Structural Biology
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Lecture – 15
X – ray Crystallography: Crystal Mounting

Hi welcome again to the course of structural biology. And as I told earlier this is the module where we have introduced the structural biology techniques I have told about the introduction of the structural biology techniques.

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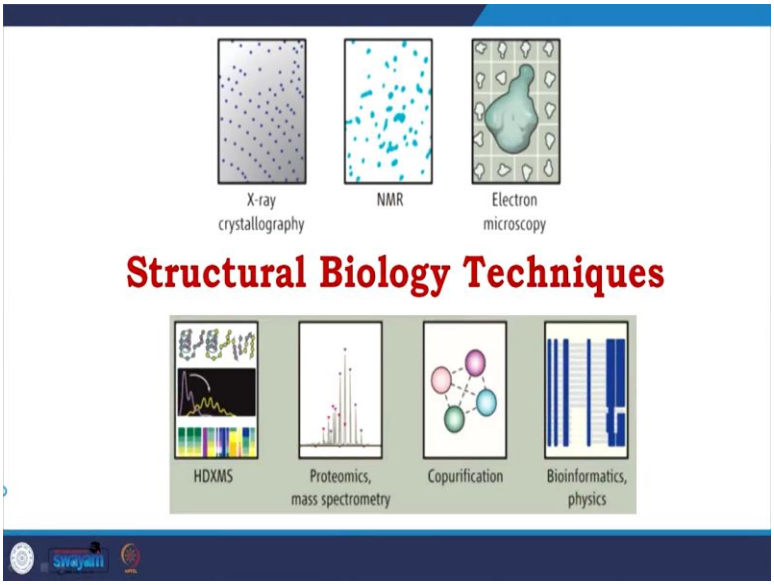

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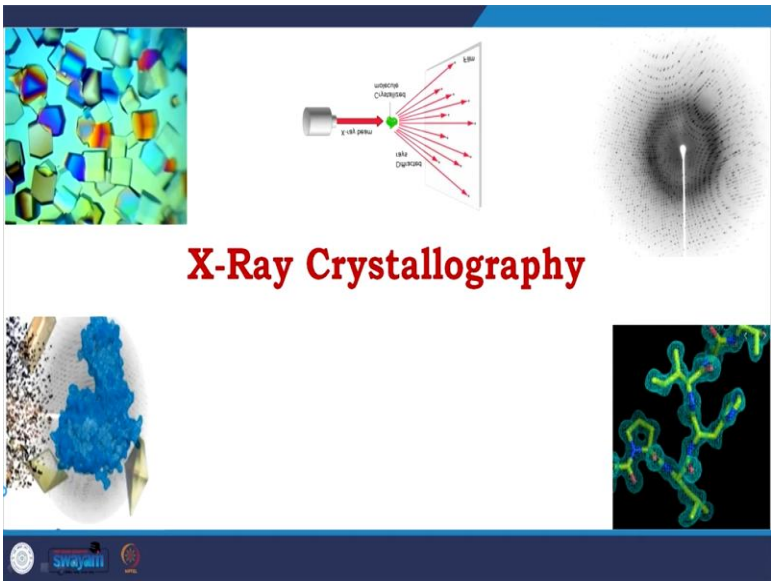

And now we are discussing X-Ray crystallography. Today we will discuss crystal mounting, what is crystal mounting? You did everything, and you get a crystal. Now you have to pick up the crystal and you have to put it on the machine so that you should get the diffraction, that is called crystal mounting.

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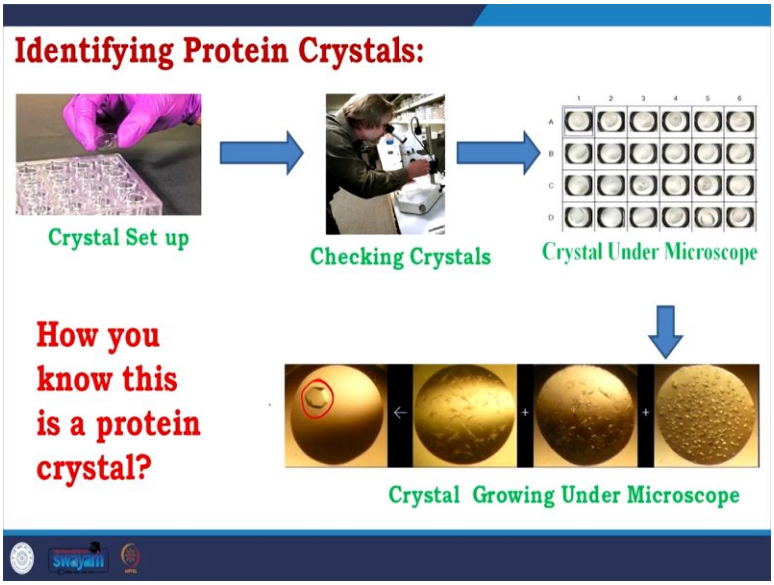

Before crystal mounting, when you get a crystal, it is always not happy news. When you are looking at crystal, it might be protein crystal, but it might also be salt crystal coming from the precipitant solution you have used to set up your crystals. So, how to check the protein crystals?

So first you do the setup, you check crystals then you go under microscope. And this is the way you see from smaller to bigger crystals are formed and you get a single crystal that is what our dream is. How do you know that this is a protein crystal? You need to check and differentiate it as a protein crystal or salt crystal.

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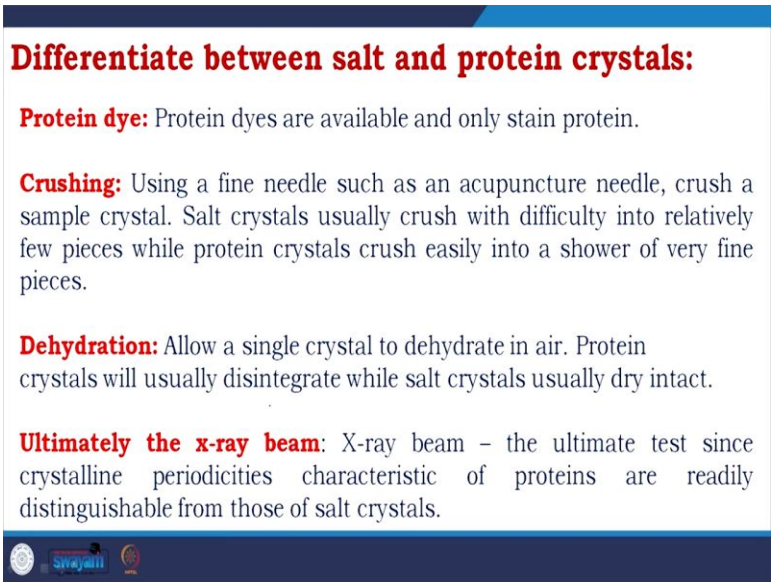

There are protein dyes, they are available, and they always stain proteins. So if you put that stain, and if it stains the crystal, then it is a protein crystal. If it does not stain the crystal, it is a salt crystal. Crushing: Using a fine needle such as an acupuncture needle, crush a sample crystal, salt crystal usually crushed with difficulty into relatively few pieces while protein crystal crush easily into a shower of very fine pieces. Remember I talked about protein crystals are having a lot of solvent channel so it is soft, it is not like the crystal you imagine. So this is a very soft and when you go through it, you get the difference; if it is a salt crystal, it is very compact and hard, and if it is protein crystal it is soft. Dehydration: Allow a single crystal pick up from the drop and dehydrate in air, protein crystal will disintegrate, while salt crystal usually dry intact.

Ultimately the X ray beam:. X ray beam, the ultimate test when you throw X ray beam and you get the beautiful diffraction pattern that is a Eureka time for a protein crystallographer.

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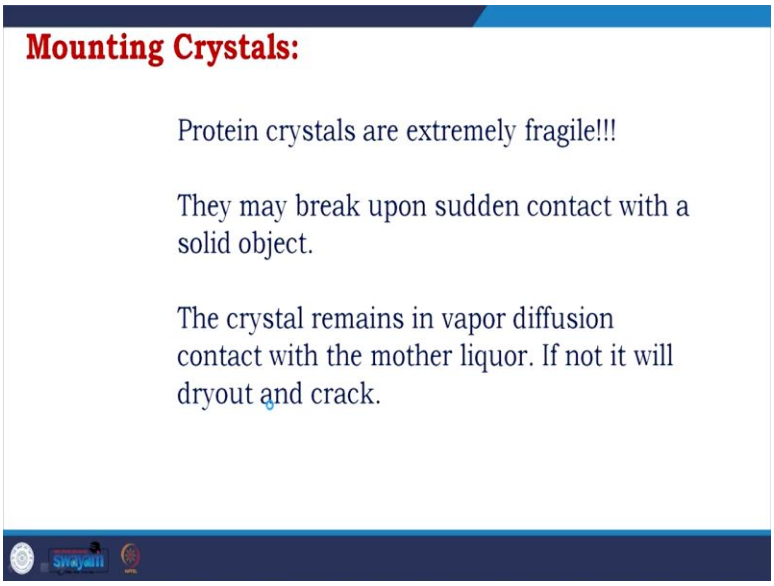

So coming about mounting crystal: I talked protein crystals are extremely fragile. So, protein crystals are soft and fragile, they may break upon certain contact with a solid object. The Crystal remains in vapour diffusion contact with the mother liquor, if not it will dry out and crack. So even if you have a crystal, now to transfer the crystal from crystal drop to the diffraction point, it is a journey which is helped by crystal mounting.

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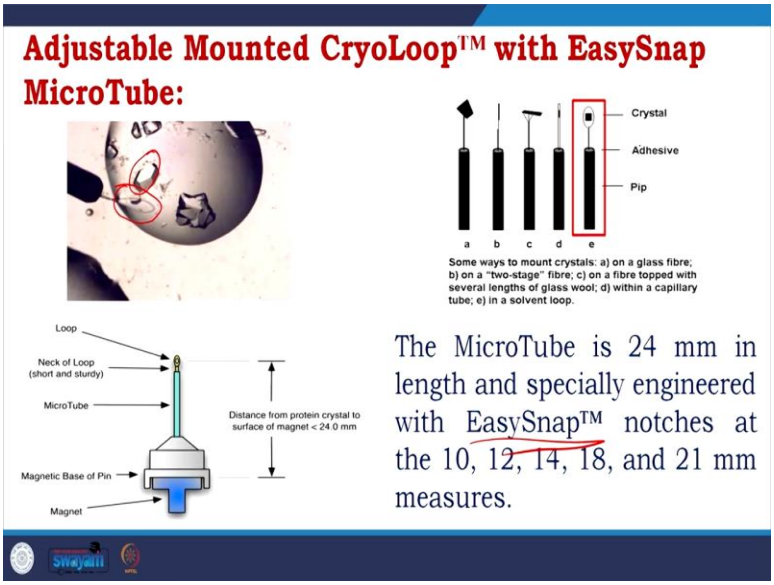
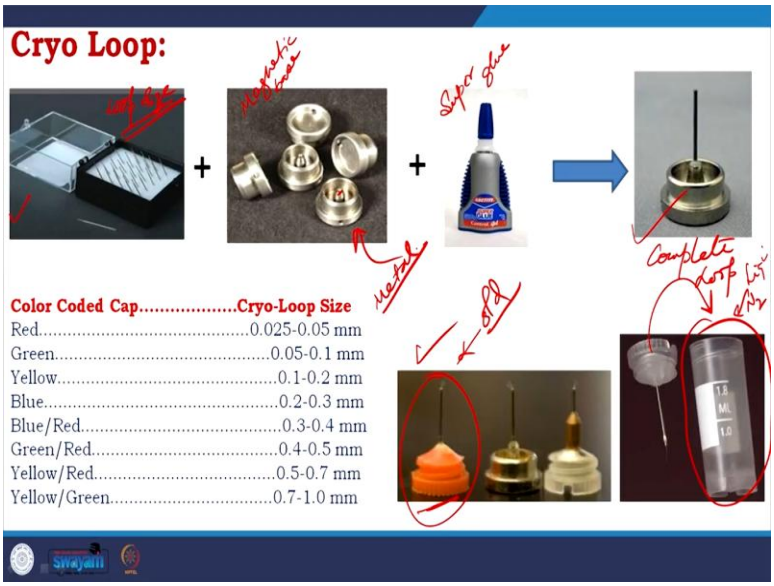


Adjustable mounted CryoLoop with EasySnap MicroTube: So this is what an ideal CryoLoop is? What is the function of the CryoLoop? Today you have a crystal, so you have to spoon it out these guys are your spoons now. So, now if you see, this is a loop and this is going to pick out a

crystal. The MicroTube is 24 millimetres in length and specifically engineered with EasySnap notches at the 10, 12, 14, 18 and 21 millimetre measures **(Refer Slide Time: 06:39)**



So this is the way we develop CryoLoops, you have these loops depending on what or how big your crystal is, you get the loop size. So there are boxes like this, this is the box where you have different loop size. So you get different boxes on different loop size then this is the magnetic base. What we do? We pick out the loop and put here in this place you see there is a hole and then we put these we calls super glue. Super glue is a glue which could help iron or metal to stick. So you pick up the loop you put it under the hole you put superglue and what you get is this, this is a complete loop. You will have liquid nitrogen, and you put your crystal containing loop, and here you see there are loop colouring system on the loop base. So red is 0.025 to 0.05 millimetre, green is 0.05 to 0.1 millimetre, yellow is 0.1 to 0.2 millimetre, blue is 0.2 to 0.3 like that, but that was old. Nowadays we use this; you do not have the colour codes, now you have these boxes. So you get these loops you put it there and you ride behind the size of the loop.

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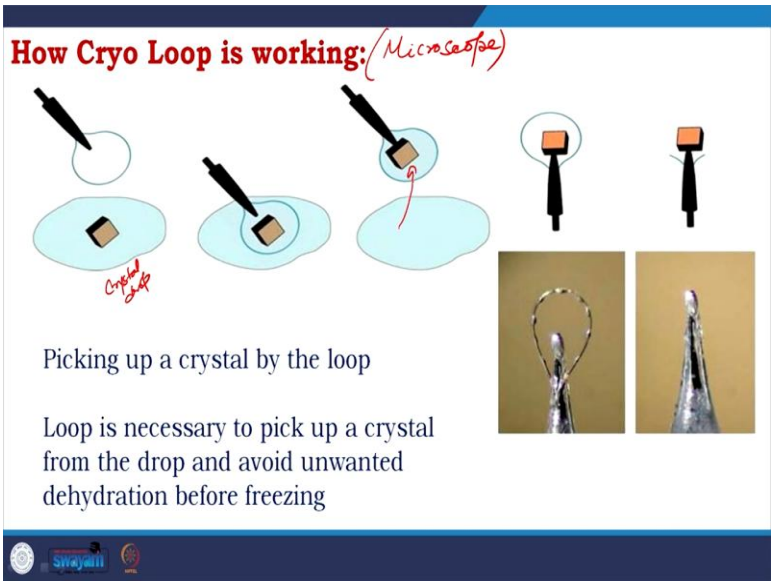

How CryoLoops work? So this is the crystal drop you bring the loop and remember this is under the microscope. So the crystals you cannot look in open eye. So you look under microscope you look at drop, you see the crystal. So you sit under microscope, you look at the drop you look at a crystal; you try to pick it out. So picking up a crystal by the loop, which is the process, loop is necessary to pick up a crystal from the drop and avoid unwanted dehydration before freezing. When you are picking up you see the mother liquor also comes here and that sets the crystal.

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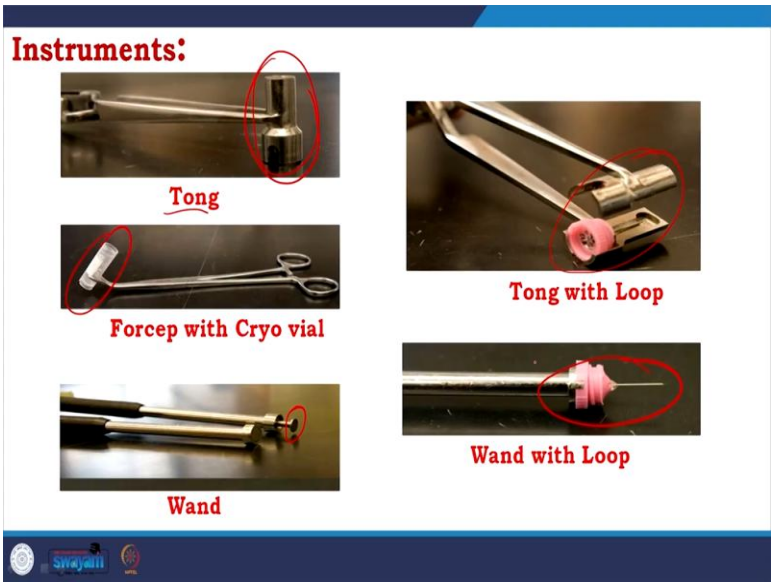

This is generally not in the course but I try to introduce those things so that you get some practical knowledge of how actually protein crystallography work. This is tong I am showing you how tong is working? You hold the loop with the tong. So tong have a loop fuller. Then you

have a forcep which could hold the cryo vial, then you have wand, wand have this iron, and remember the base of the loop have the magnet, so, wand actually hold the base.

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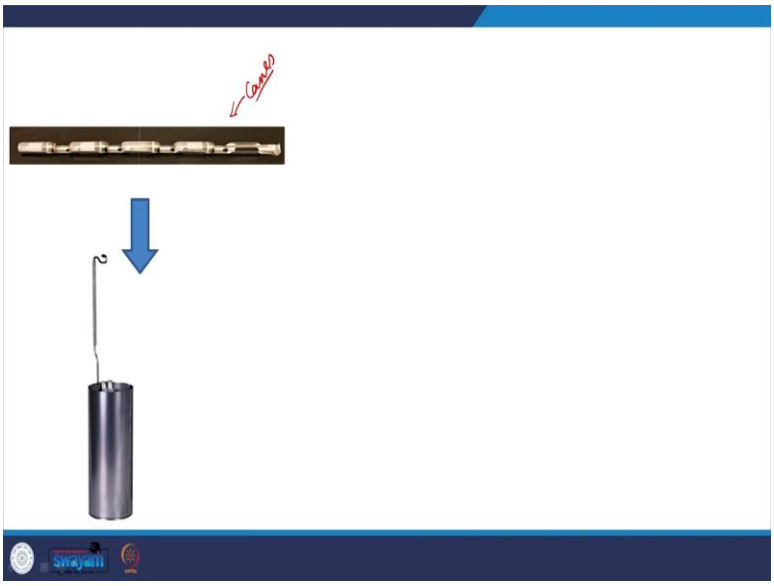
Now with these instruments I will show you what is mounting and cryopreserving the protein crystal. So this is what liquid nitrogen holder these are very lightweight you could take the whole liquid nitrogen. You hold the cryo vial then you go under microscope you scoop the crystal and when you get the crystal you put the crystal under freezing when you were freezing you might think that your crystal is getting damage with liquid nitrogen, and yes crystal could be damage by liquid nitrogen. So we use cryopreservation, I am coming into that. Now you have the crystal inside the loop and you hold the wand, and in other hand in that forcep you have the Cryo vial, now you put the loop containing the crystal inside the cryo vial and now it is safe there, cryo vial contents liquid nitrogen. So for some time it could be saved there, you could carry it in that way, if you are working inside the crystallography room that is how we do in the home source we hold this we go to the crystallography machine and set them.

But some time or many times actually we have to go to synchrotron. So I will talk about this later. There are two type of X ray sources which we use to solve crystal, one is the home source where in a institute you have your own X ray diffraction machine, if you have smaller crystals they cannot diffract, and the solution is synchrotrons, synchrotrons are national facility, I will show about what you have advantage over home source and where are the synchrotrons are established. **(Refer Slide Time: 14:13)**



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You see this is called cane. So these canes contain your cryo vials with the crystals. And then you have this thing which is helping to put the crystals. So you put that thing inside liquid nitrogen and now you put the crystal there, you put the cover, then you put it in a cryo beer instead of vial this is a big container. And now when you put it there, it is safe for a few days' even months you could carry it to anywhere.

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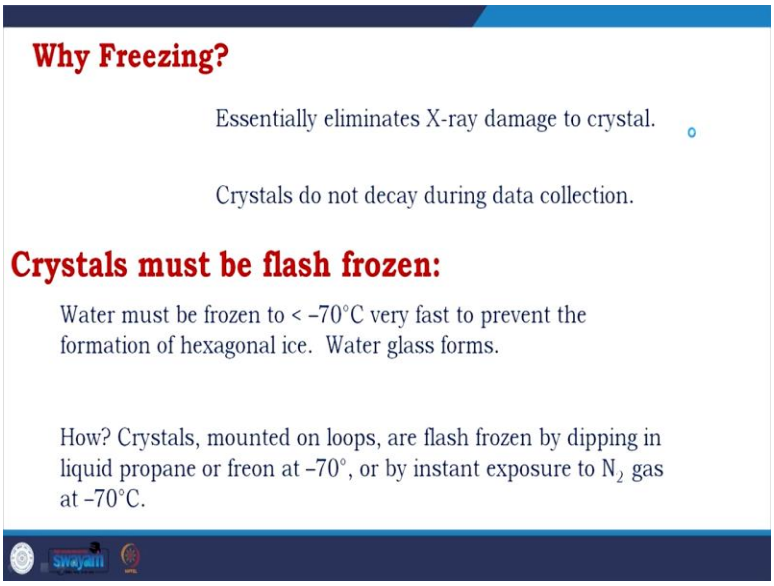

So now the question is why freezing why you need freezing? Essentially freezing eliminates X ray damage to crystal, Crystal does not decay during data collection. Crystals must be frozen because water must be frozen to minus 70 degree very fast to prevent the formation of hexagonal ice, water glass forms. How? Crystal mounted or loops are flash frozen by dipping in liquid propane or freon at minus 70 degree or by instant exposure to nitrogen gas of minus 70 degree.

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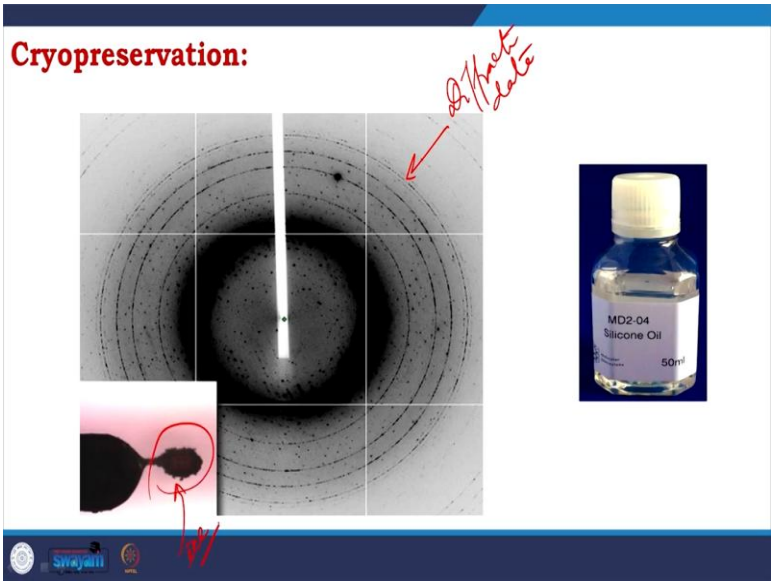

So if you do not do cryopreservation properly what will happen? You will see this dirty diffraction data and you see here, you will see that ice is there, so how you cryo preserved?

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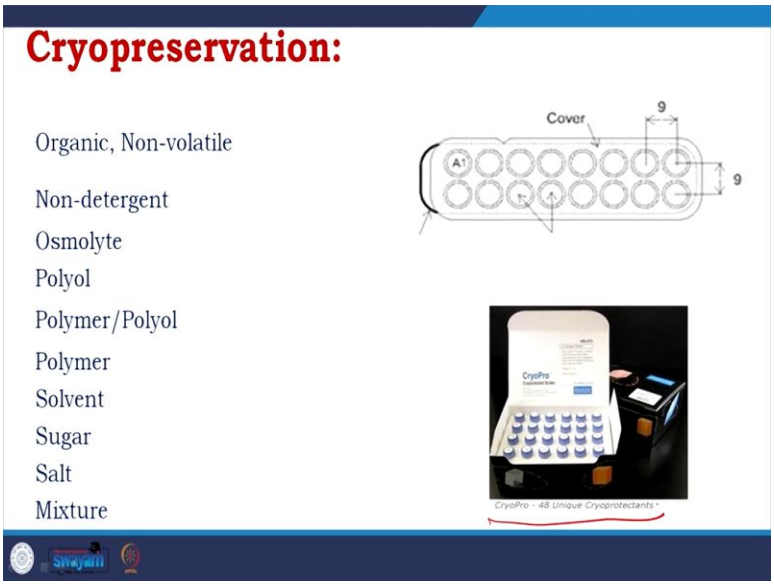

There are many solutions which could be used as cryopreservation. Organic, non volatile, non detergent, osmolyte, polyols, polymers, solvents, sugar, salt, mixture, a lot of things could be used as cryo preserver. There is also a Hampton screen which is the only screen available in the market, which helps you to cryo preserve as a screened but most of the time you will prepare your own cryo preserver.

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So when you pick the crystal, now you come to the machine, this is a demonstration of the home source, and this is your loop. So where is the liquid nitrogen? So this is where the nitrogen gas is coming out and keeps the crystal cool. That is the process; once you pick it there, you are not in

the risk of losing your crystal. And this is the goniometer, why goniometer is important and this is the screen.

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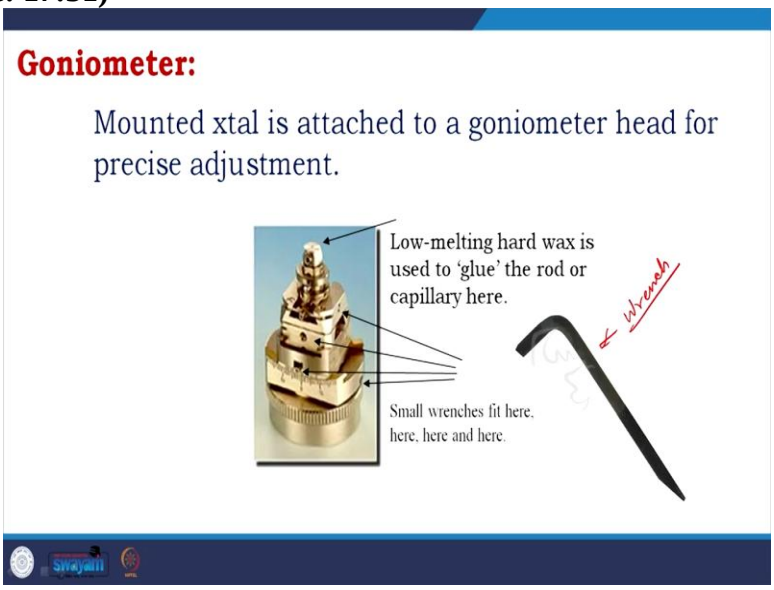

Mounted crystal is attached to a goniometer head for precise adjustment, low melting hard wax is used to glue and then you have to fix the rotations.

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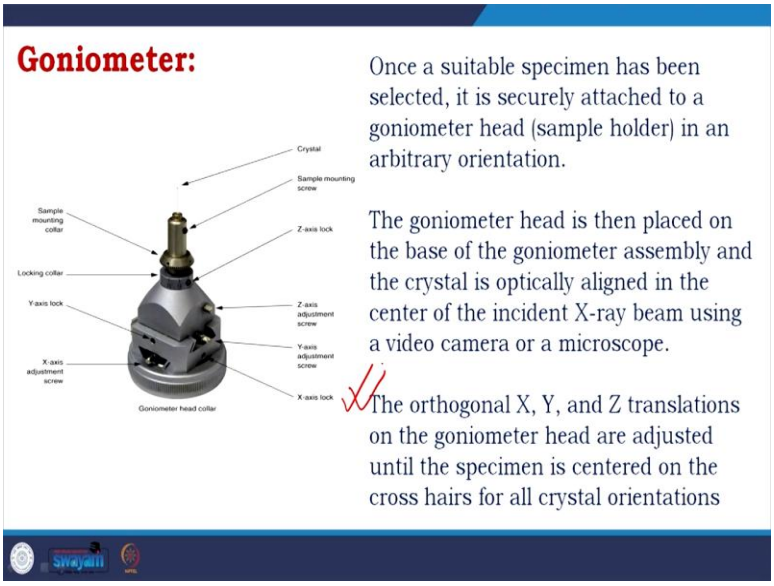

Why fixing rotation is important? So this is a goniometer head, here the crystal is, here the sample mounting screw, here the Z axis lock, here the Z axis adjustment screw and this is a range, the range is used to adjust those things. So, Y axis adjustments screw Y, X axis lock X

axis, adjustment screw Y axis lock, locking colour and sample mounting colour. So when you have done the thing you will lock them.

Once a suitable specimen has been selected it is securely attached to a goniometer head sample holder in an arbitrary orientation. That time you do not need to fix the orientation but the goniometer head is then placed on the base of the goniometer assembly and the crystal is optically aligned in the centre of the incident X ray beam using a video camera or a microscope. So in the home source machine, there is a video camera which will help you looking at the crystal because remember the crystal would only be look under microscope.

So here it is projecting your crystal. So you look at the crystal in the screen and you have to align the crystal, why you need to align the crystal? Because you have the goniometer, and the data you are collecting, you will collect at different angle. So because there is automation once you fix it, you do not need to fix the crystal after each angle of data collection. That was an older procedure where people have to do extreme hard work. They have to set up each and every angle but we are fortunate now, we do not have to do that. So once you set, you collect 90 degree, 180 degree, 360 degree and all but to make ensure the fact that with all the rotation your crystal would be aligned to the beam because for getting diffraction you have your crystal aligned with that X ray beam that is very critical. The orthogonal X, Y, Z translation on the goniometer head are adjusted until the specimen is cantered on the cross hairs of all crystal orientation that is the most critical part.

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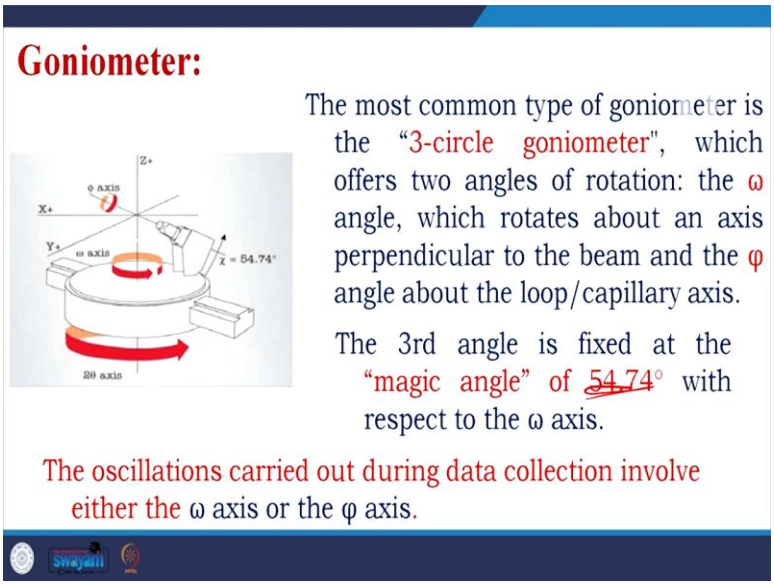

So you see that, X, Y and Z axis, the most common type of goniometer is a three circle goniometer which offers 2 angle of rotation; the omega angle which rotates about an axis perpendicular to the beam and the phi angle about the loop or capillary axis. The 3rd angle is fixed at magic angle of 54.74 degree with respect to the omega axis. The oscillation carried out during data collection involves either the omega axis or the phi axis.

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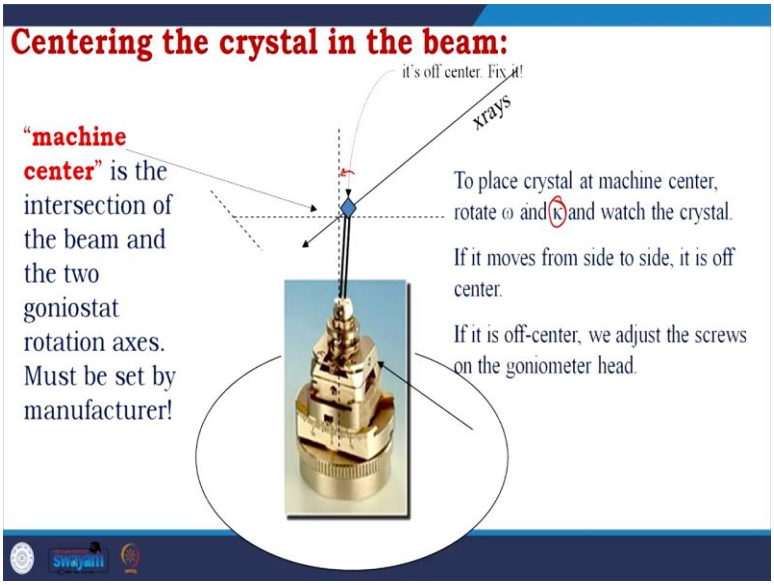
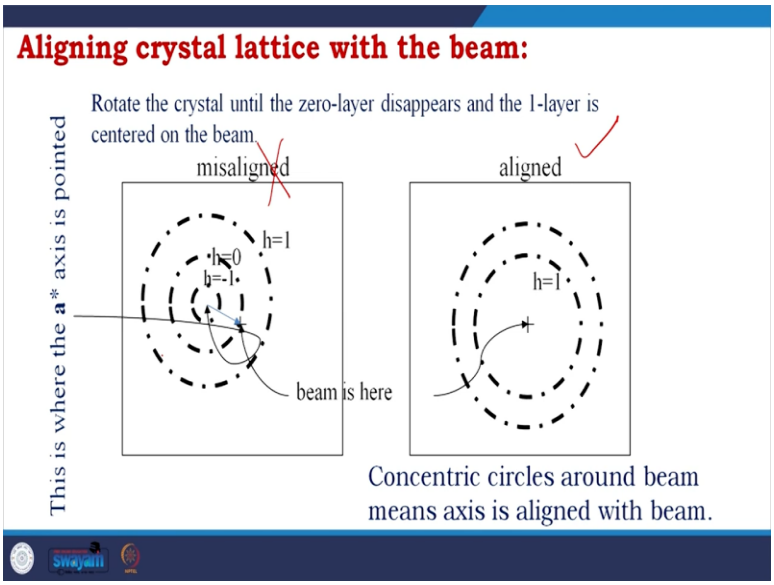


Machine centre is the intersection of the beam and the two goniostat rotation axis must be set by manufacturer. To place crystal at machine centre, rotate omega and kappa, and watch the crystal. If it moves from side to side it is off-centre., if it is off-centre, we adjust the screws on the goniometer head. **(Refer Slide Time: 21:51)**



Aligning crystal lattice with the beam: Rotate the crystal until the zero layers disappear and the first layer is centred on the beam. Concentric circles around beam means axis are aligned with beam. So now, you have the crystal and you have aligned and you do data collection.

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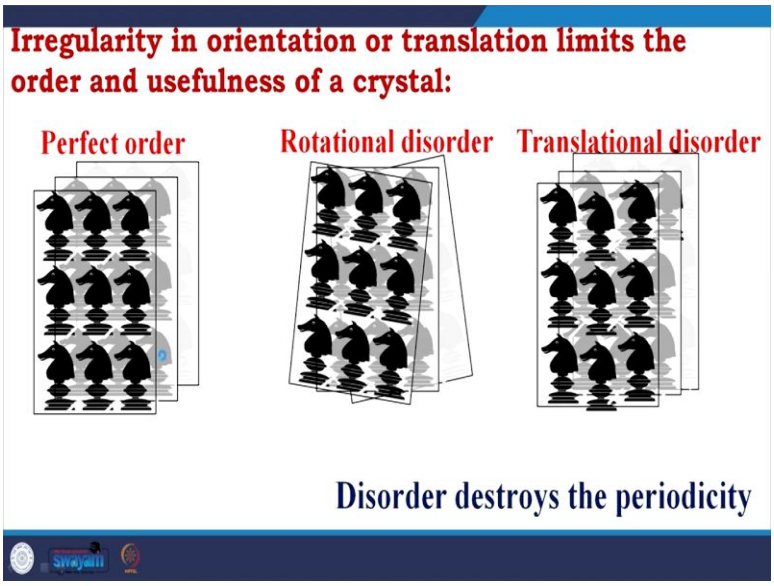

Irregularity in orientation or translation limits the order and usefulness of the crystal: you have nothing to do here; this depends on how the crystals grow? So if you look at its perfect order, rotational disorder, and translational disorder. So disorder destroy the periodicity

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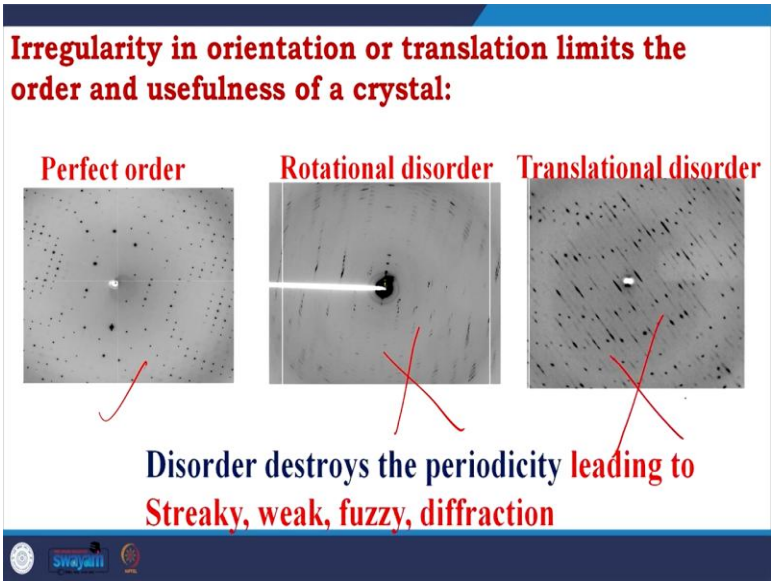

Irregularity in orientation or translation limits the order and usefulness of a crystal: When it is perfect order, you get good diffraction pattern, when it rotational disorder you do not get, when it is translational disorder, you do not get. So, disorder destroys the periodicity leading to streaky, weak, fuzzy diffraction.

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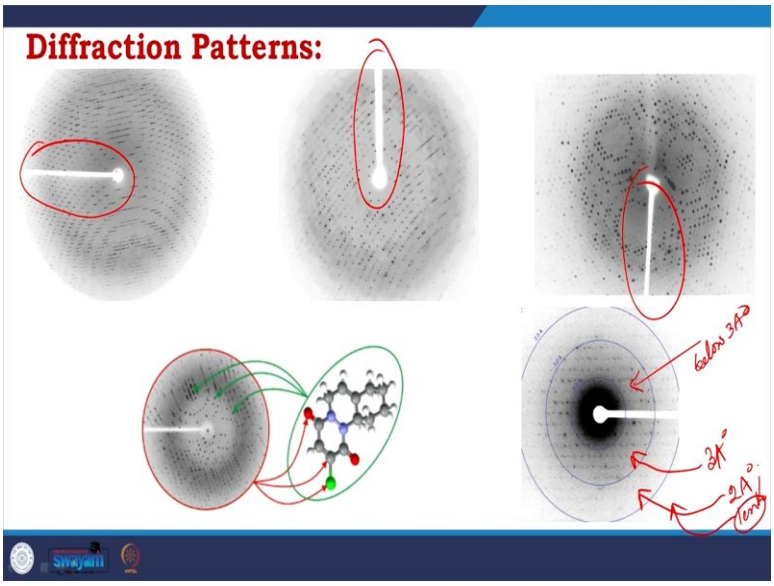

If your crystal is in perfect order, this type of diffraction you are going to get. Here you see that, there is a portion which is not getting the diffractions. Because when you go back to the machine, you do not want the diffraction to come that is why you make it stop. Also you do not want the core of the beam to come and hit the crystal, it would destroy the crystal or would say burn the crystal. That is why there is a beam stopper and because of the presence of beam stopper you will see that these things are there. Now when you have the diffraction pattern you will start calculating the data analysis. And if you see, you will get circles which will tell you what resolutions you get data.

Generally if you look lower the resolution, you will get more dense spot like you get denser spot below 3 angstrom then at 3 angstrom you get good amount and in the range of 2 angstrom you get less after 2 angstrom which is less than 2 angstrom means actually higher resolution you get even smaller spots.

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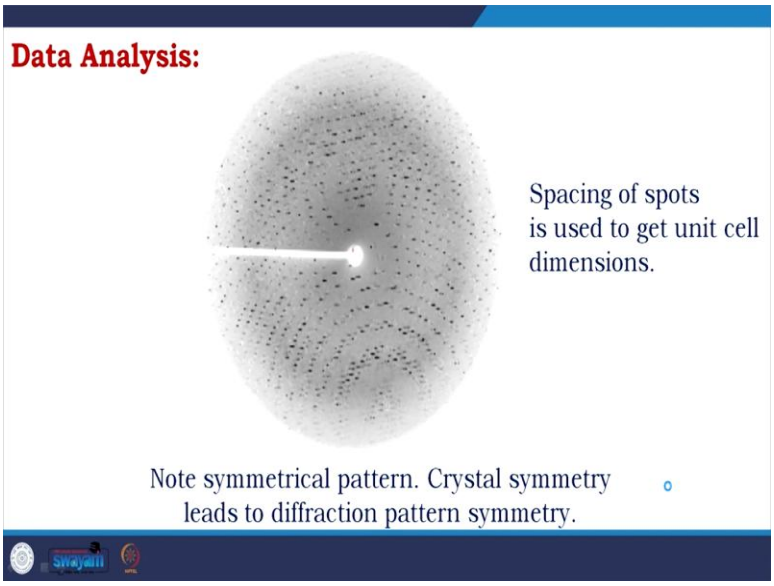

We will go for data analysis, we will look at the symmetrical pattern, and crystal symmetry leads to diffraction patterns symmetry. We will read about symmetry and spacing of spots is used to get unit cell dimensions. So we will also know about unit cells.

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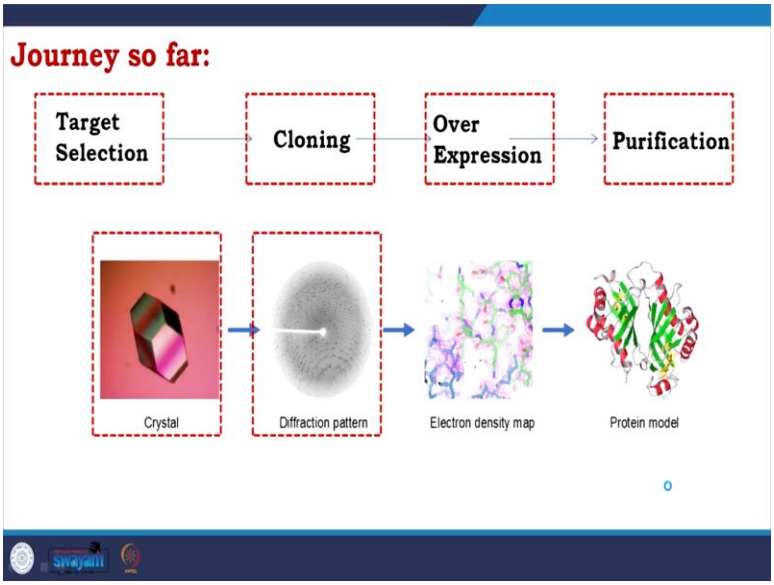

So what is our journey so far? We have done the target selection; we have to do cloning then over expression then the purification of the protein then setting up crystal. If you get a crystal, you go for diffraction pattern, you go for electron density map, and you get the protein model, in between crystal to diffraction pattern, we have things to discuss then we will go for data analysis and model development.

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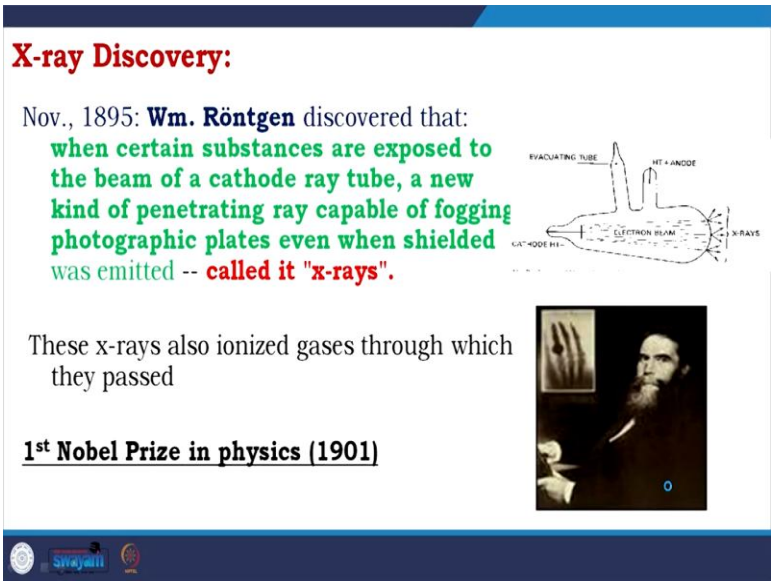

We talked about X ray crystallography, but we did not talk about the X ray. Now we will talk about X ray. X ray was invented by William Rontgen in 1895. When certain substances are exposed to the beam of a cathode ray tube a new kind of penetrating ray capable of fogging photographic plates even when shielded was emitted, he called it X rays. These X rays also ionized gases through which they passed.

And because of this invention which has changed the world science, he was awarded with Nobel Prize in 1901. And remember 1901 is the year of first Nobel Prize. So he got the first Nobel Prize in Physics. So we now know that, how X rays are produced and spatially how we could use it for X ray crystallography?

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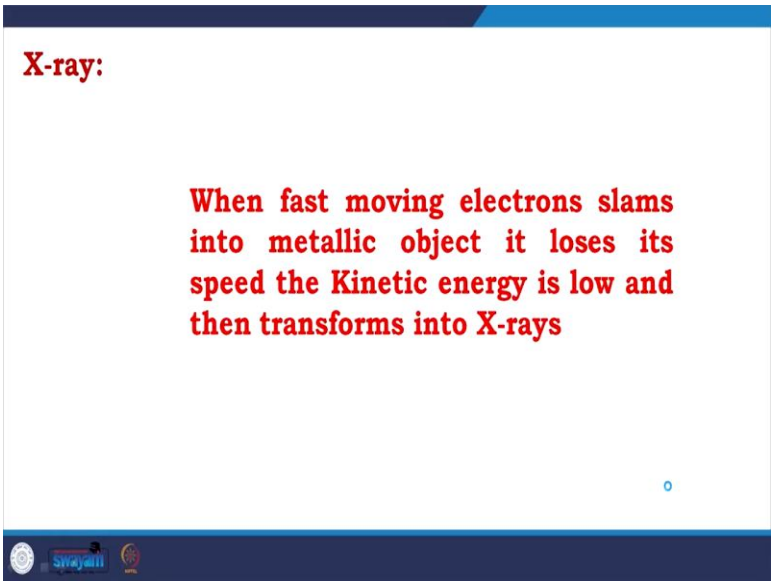

X ray is when first moving electrons slams into metallic object it loses its speed the kinetic energy is low and then transform into X rays. But how the machine works? What are the dedication of the scientists you know when William Rontgen the first invented X ray the first experiment he did on his wife he put her hand and check how the image of bone is collected this is the first X ray diffraction experiment happened.

So those amazing sciences are there those history of science dedication of people what is there, what is the principle we will discuss it in the next module in this module we have discussed about the basics of structural biology how we start to get the protein from where all the experiments would happen how we get the target how we clone it how we purify the protein and then you will come to crystallography for crystallization in NMR in cryo electron microscopy in other low resolution technique the basic part is same.

So we will discuss more on the next module. I will thank you for being an amazing audience. Please be with me in the next module. Thank you very much.