Structural Biology
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Lecture – 16
X – ray Crystallography: Production of X-ray and its Properties

Hi welcome to the course of structural biology again we are in the new module but we are still continuing with structural biology techniques.

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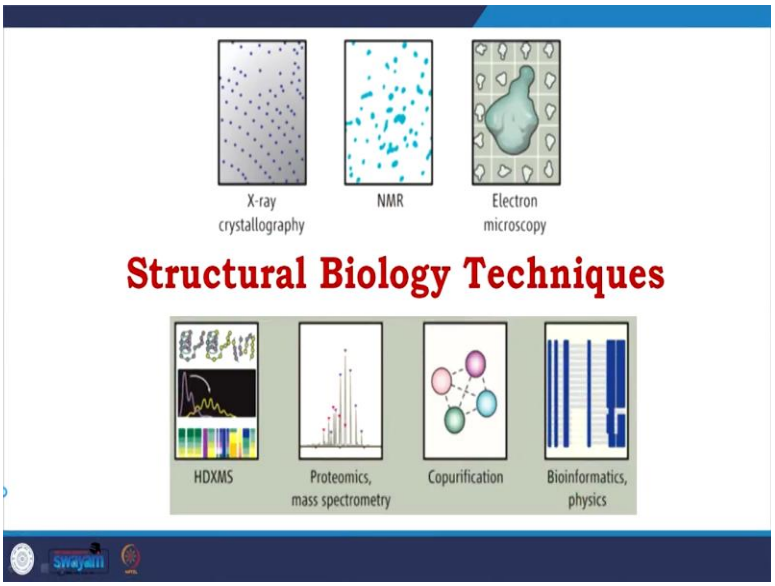

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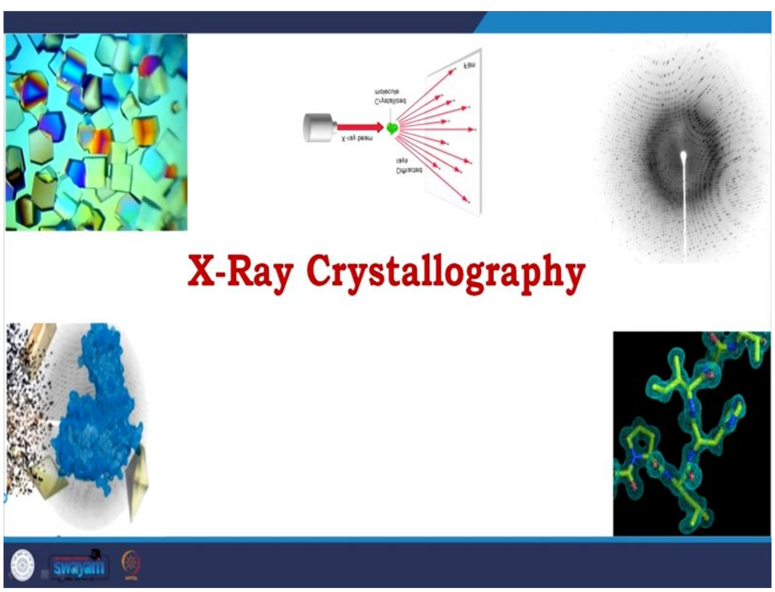

Remember we have talked about the invention of X ray, now we will start how the X ray would be produced?

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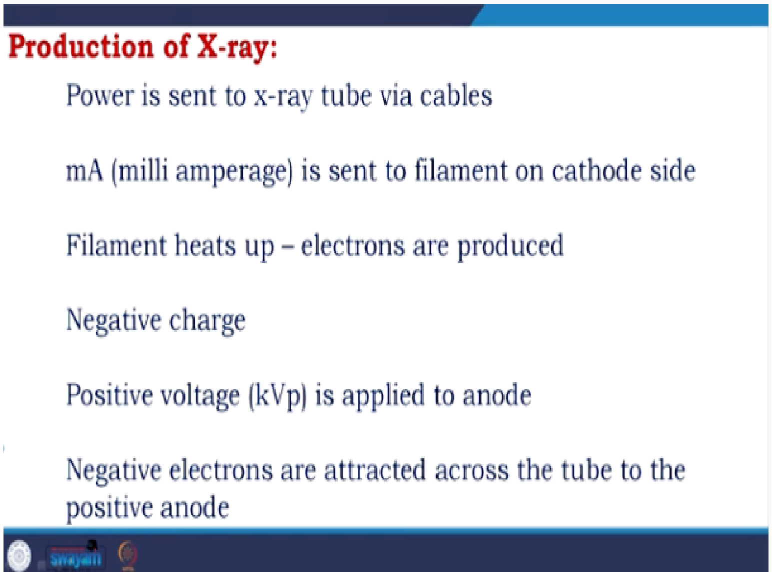

So for production of X ray, power is sent to the x ray tube via cables, and then milli amperage is sent to filament on cathode side, filament heats up and electron produce which are negatively charge and then positive voltage is applied to the anode, and then negative electrons are attracted across the tube to positive anode.

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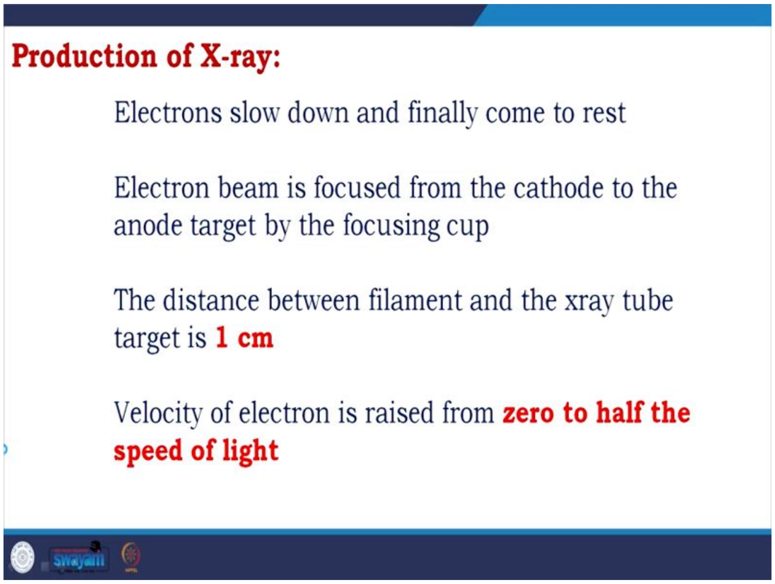

Electrons slow down and finally come to rest. Electron beam is focused from the cathode to the anode target by focusing cup. The distance between filament and the X ray tube target is 1 centimetre, and suddenly the velocity of electron raised from zero to half the speed of light. So first we produce electron, allow it to go to rest, and then focus from the cathode and the velocity of the electron is high.

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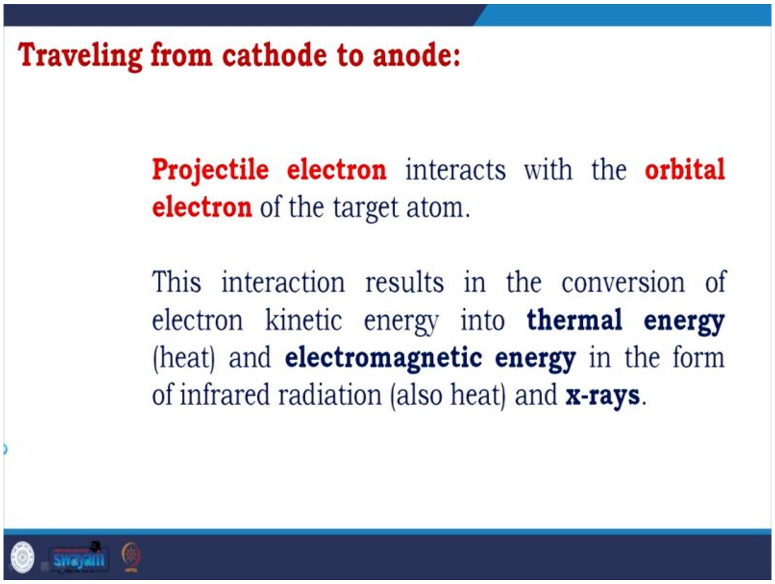

Projectile electron travelling from cathode to anode, interacts with the orbital electron of the target atom. This interaction results in the conversion of electron kinetic energy into thermal energy and electromagnetic energy in the form of infrared radiation and X rays.

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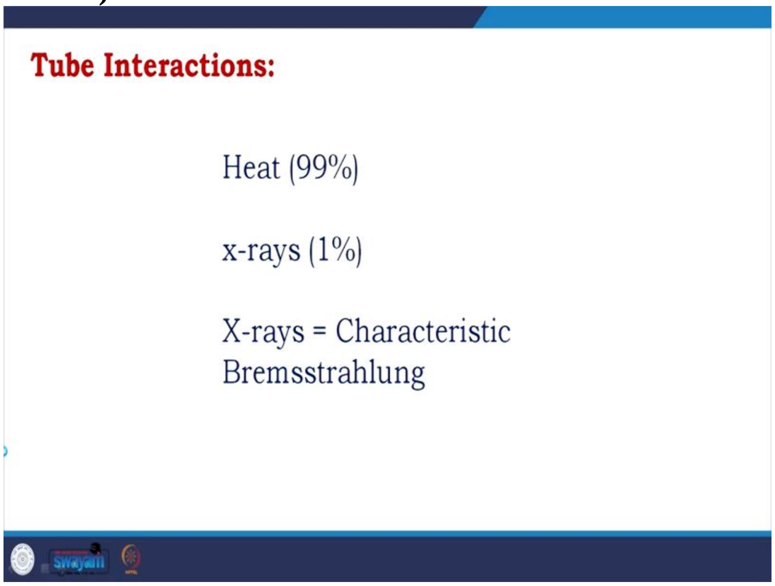

In the tube the heat produces 99% and X rays 1% and the X ray is bremsstrahlung characteristics, X ray but the main concern here the production of the heat which is 99%.

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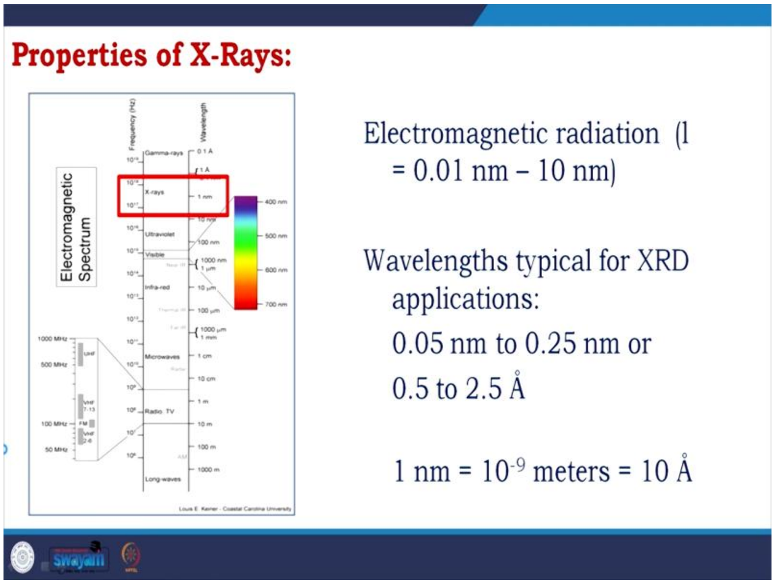

Electromagnetic radiation is generally 0.1 nanometer to 10 nanometers. The wavelength typically for XRD application is 0.05 nanometer to 0.25 nanometer (0.5 to 2.5 angstrom). That is where most of the atomic interactions exist. X-ray is used because the X-ray range is in the range of atomic distances (the bond distances). If you look at protein, there is mostly carbon nitrogen,oxygen, bonded within 1.5 angstroms around. So that is why X-ray works perfectly.

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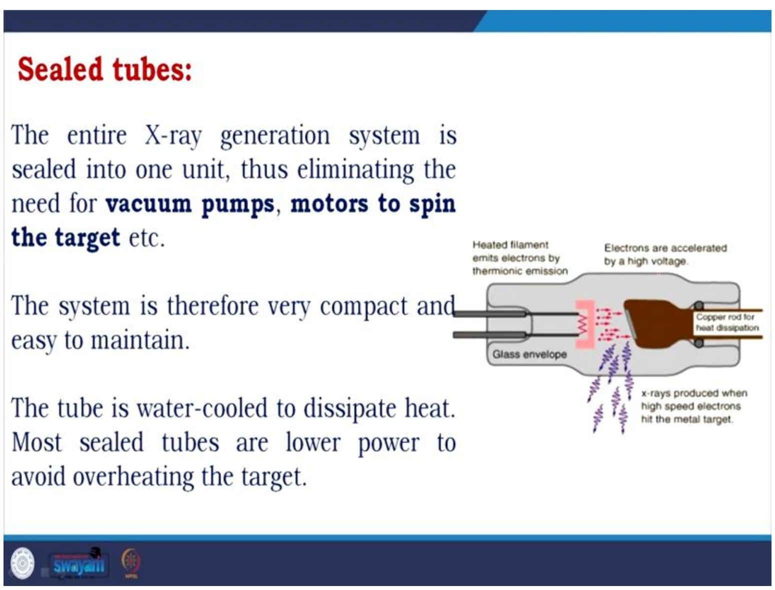

So we understand how X-ray are generate, but what is the instrumentation through which X-rays are generated? One of them is a a cooled reach tube or sealed tube. Here, if you see this picture, you see that heated filament emits electrons by thermionic emission electrons are accelerated by a high voltage copper rod for heat dissipation, and X-ray produced when high-speed electron heat the metal target.

So copper is used as a metal target, or different metal targets could also be used. The entire X-ray generation system is sealed into one unit, thus eliminating the need for a vacuum pump motor to spin the target. The system is, therefore, very compact and easy to maintain. The tube is water-cooled to dissipate heat. Most sealed tubes are lower power to avoid overheating the target. So you cannot generate powerful X-rays because with the increasing energy requirement, the heat generation would also be enhanced. That is not good for the instrument's safety, hence safety of the experiment.

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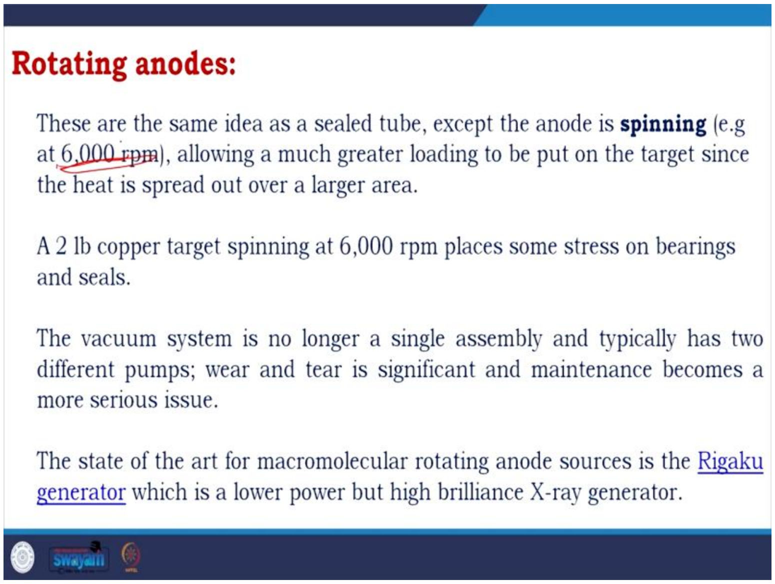

Rotating anodes. rotating anode is a derivative concept. It is the mostly used X-ray source here the modification the critical modification is instead of making it static there is a rotation happened and because of the spinning which is around 6000 RPM.

A 2-pound copper target spinning at 6000 RPM places some trace on bearing and seal. So the target is 2 pound, and it has to rotate at 6000 RPM continuously.

The vacuum system is no longer a single assembly and typically has two different pumps; wear and tear is significant and maintenance becomes a more serious issue.

The state of the earth for macromolecular rotating anode sources is a Rigaku generator which is a lower power but high brilliance X ray generator for long time Rigaku generator is giving us good data collection in home source.

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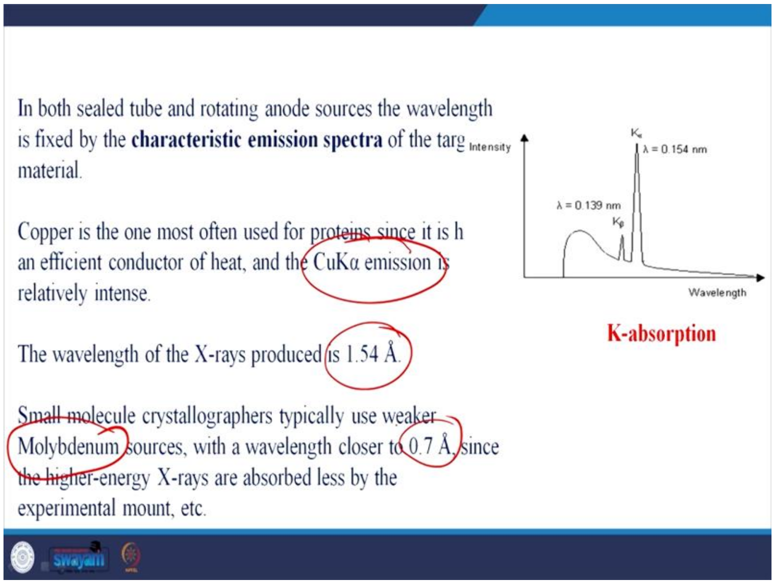

In both sealed tube and rotating anode sources, the wavelength is fixed by the characteristic emission spectra of the target material. Copper is often used for protein since it has an efficient conductor of heat and the emission (CuKα alpha emission) is relatively intense than other metals. The wavelength of X ray produced by copper is 1.54 angstrom. Again very much matches with the bond length. Small molecule crystallographers typically use weaker molybdenum sources they do not need that much intent source what we need to solve the protein crystals, with a wavelength closer to 0.7 angstroms. Since the higher energy X rays are absorbed less by the experimental mount.

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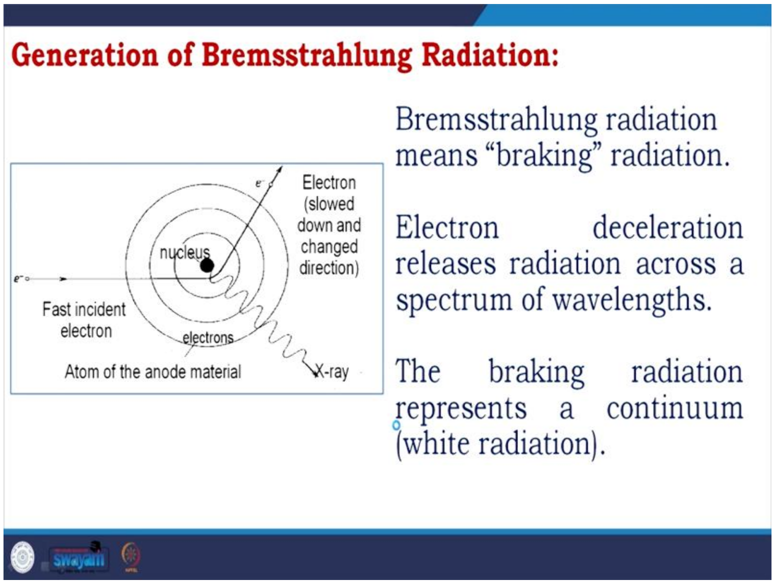

Bremsstrahlung radiation means breaking radiation. Electron deceleration releases radiation across a spectrum of wavelengths. The braking radiation represents a continuum (white radiation)

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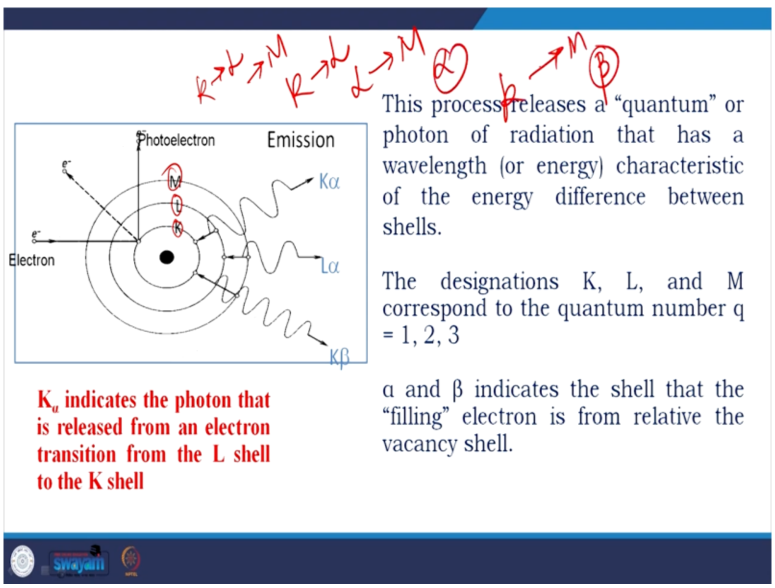

This process releases a quantum or photon of radiation that has a wavelength characteristics of the energy difference between the shells. So when it heat, electron release, and the other electron comes and match the hole. The designation K, L and M correspond to the quantum number 1, 2, 3. Alpha and Beta indicate that the filling electron is from relative to to the vacancy shells. Kα indicates the photon that is released from an electron transition from the L shell to the K shell.

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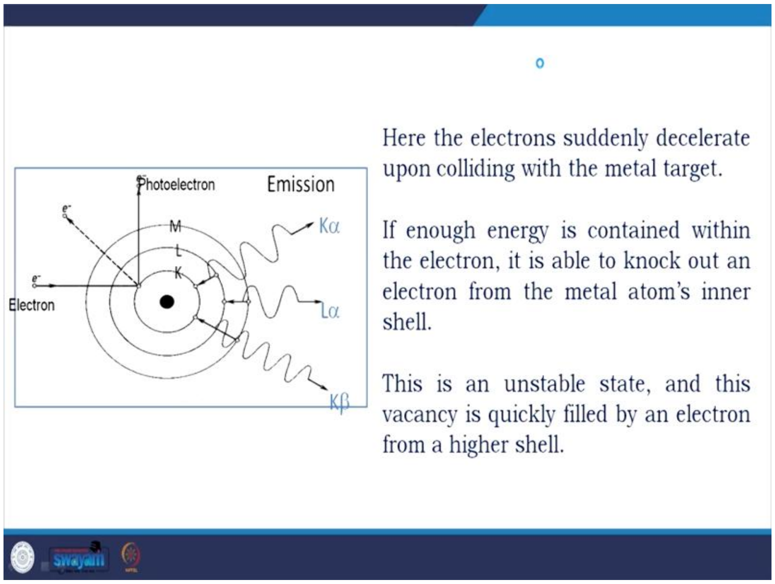

Here the electron suddenly decelerate upon colliding with the metal target. If enough energy is contained within the electron, it is able to knock out an electron from the metal atoms inner shell. This is an unstable state, and this vacancy is quickly filled by an electron from the higher shell

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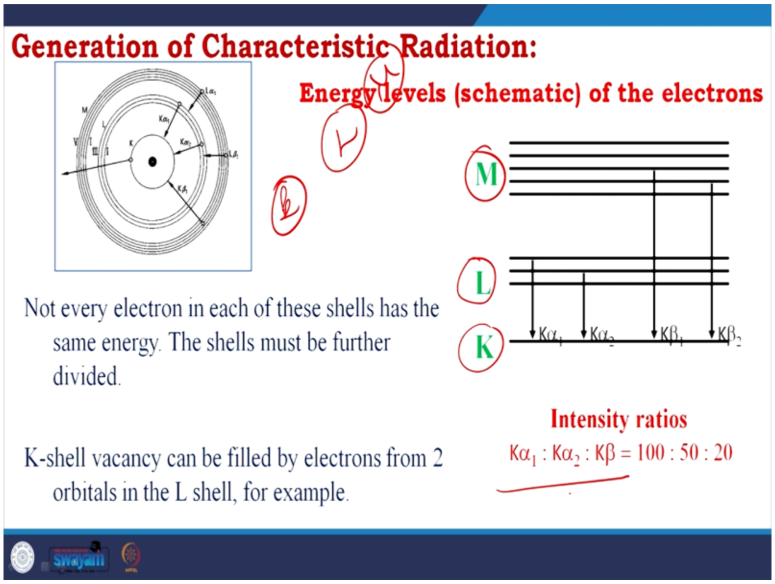

Generation of characteristic radiation: Not every electron in each of these shells has the same energy. The shells must be further divided. K shell vacancy can be filled by electrons from 2 orbital's in the L shell. When there is a vacancy in K, this would be filled by L and then M. So when one jump, it is called as alpha, when two jumps, it iscalled as Beta. The intensity ratios $K\alpha_1$, $K\alpha_2$ and $K\beta$ = 100 to 50 to 20.

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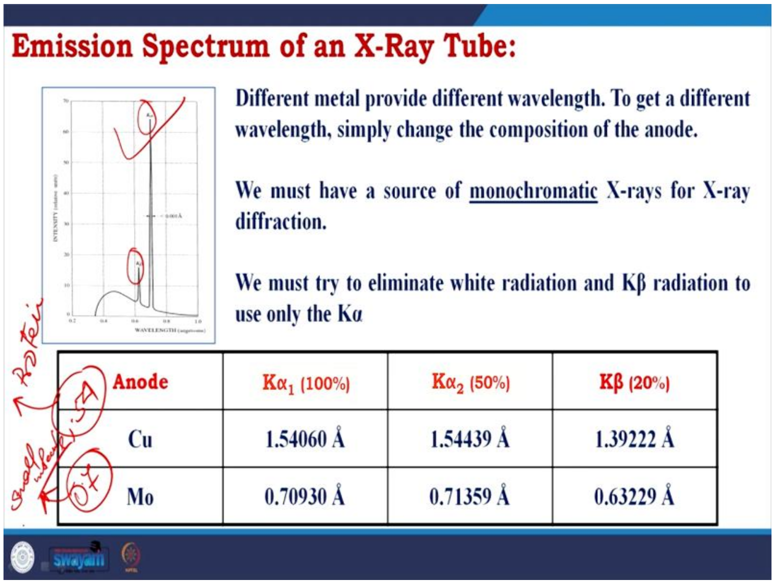

Emission spectrum of an X ray tube: Different metal provide different wavelength. To get a different wavelengths, change the composition of the anode. We must have a source of monochromatic X rays for X ray diffraction. We must try to eliminate white radiation and Kβ radiation to use only the Kα. When you use copper as anode, $K\alpha_1$ is 1.54, $K\alpha_2$ is almost 1.54, and Kβ is 1.39, whereas when you use molybdenum, $K\alpha_1$ is 0.70, $K\alpha_2$ is 0.71 around, and Kβ is 0.63. 1.54 is used for protein crystal and 0.7 is used for small molecules.

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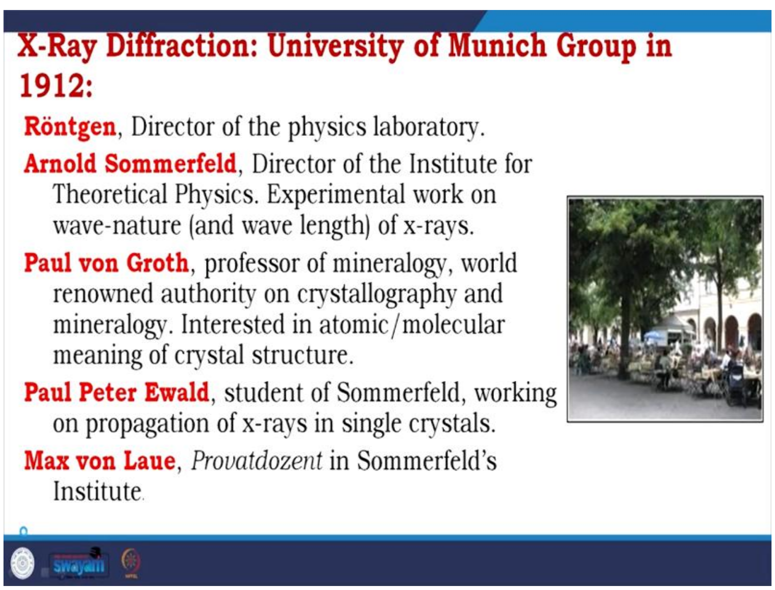

In this slide I will not talk about science, but I want to let you know, what a beautiful group was there in university of Munich in 1912? Rontgen, you already heard about, he was the director of the Physics Laboratory, he invented X ray and change the world's recognition or detection or device development. Arnold Sommerfeld, was the director of the Institute of theoretical physics. He did experimental work on the web nature of X rays, and his contribution is greatly helped us. Paul von Groth, professor of mineralogy, starting of X-ray related research started on minerals and then looking at the characteristic foundation, people, have also started exploring protein.

Paul Peter Ewald was a student of Sommerfeld working on propagation of X rays in single, and Max von Laue was a provatdozent which means a lecturer with not a permanent position, in Sommerfeld's institute.

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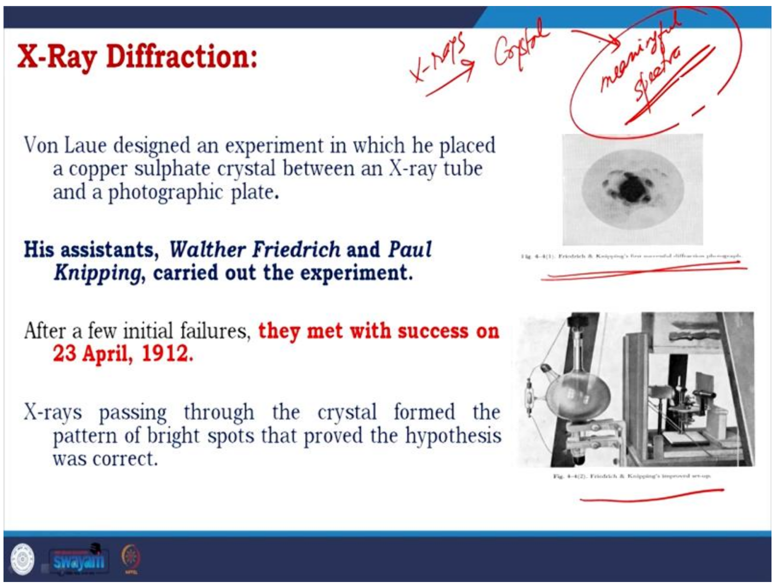

X-ray diffraction: Von Laue designed an experiment to place a copper sulfate crystal between an X-ray tube and a photographic plate. You could see that this is Fredrich and Knipping's improved setup. After a few initial failures, they met with success on 23rd April 1912. X-rays passing through the crystal form the pattern of bright spots that prove the hypothesis was correct. When you have a crystal, you pass X-rays you get meaningful spectra that is the kind of the birth of the X-ray crystallography diffraction.

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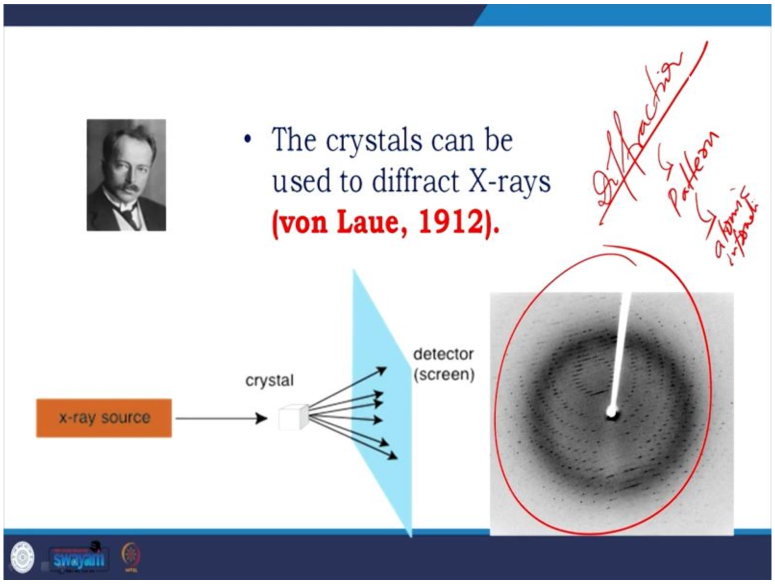

The crystals can be used to diffract X-rays. So you have an X-ray source, you have crystal, and you have a diffraction pattern. This is diffraction, from where you get the spots and calculate the pattern to get some atomic information.

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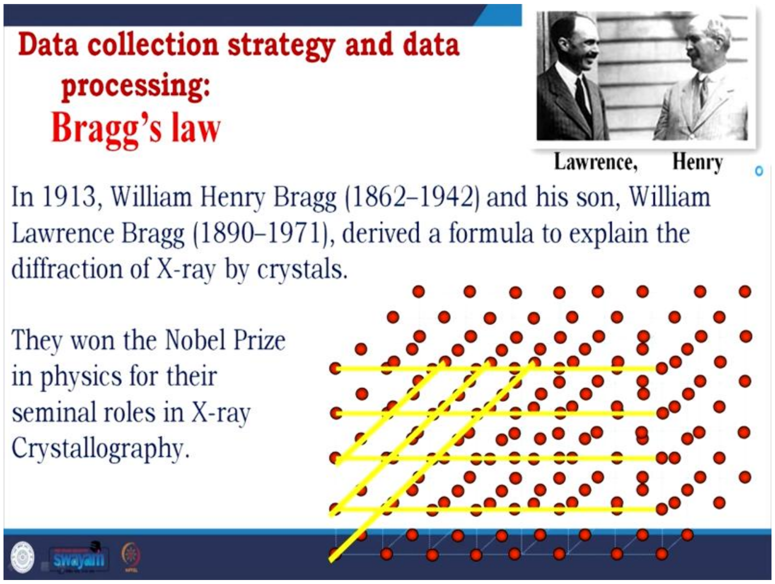

Data collection strategy and data processing: So now, we have come far away from where they have heated crystals and got diffraction. They need to collect more data and process the phenomenal development with this Father and Son Henry and Lawrence Bragg. It is called the famous Bragg's law. In 1913 William Henry Bragg and his son William Lawrence Bragg derived a formula to explain the diffraction of X rays by crystals.

They won Nobel Prizes in Physics for their seminal role in X-ray crystallography.

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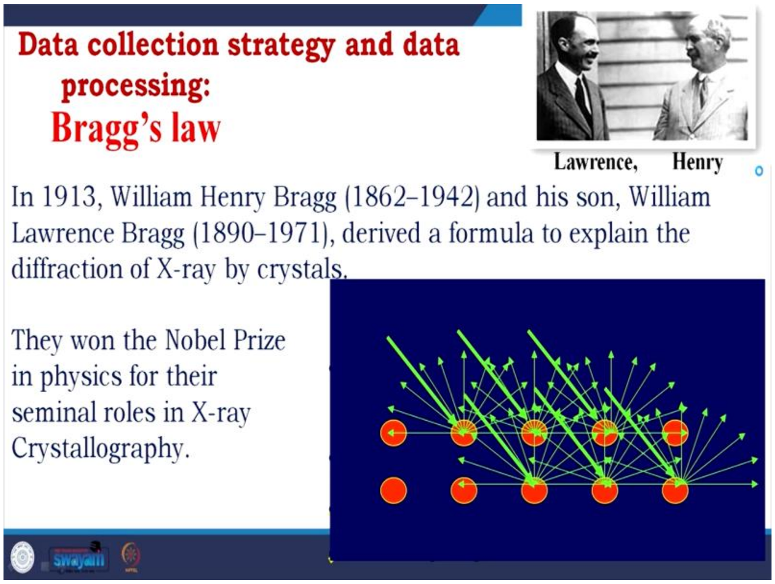

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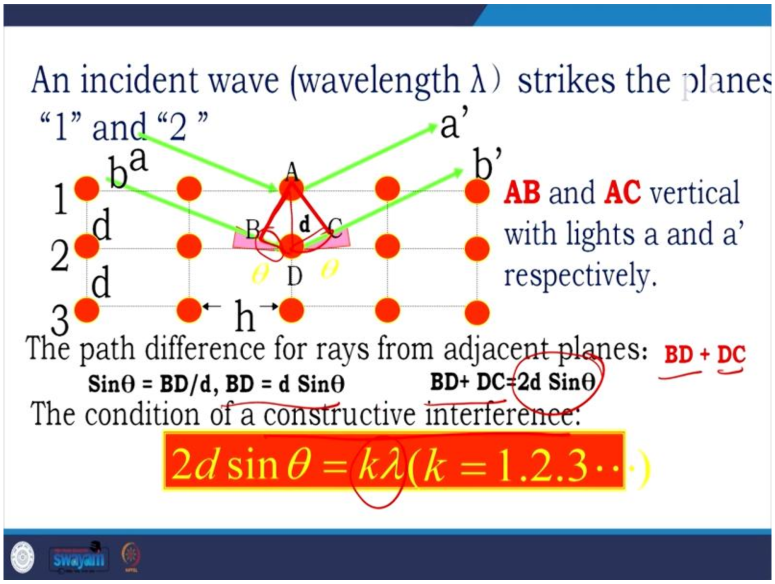

The light comes, and the ray will be reflected in different directions. Instead of a crystal, you consider incident planes. So an incident wave with a certain wavelength strikes planes 1 and 2. And as in a crystal, every plane is equidistance, and the distance is D. If you see, they come, and they are reflected. So if you see the angle, the angle is θ, and the path distance is AB and AC.

So AB and AC vertical with lights A and A' respectively. The path difference for rays from adjacent planes is a perpendicular triangle formed, and you get an angle which is θ. So the distance is D, and this distance is BD. So the path difference for rays from the adjustment plane is BD plus DC (BD + DC).

Now if you put trigonometric formula

$\sin\theta = BD/AD$, $AD = d$, so

$\sin\theta = BD/d$, so

$BD = d\sin\theta$

so $BD + DC = 2d\sin\theta$

when the waves are coming, they could be in destructive and constructive interference, and you need constructive interference for the work to continue for the detection.

So $2d \sin\theta = k\lambda$

where k is 1, 2, 3 like integers, which is Braggs's law. This relation helps us tremendously in the field of crystallography.

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This is the home source from Rigaku for protein crystallography, you could see that here is the detector, and here you could put the crystal, this is the camera where you see the crystal, and you could align.

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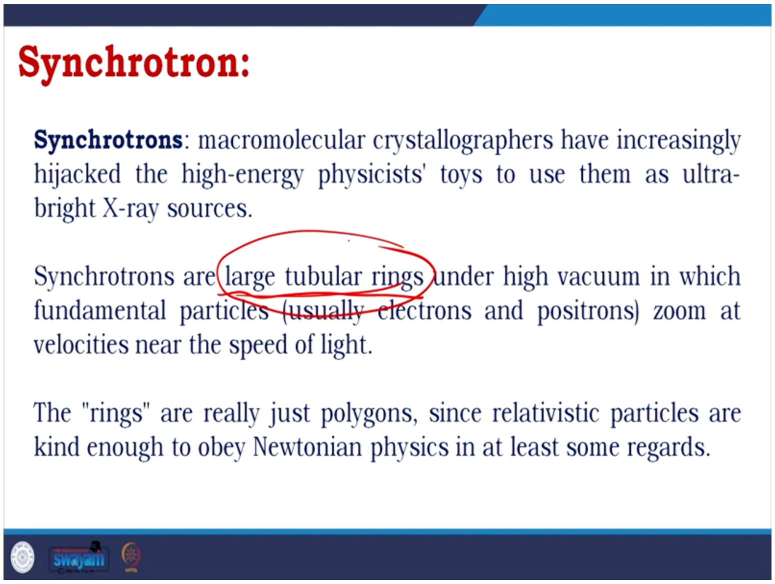

I talked about home sources as are relatively recent development before the data used to be collected in a synchrotron. Still, now synchrotron is even more and more coming useful to crystallographers. So it is called that macromolecular crystallographers have increasingly hijacked the high energy physicist's toys to use them as ultra-bright X-ray sources. Synchrotrons are large tubular rings under a high vacuum in which fundamental particles, usually electrons and positrons, zoom at velocities near the speed of light.

I repeat, synchrotrons are large tubular rings under a high vacuum in which fundamental particles, usually electrons and positrons, zoom at velocities near the speed of light. I say large tubular ring. Can you imagine how big this large tubular ring is? Generally, where synchrotrons at developed, it is a tiny town, and the beam covers the entire town. Now you could understand if you are in a small town and your town contains a synchrotron.

So you will get a circle. The whole circle surrounds your tiny town, and that is big the magnet is. The rings are just polygons since the relativistic particles are kind enough to obey Newtonian physics in at least some regards.

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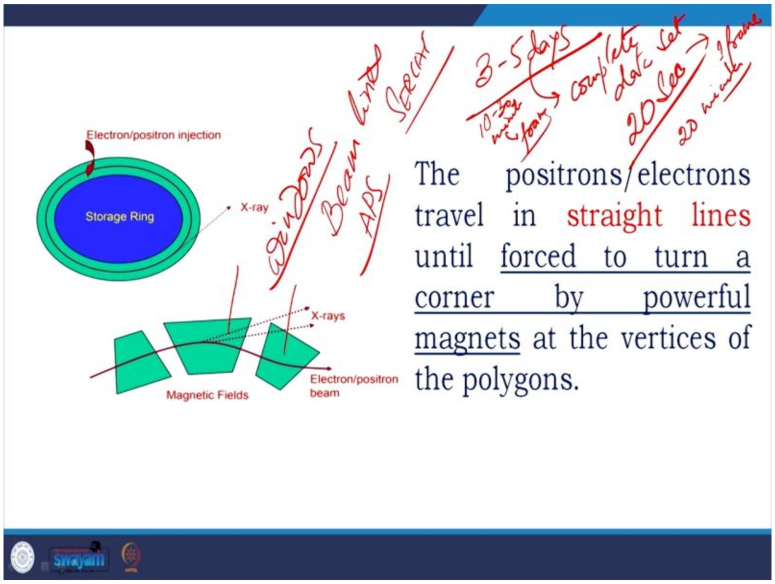

Why? So electron-positron will be in a perfect ring rather than this broken ring from where these are the magnetic field and X rays coming out through windows. The positron electrons travel in straight lines until powerful magnets are forced to turn a corner. And those corners are called windows. So in the windows, there are beamlines. I worked in Argonne National Lab APS, where we used to work on circuit beamlines.

So in every synchrotron, they have certain beamlines where crystallographers work, go there, and collect their data.

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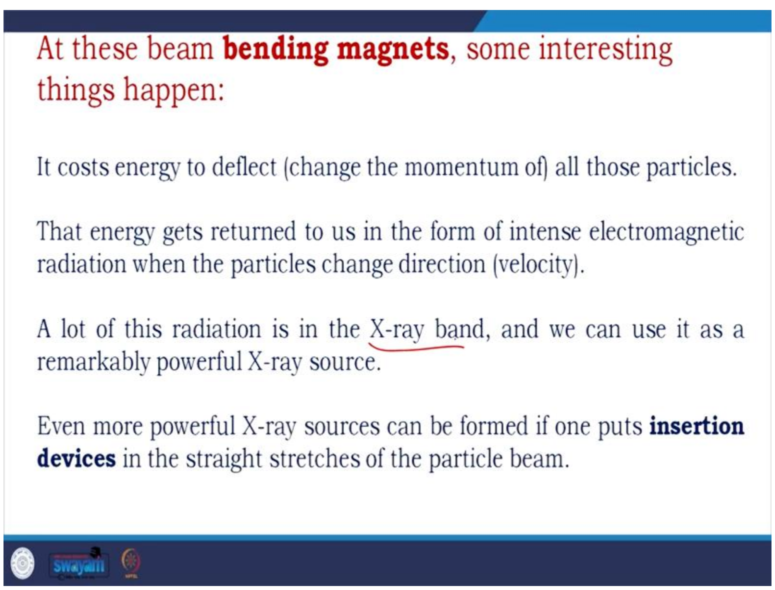

Fortunately, the difference is also getting reduced day by day. At these beam bending magnets, some interesting things happen. It costs energy to deflect change to the momentum of all those particles. That energy gets returned to us in the form of intense electromagnetic radiation when the particle changes direction. A lot of this radiation is in the X-ray band. And we can use it as a remarkably powerful X-ray source. Even more powerful X-ray source can be formed if one put insertion devices in the straight stretches of the particle beam.

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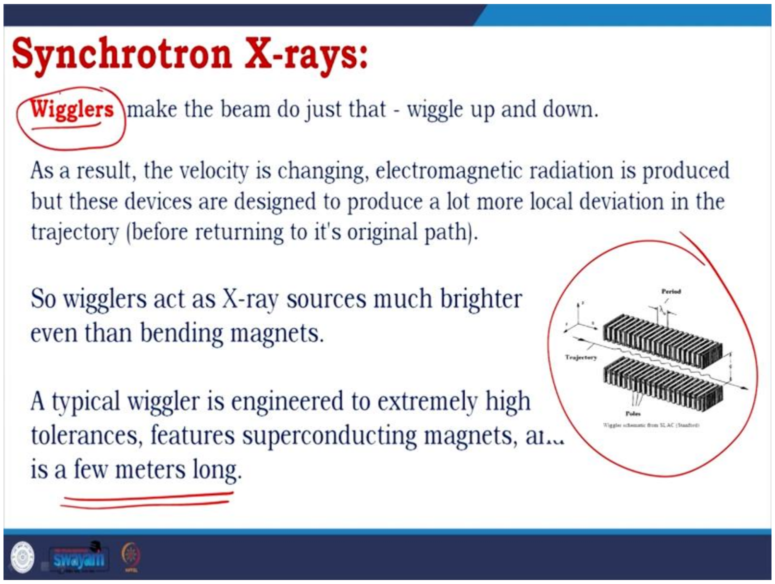

So generally, it is powerful, but what do people do? What does advancement do? They use something called wigglers. Wigglers make the beam do just what wiggle up and down. As a result, the velocity is changing electromagnetic radiation is produced. But these devices are designed to produce a lot more local deviation in the trajectory before returning to the original path. So Wiggler acts as X-ray resources much brighter even than the bending magnets.

If typical Wiggler is engineered to extremely high tolerances features superconducting magnets and is a few meters long, so you see the change while the bending magnet is covering a whole tiny town these are few meters long. So that is the beauty of the wigglers.

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Here I am giving you some examples of primary synchrotron sources in the US, the national synchrotron light source NSLS at Brookhaven National Lab. BNL at New York, the advanced photon source APS at Argonne National Lab in Chicago Illinois, the Cornell high energy synchrotron source changed at Cornell College Advance Light Source ALS in Berkeley California, Stanford synchrotron radiation lab SSRL in Stanford California.

Other notable ones include diamond from United Kingdom, ESRF from France, photon factory from Japan and ANSTO from Australia.

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I talked about a synchrotron beam is like covering a tiny town. This is Argonne National Laboratory; I did my Ph.D. at the University of Illinois at Chicago. So we used to go there, and as you see, this beam covers the whole kind of small town.

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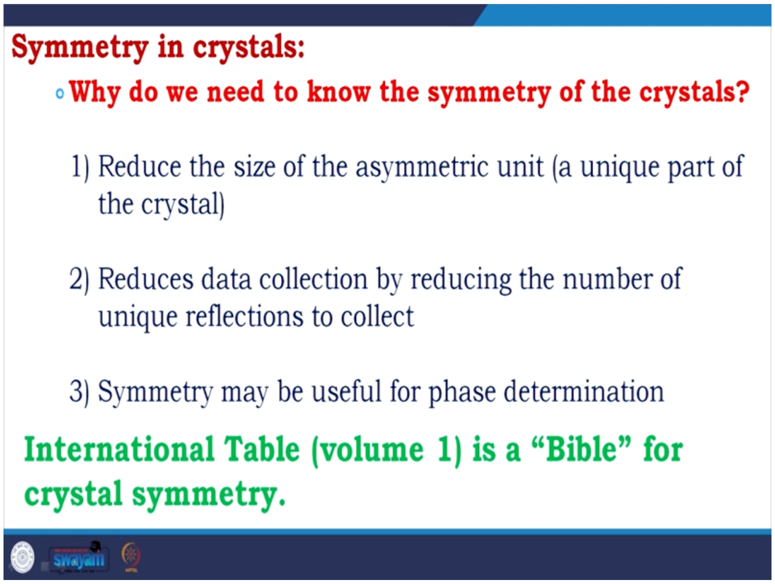

Coming to symmetries and crystals, why do we need to know the symmetry of the crystals? Because we reduce the size of the asymmetric unit, which is a unique part of the crystal, we will discuss what the asymmetric unit is, reducing data collection by reducing the number of unique reflections to collect. Symmetry may be useful in phase determination. International table volume 1 is considered the Bible for crystal symmetry.

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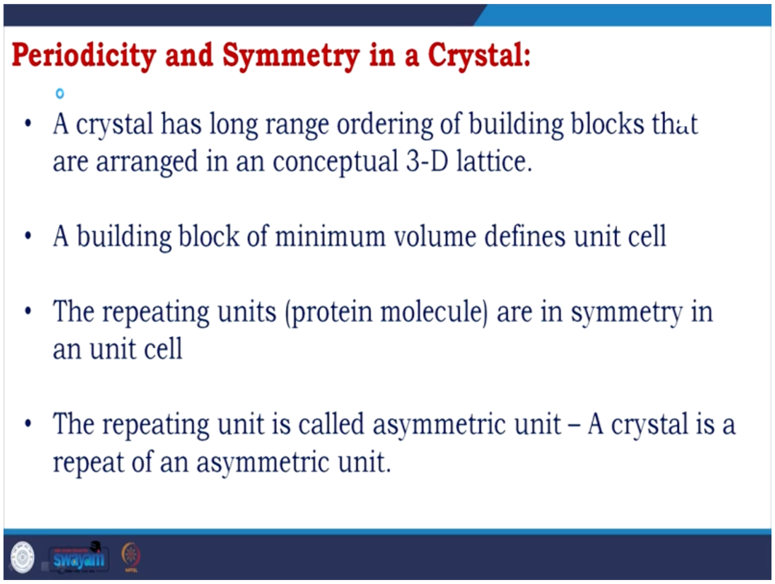

Periodicity and symmetry in a crystal: A crystal has a long-range ordering of building blocks arranged in a conceptual 3D lattice. A building block of minimum volume defines a unit cell. The repeating units of a protein molecule are in symmetry in a unit cell. The repeating unit is called an asymmetric unit, and a crystal is a repeat of an asymmetric unit.

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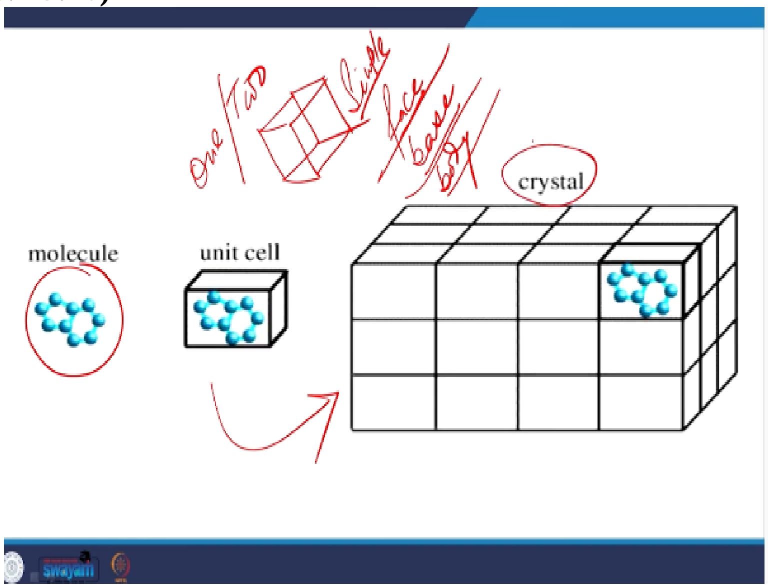

So if you see you have a small molecule or a protein molecule, this protein molecule might be presented as 1 in the unit cell two or many they could be developed many symmetries like if you see it could be a cube, in the cube the molecule might present on the corners they might present on the face, they might present on the base, they might present on the show its face base and body. So you got simple. So simple cubic development, face centred cubic development, base centred cubic development and body centred cubic development.

And they will be repeated to develop a 3-dimensional array of our crystal. So we have discussed the X-ray source, how X-ray is generated, what the home source and high powerful synchrotron source are. Next, we will discuss in detail how this unit cell and symmetry is there and how it is different from normal molecule and protein molecule?