Symmetry and Structure in the Solid State
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Lecture – 42
Limits of Resolution

A couple of more points about this limit of the reciprocal space, particularly in terms of how much we can cover in a typical experiment, this is crucial particularly as the size of the unit cell increases.

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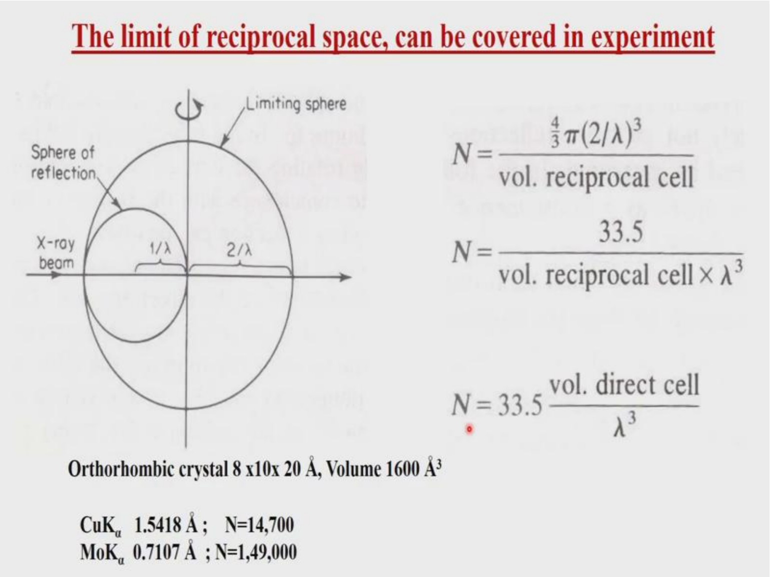

The limit of reciprocal space, can be covered in experiment

$$N = \frac{\frac{4}{3}\pi(2/\lambda)^3}{\text{vol. reciprocal cell}}$$

$$N = \frac{33.5}{\text{vol. reciprocal cell} \times \lambda^3}$$

$$N = 33.5\,\frac{\text{vol. direct cell}}{\lambda^3}$$

Orthorhombic crystal 8 x10x 20 Å, Volume 1600 Å^3

CuK_α 1.5418 Å ; N=14,700
MoK_α 0.7107 Å ; N=1,49,000

Suppose you see we have talked about the change in the wavelength up here which we discussed in the previous discussion and we found that changing the wavelength will give you larger numbe of reflections. Of course, not all 1,49,000 will be independent. It depends upon the symmetry associated with the system, but it maybe substantially reduced by one-fourth or one-eighth of the total number; however, it is a very large number of reflections.

And one once we have the access to all these measurements we can, we indeed can measure experimentally all these individual reflections. And therefore, if they are symmetry equivalent we can take the average of the 2 so the accuracy of the experiment increases.

The other issue is that the limit of the limiting sphere is defined by the value of sin θ, sin θ going up to 1, but it is not necessary that the diffraction is limited. The diffraction can go beyond sin θ/λ and that is something which we will discuss towards the end of this course where we can use such data to determine the bonding densities. We can also determine what happens to the density, electron density between two molecules, if it is let us say having intermolecular interactions, so that particular data is of extreme consequence for analyzing accurate datasets.

However, in the context of what we are discussing now, the limiting sphere. Not only limits, the number of reflections depending upon the wavelength. It also depends upon the volume as you can see from this expression N is 33.5*(volume of the direct cell/ λ^3). So, there are two variables here; one is the λ and the other is the volume of the direct cell. In the example which you have taken here, we have taken the volume of the direct cell to be 1600 $Å^3$, but if you are now crystallizing a protein or a virus or whatever, the volume of the unit cell will be something like 100 $Å^3$ on an average, so the volume will be enormously large. So, the larger the volume, larger are the number of reflections.

So, effectively when we now compare this to the multi slit experiment which we discussed as the number of slits, increases the number of scatterers also will increase. And therefore, in the case of a protein crystal structure the number of scattering points associated with the electron density which happens to be the atoms in the unit cell, they will increase enormously. And since they increase enormously, we will have the diffraction pattern luckily for us, it is a reciprocal relationship. So, we do find the diffraction pattern over crowded, but then at the same time reasonably separated from each other. So, we have to have individual reflections, separated from each other in order to estimate the intensities.

So, what really therefore happens, is that when we have to talk about such situations larger, smaller molecules or even the case where we have very accurate datasets. We also talk about an issue which is extremely important for experimentalist and this is something based on which your results can be evaluated. For example, you are let us say studying a very large molecule like a protein or a virus then it so happens that we have to worry about this value d_{hkl} is λ/2sin θ.

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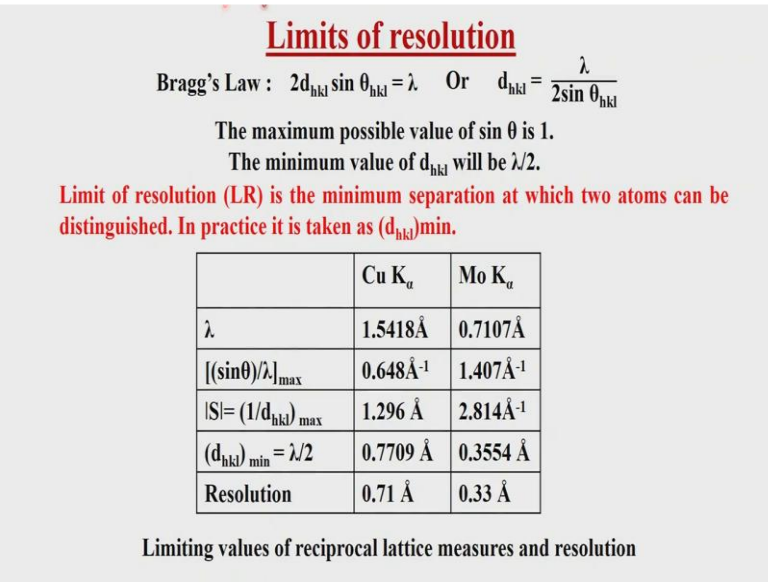

Limits of resolution

Bragg's Law : $2d_{hkl} \sin \theta_{hkl} = \lambda$ Or $d_{hkl} = \frac{\lambda}{2\sin \theta_{hkl}}$

The maximum possible value of sin θ is 1.

The minimum value of d_{hkl} will be λ/2.

Limit of resolution (LR) is the minimum separation at which two atoms can be distinguished. In practice it is taken as (d_{hkl})min.

	Cu K_α	Mo K_α
λ	1.5418Å	0.7107Å
$[(\sin\theta)/\lambda]_{max}$	0.648Å^{-1}	1.407Å^{-1}
$\lvert S\rvert = (1/d_{hkl})_{max}$	1.296 Å	2.814Å^{-1}
$(d_{hkl})_{min} = \lambda/2$	0.7709 Å	0.3554 Å
Resolution	0.71 Å	0.33 Å

Limiting values of reciprocal lattice measures and resolution

What also happens is that in the case of the biological molecules, particularly there is a large amount of space which is occupied by what we call as solvent water mostly it is water in many of the cases in biological molecules. And therefore, this solvent water has some kind of a structure, but not a realistic structure associated with the actual crystal structure of the protein and therefore, this solvent protein will give you some kind of diffuseness associated with the diffraction. So, much that the actual even though the crystal is of a very high quality the extent to which it goes in sin θ is severely limited.

In other words the extent to which we can get reflections beyond let us say a certain θ value is limited. If the θ value is limited you see that is in the denominator. So, the corresponding d value is therefore, dependent upon the value of sin θ. So, as we go to higher and higher sin θ values we can get to better and better d_{hkl}, because what is relevant is $1/d_{hkl}$ from the reciprocal lattice point of view and this $1/d_{hkl}$ will identify the distance from the reciprocal lattice origin to individual reflections and that d value is very crucial.

So, we call this d value as the resolution. So, when we refer to this d value of a given experiment and if the d value of the given experiment let us say is limited to 2Å; that means, the resolution is limited to 2Å; that means, anything beyond 2Å level is not a reliable conclusion. Suppose we are looking at let us say the distance between two carbon atoms. The distance is 1.54 or something. If we are now talking about a 2Å resolution data this we call as the 2Å resolution data depending on the d value that depends again now only sin θ when once the λ is fixed.

So, one once the λ value is fixed we therefore, decide on the resolution of the experiment. In a typical small molecule experiment these resolution values are not so very critical as you can see down in this table here the minimum value of d_{hkl} we can reach with the wavelength that we let us say we use copper or molybdenum with when we use the copper radiation the minimum resolution we can get is 0.77 that means, in principle we should be able to see the carbon hydrogen distances just to give an example because carbon hydrogen distance is around 0.9 also.

So, this is the resolution one would expect in a typical experiment on a smaller molecule. The problem with the larger molecules is even though they also have the $(d_{hkl})_{min}$ associated with the λ value. See the minimum value of d_{hkl} is associated with the wavelength we are using.

So, shorter the wavelength the lower is the d_{min}. For example, in this case of molybdenum it is 0.35; obviously, using the molybdenum data to determine small molecule structures are much more accurate than the much more reliable in terms of the resolution compared to the resolution we obtain from copper. But there are other limitations because copper gives higher intensities, the intensities which we get from the copper diffraction is much higher in intensities therefore, the measurements become more and more reliable than the measurements we can get from molybdenum at this resolution.

When we go to this resolution we need a very accurate measurement technique and most often they are not since we now use high resolution CCD cameras or CMOS detectors or whatever this kind of resolution limits is possible to reach one once you have a good diffraction condition and a very stable λ and particularly a tunable λ, which can be obtained from synchrotron radiation.

So, essentially what we are now discussing is the fact that we have now a diffraction measurement possible. We have found out how the diffraction occurs in reciprocal space. We know the Bragg's law in reciprocal space. So, we can identify where these reflections come from and how many reflections we can calculate also has been a evaluated in terms of the limiting sphere response; however, the quality and the quantity of data depends upon the value of the d, the minimum value of d we can reach. So, in a typical small molecule experiment these are the values, but for larger and larger molecules this value keeps going up.

So, when you see literature where structures of proteins and complexes of proteins and all are reported you will see that in the title of the paper itself people say at what resolution this work has been done. So, you will say 2 Å resolution structure, 4 Å resolutions structure, 1 Å resolution structure which means that the accuracy with the 1 Å resolution structure is enormously high. So, your reliability on the positions of the atoms which you have determined becomes higher and higher. So, that way the value of the limits of resolution becomes an important quantity to evaluate.

There are other definitions associated with the limits of resolution see we have said here the maximum possible value of sin θ is 1 that depends and therefore, the value of sin θ/λ , sin θ is 1, but sin θ/λ where λ is let us say 0.7 is much higher than the value of sin θ and therefore, we can get access to diffraction beyond sin θ/λ, 1 and the minimum value of d_{hkl} as we have seen is λ/2.

The other measures of accuracy or the resolution which we can reach are in terms of this table where we have the λ we also have a quantity called sin θ/λ. So, we can calculate the sin θ/λ by taking this value down here and that will be one over of d_{hkl} and that value is given in terms of what we see here.

So, the maximum value of sin θ/λ corresponds to 0.64 in the case of copper so; obviously, if you are looking at the measurements which you want to do for covalent interactions and intermolecular interaction understanding in molecular crystals copper is a “No”. You have to use the molybdenum and other lower wavelength diffraction wavelengths, so, that we can essentially get to the accuracies which we are looking for.

So, you see that the sin θ/λ max is also a very crucial factor. So, if you are getting a data up to 0.648 in copper, it is equivalent to getting a data of 1.407 in sin θ/λ in molybdenum; that means, with the molybdenum data the reciprocal lattice is effectively in principle expands and these are issues which one has to remember when we choose the wavelength that is required for the given experiment. For example, if you use molybdenum radiation and try to collect the data on a protein, more often it is very difficult, because the intensities we get out from the molybdenum k radiation are much lower than the intensity we get for the copper in case of the diffraction conditions being satisfied under the conditions in which we do the experiment. The other issue which has not been mentioned here is the fact that the temperature of data collection. So, that is also a limit of resolution, because at any given temperature, let us say at the ambient temperature when we measure the data the reciprocal lattice is distributed in such a way that the a b c α β γ values or for that matter a^*, b^*, c^* values we will have a certain particular value.

Now, as we cool the crystal if we go down to let us say 100 Kelvin there is a little contraction of the cell. So, since there is a contraction of the cell, there is an expansion of the reciprocal lattice. So, the expansion of the reciprocal lattice will allow us if we can get into these kind of limits associated with the d_{max} , $(d_{hkl})_{min}$ then we can access more and more data.

So, the fact that the temperature factor gets reduced is one advantage, the second is that if we collect low temperature data with the molybdenum radiation we can reach higher accuracies and also the higher angle of diffraction associated. So, these are all issues which are practical, which are very important if somebody is practicing crystallography as a tool just out of curiosity or crystallography for a living.

So, in both ways these points should be borne in mind and at the same time when if people are in industry or using crystallogra φ c results they should ask the questions like what is the resolution limit and what is the resolution of the experiment one has done here particularly if they interact with some collaborators they should ask those questions.

The resolution of the data set, the temperature at which it is collected, the extent to which they can rely on the results which they get in terms of identifying the atom positions, finding the bond lengths angles and intermolecular interaction features. And in case of protein structures the nature of the fold, the alpha helix, and the beta sheets and to extent to which the reliability can be placed on both the secondary and the tertiary structures. Quaternary structure is also an issue which can be addressed at this stage.

So, this kind of brings us to a state where we can wind up on the discussion on the let me see what the next slide is yeah we can wind up on the discussion on the reciprocal lattice. So, I think this is by far the extent to which anybody who have any textbook would cover the reciprocal lattice information. So, the overall take-home is that when we have a crystal which is kept in the path of an X-ray beam we get the diffraction; the diffraction gives us the reciprocal lattice information. From the reciprocal lattice information we get the information of the crystal, the nature of the crystal; the crystal system and then we also get something more and that something more is what we will now address.

The something more is to find out the point group and the space group information from the diffraction measurements because the diffraction measurements see the atoms and molecules inside the unit cell they also see the symmetry that is relating one molecule to the other or one part of the ion to the other and so on in the given crystal. And therefore, it becomes crucial that we not only get the highest resolution data, the examination of the reciprocal lattice values that is in terms of the number of *h k l* we have got the way in which *h k l* distribute themselves in other words whether *h k l* are all in one side and *–h,-k,-l* on the other side and so on.

So, the question is now what is the way in which we can get information out of the measured reflections regarding the issue of a space group can we uniquely determine a space group by looking at the reflections and the distribution of the reflections that statistical distribution of the reflections? How many are in the projection reflections? How many are in the axial reflections? What is the way in which the axial reflections and the projection reflections come up compared to the overall reflections and in what way the overall *h k l* planes behave with respect to the incoming radiation? So, that will take us to another part of the discussion where we will wherein we will now see how this all develops.

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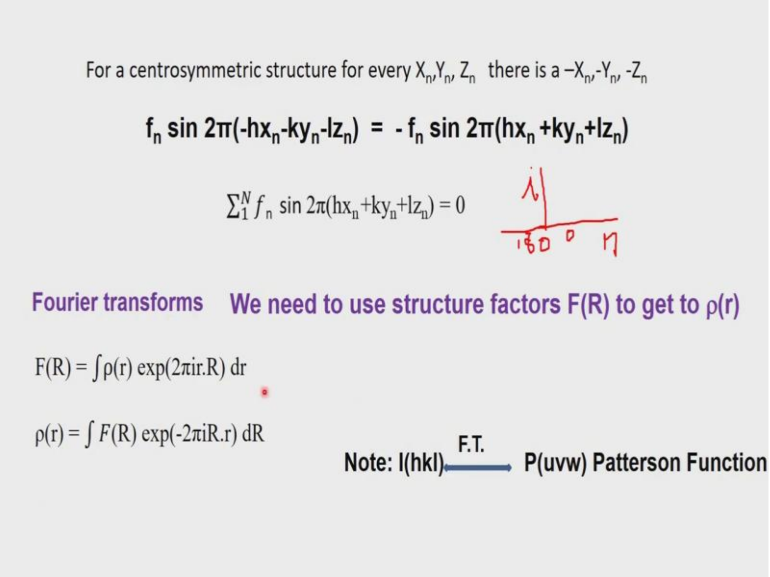

For a centrosymmetric structure for every X_n, Y_n, Z_n there is a $-X_n, -Y_n, -Z_n$

$$f_n \sin 2\pi(-hx_n - ky_n - lz_n) = -f_n \sin 2\pi(hx_n + ky_n + lz_n)$$

$$\Sigma_1^N f_n \sin 2\pi(hx_n + ky_n + lz_n) = 0$$

Fourier transforms **We need to use structure factors F(R) to get to ρ(r)**

$$F(R) = \int \rho(r) \exp(2\pi i r.R)\, dr$$

$$\rho(r) = \int F(R) \exp(-2\pi i R.r)\, dR$$

Note: $\mathbf{I(hkl)} \xleftrightarrow{\text{F.T.}} \mathbf{P(uvw)}$ **Patterson Function**

One of the immediate things which we notice is that for a centrosymmetric structure we know that for every x_n, y_n, z_n there is a corresponding $-x_n$, $-y_n$, $-z_n$. So; that means, that the f_n if you look at the expression for the sine part of the cosine + sine which we wrote in the intensities. If you recollect that the intensities are written as the f_n cosine term first and square of that, sine term first and square of that next.

So, the sine term we said it vanishes in case of when we took the example of caesium chloride. Now that is because the sine value disappears depending upon where the atoms are sitting, the atoms are sitting there at 0 0 0, ½ ½ ½ but when we say the atoms are sitting at 0 0 0 and ½ ½ ½ we did not say at that particular state moment that the 0 0 0 is also the the position for the inversion centre in caesium chloride. So, caesium chloride is a centrosymmetric structure and therefore, in centrosymmetric structures you will have this sine function always becomes 0 as is written here.

The reason why that becomes 0 is because of the fact that the $f_n \sin 2\pi(-hx_n - ky_n - lz_n)$ which comes under the summation is equal to $- f_n \sin 2\pi(hx_n + ky_n + lz_n)$. So, when we take this expression combine it with the $f_n \sin 2\pi(hx_n + ky_n + lz_n)$ they each other cancel and therefore, we get to a 0. So, the summation over 1 to N all the atoms in the sin terms in any centrosymmetric structure is equal to 0 which means to say that the phase associated with centrosymmetric systems is always a real value, it cannot be in the an imaginary value.

So, in other word in principle what we are talking about is if you now consider the coordinate system to be like this and this is the real axis and this is the imaginary axis. The component of the phase along the real axis alone will be there in case of the centrosymmetric system. So, it will be either 0 or 180^0, so, it is either 0 or 180. So, we also associate that as + and -. So, the phase factor associated with centrosymmetric structures can be only real quantities and therefore, in other words we use only the cosine function in order to calculate the intensity.

We will examine the reason why this becomes very crucial and also of extreme importance to solve the crystal structures later on, but at this particular moment we should remember that in centrosymmetric structures the phases are either 0 or 180^0 associated with every *hkl* reflection. So, on the other hand in case of non centrosymmetric structures it can be anywhere in this 0 to 360^0. So, it can be now having an imaginary component as well because the value of the sin x in the expression of intensity calculation will also have a certain value.

Now, one of the things we must go back and forth and always keep remembering are these two equations. These two equations are the bread and butter of our study. The first one is the structure factor, which now gives us the electron density and the second one is the electron density, which gives the structure factor. These two are the so called Fourier transforms of each other.

So, the way in which this develops is the fact that if we know the electron density and we know where the atoms are sitting; that means, the vector R then it is possible to calculate F(R). And if we know if F(R) and where the R vectors come that is our reciprocal lattice vectors come from the reciprocal space measurements then we can calculate where the atoms are sitting and the corresponding electron density distribution.

So, these two equations; therefore, as I mentioned are very important for structure determination. So, it is also very clear that one once we have got this information about F(R) it is in fact, the scattering structure factor and that structure factor is square root of the intensity. So, in the typical X-ray experiment we send in an X-ray beam get the diffracted diffraction done and we identify the coordination of each and every spot in the reciprocal lattice in terms of *hkl* and we measure the corresponding intensities of *hkl* with a detector.

So, this intensity of *hkl* will give us the modulus of F(R), but the phase associated with the F(R) has to be calculated. We actually estimate these phases by various ways means because phase by itself cannot be experimentally determined nor it can be accurately estimated to the accuracy that we require in a structure determination. So, therefore, this is always an approximation which will result in a nearby phase being estimated. So, in that context the previous discussion^{0} we had on the possibility of a centrosymmetric molecule having a phase of 0 or 180 gains a lot of importance because we have only two possibilities for every reflection and as a consequence the structure solution protocols become easier.

We will evaluate them little more closely later on. So, the Fourier transforms we need to use the structure factors F(R) to get to ρ(R). So, that is our aim when we say structure determination in the solid state using the symmetry information and on the way in which the diffraction pattern appears then we have to use the F(R) to get to ρ(R). The Fourier transform will give us the final result. At this time I just want you to make a note you see we have in an experiment we have the intensities measure.

Now, why cannot you use intensities directly and go to the structure? In principle it is possible what we get when we use the Fourier to the in fact, the I(hkl) and P(uvw) this Patterson function which is essentially a vector map, it depends upon the vector distance between atom one and atom two. So, if there are two atoms the distance between the atoms is the characteristic of u, v, and w. So, u is equal to $x_1 - x_2$, v is equal to $y_1 - y_2$, w is $z_1 - z_2$. So, the inter atomic distance is the function which goes and becomes the so called Patterson function.

Here there is no phase problem, so we can use the intensities in principle to get to the Patterson function and if we interpret the Patterson function properly then based on the Patterson function because we have all the atoms there, but not as atoms, but as inter atomic vectors. So, there are lots of limitations in this approach, but this particular approach which was developed by Patterson in 1925 can also be utilized under very special circumstances, which we will discuss when we go for the structure determination.

So, essentially Fourier transform is the key and the Fourier transform on F(R) is the one which is required to get to the electron density which we are seeking. So, if you use this

I(hkl) we get P(uvw) then we have to treat P(uvw) in such a way that this eventually reach to ρ(R).

So, how to go from P(uvw) to ρ(R) will be a part of the discussion when we discuss structure determination protocols. So, at this moment therefore, we see that the phases associated with the centrosymmetric systems become all real. So, that the functions become cosine functions only when we do the summation to calculate the structure factor.

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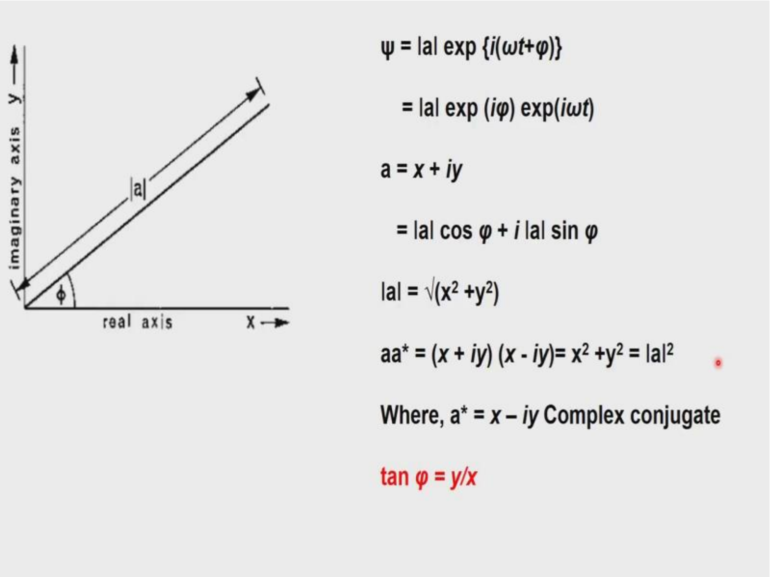

$\psi = |a| \exp\{i(\omega t + \varphi)\}$

$= |a| \exp(i\varphi) \exp(i\omega t)$

$a = x + iy$

$= |a| \cos\varphi + i\,|a| \sin\varphi$

$|a| = \sqrt{(x^2 + y^2)}$

$aa^* = (x + iy)(x - iy) = x^2 + y^2 = |a|^2$

Where, $a^* = x - iy$ Complex conjugate

$\tan\varphi = y/x$

Now let us understand therefore, the structure factor in more detail, because the structure factor is the one which we need and the structure factor is a quantity which depends upon the wave nature of the material. So, the suppose we consider a disturbance the you know it is the electromagnetic wave, which is now related by this expression the $e^{i(\omega t + \varphi)}$. We are going to take $e^{i(\omega t)}$ out this is equivalent to the expression we wrote earlier on in terms of $E_0\, e^{i(\varphi hkl)}$ the φ_{hkl} is the phase that is associated with individual reflections. Modulus of a is nothing but the modulus of F(*hkl*) which we get from the intensity measurements.

The $e^{i(\omega t)}$ we are going to drop completely because the diffraction is assumed to be coherent. There is no change in the frequency. The fact that there is no change in the frequency we remove this. In fact, if we had considered this the possibilities are very immensely interesting. If you had consider this and then involve the expressions for the structure factor calculations what we will get is the behaviour of what is known as

Compton scattering and Compton scattering analysis will give us the momentum densities and so on, but that is not a part of our discussion here. So, we are going to remove this $e^{i(\omega t)}$.

So, what we require is therefore, this quantity "a". So, for a single wave which is coming out from the experiment we have a modulus of "a" which is nothing, but our modulus of F(*hkl*). We will bring in the F(*hkl*) later, but we will understand the mathematics in terms of the disturbance which is associated with φ which you can call as a wave function if you wish.

So, the wave function depends upon the modulus of the amplitude, "a" is the amplitude here, $e^{i\varphi}$, $i\varphi$ represents the phase. So, this is the amplitude, this is the phase, amplitude and phase together give us the wave function. So, therefore, we have a wave which is coming out, this wave of course, can be a constructed based upon the overlap of several other waves as we know depending upon where the atoms are in the unit cell and so on.

So, at this moment for our simple discussion and understanding we will assume that there is a wave single wave which is coming which probably could be the resultant wave of all the waves overlapping in a given direction. So, if we consider that as ψ then we have a * $e^{i\varphi}$ forgetting $e^{i(\omega t)}$ that will be the simplest expression. So, this expression is if you consider this quantity "a", the quantity "a" as we already saw has two components, the cosine component as well as the sine component. So, we expressed therefore, the value of a is equal to x + iy.

The value of x is modulus of 'a' cos φ and the value of y is modulus of 'a' sin φ. So, the imaginary component is up here. So, this therefore, now gives us the definition of the amplitude. So, the amplitude has got a real part and it has also got an imaginary part. So, the real part has a certain modulus of 'a', the same modulus of 'a' is associated with the imaginary part as well and 'φ' is the angle which is shown here.

So, if you take this simple Argand diagram this is known as Argand diagram by the way you all know this. So, it is that this is the real axis and that is the imaginary axis. You have this value of 'a' which is what we would like to determine and that depends upon the value of 'φ'. So, whatever is the value of 'a' depends on the value of 'φ'.

So, in terms of the projections we have written ‘a’ is equal to x + iy. So, if you take the projection of this onto this, this gives you the value of x, the projection of this onto the imaginary axis gives you y, but since it is on the imaginary axis we have a = x + iy and now what we do is this modulus of ‘a’ since it is x + iy we can take it take the square each of these quantities. You remember how we did the intensity calculation?

When we did the intensity calculation this which took it and squared it and that value we took it and squared it and therefore, i became - i and so we had this value, which is not having any imaginary component. So, that is how the intensity developed. So, we if we now, therefore, draw the analogy with that one, we have modulus of ‘a’ is equal to square root of $(x^2 + y^2)$ so; that means, it in mathematics we can write this as $a \cdot a^*$, this a^* is what we call as the complex conjugate, if ‘a’ is x + iy then a^* will be x - iy.

So, the product of $a.a^*$ will give us the modulus of a^2 which is our intensity. So, the intensity therefore, consists of not just the structure factor alone it also consists of the product of the structure factor with its complex conjugate. So, the structure factor with its complex conjugate will give us $(x^2 + y^2)$ which is the modulus of a^2.

So, you also see that if you want to calculate the phase associated with this ‘a’ value then you see that it is very straight forward. Now tan φ = y/x. So, what we have shown try to show here is a simple mathematical way in which we appreciate and understand the ways and analyze them when they are in the Argand diagram. And then see whether we can express the amplitudes in such a way that we have the x component which is the real value component, the y component which is the imaginary value component and then get to the final value of the way in which we can describe the intensity.

So, the intensity is what we measure, but what we want is the ‘a’ value and intensity happens to be $a.a^*$; a^* is the complex conjugate and the angle φ which is the phase angle can be determined by this tan φ = y/x. So, this essentially tells us that we can go ahead now and it study the structure factors with respect to how they develop and how they come up.

So, there is a little more of mathematics coming your way and I will try to make it as simple as possible. So, that everyone in this in this group will appreciate because if this is understood then I think the basics of all diffraction will be understood fully. One once we have a full understanding of it we do not have to depend upon what the pressing of the button on the machine gives because what all you have to do as, as you have many of the pharma industry and probably people who use diffractometers or have realized is that you have to just put the crystal on the diffractometer measure the diffraction that is coming and then analyze the diffraction in terms of what the machine says and press a few times the enter button before you get to the final structure.

So, the background mathematics which is required here, the background understanding of both physics and mathematics. The scattering processes, the diffraction process and all that you have now started to understand fully. And one once you appreciate this I would welcome you to do that and enter operations to determine the structure, but otherwise do not determine the structure yourself because you do not know how it is coming and obviously, you can make many mistakes and those mistakes can be quite costly there are a large number of structures which are published in literature which go with an error and one has to be extremely cautious when you conclude.

For example, particularly pharma industries when they conclude whether it is a conclude cocrystal or a salt they will make enormous mistakes and then this leads to all kinds of legal issues and problems and so on, which the pharma people will face. So, if some pharma people are taking this course then it is addressed to them that this what you have learned today and out what you are going to learn over the last few days and also what you are learning in the next few days is rather looking very complicated, because it has some mathematics.

I am trying to make it mathematics as simple as possible, but you will be able to appreciate why we do this because one once you do that you will not make such mistakes where you cannot do it not only costs a lot of money for your company, it also costs lots of other problems, because you know people take your drugs. For the pharma people it has to be taken with seriousness, but what we call as a structure as cocrystal or a salt or a polymorph all these issues one has to worry about when such a thing happens.