Symmetry and Structure in the Solid State
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Lecture – 48
Experimental Aspects of Data collection

So, at this time we are in a position to look at the Experimental Aspects.

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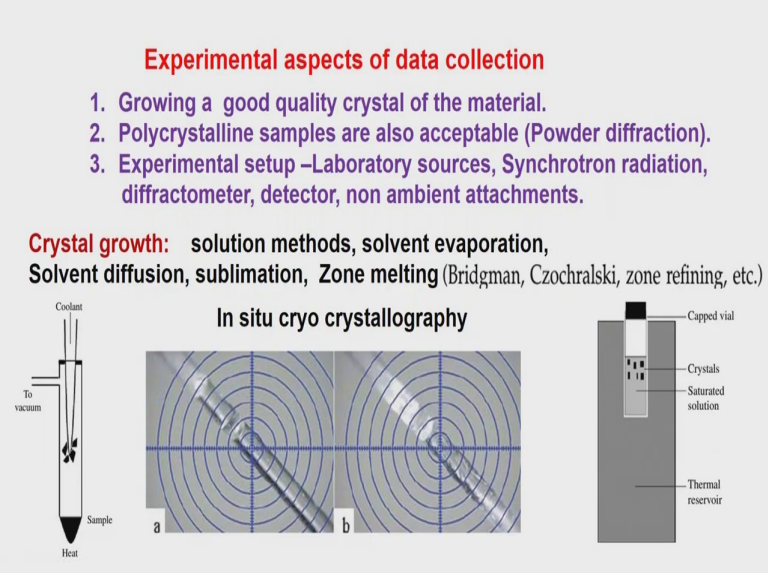

So, this is this is now becoming a little more critical from the point of view of what we can do from knowing all the rules of symmetry and details of how the molecules arranged themselves in the given space group and so on. Obviously, the job would be to see where the atoms are and determine the positions of the atoms and eventually find out how the atoms are connected to each other, look at their properties and eventually relate the property of the crystalline material to the external physical property it exhibits.

So, there are crystals which exhibit different kinds of properties some of them may be fast ion conductors some of them may be pharmaceutically important compounds some of them may be ferroelectric materials and so on. So, basically now is we are we get into the stage where we are now going to look at the experimental aspects in more detail. What are the major requirements which comes across is to grow a crystal and we have to grow a good quality crystal.

Now, growing a good quality of the crystal is in fact, can be considered a rate limiting step because very often some of the molecules have the property of not giving us good quality crystals. By good quality crystals I mean the diffraction extending to as much extent as possible on the limiting sphere reciprocal lattice space. So, if you get as many reflections then the issue of not having the required resolution limits becomes an issue.

So, growing a good quality crystal therefore is what we will concentrate on. Of course, if a material does not grow into a good quality crystal, but remains polycrystalline then the poly crystalline samples are also acceptable because one can use the so called technique of powder diffraction. We will be spending a little bit of time on powder diffraction as well.

So, apart from that what is the experimental setup we look for? We can look for laboratory sources where we can have a tube x ray or synchrotron radiation which is available in several centers around the world including the one in Indore in India then of course, we need a diffractometer which is with a detector of a high quality. So, diffractometer is the one in which we mount the crystal on a goniometer and things like that and then of course, we also need non ambient attachments like low and high temperature attachments, high pressure attachments and things of that kind so that we can study the variation in the property with respect to these non ambient conditions.

So, particularly some of the compounds become highly conducting at higher temperatures and some of the compounds at low temperatures shows specific properties which are different from the room temperature property basically phase transition studies associated with these materials will be of consequence. In context with the pharmaceutical industry, the requirement that the structure is known fully and it is behavior is known in non ambient conditions becomes an issue because it is it depends upon how the how and why the crystals grow and the so called depiction of polymorphism associated with crystalline materials will also bring in the issue of dichotomy in pharma industry.

So, considering all these this is the basic experimental setup that is required all these together and of course, we can either have a good quality single crystal or a polycrystalline sample. Now what are the methods we use for growing crystals? One is the solution methods where in principle we take the material and then dissolve that

material in a solvent and then eventually control the evaporation of the solvent. This is the usual approach which is shown here.

So, we have a saturated solution and then the crystals will start appearing depending upon the thermal reservoir. So, eventually the temperature associated with this we can control so that the evaporation of the solvent is slow enough so that we get good quality crystals.

This can be controlled in various ways we can have more than 1 availed more than 1 thermal reservoir and things like that. So, this is basically the solution method. There is no time to, in this particular course we are not going to spend too much time on crystal growth mechanisms and so on, that is not the purpose of this particular course. However, we should know how we grow crystals.

So, we have solution methods when the solvent goes away or straight forward solvent evaporation we can take the saturated solution heat it up or make a solution which is near saturation and then just heat it up, evaporate the solvent and hope that the crystals will form at the edges of the tube. The other approach is of course, using solving diffusion. Allow the solvents to slowly diffuse out of the material so that crystals will form.

Another approach is the so called sublimation method where we heat the sample, the sample sublimes, and then you provide a thermal gradient along this tube and this eventually we send in a coolant here and then this is vacuum chamber. So, after the sample evaporates, it now condenses on to the tip here which is carrying the coolant and you will get crystals. So, this is the approach of sublimation because in case the compound sublimes this is a very good method to prepare good quality crystals because now we can control the coolant temperature in such a way that the temperature associated with this can be monitored and the size of the crystal thus can be controlled; size and shape of the crystal.

The other approach which has essentially developed in very few laboratories and one of the laboratories happens to be our own where is the use of in situ cryo-crystallography. Here what is done is the you know there are lots of solvents which are used in crystallization process and the solvent structure becomes therefore, very important whether it is having a certain property related to it like the associated dipole moments

and so on, if it is a sub polar solvent or a non polar solvent, what is the influence of those solvents.

So, in order to know about that we have to crystallize the solvents themselves. So, one can crystallize the solvent by this procedure. What is done is the solution is taken in a capillary tube and there is a thermal gradient which is created in such a way that at one time the solvent inside the capillary tube freezes and becomes a solid. Now, by repeated reorganization of this like what they call is the zone melting we keep melting the solid by heat it providing a certain amount of heat and again freezing it up. So, as this process is repeated over and over again a good quality crystal will develop as is shown here. This is a good quality crystal which eventually develops and the quality of the crystal can be checked on a diffractometer by checking the diffraction spots which come up come out of this experiment.

So, these are various methods and several others, but these are the basic methods which are essentially used. In order to crystallize proteins there is a total new methodology, there is a total clearly different kinds of approaches. They saw they saw is these the crisp the material is taken in the form of a inside a solvent and then as drop of the solvent is made to hang on upside down against the gravity on a glass plate and because of the gravity eventually the material sort of sinks in and you will get the crystal growth in the solvent drop method.

There are several other methods. In fact, we can also have the zone melting which is very popular among the metallurgist, where they can heat and cool, heat and cool the system. There are several apparatus that are available to grow quality crystals so much that some of these methods will allow us to grow crystals which are very large in size that brings us to the size of the crystal we want to use in our experiments. Most of the experiments which use x-ray diffraction would like the size of the crystals to be about 0.1 mm^3.

So, we do not want any bigger crystals because of absorption of the x-rays within the material and at the same time we do not want too very thin crystals because the quality of diffraction suffers. So, there is the optimum size estimated for either organic or inorganic materials is about 0.1 mm^3. In case the crystal is a big crystal then we have to apply absorption corrections; in case the crystal is a very small crystal then hardly diffraction comes. You see the whole idea is to get the reflections in the entire reciprocal lattice

frame; that means, the the all possible reflections which come within the limiting sphere must be collected the larger, the data that we get the better it is. And, that is where we decide on the quality of the crystal. The quality of the crystal is not by the looks of the crystal, but by the extent to which it diffracts.

So, as we discussed earlier, we discussed about the resolution limits we calculated the value of d from 2d sinθ λ and the value of d now decides what is the resolution the data of the data we are going to expect by checking out what is the extent to which the diffraction occurs in sin theta. So, the larger the sine theta up to 1 degrees 1.0 sinθ value the better the data quality and also the quantity of data, that is both are required in order to do a good structural determination protocol.

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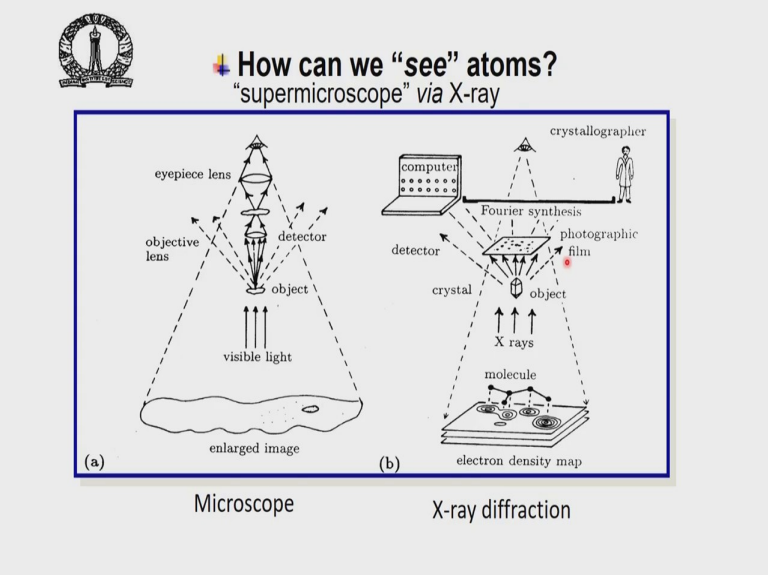

So, as we saw earlier also this slide that it is taken from the book of Glasker and Tubuler it is a old book and that is how we have now shown here a photographic film in that place we put now the CCD camera or the CMOS detector. So, what essentially it does is comparison between a microscope and a x-ray diffraction experiment. In case of a microscope the object now sends scattered radiation. Here the radiation is following the Braggs condition because it is a crystal.

And, all these Braggs conditions will satisfy, we get the reciprocal lattice image. This reciprocal lattice image the intensity can be measured and the measured intensity now converted to the modulus of f of h k l, the structure factor. The phase problem is solved

either by the crystallographer or by the computer and then we do a Fourier synthesis. The Fourier synthesis we will reconstruct the image of the object and in terms of the electron density distribution.

So, we do get an electron density map, as we call it and this electron density map not only identifies the positions of the atoms. In terms of its strength it also identifies the nature of the atom whether it is a carbon or a bromine or a nitrogen or whatever. So, that identity of the molecule can be obtained from what we call as the electron density map.

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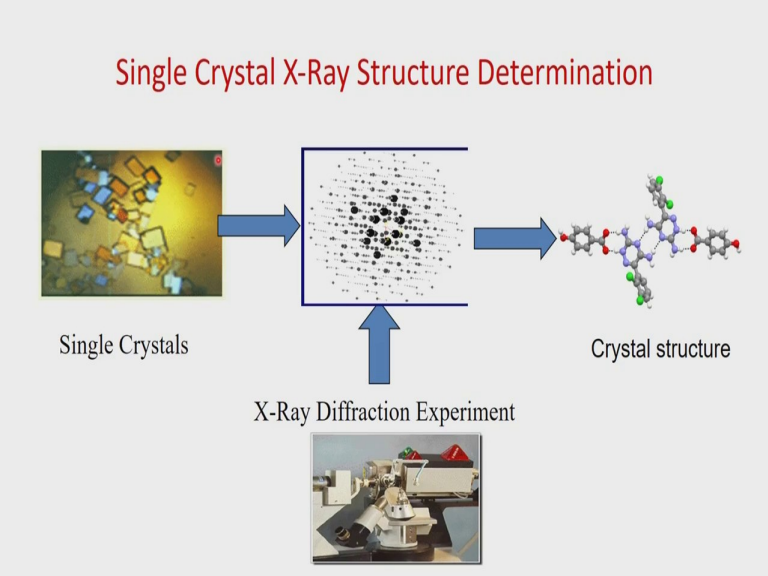

So, this is the exercise we will have to do in the experiment and normally therefore, this is the protocol, you grow single crystals, pick the best of the single crystals. We will see in a minute how we pick them up and how we mount them on the on this machine which is a 4-circle diffractometer, that is a in-house diffractometer which we have and this particular diffractometer we will we have several parts. The moving part is the this part is the goniometer. The goniometer is mounted along this what we call as the omega axis and on that omega axis the crystal will can rotate and therefore, we now identify the position of the reflection in terms of 4-circles.

The circles are the rotation about crystal itself which is the phi rotation, the rotation of the of the 2 theta arm which is the this is the 2 theta arm the rotation of the 2 theta arm that is theta. So, we have phi, theta and then this is the angle which the goniometer forces itself to go, that is the kappa angle and then the base plate rotation is the omega. So, we

have therefore, phi, omega, kappa and 2 theta. These are the four angles and that is why it is called a 4-circle automatic diffractometer.

This gives us the reciprocal lattice image a good crystal will give very nice spots as is shown here separated from each other. So, we now next have to do what is known as the indexing of this pattern and when once we do the indexing we will get identity of each one these h k l and we measure the intensity of each of spot. So, that is our data. So, when once we have this data we take it through the phase problem analysis and then the Fourier synthesis, we will end up with the electron density distribution and this represents therefore, the crystal structure. So, this view graph essentially tells you the basic experimental methodology one has to adapt for determining the structures.

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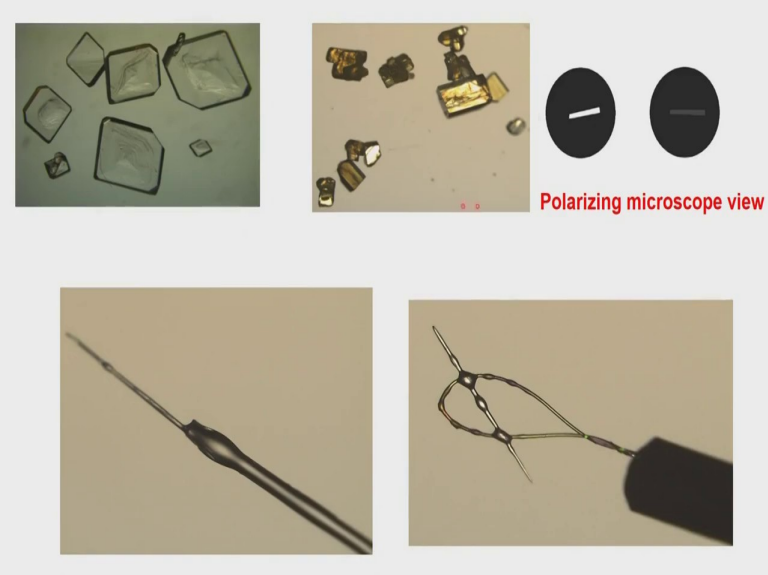

Now, the crystals can be of different shapes and sizes here are some of the crystals which are good crystals in appearance are shown here. These crystals are not so good in appearance, but they are also shown here just to tell you the variety of crystallinity we can impart on these materials. What is more important is not the appearance of good crystals or a bad crystal; what is important is this part which is now the polarizing microscope view.

So, if we put the crystal under a polarizing microscope and then move the polarizer, the Nicol prisms in such a way that we will have an extinction of the light which is coming from this crystal. So, the crystal is in this orientation. Different polarized light is now

sent in and as the polarization polarizer is rotated we will have extinction. So, if the complete darkness comes up and again when we rotate another 180 degrees of the polarizer, we will get the crystal picture like this.

So, this is very important before we decide to take the crystal on to the machine. So, if this is not happening and this remains same as let us say same transparency as you seen here with respect to light then it means that the crystal is not a very good crystal. So, the crystal quality is tested under the polarizing microscope. It is not a guaranteed test, but basically it tells you the possibility of using that as a quality crystal onto the diffractometer.

Now, on the diffractometer, on the goniometer we can have different ways in which we can mount the crystal. One of the ways in which normally it is mounted is to take a glass fiber and that the tip of the glass fiber use the epoxy of some kind and then mount your crystal. This is a needle crystal which is mounted up here. If you are doing non-ambient crystallography and particularly variable temperature crystallography and so on, then the crystal has to be protected because we are going to change the temperature and pressure.

So, for such a purpose we can enclose this crystal inside a capillary called the Lindemann capillary. This Lindemann capillary has the property that it will not absorb our diffract x-rays and as a consequence they will just be the containers which hold the crystal. So, whatever comes out in the form of diffraction is that from the crystal. So, we get different signs of these Lindemann capillaries which can be mounted up here. And, so, that is these are the two ways in which you collect the data.

Of late the data collection particularly if the crystal is small and also if the crystal is protein crystal or whatever we have these what are called the loops. So, this is a loop mounting with the help of a oil. In this particular case we use different kinds of materials when you are doing let us say a data collection at 100 K you can use a oil which is now holding the crystal at this point and then at 100 K it will freeze. And, this is essentially it is oil and the oil that we use is very special and that particular oil, paratone oil. So, it is the paratone oil which we use in order to mount the crystal.

So, we make use of the paratone oil and we mount the crystal on this loop. So, this is now the most common method which is used because there is no interference of either glass or any other material with the system and also at the same time the crystal is now

completely bathed in the incoming beam which is a requirement for a quality diffraction. So, we will not go into the unnecessarily further details of experimentation because that is not the purpose.

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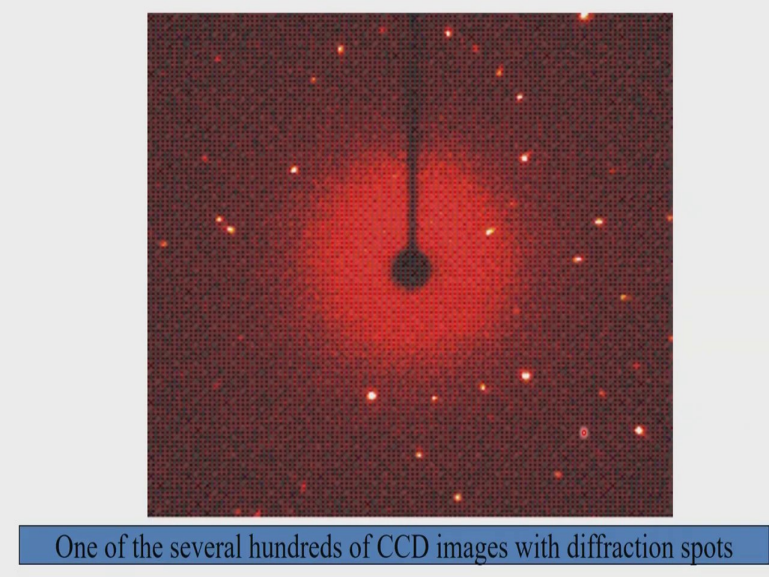

The purpose of this is to see what happens when we do the diffraction. So, we do the diffraction in this one of the CCD images which shows the reciprocal lattice images. So, these are the reciprocal lattice points which are seen here dark and white. We know why some of them are dark and some of them are not so very dark as are bright as we have seen from our positioning of the atom with respect to the planes.

So, having got this is one of the several hundreds of images which we can collect for a given crystal depending upon the orientation of the 2 theta, phi, psi and kappa. So, these 4 values, the 4 circles now decide the geometry of the reciprocal lattice which is now 3-dimensional in space. So, we collect the reciprocal lattice image which is 3-dimension in space and make use of these determine their intensities from the experiment by using a measurement technique which is now converting these photons into electric fields in the CCD detector and we measure the intensity directly.

The there are other detectors which one can use the detectors could be image plates, the detector could be CMOS detector which is essentially used in space technology and CCD which is very commonly used nowadays. Image plate is very commonly used in case of macro molecular structures because it is possible to wipe the image on the image plate

before we re-expose. So, these are some of the advantages in an image plate where the reflections are very closely spaced. Those materials which are not so very closely placed can be collected both on CCD as well as CMOS. The time required and the amount of time we spend to collect each data point is pre decided by us and that depends upon the quality of the crystal and the extent to which we are looking for reflections in the reciprocal plane.

So, if we want to go to the end of the reciprocal lattice dimension which is the limiting sphere we would like to have a good time of exposure and these exposure time also depends as we know as we have already seen on the f versus sine theta by lambda curve. So, at lower angles we will have higher intensities at smaller angle the larger angles we will have lower intensities. So, the exposure time accordingly we can change we can go from smaller 10 second data collection to a almost a minute data collection if you are collecting at very high temperature and thus very high angular range.

The other thing is as we increase the temperature on the crystal, the crystal the atoms in the crystal vibrate a little more than necessary and those vibrations can cause the intensities to go down. So, on the other hand if we cool the crystal the spots become sharper so, because the vibrations of the atoms are minimized. So, all these are experimental aspects which we have to optimize before we collect a good data set.

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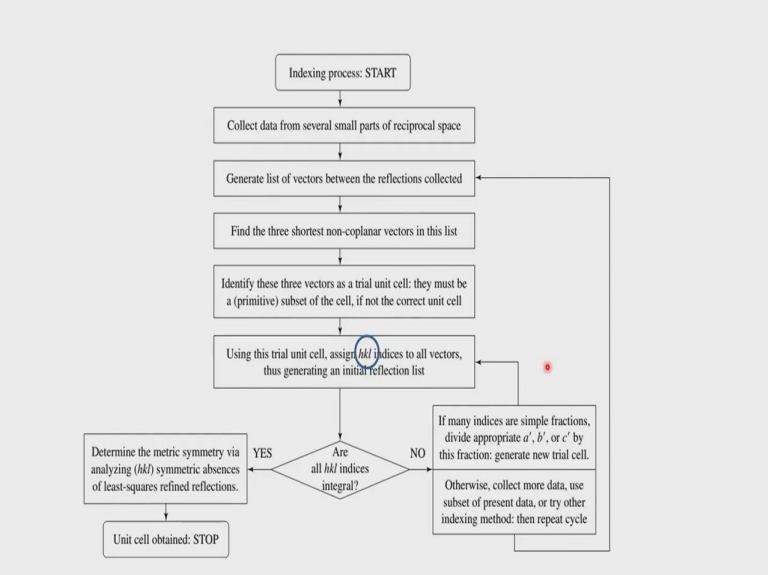

Having got this kind of a thing we start now with what we call as indexing this is the backbone of the entire structure determination. So, this is a process which I have given a flow chart from the textbook of William Clegg; edited by William Clegg, several other authors have written this. William Clegg is the editor of this particular book which I will of course, in the end of this course I will give all the list of all the books with their publishers etcetera with very special acknowledgments to each one of these authors who have written the books because I have taken their pictures, their equations and their flowcharts as you can see here. This is something which we must be very happy about because these are already available and we can use it for our presentation.

So, the indexing process will start by collecting the data for several small parts of the reciprocal space. You saw in the previous picture a position a portion of the reciprocal space and in this reciprocal space you see there are a few reciprocal lattice points which intersect the Ewald sphere. And, those which intersect the Ewald sphere come up with intensities like this that is how you see the spread in the case where there is cell dimensions are very large you see the spread becoming closer closer. So, larger the cell dimensions the spacing between these spots will be much much closer. So, it is a reciprocal relationship. So, if the cell dimension is 100 angstroms like in a protein, then these parts will be very close to each other; whereas, in this case it looks like a data from a very not so very strong reflecting material and at the same time a smaller unit cell damage.

So, you collect the data from several small parts of the reciprocal lattice. So, do not keep the detector in one particular place you change the angles; the other three angles the phi, psi and omega. So, if you change the phi, psi, omega and then keep changing also the 2 theta in go in steps of 2 theta keep the let us say collect data 10 degrees, 12 degrees, 17 degrees, 18 you can program it in when you do the data collection protocol. So, collect the data from several small parts of the reciprocal lattice. So, we have a representative number of reflections just as we saw in the previous viewgraph. We have a representative number of reflections coming from the same material in different parts of 2 theta space.

So, having got that you generate a list of vectors between the reflections collected, vectors between the reflections collected and then in such a way that you find three shortest non-coplanar vectors in the list. They should not be in the same plane. So, it should not be coplanar and then find among the set of spots which you have got. Find the

smallest or the shortest non-coplanar vectors from the list. It is believed that these three vectors will give us the trial unit cell it may not be the correct unit cell, but you just select the three shortest non-coplanar vectors. And, these three short non-planar it is can be now used there must be a primitive subset of the cell if not the correct unit cell this is something which we have to examine.

So, to find three vectors which are non-coplanar, identify these three vectors as the trial units. When once you find that out you say well this could be the possible unit cell some a, b, c, alpha, beta, gamma between them. So, these three vectors are taken as the trial unit cell. If this unit cell is a correct unit cell using the trial unit cell assign hkl indices to all other vectors. So, if you take this three as the trial unit cell, then you know the endpoints with these 3 vectors are defining now a, b and c. So, the endpoints with these three vectors are a, b and c.

So, we now have the unit cell dimension and the interactional angles between them because we are identifying the vectors. So, using that you now assign hkl values to the rest of them. So, suppose this is 1 unit, 1 unit, 1 unit along a, b and c and something else which is coming farther away could be a multiple of this something which is coming below this could be a fraction of that, but the chance of something coming shorter than this is less because we have taken the three shortest non-coplanar vectors in from the list.

So, these are the three star shortest non coplanar vectors. So, it could be a correct cell, it could be a not a correct cell that is that is checked by this questionnaire here or all hkl indices integral value. So, then once we have this thus it come the rest of the spots which we have. We may have a 100 spots. Let us say the 100 spots which we have are these hkl indices also integral values. So, we check on these hkl indices integral values and if these integral values all appear to be good in the sense you may not get exactly 1, 4, 5; you may get 1.2 at the most and on the other side 0.8, then you can round it off to 1 effectively or you may get 4, 4, 7 and 8, 4, 3.9 and 4.1 and things like that.

So, you give a tolerance limit within which all the hkl integers are integral. If they are really falling in that zone then you say that you are determine the unit cell dimension. So, use this unit cell dimensions and do what is known as a least squares fit for this to identify the unit cell a, b, c. So, unit cell obtained and you stop it here. So, this is the indexing process.

So, in the indexing process the major requirement is that you have to now take every point in the reciprocal lattice for which you have measured the intensities. Take those spots and then make sure that these spots are generated by three non-coplanar vectors which are the shortest set of vectors in this representative handled or reflections. This can be easily done programmed and the machine will do this work and so, when once that is done if they are all integers, it is fine. If they are not integers then what you do is if they if they come out as simple fractions let us say one fourth, one half then we can multiply by the 2 and 4 and then eventually decide this would be the cell.

So, if many indices are simple fractions then divide appropriate a, b, c by that fraction. So, you will get the new trial cell. When once you have the new trial cell go back here and then again using the new trial cell assign hkl and now they will become all integral. If this process happens there is no problem. But, if this also fails if you do not get a hold of it, then you can do two things – one is to collect more data and use the subset of present data or try another indexing method then repeat the cycle. There are different ways in which one can index the methodology there are programs which are available different kinds of programs approaches it in different ways. And, these therefore, can be now put in such a way that we go back in and then again generate the list of vectors.

So, this flowchart essentially tells us how to get to the cell dimensions. So, the way how to get to the cell dimensions and how to get the hkl values assigned to individual reciprocal lattice points. The appearance of the individual reciprocal lattice points thus is very important. So, when you have a good crystal the appearance of the individual reflections should be as spherical as possible. It may not be always of spherical, but as spherical as possible that is because that we will tell us the that the crystal is rightly bet in the beam of x-rays which are coming in because the normally the x-ray beam which comes out is a is a circular path. It has a circular diameter, it has got a circular what should we say circular shape which now falls on the crystal and therefore, the reciprocal lattice points should mimic the shape of the incoming radiation.

The incoming radiation is collimated normally. So, the x-rays which are coming from this various sources they pass through a collimator, the collimator will adjust it to the size accordingly. So, we can change the size of the collimator. We can have 0.5 mm, 0.3 mm, 0.1 mm depending upon the size of the crystal. If the as the crystal becomes bigger and bigger you can use bigger collimators, but then very big crystals are no no because

they will absorb all the radiation which comes in. So, there is an optimized value again. So, normally the ones which are used on the routine diffractometer laboratory setup the collimators are 0.05 and in some special circumstances 0.03 is also a used diameter for the collimator.

So, the one once we have this hkl indices identified the initial reflection list is generated we have the unit cell. So, when once we have the unit cell now you collect a few more frames of data that is reorient in different 2 theta directions and collect some more reflections. Use these cell dimensions, this is an additional check which we can do which is not indicated in this flow chart use these a, b, c alpha beta values and then work out their corresponding hkl's. So, go backwards and determine the hkl's and those should be integral values. This will now doubly verify the nature of the unit cell dimension which you have determined.

So, the unit cell three vectors non-collinear vectors will also have a, b, c, alpha, beta, gamma information. So, the moment you have the first three shortest non-coplanar vectors as identified as a unit cell you know the crystal system because you know a, b, c you know alpha, beta, gamma, so, you know the crystal system. So, the crystal system is already identified by just doing a diffraction experiment and selecting the reciprocal lattice points in such a way that there are three directions in which the shortest vectors which will generate the rest of the reciprocal lattice points are identified.

And, this process can be repeated over and over again until the final a, b, c, alpha, beta, gamma values are depicted on the screen. One once that is done any data points you collect you can go ahead and collect the full data full sphere of data, every lattice point in principle should be indexable that means, we can give a hkl value based on this a, b, c, alpha, beta, gamma. So, that way this is a very useful approach by means of which you identified the cell dimensions.