**

Hello everyone, today we are going to learn X-ray diffraction. This is an important technique in nanomaterial characterization. In today's lecture, we are going to learn the working principle of XRD, Bragg's law and applications of XRD. At the end of lecture, we will also have a practical demonstration to understand this technique more in detail. Let us see what is X-ray diffraction.

X-ray diffraction that is XRD is a non-destructive analytical technique used to analyze physical properties such as phase composition, crystal structure and orientation of powder, solid and liquid samples. It can analyze all kinds of amorphous powder and crystalline materials. It is ideal for probing the interplanar distance in the crystalline materials. Interplanar distance means it is the distance between any two parallel planes in the crystal. XRD can also distinguish between crystalline and amorphous phases as well as the different crystal polymorphs

and XRD provides data regarding crystal structure, phase identification, lattice parameters and crystallite size and strain. Let us briefly see the history of XRD. X-ray was discovered in 1895 and for the discovery of X-ray, the Nobel Prize was given in 1901 followed by the discovery of X-ray. So, the X-ray diffraction was discovered in 1912 and Nobel Prize was given in 1914. So, based on the X-ray diffraction, you can see that there are so many Nobel Prize. So, which shows the importance of this technique including the structure of DNA which was illustrated using this XRD and protein structure.

So, this technique played a very important role not only in material characterization but also in biological science. Let us see what X-rays are. X-rays are electromagnetic radiation with wavelengths in the range of 0.01 to 10 nanometers. These X-rays possess high energy and are capable of penetrating most materials. You can see this is the first X-ray apparatus used by Röntgen in 1895 while experimenting with cathode rays.

These X-rays can exhibit absorption, scattering, and diffraction upon interaction with matter. Let us see how these X-rays are generated. So, these X-rays are generated by striking accelerating electrons onto an anodic metal target coming from a cathode metal under a closed vacuum. This anode is mainly made up of copper or molybdenum. If you see, the electrons emitted from the heated cathode, which is a tungsten filament through thermionic emission, are accelerated by high voltage towards the anode.

Upon striking the anode, the high-energy electrons interact with atoms, producing X-rays through two main processes. One is Bremsstrahlung radiation, and the other is characteristic radiation. So, this Bremsstrahlung radiation is also known as braking radiation. It is a type of electromagnetic radiation that occurs when a charged particle like an electron slows down or is deflected when it interacts with matter.

So, you can see here the target nuclei it is slowing down on the oncoming electrons and which release their energy as X-rays. So, it occurs when fast moving electrons are decelerated by the electric field of atomic nuclei converting their kinetic energy into X-ray photons while conserving the energy. The next one is characteristic radiation. Here you can see the inertial electrons of the metal target are knocked out by the incident electrons beam. Then the vacancy is filled by a higher energy electron and the energy release is emitted as X-rays.

From this picture, we can understand how these X-rays are generated. So, as I told earlier these X-rays are generated when high energy electrons from a heated cathode strike a metal anode that is a target and here the due to the rapid deceleration of electrons near the nucleus of the target atom which produce a Bremsstrahlung radiation that is a continuous spectrum. Additionally, when these high energy electrons are ejected the inertial electrons from the target atoms what happened this outer shell electrons fill these vacancies. and releasing an extra energy as characteristic radiation which will have a discrete wavelength.

So, both types of radiations like Bremsstrahlung radiation as well as the characteristic radiation combine to form the X-ray. Let us see the geometry of crystals. A crystal structure refers to the organized arrangement of atoms, ions or molecules in a crystalline material. In a crystal, the components are arranged in a repeating pattern that extends in all three spatial dimensions forming a lattice. So, this is the unit cell.

So, each point in the lattice represents the portion of a particle. It can be an atom, ion, or molecule. The smallest repeating structure of the crystal lattice is called as unit cell and the dimension of unit cell includes the length of the edges that is the  $a$ ,  $b$ ,  $c$  and their angle is

alpha beta and gamma of the unit cell. These lattice parameters can be determined by XRD, which can further be used to identify the crystal structure. So, this unit cell is like a kind of brick; for example, we use bricks to construct homes of different designs.

For crystal structures, this unit cell arrangement is very important. So, we have different kinds of crystals. This crystal structure can be studied or determined by XRD, and we can also understand the lattice parameters using XRD. Let us see what Miller indices are. Dr. Miller devised this mathematical system for describing any crystal phase or group of similar forms.

These Miller indices are a set of three numbers (HKL) used to describe the orientation of a crystal plane. Or a set of parallel planes within a crystal lattice. These Miller indices are determined by first identifying the points where the plane intersects each axis. Each number is then reciprocated and converted into a whole number. I will explain this in detail in the next slide.

So, let us see the significance of these Miller indices. So, these Miller indices are useful for identifying specific planes or directions in the crystal lattice, and they also help in understanding the properties of materials. We can identify different planes in a crystal, which may be related to different physical properties of a material. It can be relevant to electrical conductivity or to the hardness of the material. These are various examples of the Miller indices.

And we are going to take an example of this one: 100. How do we get this 100 using the Miller indices? So, let us see an example to understand the Miller indices. So, in this case, let us take a cubic unit cell with equal edge length, that is,  $a$ . The first step is to determine the intercept. So, identify the location where the plane intercepts the  $x$ ,  $y$ , and  $z$  axes.

Once the plane cuts the  $x$ -axis at  $a$ ,  $0$ ,  $0$ , and since it is parallel to the  $y$  and  $z$  axes, the respective intercepts will be infinity. So, that is why the intercepts for the  $x$ ,  $y$ , and  $z$  axes are  $a$ , infinity, and infinity. So, the next step is to convert to fractional coordinates. Now, we will convert them into fractional coordinates by dividing each of them by the respective edge length. Since it is a cubic lattice, each edge length is the same. So, we will divide each of them by  $a$ . Here, the fractional coordinates for the  $x$ ,  $y$ ,  $z$  intercepts become  $a/a$ , infinity/ $a$ , and infinity/ $a$ , which equals  $1$ , infinity, and infinity.

Then, the third step is to take the reciprocals. So, the final step is to take the reciprocal of each coordinate and convert them to whole numbers. The reciprocal of one, infinity, and

infinity will be one by one, one by infinity, and one by infinity, which is equal to one, zero, zero. So, the Miller indices for these planes become one zero zero. Let us see what is refraction. Diffraction is a phenomenon where waves, such as light, sound, or X-rays, bend around obstacles or spread out after passing through narrow openings or slits. This occurs when the wave interacts with structures or objects that have dimensions comparable to its wavelength, resulting in constructive or destructive interference.

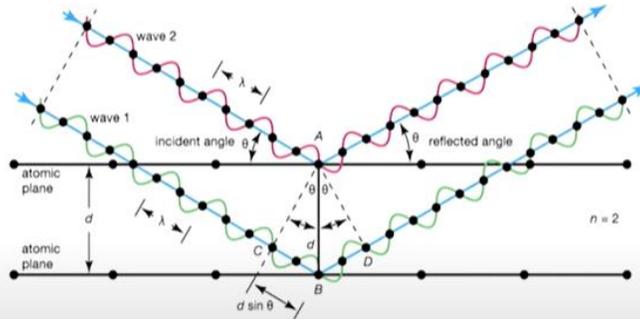
The atomic planes in the crystal act like a set of closely spaced slits. Causing the X-rays to diffract. So, if we take a crystal, these atomic planes in the crystal act like a slit. So, it makes the X-rays diffract. And let us see what constructive interference and destructive interference are.

So, constructive interference means the waves in the same phase lead to a peak in the diffraction pattern. We can see the waves are in the same phase. So, we will get the peak. While out-of-phase waves undergo destructive interference and will cancel out each other. You can see here the waves are out of phase.

So, due to which, they will cancel out each other, and that is called destructive interference. And from this picture, you can see this red-colored ring. So, the red-colored ring is the constructive interference, and the black-colored one is the destructive interference. So, whatever we are getting, this red-colored ring is constructive interference. And where you are not seeing the red-colored ring, that is the black-colored one, which is destructive interference.

Let us see what Bragg's Law is. Bragg's Law explains the condition for constructive interference of X-rays scattered by a crystal lattice. It relates the X-ray wavelength to the interplanar spacing in the crystal and the angle of incidence. So, Bragg's Law that  $n\lambda$  is equal to  $2d \sin \theta$  where  $n$  is the order of diffraction and the  $\lambda$  is the wavelength of x-ray and  $d$  is the interplanar spacing, which is the distance between the crystal planes, and  $\theta$  is the angle between the incident X-ray beam and the crystal plane.

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



Where:

- $n$ : Order of diffraction (integer)
- $\lambda$ : Wavelength of the X-rays.
- $d$ : Interplanar spacing (distance between crystal planes).
- $\theta$ : Angle between the incident X-ray beam and the crystal plane.

Let us see the parts of XRD. The first one is the X-ray source, which produces the X-rays, typically using a vacuum-sealed tube, and commonly used metals are copper and molybdenum targets for characteristic X-rays. Then we have the monochromator, which filters unwanted wavelengths and isolates a single wavelength for precise diffraction analysis, and we have the sample holder, which securely holds the sample in place and ensures the correct alignment for interaction with the X-rays.

Then we have this goniometer. So, this is a mechanical device that precisely positions the sample, detector, and the X-ray source at specific angles. And we have the collimator. This ensures that the X-ray beam is narrow and well-aligned, reducing the divergence to produce a parallel beam for accurate diffraction measurement. Then we have the detector, which measures the intensity of the diffracted X-rays.

Common types include scintillation counters and CCD detectors. Finally, we have the data processing unit. That is a computer with software for recording, processing, and analyzing the diffraction patterns. Let us see how the diffractometer works. Here, the X-rays are produced by bombarding high-energy electrons onto a metal anode, such as copper or molybdenum, as I mentioned earlier.

The sample is mounted on a holder and aligned precisely. Then, a collimated X-ray beam is directed onto the sample. The X-rays interact with the crystal lattice, producing diffracted beams based on Bragg's law. The diffracted X-rays are detected by a rotating detector at various angles. The intensity of the diffracted X-rays is recorded as a function of the angle.

Then, the diffraction pattern is analyzed to determine the material's crystal structure, phase, and other properties. So, from this slide, you can understand how crystalline polymers can be identified by XRD. You can see these: the quartz, cristobalite, and amorphous SiO<sub>2</sub>. All are made up of SiO<sub>2</sub> but produce different diffraction patterns because of the different periodicity and arrangement of atoms in the material. From this picture, you can understand that this SiO<sub>2</sub> is amorphous in nature, so there are no peaks.

Whereas, in the case of quartz and cristobalite, you can see the peaks, but the positions of the peaks are different. So, based on that, we can identify the polymorphs of the crystal. Let us see another example to understand the X-ray diffraction pattern. So, here we are going to compare graphene oxide and reduced graphene oxide. In the case of graphene oxide, you are able to get a peak around 11.01, and the plane is 001.

But this peak diminishes after reduction. In the reduced graphene oxide, you are not able to see this particular peak. But there is a new peak at around 26.8, and the plane is 002. So, the increased interplanar spacing in graphene oxide is due to the intercalation of oxygen-containing functional groups between the graphene layers. Whereas, in the case of reduced graphene oxide, it exhibits an interplanar spacing similar to graphite.

So, which indicates that the reduction process decreases the interlayer distance by removing these functional groups. Let us see the importance of proper sample preparation. This is an example of ideal powder sample preparation, and here you can see the complete rings corresponding to an ideal powder sample with a large number of small, randomly oriented crystallites. So, you will get small and randomly oriented crystallites, and this ensures the diffraction contribution from all possible lattice planes. The measurement from this type of ideal sample can accurately represent the material's crystal structure, whereas in this case, you can see here the sample has a small number of large crystallites. The spotty or incomplete rings can be due to a sample with a small number of large crystallites and this incomplete representation of diffraction due to the lower randomness of crystallite arrangements. So, this is not an ideal measurement and cannot be relied upon to accurately determine the crystal structure. So, that is why a finely ground powder sample gives a better diffraction pattern.

So, the sample preparation is very important in XRD. Let us see some of the applications of XRD. In material science, XRD is useful for understanding the crystal structure as well as phase identification. In nanotechnology, we can use it for nanoparticle size estimation, lattice strain analysis, and characterization of nanocomposites and thin films. In

pharmaceutical industries, it is useful for understanding drug polymorphs, crystallinity, and stability to optimize bioavailability.

For example, the different crystalline forms will have different solubility as well as absorption. So, based on that, we can understand their bioavailability. In the case of geology and mineralogy, it will be useful for minerals and soil composition and crystal phases in the rocks. In environmental science, it will be useful for analyzing the soil, pollutants, and particulate matter in the atmosphere. For example, it can be useful for detecting toxic metal oxides in the soil samples. In the case of forensic science, it will be useful for the examination of materials like paints and also for identifying unknown substances.

Let us see an example to understand how this XRD can be useful for the identification of these gold nanoparticles. You can see there are four prominent diffraction peaks for the gold nanoparticle, and this is mainly the reflections of the face-centered cubic of gold metal nanoparticles. This indicates that the synthesized tannic acid-capped gold nanoparticle comprises pure crystalline gold. This can be compared with the standard, which is the JCPDS, the Joint Committee on Powder Diffraction Standards. We can compare with this standard and confirm the gold nanoparticle.

The intensity of the peak that corresponds to this 111—you can see this peak's intensity is very high. So, when compared to the other three peaks, that indicates this is the plane which is the predominant orientation for the growth of the nanoparticles. Let us see some of the problems we face when doing XRD and how to overcome those challenges. So, the first problem is weak or no diffraction peaks. So, the reason may be the sample is not properly aligned.

So, recheck the sample alignment. And it may be due to the insufficient sample quantity. So increase the amount of sample or ensure uniform distribution. And the next problem is broad diffraction peaks. It may be due to the

poor crystallinity or small crystallize. So, prepare a better crystallized sample and it may be also due to the instrumental broadening, perform calibration using a standard sample. And if you are facing like overlapping of peaks, so that is due to the multiple phases or complex structure, use advanced data analysis tools or complementary techniques like rietveld refinement. And if you are getting the background noise in pattern, it may be due to the contaminants in the sample. So, clean the sample holder and ensure no external materials interfere

and if you are getting the peak shifts that may be due to the misalignment of the sample or instrument so realign the sample and recalibrate the instrument or it may be due to the residual stress or strain so you have to check it whether is any account for these effects during the analysis and if you are getting the low resolution it may be due to the incorrect scan speed use a slower scan speed for better peak resolution and if you are getting the unstable intensity or fluctuation so that may be due to the old X-ray tube or unstable power supply replace the X-ray tube and ensure stable power supply. So, if the data is not reproducible it may be due to the inconsistent sample preparation ensure uniform thickness and density of sample and also it may be due to the temperature or humidity variation.

So, always perform the analysis in a controlled environment. I hope you got the overall idea about XRD. Let us go to the lab and learn this technique in more detail. Today, we are going to perform XRD analysis of powdered graphene oxide. For this, we require a sample holder.

The sample is placed in the center cavity of the holder. The powdered sample should be properly leveled to get good diffraction of the X-rays from the sample. For this purpose, we use a clean glass slide to level the sample in the holder. Additionally, we will need a spatula, tissue paper, a beaker to discard the waste, and isopropanol for cleaning. We cannot use solvents like acetone for cleaning, as it can damage the sample holder.

First, using tissue paper and isopropanol, we will clean the spatula to remove any residual material. Then, we will wipe the center cavity of the sample holder with isopropanol. Using the clean spatula, we will completely fill the center cavity with our desired sample. Next, we will wipe the glass slide with isopropanol, which we will use to level the sample in the holder. Place the glass slide on the holder and ensure that the sample cavity is completely filled with no space between the sample and the glass slide.

This is Bruker D8 Advanced XRD machine which we will use for our analysis. First, turn on the machine. Let us briefly learn the instrumentation part. This is the X-ray source, which generates the x-rays for analysis. Next is the collimator which narrows down the beam of x-ray which is then passed through a shutter and a slit to focus the x-rays onto the sample.

The instrument also has an anti-scatter screen with a knife edge at the end to block the stray x-rays. And we have the sample stage where the sample is kept. The system is coupled with a goniometer that can independently move the source and detector and control the angle of incidence of the X-rays beam onto the sample. On the other side we have another

collimator and slit setup which focus the X-rays that are reflected from the sample onto the detector and finally the detector detects the reflected X-rays to generate the data.

This machine is paired with a robotic sampling system which can automatically load and unload the samples. We can place up to 15 samples in one holder and there are total of 6 holders. So we can analyze 90 samples at a time in this machine. We can keep the sample in any of these sample positions which we have to specify in the control software.

We have prepared the sample. You can see that the sample is filled in the center cavity of the sample holder. We are placing our sample in the A10 position of the holder. According to which we will give the command to the automatic sampler. Close the machine door and then turn on the machine.

You can see that X-rays are switched on which is indicated by the indicator lights. But these X-rays will not pass on to the sample because the shutter is still closed. which will open only on giving the start command. After the X-rays are turned on, the door will not open to protect the user from X-ray radiation. This is the commander software that controls the operation of the XRD machine.

Here you can see that the X-ray source is on and the shutter is closed till the process starts. First, we need to set the voltage and the current for the X-ray source. We will set the voltage and current to 40 kW and 40 mA respectively by increasing the voltage and current values alternatively with increments of 5 units at a time to prevent damage to the X-ray source. Once the voltage and current values are set, we will give the command to insert the sample onto the sample stage. You can see that the robotic arm takes the sample from the instructed A10 sample position and keeps it in the sample stage.

On the right hand side, we have the setup of the analysis parameters where the scan type has been set to coupled 2theta or theta setting. The source and the detector will move simultaneously. The time taken for measurement at each step has been set to 0.1 seconds. The total steps according to the 2theta values are around 4600 and it will take around 475 seconds to complete the measurement. The 2theta values of our measurement have been set from a start position of 5 degrees to a stop position at 100 degrees with an increment of 0.02 degrees at each step.

After setting the parameters, we can move on to the measurement. Once we press start, the source and the detector will move to the starting 2theta

position which we have set at 5 degrees. You can see that after starting the measurement the sector has opened which means that the x-rays are falling on the sample and going to the detector. The machine has started the measurements and the diffractions are being displayed in the form of peaks. After process completion, we will unload the sample by clicking the sample unload button.

The robotic hand automatically takes the sample and place it in the initial place. After this we have to decrease the voltage and current to their initial values. Now switch off the X-rays. using the X-ray toggle button. Now we can safely open the door of the machine and take out the sample.

Close the door and switch off the machine. From this data, you can understand that the diffraction peak at 10.67 and the reflection plane (001) confirm the characteristic peak of graphene oxide. As a summary, in today's lecture, we learned the basics of X-rays and diffraction, and we also learned the principles of XRD and its applications. Also, through practical demonstration, we learned about XRD in more detail. Thank you for your kind attention.

I will see you in another interesting lecture.