Chemical Crystallograhpy
Prof. Angshuman Roy Choudhury
Department of Chemical Sciences
Indian Institute of Science Education and Research, Mohali

Lecture – 39
Structure Solution using Apex II (Bruker Diffractometer)

Welcome back to the course of Crystallography. In the previous lecture we have discussed about how to go about with a data recorded using a Rigaku diffractometer, it was recorded using the Rigaku X-ray lab mini table top x ray diffractometer and now we will go through the similar process using the Bruker software. Bruker AXS is the other company which provides supplies different variety of diffractometers with different sources and detectors. So, the data collection part is very similar. So, in that also we have about 20 to 30 images that are collected.

So, in those 30 images with different orientation of the crystal, a certain number of reflections could be obtained and from that one can index it and then do a data collection based on the crystal system that is based on the Levy group and then one can collect the data at 100 Kelvin or room temperature depending on the what the need is. So, once again the data collection time on this type of diffractometer with the sealed tube source ranges from 2 to about 12 hours depending on the crystal quality and crystal system that we have.

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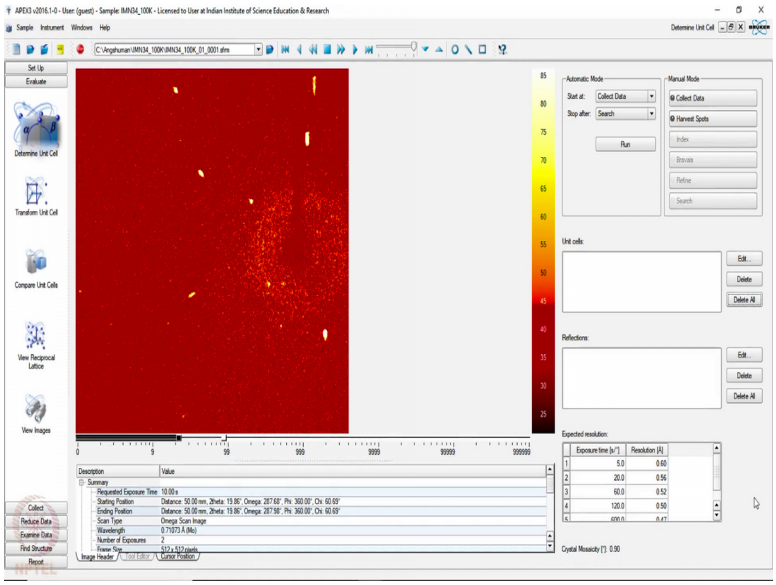

This case we have the software as I have opened is the Apex 3 version of the software. This Apex 3 is again a licensed software with Bruker just like (Refer Time: 01:52) probing licensed with Rigaku. So, if you are user of Bruker or Rigaku then only you can get use get use this packages.

So, here the first point after data collection is to see the data as usual here what we are seeing in this screen the red screen with a with squared type red screen which is the surface of the detector which records the x ray diffraction data as spots. You can see this spots are not really circular they are like streaks. So, they have some physical significance. This dark point is the shadow of the beam stop. What is a beam stop? A beam stop is a metal piece which is placed in front of the x ray source that is beyond the crystal, the direct beam should not go and hit the detector.

So, there is a small piece of lead held by a some, by a holder like this. So, that the direct beam is blocked from the main from the detector. So, now, if we quickly run through the images, you can see that the images are like that. There are different sets and each set has a certain number of reflections and you can see the all the reflections are like streaks instead of spots. So, now the first thing first we would like to see the indexing of this crystal with if possible all the images, but sometimes the number of images is so large that we do not want to process everything.

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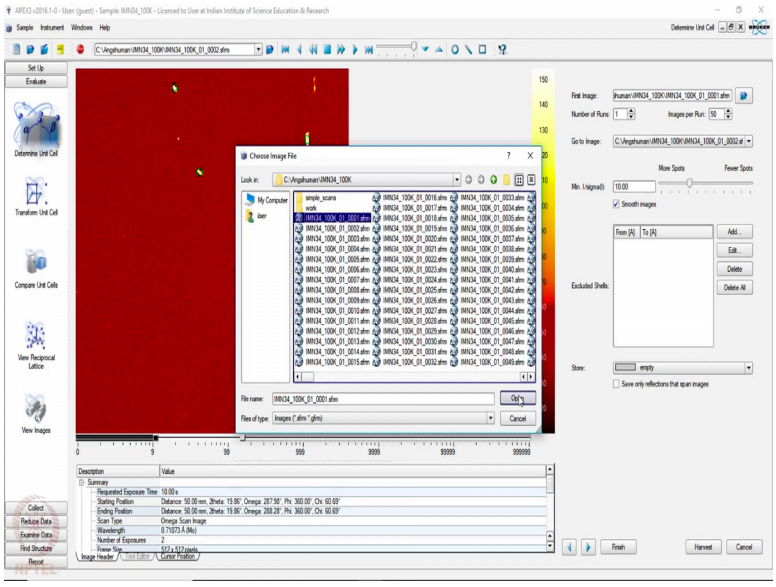

So, then we try to do harvesting of a certain number of frames or images from each run. So, we choose the first image that is 0 0 0 1 SFRM is the first image file here and open it. We have 5 such datasets; 5 such runs. So, we make it 5 and maybe we will go see 150 frames of each set of data each with different orientations.

You can see how what was the crystal details like for example, on the bottom here which where I have showing the pointer, it is a 10 second exposure data, sample to detector distance is 50 millimetre, 2 theta was kept at minus plus 19, omega is changing, phi is fixed and chi is 16.69, which indicates it is a fo4ur circle diffractometer.

So, from one run to another run you can see that the value of phi has changed, 2 theta and omega did not change to start with. And then if we go to a different set set number 3, omega has changed, phi has become 102 and chi is 54.74 and then in case of set 4, you can see that the omega is 279 and here it is 360 and 23 degree chi.

So, you can see here the data is collected in several batches with different values of omega phi and chi and this is possible only when you have a 4 circle diffractometer. So, now, after doing this I am choosing that I by sigma that is mean I by sigma for the reflections to be considered for indexing is 10; that means, we have only considering significantly strong reflections only. And then if we say harvest, but it does is you will see it is going to read all these frames one after another and then collect the set of reflections that are there in these frames.

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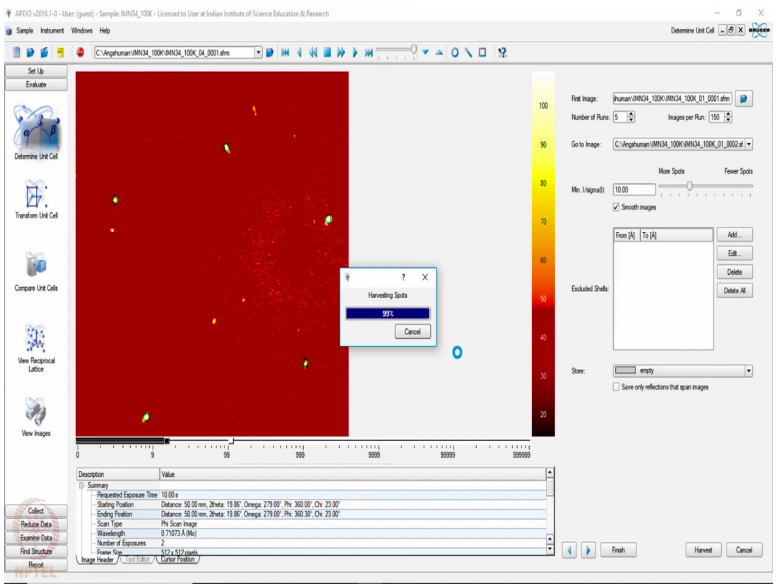

I am showing you this in real time because you also realise that it takes a bit of time to go through all these frames and data, collects information these are all digitally recorded images from which it is recording the positions of its diffracted X-ray beam and collecting the information about the intensity of those diffracted beams as well. So, what has happened is it has found 758 reflections in these 5 set of 150 frames. So, our next job is to do the indexing.

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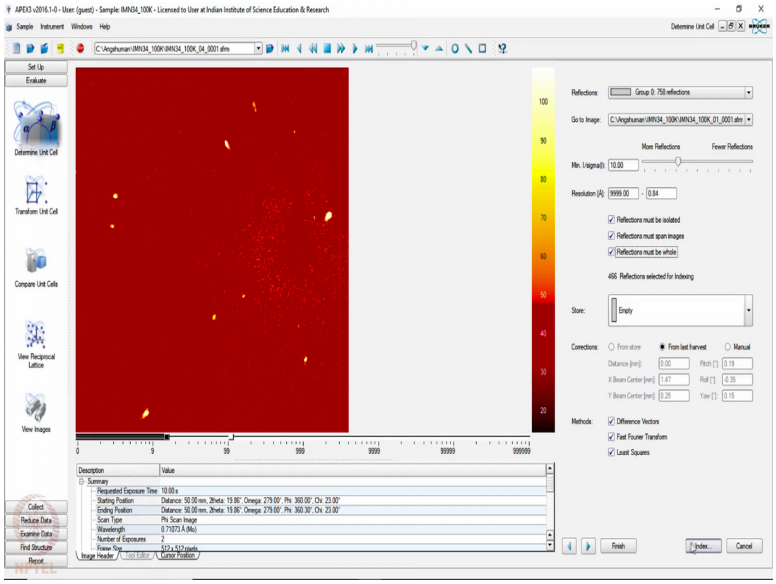

So, in indexing there are 3 different methods that it uses; difference vector, fast Fourier transformation and least squared method. So, we can choose all the 3 methods to see even all of them are giving you the same cell or that giving different cells, what are the percentage of number of reflections that are being indexed in each of those methods and based on that we will decide which is the best cell. Here, we can also apply conditions like reflections must span images; that means, it should not be the case that 1 reflection only appears in 1 frame and reflections must be whole; that means, it should not be partially recorded.

So, immediately the total number reduces to 466 which are now going to be considered for indexing, otherwise without that this was 744.

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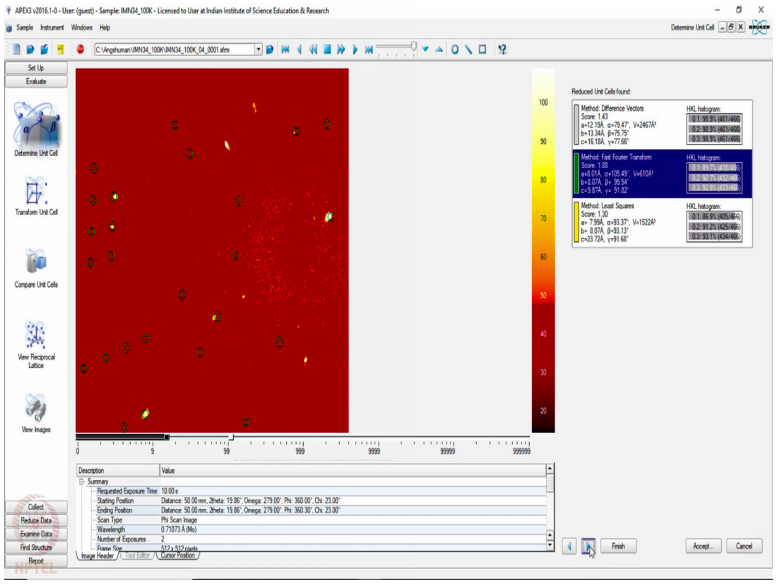

Once again this may take a few minutes and it will show you that there is a score 4.431, 1.88 and let us see what the other on gives. Here, what it shows that what is the percentage of reflections that are being indexed with the HKL being close to integer. So, here clearly the first one although has a lower score has a better percentage of reflections indexed.

And it this last one least square method is still moving on. Sometimes it may not yield a good result and as we see here it is only indexing about 86 percent and giving some cell which is probably not the right cell because you see here this is 600 and that is 2400. So, there is f 1 is 2 4 relationship, but it is not related to the 600 cubic angstrom cell. So, in at the moment the software is suggesting us to take this cell which is marked as blue because it has a higher score. So, let us try to take that unit cell and see what happens.

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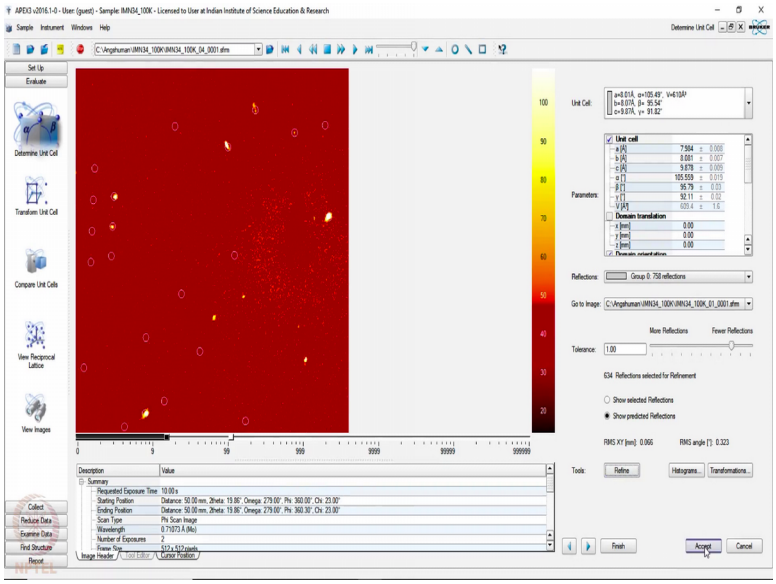

So, now we should refine this particular unit cell and for refining we set the tolerance to 1. So, that it includes a large number of reflections and then click on refine, what it does is then it gives you the corresponding standard deviations on the right hand side. So, you refined a few times to improve the unit cell, maybe it will give you a better standard deviation and then you accept the unit cell.

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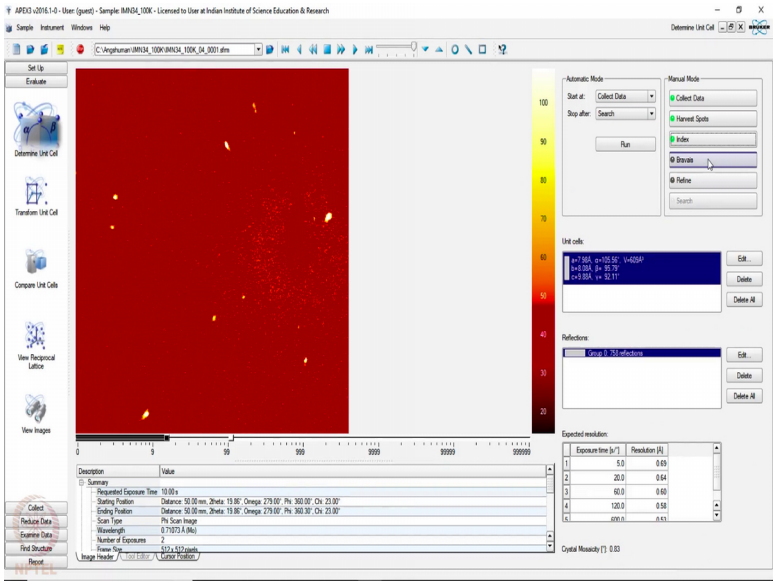

So, next step is to find a suitable Bravais lattice for this unit cell.

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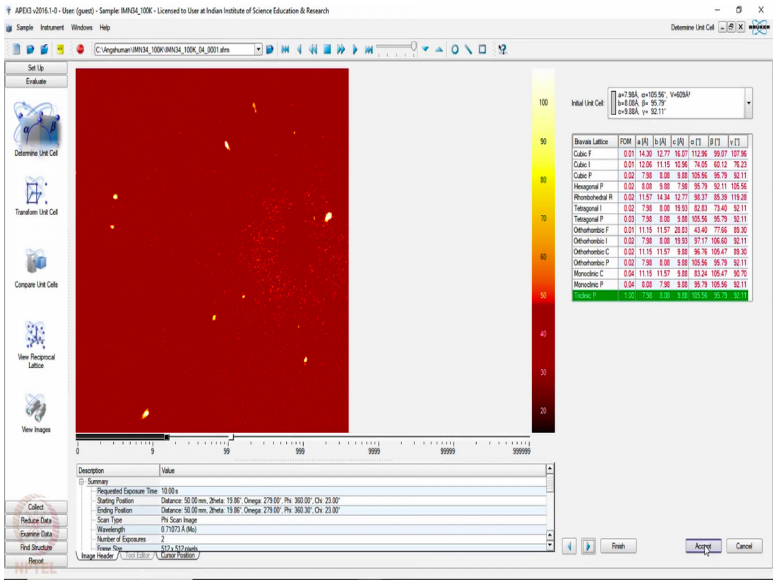

See it does not provide anything else other than a the same triclinic lattice and then we checked it and refine it once again and make that tolerance 1 as usual and then refine.

So, this tolerance 1 make brings in a large number of larger fractions of reflections in this refinement. So, we accept it and then as before as we have seen the in case of free (Refer Time: 11:37) that one can view these reflections in reciprocal lattice.

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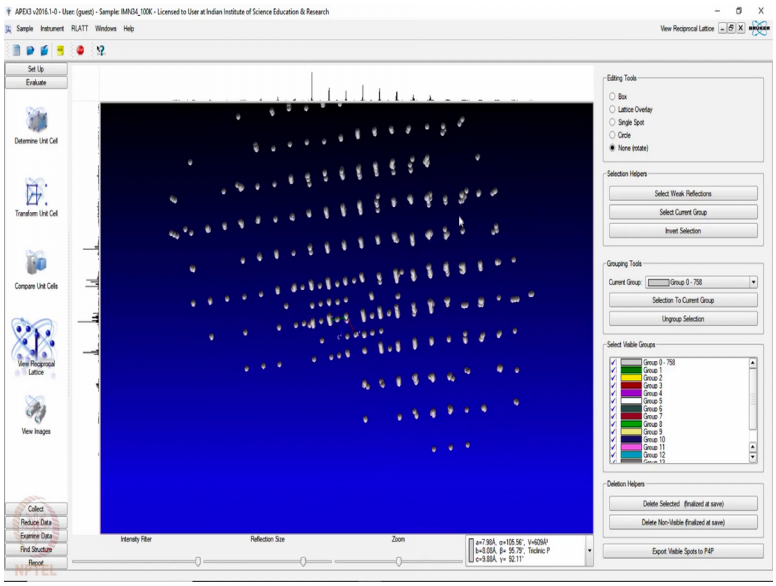

Simultaneously, here also one can view these reflections in reciprocal space. So, what we have seeing here is that the reciprocal lattice is displaying some problems. For example,

there are some reflections which are abruptly coming in the middle which are not the case with the other ones. So, we need to eliminate some of those reflections and try to do the re indexing.

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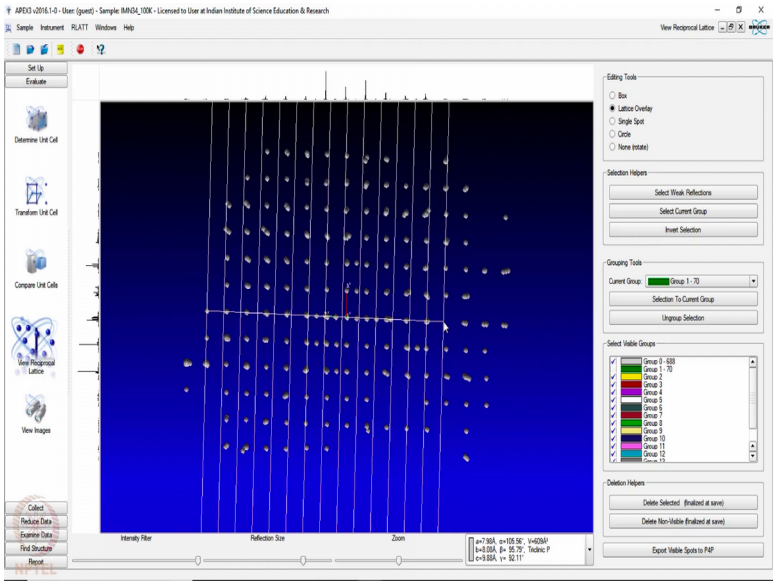

So, I am doing it using the lattice tool. So, we are trying to see the periodicity in this data and the reciprocal space and taking the reflections which are just falling in line as the selected reflections then we just invite the selection and remove the selected once to a different group in green and remove that green set of reflections. Once again we choose the lattice tool, Remember here we do not need to include everything up to very high angle. What we need is we need only the reflections which are falling in a given array.

So, these arrays of reflections are going to be collected and rest are going to be removed.

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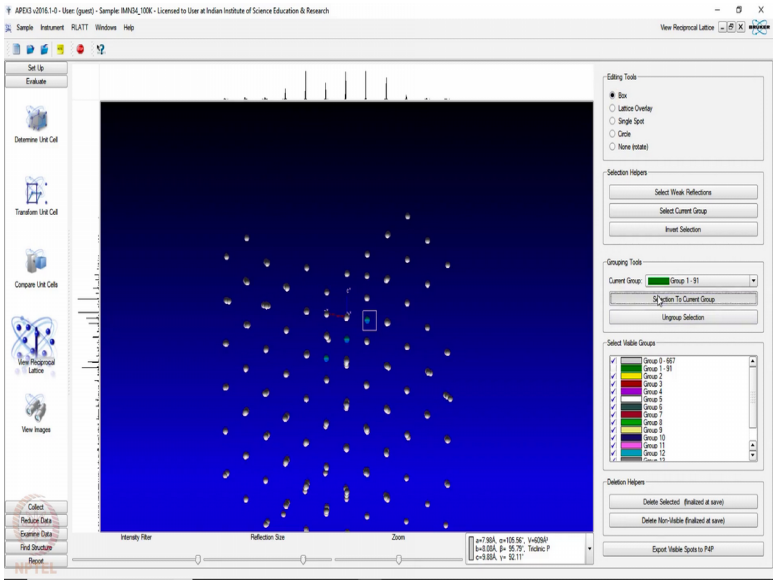

So, suppose this part this part the once which have coming in middle I am trying to remove them. So, now, in a different orientation if we see there maybe some more reflections which could be removed; so, I can choose those reflections specifically and remove them. As you could see there are many such reflections which are not exactly falling in line which maybe indicate you of the fact that the crystal was moving during the data collection.

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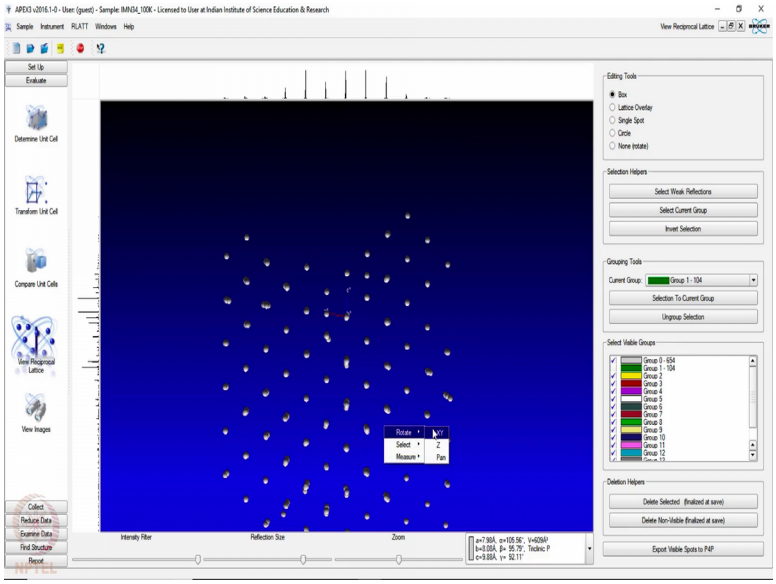

So, now, with these reflections which are in gray, I am going to re index and remove those which are bad. So, now, again we should do a fresh indexing and see what we get out of these modified reflections. So, now, you see in both the cases we are getting similar reflections and the third one has given you 668 305 which is about 94 percent indexed, but here the score is smaller, but it has 96 percent indexed. So, we take that as the unit cell.

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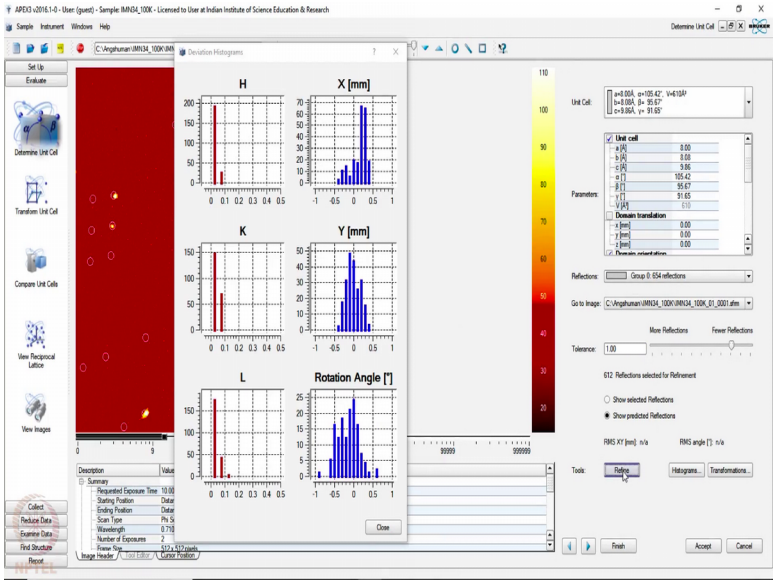

And then refine it.

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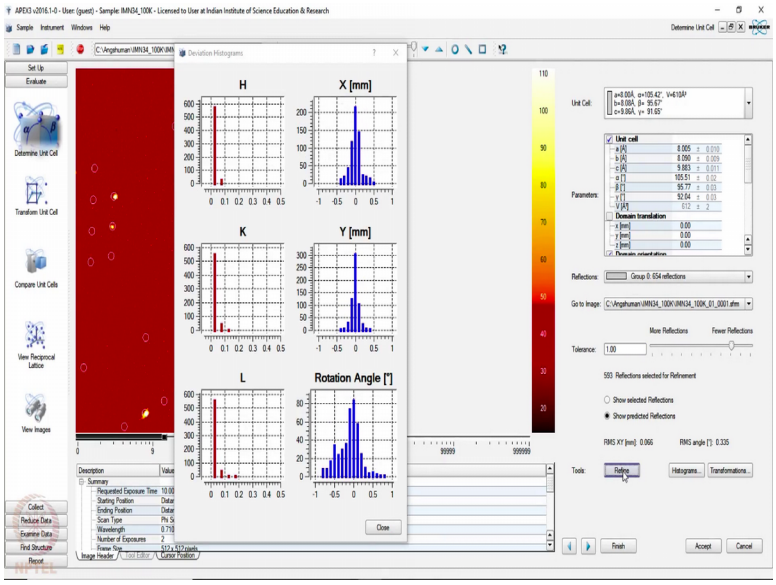

A few times this particular window which is now open it shows that whether the centering was fine. And when we see the rotation angle and it has a spread that indicates the crystal was moving during the data collection. So, we now know the data is not a very good quality data, the crystal was moving. So, we will have to face the consequences and that is why it is immensely important that one does a centering very carefully.

Next your; that if the crystal is not loose or the sample holder is not loose and it is not moving in the beam during data collection. So, now, with this we will try to do the data reduction as usual and you see the data reduction happens in the same folder with a new subfolder generated.

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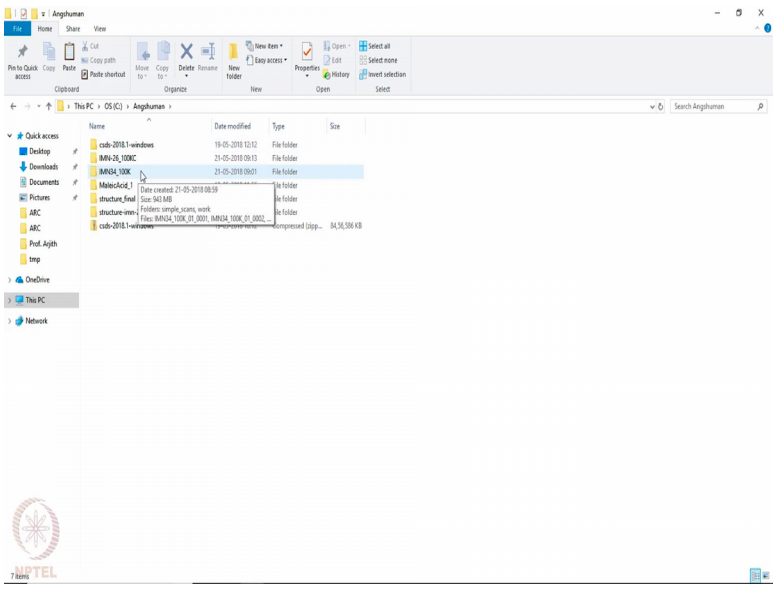

This is the folder in which we are going to do the data reduction. It will generate a new folder called work.

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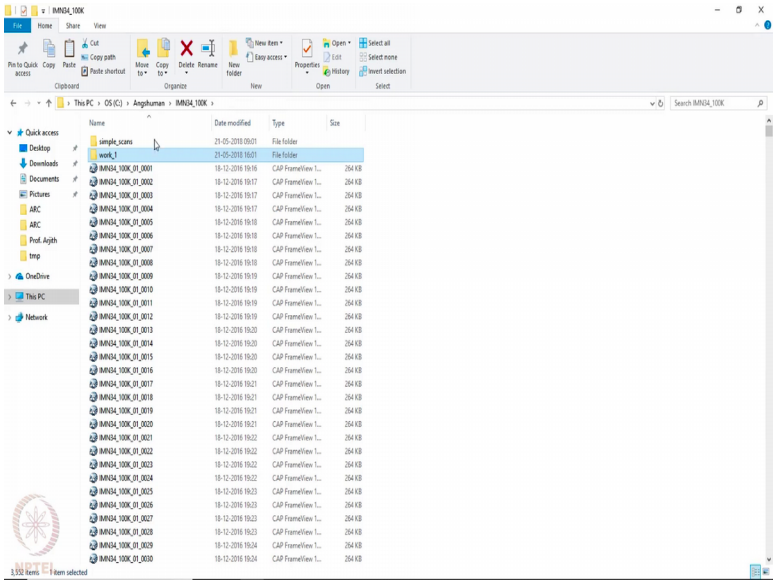

So, the previous work I am renaming as 1 and storing it for future reference.

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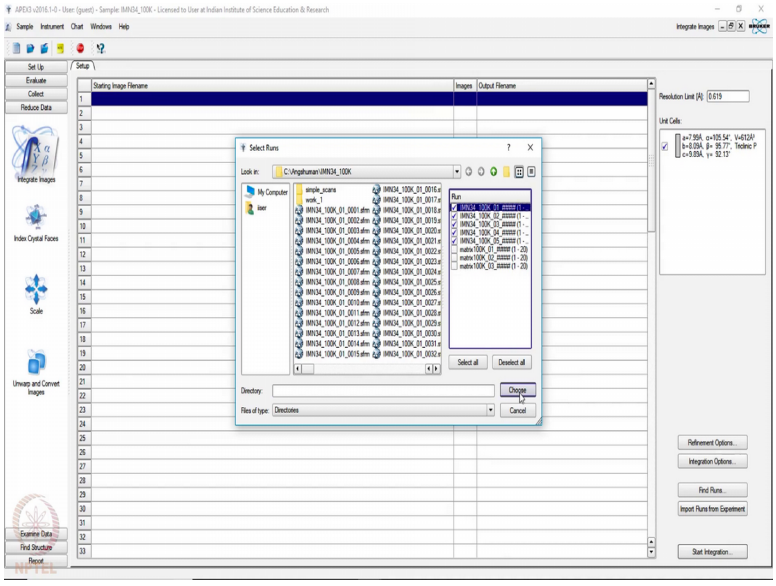

So, here we need to locate the runs those are the runs and here you see there are 20 20 20 total 60 frames which were recorded for the unit cell determination in this case it is 60 frames.

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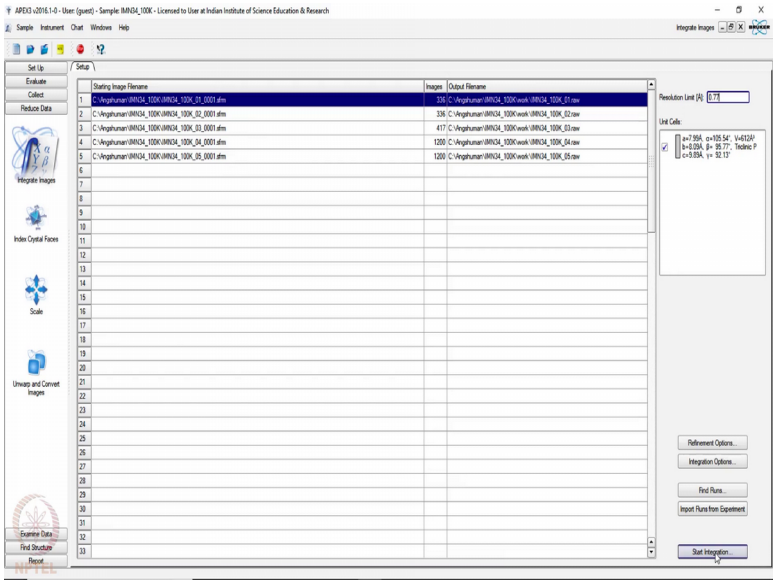

So, now it has total so, many runs, a large number of runs and we restrict ourselves to 0.77 resolution in angstroms.

So, the unit cell will be determined is given here and then we start the integration. Once again like before this integration will take a while.

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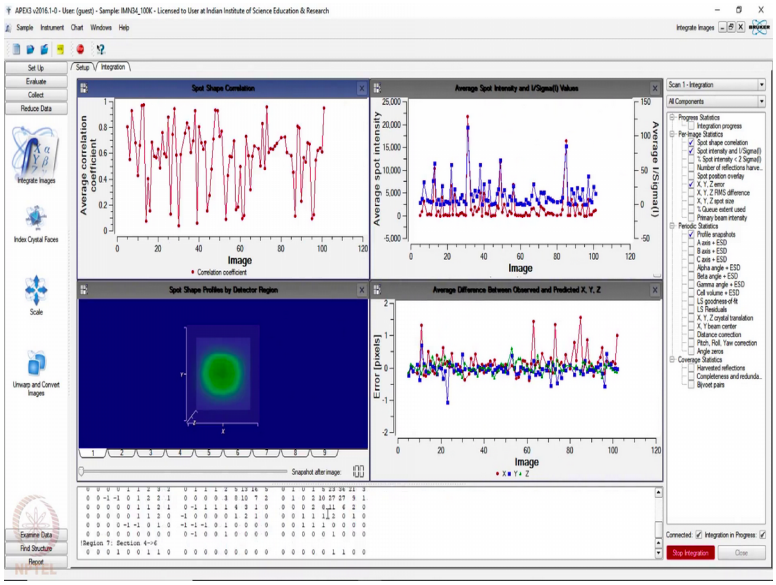

It will go through all the images, generates lots of plots and read the frames for every reflection and write those reflections as raw file. So, this may take a while. See now, we have reached the end of this data reduction program and it shows it says end of global

refinement. So, now we can close these four statistics windows and see what it has given at the end.

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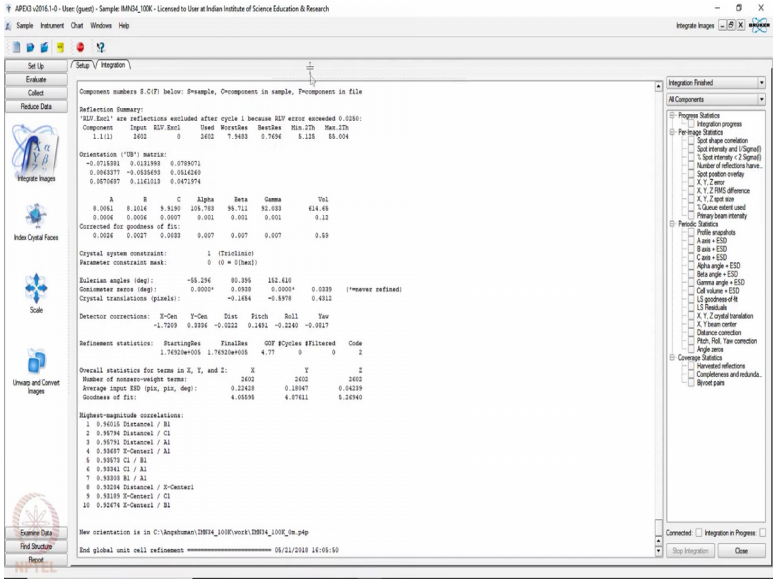

It has it is giving A, B, C, alpha, beta, gamma as usual, triclinic 614 volume and the corresponding yes these and these are the corrected standard deviations.

And from here we can go forward and do the scaling. Scaling is a process as you we discussed where we join these different groups of datasets and then apply all corrections.

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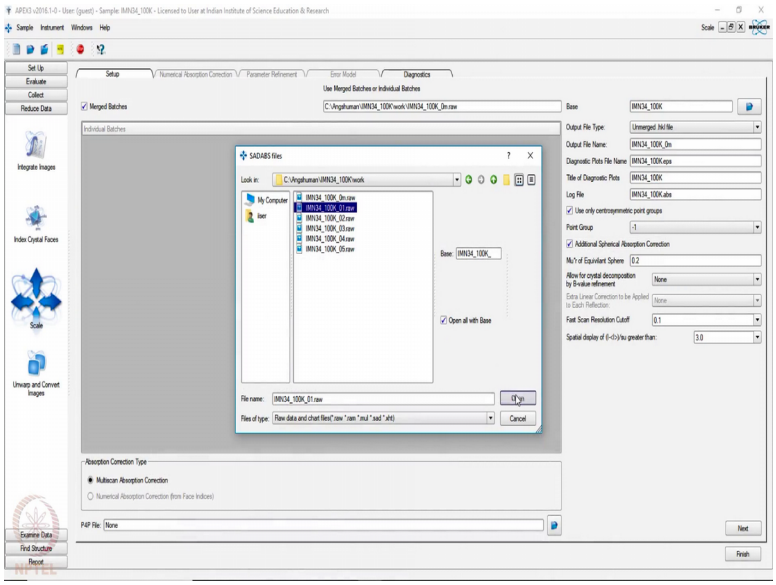

So, these are the raw files which now got generated in the new work folder. You can see I renamed it as work 1 and now it has created a new work folder and inside that it has written all the data reduced data in form of dot raw. So, we have choose 1 and open with all base numbers it opens and we do the standard processing here.

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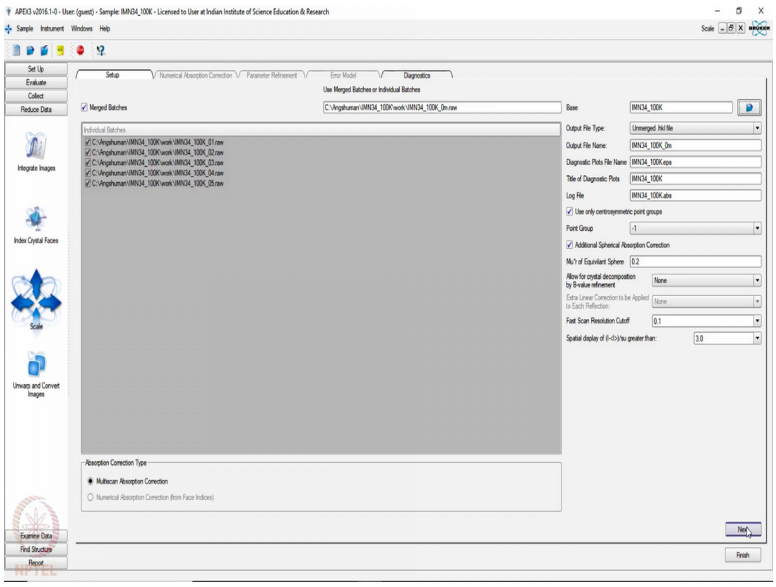

I am going with first in this because these are the one thing which you get training also when you collect data and handle the software; this is just the routine process that we follow.

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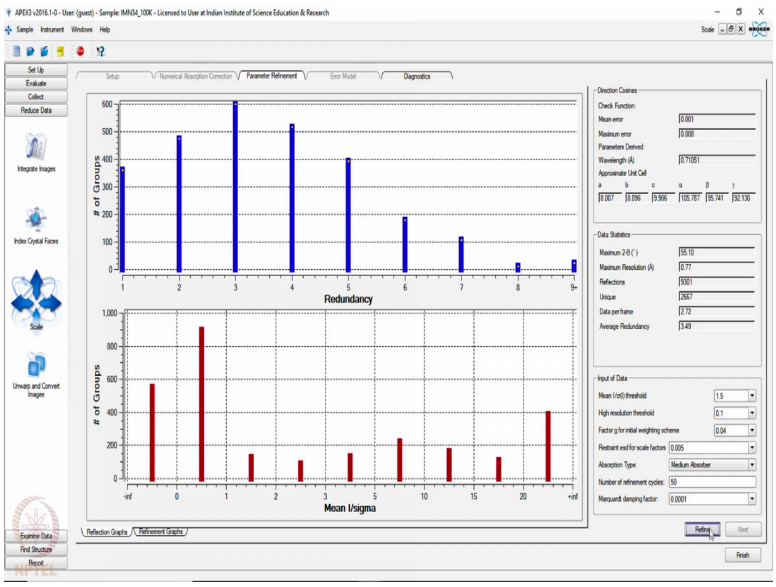

And here it is giving you the number of groups with redundancy, number of groups with mean I by sigma and this statistics shows that this is not a very very good data because the mean I by sigma is in the lower region; means, the number of reflections number of groups having this kind of I by sigma is less and groups with low intensity is quite high.

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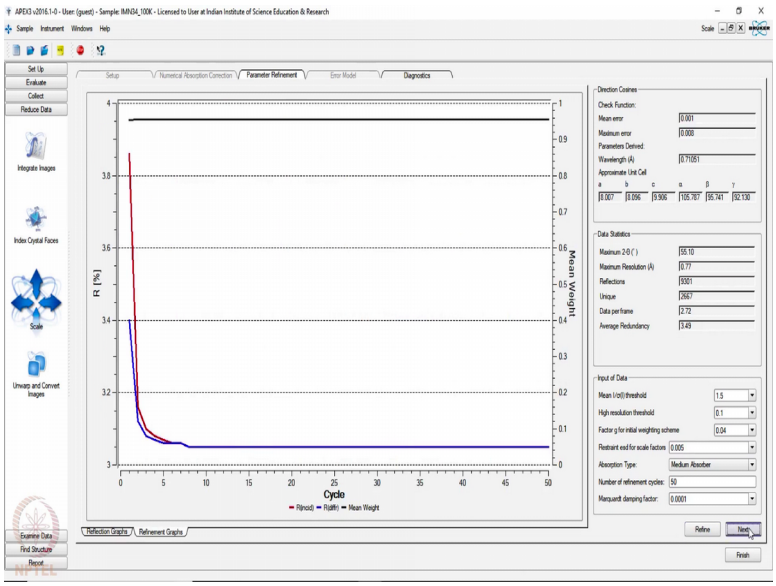

So, then we refine the HKL file.

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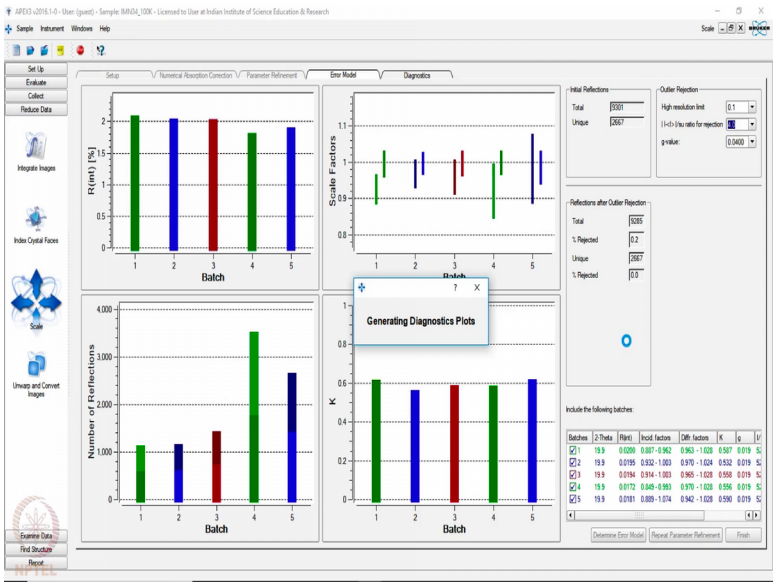

And then we continue, it gives us some more statistics on R int scale factors, number of reflections and the parameter K, which we have discussed before in the class and we see

here the R int is running around 1 to 2 percent with 2 theta value here and for all the different sets.

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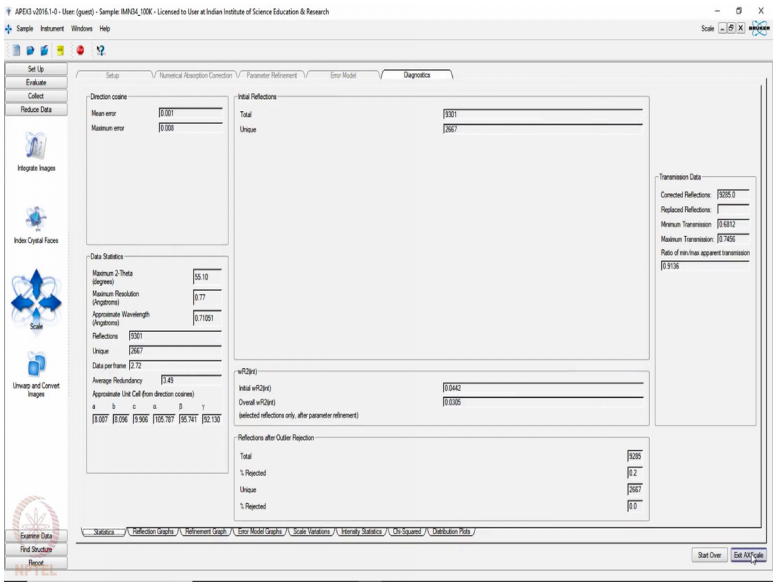

So, if we finish it will generate the corresponding HKL and it will generate the HKL file in the folder. So, we should exit. So, now, the HKL file is there ready for the next part of processing that is the structure that is the space group determination.

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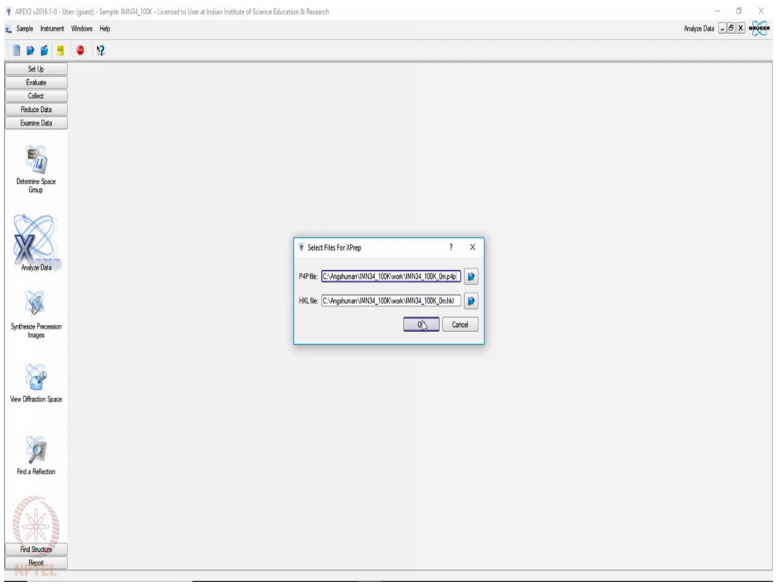

So, here we can do this space group determination using different ways. I am going to use the option XPrep, where it is asking us to input name of P4P file and here the name of HKL file which has been generated in the previous step. We click ok.

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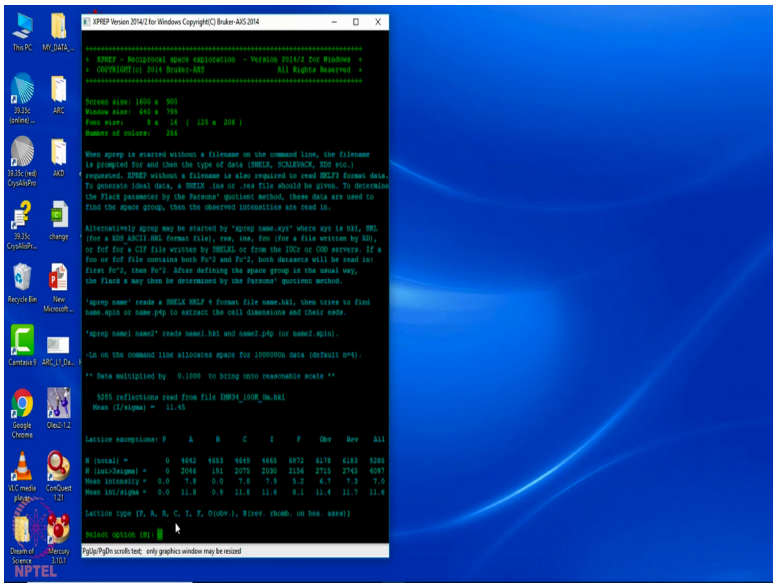

So, immediately it should open the XPrep window, I minimize the back side here and here you can see it is now showing that the systematic absence conditions and here it shows absence for primitive. So, I should write here primitive P and then enter.

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When it says the next option to select is a H; H means, search for higher symmetry if at all and it says that there is no other higher symmetry than other than the triclinic. So, we take that.

And then we go for space group determination. You see in triclinic there are 2 fresh space groups possible; P 1 and P 1 bar. So, it will look for the possibility of centre of inversion only and then it will give you the possible space group and for whatever reason this is unable to sorry it is you have to choose the option A and then I have to choose the option A again, sorry P and then it could gives you the possible space groups and here we can see that P 1 bar has the higher much sorry P1 bar has the lower figure of merit, P 1 has the higher figure of merit.

So, we choose the option B and then we generate the final HKL file. Here its shows you the statistics for the data intensity, statistics for data and it says it is pumped only about 82 percent complete up to 0.77 angstrom.

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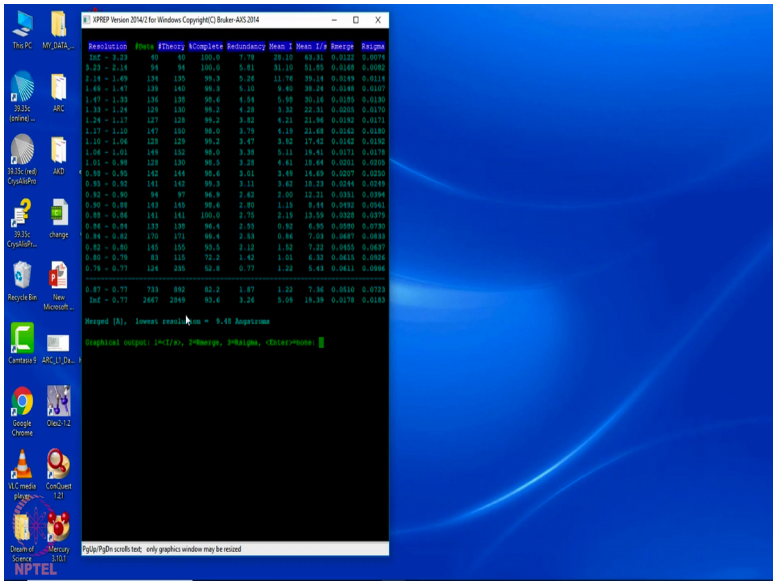

So, we apply high resolution limit to 0.84 and then again this were the statistics, then it is about 98 percent complete.

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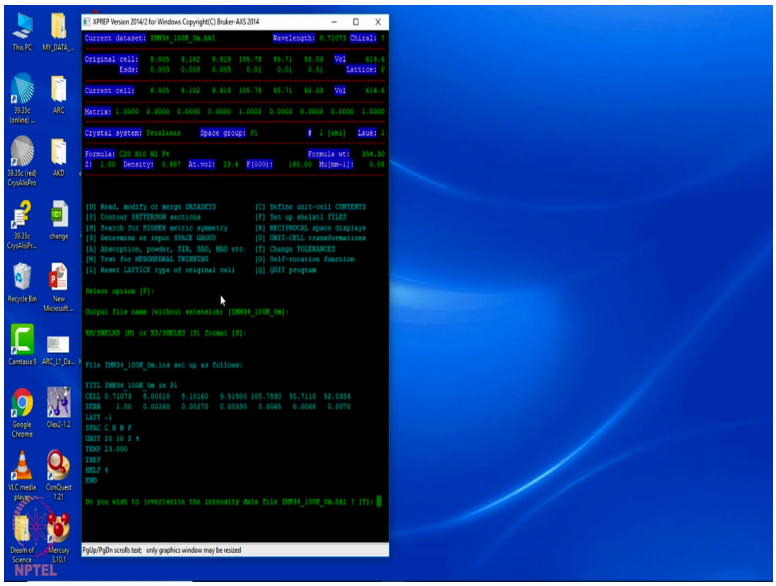

So, now we again need to write down the formula. It immediately calculates the Z and it will write the corresponding new HKL and INS files as usual and we will exit the program.

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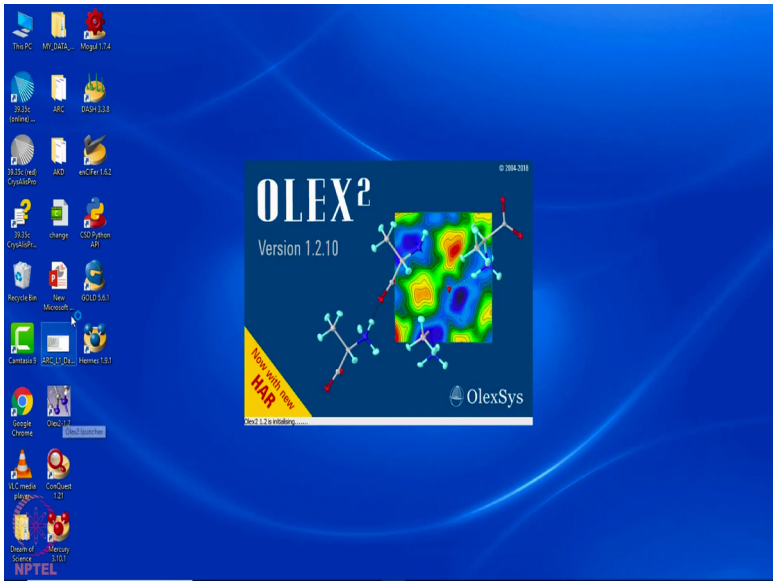

So, now using Olex 2 once again on the HKL and INS file, we should be able to solve and refine the structure. As I indicated in the previous lecture, Olex 2 is a come is a switch can which allows you to do a lot of things, it allows you to use ShelX l packages or structure solution and refinement.

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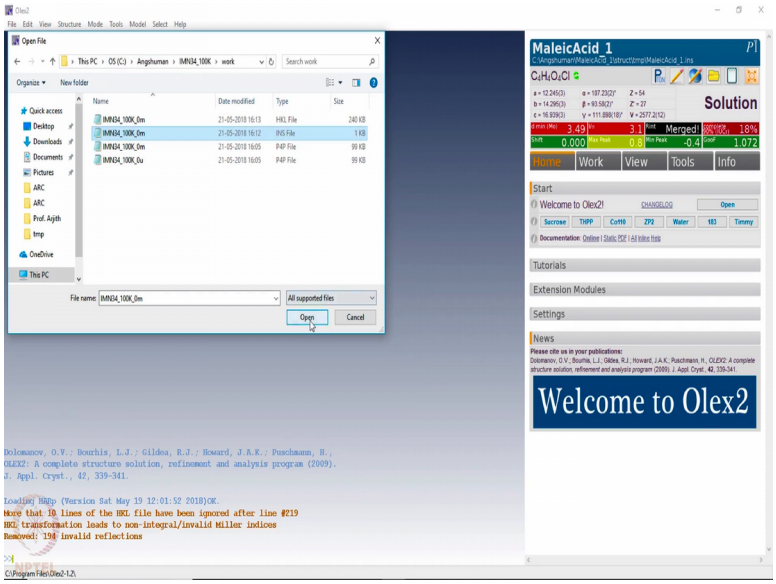

So, here first we need to load our data from the corresponding work folder, the INS file should be selected and then we open.

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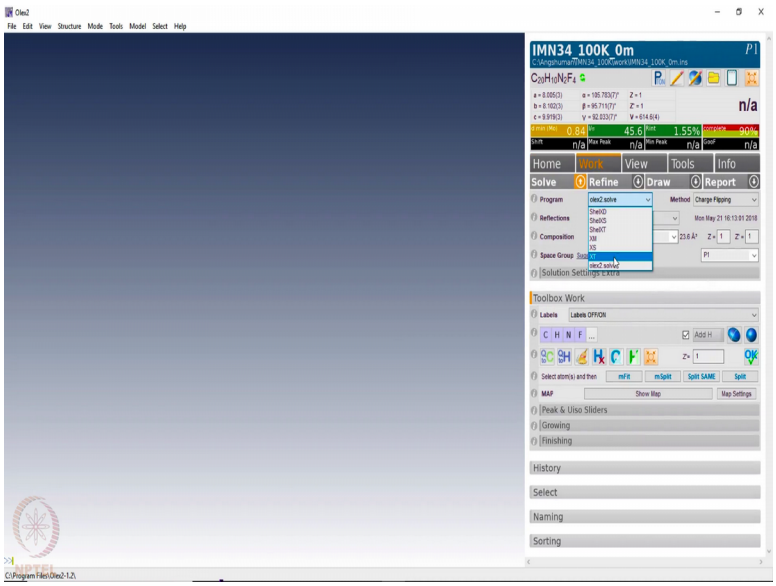

Go to the work folder and then click the solve button down just like before, this you should be familiar with because you may be collecting data somewhere else and one person giving you the data, giving you the just the HKL and INS file and you should be able to solve it from there.

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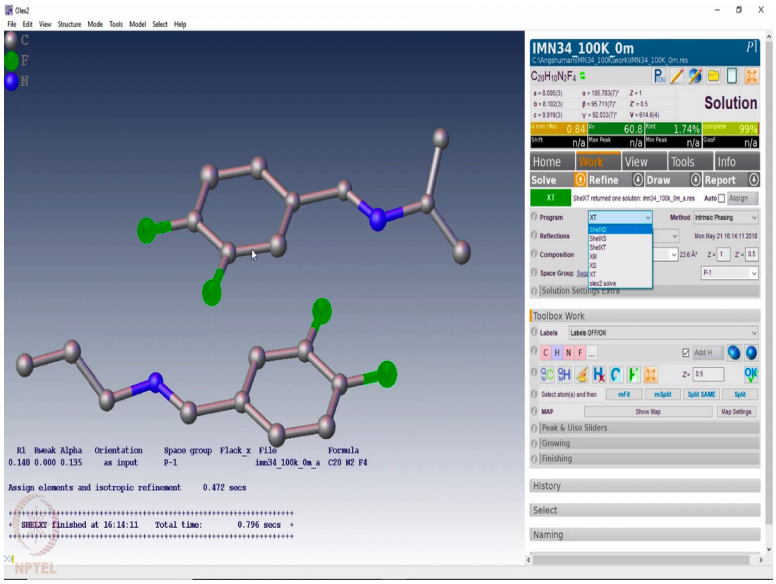

So, what we have here? A 2 half parts and you see what I have done now. I have used XT. When I use XT, it is little smarter than XS where it can identify the atoms by carbon hydrogen nitrogen and oxygen. If you remember, in the previous class we used the option XS or ShelXS, it was not able to identify the atoms, it had thrown the atoms as brown circuits or brown spheres.

So, with my chemical knowledge I believe that the atom identifications are right. Here, you see that one molecule is half in such a way that there is a centre of inversion here it generates the other half and here also there is a centre of inversion here we generates the other half of the molecule.

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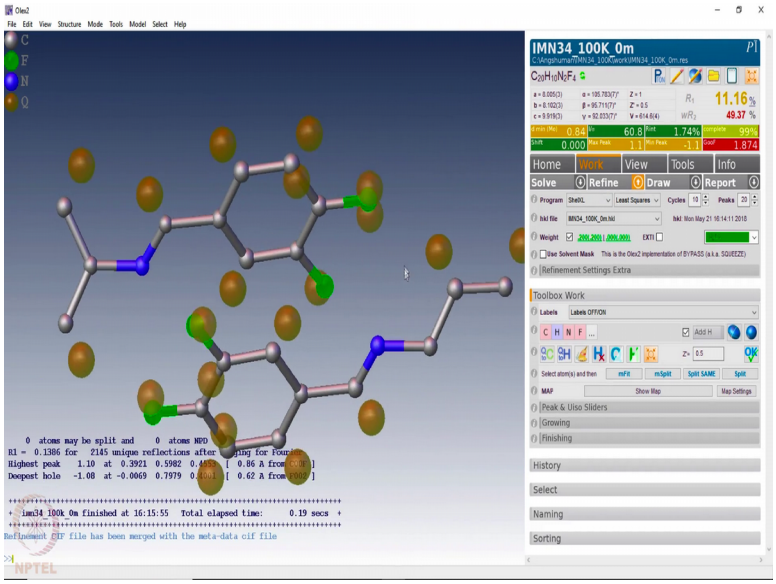

So, now if we do the refinement, we ShelXL and least squares and as I indicated we should do it at least about 10 times and do it with the information acta. So, it will generate the C file in acta crystallographica format. So, if we refine it a few times we will see that the ones which are coming as red should become green which means, the refinement is converging. Once the refinement has converged, then everything will be green. Converged means, on further refinement no parameter is changing significantly and hence we have reached a level of convergence.

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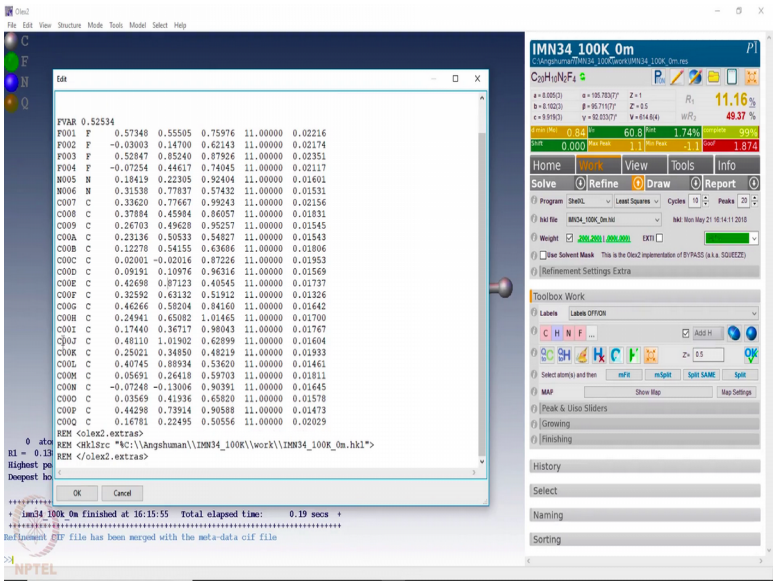

Now, as usual if we try to see the atomic coordinates, so, here you have the fractional coordinates and here you have the occupancies and the thermal parameters. So, now, you see the atoms are not numbered appropriately, one has to do the numbering proper.

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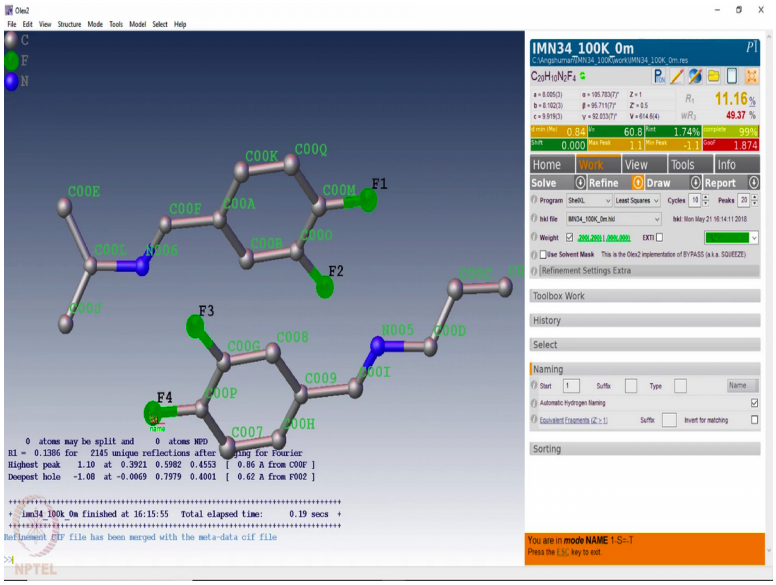

So, we should go for naming and then you start naming them as F 1, F 2, F 3 and F 4 and then we name the carbon skeleton sorry I should have done it in the in opposite direction. So, that the fluorene get the lowest numbers carbon atoms and then number these 2 carbons as well. Similarly, in this ring we should do it (Refer Time: 28:13)

And then again number the nitrogen atoms and that is it. So, now, if we see we will see that the atoms are numbered properly. So, then one can do a sorting. If you just sort in terms of part, Z, level and that is it, it has sorted. So, after sorting you can see that it is numbered C 1, 2, 3, 4 to 1920. So, now, we again go back to refinement toolbox and then refine it anisotropically because till now it is refined isotropicallly. So, we have only spherical thermal parameters. So, once again after anisotropic refinement you can see the atoms have generated ellipsoids instead of spheres.

And these are not converged yet. So, we do a few more cycles of refinement till we reach a state where all these parameters have reached a convergence. Since, we see that it is not converging and also the refinement is not complete, let us now add the hydrogen's. You can keep an eye on the R factor when it is being refined stepwise. The R factor has

reduced now from about 8 percent to 4.6 percent before and after hydrogen fixing. So, now, things have improved, these all are green, the goodness of it is very good 1.06.

And all the residual electron density is are appearing close to the bonds which is the correct representation.

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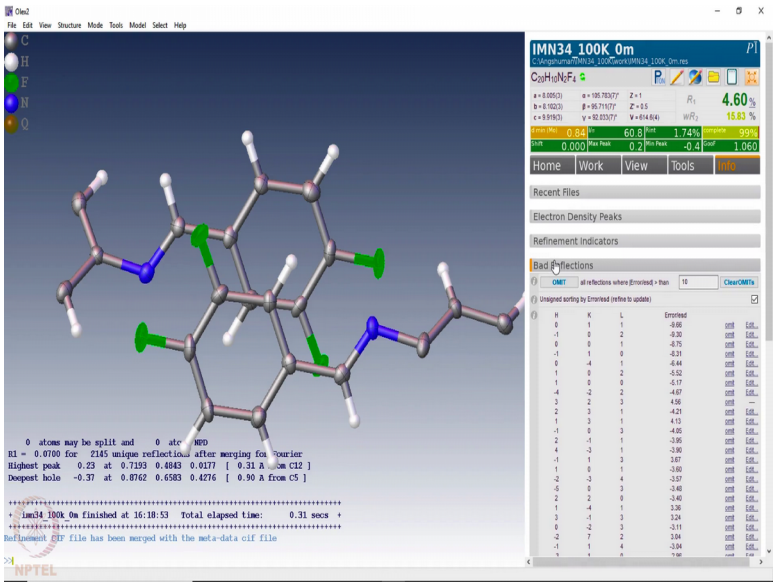

So, now let us see as before go to the information and see if there are some bad reflections, yes there are some because see here the numbers are above 5, there are few of them. So, we remove reflections which has higher error for standard deviation which higher than higher than 5 and then go back and refine a few cycles. The R factor has not reduced a lot. We should do a few more cycles to convergence.

See there are some more which can be omitted. These omit does not mean that we are removing the reflections from the HKL file and it means that we are not considering those reflections for further refinement cycles. So, now, there is one refresh button here. So, if we refresh it then calculates the total molecular formula for this particular system, correct and then you do a few more cycles of refinement and then you reach the convergence.

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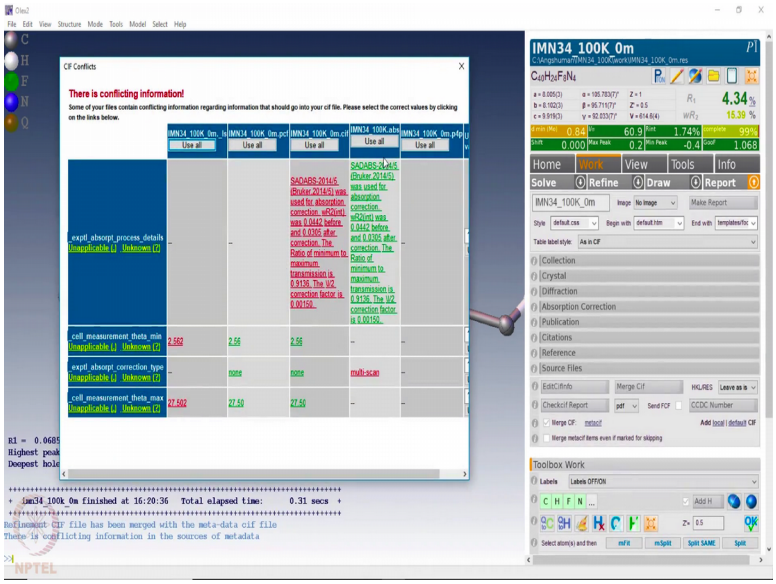

And from here we go down and do the report generation.

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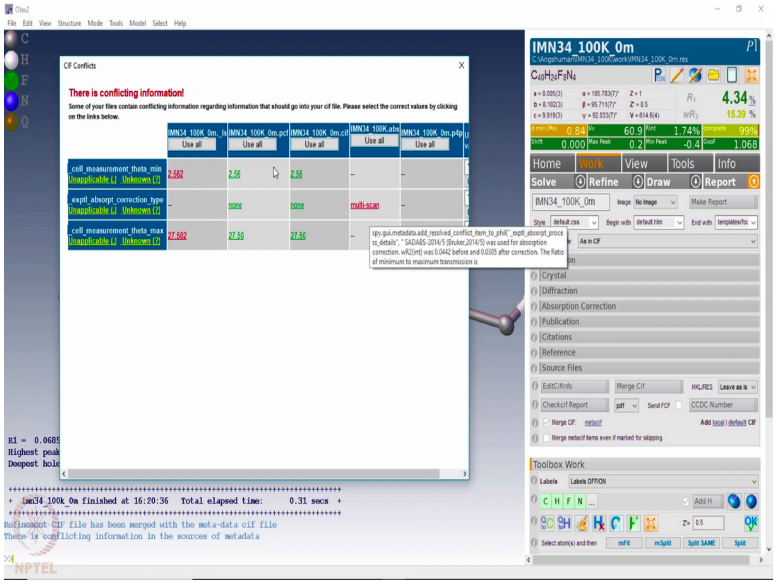

We take the ones which are green.

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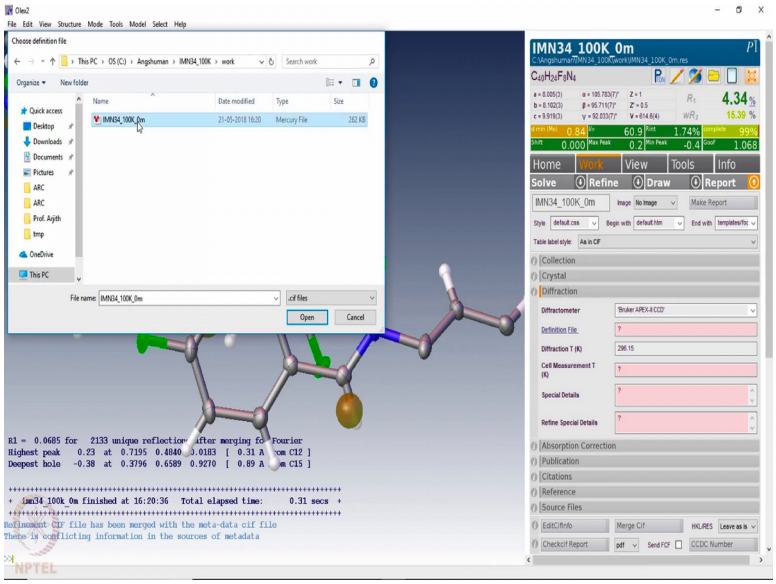

And then these are all different parameters which we should incorporate.

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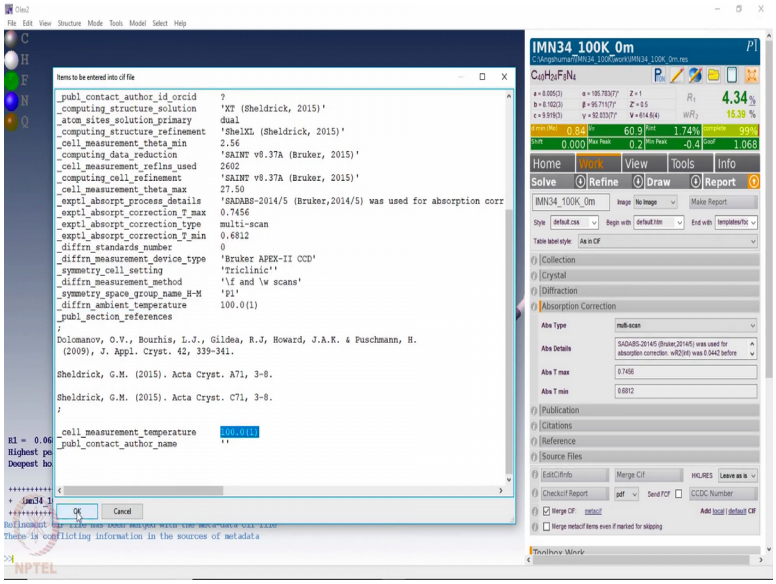

This is a 100 K data. So, we should incorporate that as well and the corresponding standard deviation should be incorporated. Then the information for absorption correction is there which we do is a multi scan absorption correction; T min T max is there and then we see the data correction file as usual as I have shown you before. This also generates the CIF with the information about the software that was used for this process and the corresponding temperature that we have incorporated.

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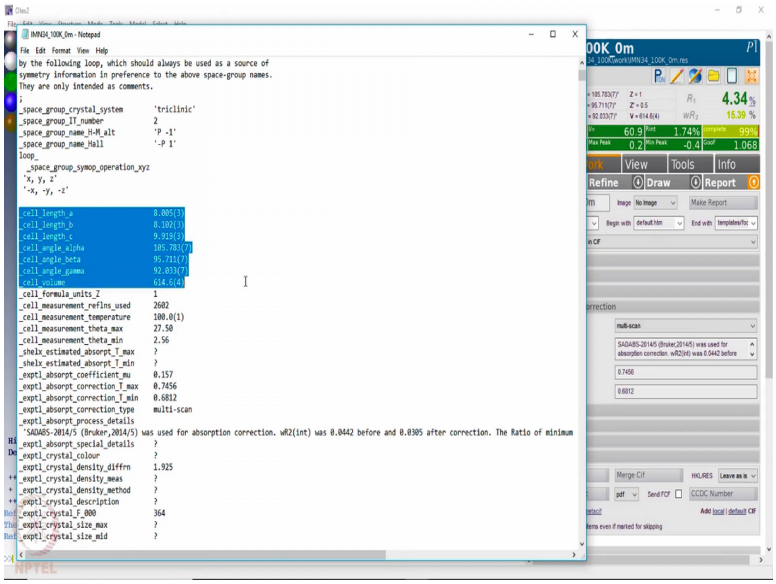

Then we merge this data collection schedules data, collection details with the structure solved in the previous steps. Here again you could see the unit cell parameters, volume then you have the number of reflection used for indexing and all the structural parameters that were generated during the data reduction process and then the information about the software and then all the information about the structural refinement and finally, at the end you have the atom coordinates, fractional coordinates with the isotropic thermal parameters.

Here, you have the data for anisotropic thermal parameter, then you have all the bond angles sorry bond lengths bond angles here and then the corresponding final REX file is embedded here along with the HKL files which is also pasted at the bottom of the CIF for checking purpose.

So, when we have all these data we click along with FCF, which send the information to IUCR. So, for that we need to connect to internet and then send the data. So, once we send there is a sending confirmation and in a while it should revert back with the report. If it does not revert then we should again go to the website of IUCR, the check CIF page and then submit the required CIF file.

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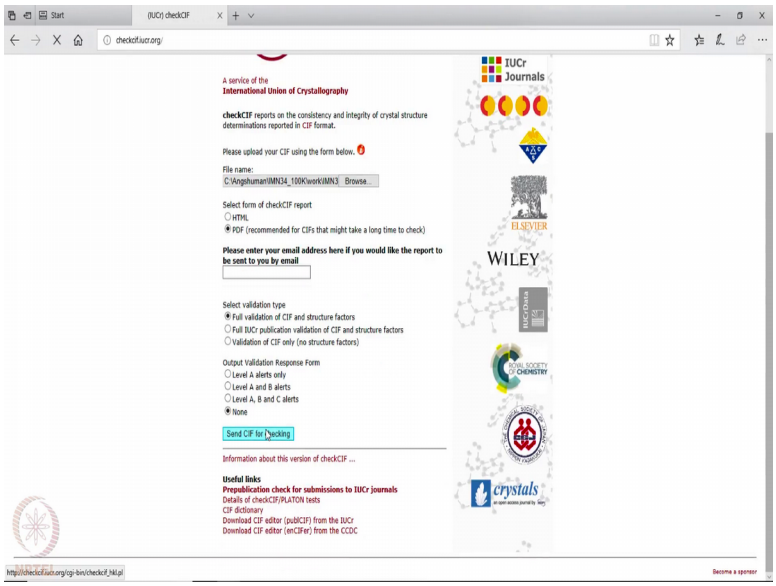

And then generate the pdf, send it for validation as usual it immediately checks for the validity.

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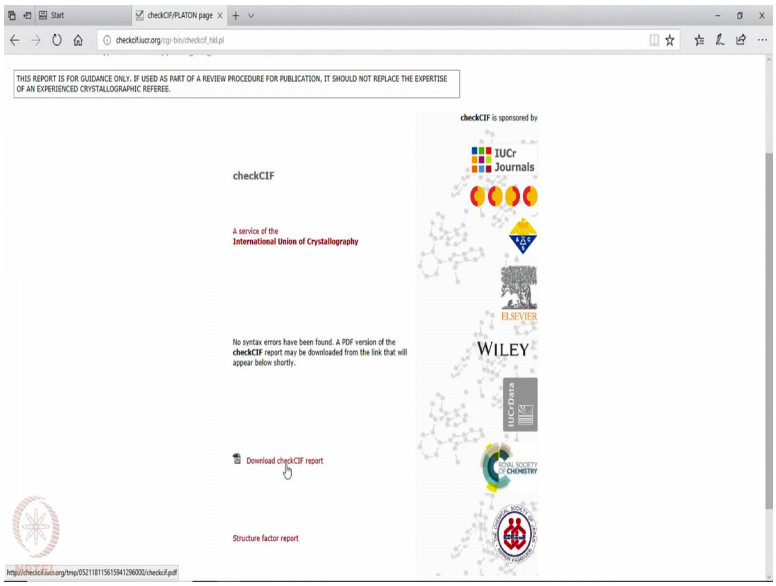

And it will give you the pdf file which will contain the report of this validation process and the validated data is now here.

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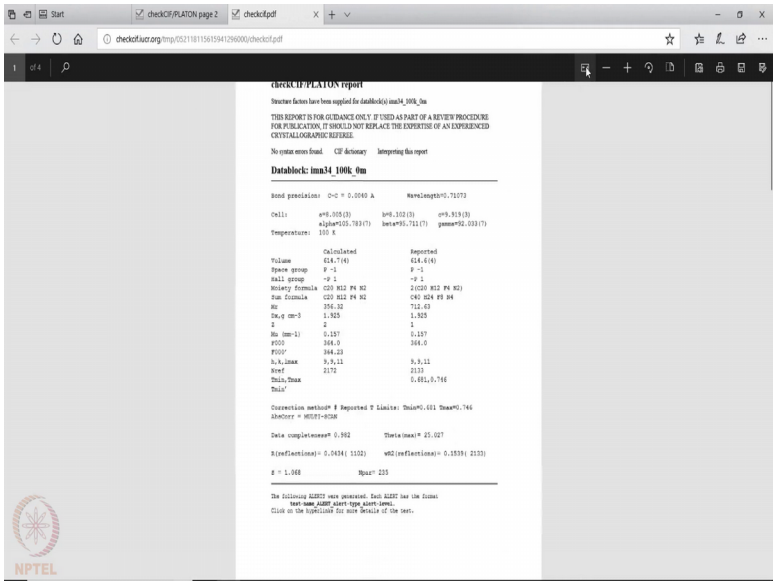

So, it shows that the bond precision is in the range, the wavelength that is used is incorporated. Here it gives you the a, b, c, alpha, beta, gamma, the temperature and all other parameters has calculated and reported.

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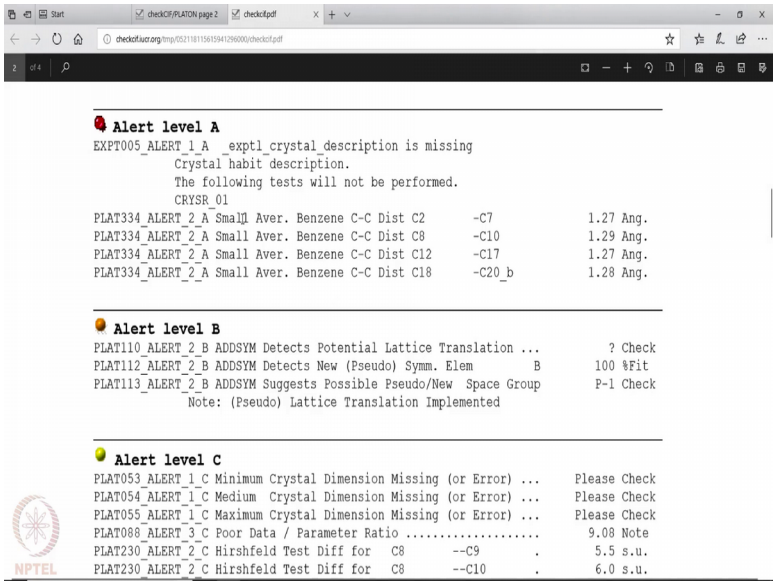

And here there are some A level alert, which one of them says crystal experimental description is missing that means, we have not indicated the size and shape of the crystal. So, that gives you an alert and here it says small average benzene C C distance. So, the average benzene C C distance in benzene is on the lower side.

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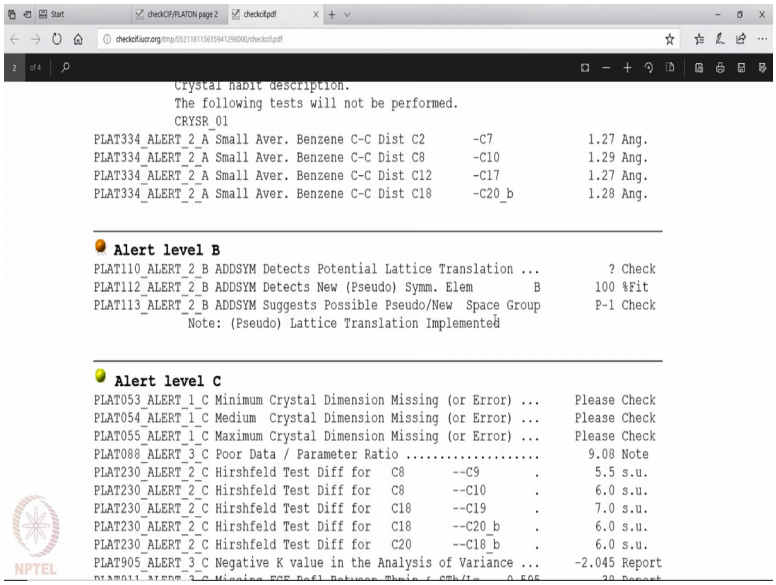

So, that there is one alert for that and the symmetry that we did for structure solution is P 1 bar. Sorry we have done it in P 1 and it is suggesting that there may be possibility of P 1 bar space group. So, this we need to check. So, this is how one can see if there is something wrong done during the process of space group determination.

So, here it is an indicative that probably we have solved the structure in a wrong space group and instead of P 1 it should have been P 1 bar. So, by looking at this validation report, one can go back and re select the correct space group and come back and do the solution once again and then if one would see that this space group problem is then avoided.

So, with this we could conclude today's this lecture that we have tried to manipulate or you have tried to solve a data collected on a Bruker diffractometer using their software and then followed by Olex 2 which allows us to use different models of SheleX as we have seen.

And here in case of this particular data, it was a wrong space group determination which leads us to a situation like this. So, we understand now that the importance of space group selection during the process of data processing and if we have chosen some wrong space group that wrong space group will be indicated at the data validation step as well.