One and Two-Dimensional NMR Spectroscopy for Chemists
Prof. N. Suryaprakash
NMR Research Centre
Indian Institute of Science – Bangalore

Lecture – 50
Two - Dimensional NMR

Welcome back in the last class we started discussion about two dimensional NMR where I briefed you about the need for a two dimensional NMR and limitations that are present in the 1D NMR; and I explained what happens if I have a complex molecule with a complex spectra why 1D is difficult to analyse. I do not think it is impossible, but it is difficult to analyse. What is the resolution problem that you are going to get and simultaneous detection of two nuclei and forbidden transition like multiple quantum, etc that cannot be detected. Several limitations were there. Then I showed what is the need to do a Two-dimensional NMR.

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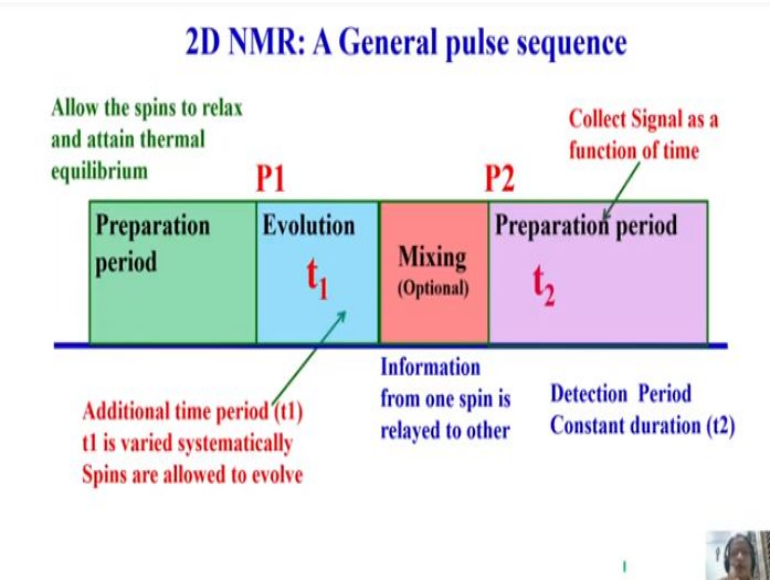

So, this is the reason why we have to start using 2D NMR. So this is general pulse sequence, in fact, in the last class I showed you. A 1D NMR is the one, where we apply the radio frequency pulse, bring the magnetisation to the xy plane, start collecting the time domain signal; and do the Fourier transformation. Single time domain data; single Fourier transformation gives you single frequency domain spectrum, it is the one dimensional NMR.

The 1D NMR you get the chemical shift information on the x axis and the intensity on the y axis. Of course, we are not very much worried about the intensity at the moment. We are worried about only time period to discuss the dimensionality. So, one dimensional NMR is

simply remember, it is because of one-time period and you do single Fourier transformation and you get a single frequency domain spectrum.

Now coming back to the two-dimensional NMR, it is a general pulse sequence I have written. Any two-dimensional NMR for that matter, any two-dimensional NMR consists of basically 4 time periods like this. It has 4 time periods. So, what is happening here? Look at it this 4-time periods has been divided into preparation period, evolution period, mixing period and preparation, I am sorry detection period, it is a mistake it is a detection period okay.

Okay so this is detection period; these are the 4 periods that any 2-dimensional sequence will have. What is happening in each of the pulse blocks? First in the preparation period what happens is we allow the spins to relax and attain thermal equilibrium. So, we prepare the spin system for doing a specific experiment; we allow the spins to relax here and attain thermal equilibrium and do whatever you want for the next pulse to be applied and what information you want to do get, in the pulse sequence.

So correspondingly so we will prepare the spin system. basically in most of the cases, the preparation period is for allowing the spins to relax and attain thermal equilibrium. Of course, P1 and P2 are the pulses we apply for this and this time, let us not worry about it, we discuss only the time periods. After the preparation period you will apply a radio frequency pulse and bring the magnetisation to xy plane. Here the spin starts evolving; evolution takes place like we talked about human life evolution. The magnetisation starts evolving and evolution depending upon the information content that is already present here; for example it can evolve due to chemical shifts, it can be due to relaxation; so various information content is there and it starts evolving here. And what we try to do here is this time period is additional compared to normal one dimensional experiment. One dimensional experiment only this part we have a pulse here and collect the signal and do the Fourier transformation.

But we have extra period here. This period is not constant unlike detection period which is constant. This evolution period is not constant and this additional period is systematically varied such that spins are allowed to evolve as a function of t1 period. So, this is an important point remember this is not a constant period. And the next period is what is called mixing period. This period is optional, the third period is mixing period.

We can have it or we need not have; it is our choice depending upon the type of experiment we are designing. In this case if at all it is present also; what happens is the information from one spin is related to other also in this case. Of course same thing happens here also. But here this is another thing where in the mixing period the information from one spin is related to other here; that is the role of the mixing period.

The last is the detection period. Of course we apply a detection pulse of varied angle. For example, in the case of DEPT experiment we have a different flip angles, like that. So we can have different pulse sequences; it can be 90-degree pulse; 45-degree pulse whatever it is; finally it is the detection pulse we apply and start collecting free induction decay.

And signal is collected as a function of time and we do the Fourier transformation here. And this detection period is a constant duration; here t2 is constant, we are not varying t2. Only we are varying t1. Understand, there are two time periods one is a constant period; and other is a variable period which is constantly varied systematically.

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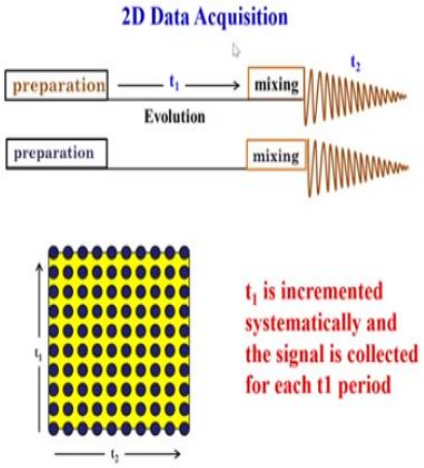

How we would acquire a data? For example, when I said t1 is systematically varied how do I vary t1 here? how do we acquire data? And diagrammatically it is shown here now, we have preparation, this is the evolution, mixing and collection of the FID; we start acquiring FID. How we increment t1 initially, let us say, there is no delay, t1 is 0. Absolutely no delay at all; Now we give a small time period here; t1 is put and here FID is collected; signal is collected here. Now as a consequence, there was no delay, now there is a delay and the gap increases.

Now we add some more delay, increment the delay; like initially it is a 5 micro second, next we have 7 micro second, 8 micro second like that. What is happening? every time I am increasing the time delay t1. But notice one thing, this is constant. I am not incrementing this; only this period incremented. We keep on incrementing like this. Now we completely acquire the signal in two dimensions in t1 dimension also we acquired signal, this is one dimension.

This is two dimensions, where we have a data acquired which is the constant period of time; but systematically we varied t1 period collected signal here and also collected it. This is basically what we do generally in most of the 2D sequence, okay?

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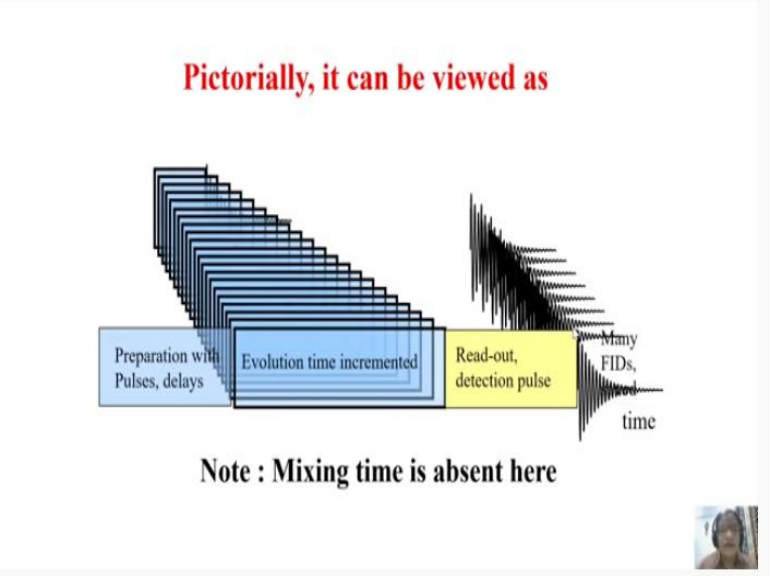

If you see pictorially like this. Look initially there was a small time period in the evolution which, keeps on getting incremented, small increase; small increase, small increase, … that is what I showed. Finally we have whatever the time period we wanted; so it keeps on increasing; and we have the mixing pulse. After the mixing time, we have read out pulse; and start collecting FID. See here this is getting changed, but here this is always constant; t2 is a constant.

In this particular example where read out pulse is 90 degree pulse; that itself we can consider as a mixing pulse and also as a detection pulse, no problem. But here the mixing pulse is absent you apply only 90 degree pulse and collect the signal. This is similar to the pulse sequence what you see in the coming slides today called a COSY. It is similar to COSY. You understand? This is pictorially how we can see the two-dimensional time domain data. So

how many time domain data we have here; one is a time domain data here other time domain data here.

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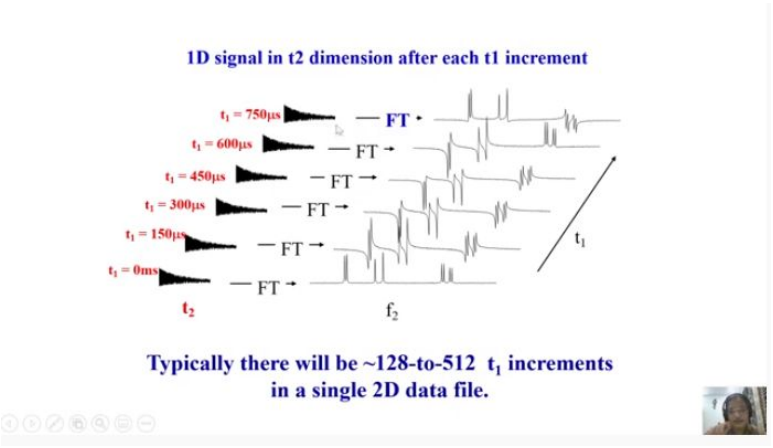

I said time and frequency are Fourier pairs; we have to convert them to frequency domain, we will do that. So now one-dimensional signal in t2 is collected for each t1 increment like this. What happens? like this if we keep incrementing t1, the FID will be like this; and do the Fourier transformation. In the F2 period the signal appears with the different phases like this, why? Because when there is a delay in sending the pulse and start collecting the data, there is always a phase delay.

There is a phase distortion. But in this case phase is uniformly changing, because we are uniformly changing this. Look at this one; this is a signal intensity, it is positive like this; phase goes like this, like this and finally it goes like this. So, you can have typically a 100 to 512 points or even 1k data points depending upon size of memory and information content you want. Depending upon what type of information you want you can have that many numbers of t1 incremental points; that is fine.

Now look at this; how do we visualize FID in t1 dimension. So far, remember in this case, I was showing you only t2 domain FID which changes phase as you keep on incrementing the t1. Every time you collect the signal, just let us say I collected this signal, you get this FID this spectrum. I collected this FID after 150 microsecond this is a spectrum, no phase correction is done, simply we collect the FID and do Fourier transformation this is what we have got okay.

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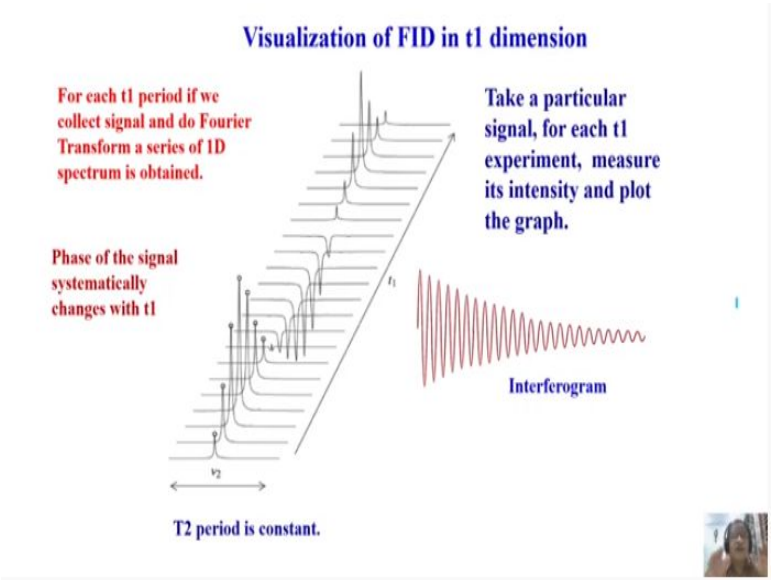

So now what we can do is for each t1 if you collect signal and do Fourier transformation you get a spectrum. Let us do one thing, collect only one of the signal, let us say, in this case you have collected one signal, like this. anyone of them. See how it is varying; if you collect only one of them ,follow the intensity pattern as a function of t1, this being constant here, now what is happening? It keeps on going like this.

Look at it, trace the top of the peak here. It keeps going like this, comes down, goes negative zero, what is happening here? If you look at it very carefully, this is an oscillatory function. Look at it, it is like a regular free induction decay what we collect is an oscillatory function. Is it not? Exactly in the t1 dimension indirectly we have created a free induction decay, a time domain decay oscillatory and decaying signal you have created; in the time domain?

That is precisely what you are going to have. You are having a FID; free induction decay with t1 domain; and also you have a FID in the t2 domain. That is how you indirectly created. So finally, actually if you have many such frequencies in the t1 dimension you also have an interferogram; exactly like this. I took an example for only a single frequency; how it is varying to show it an oscillator function and also as a decaying function like this. It is a decaying FID; dampened oscillations are there for one frequency.

If you have n number of frequencies n number of peaks present, you have an interferogram like this. And that is what happens in a 2D data acquisition. You created the FID indirectly; in the indirect dimension by systematically varying with t1 time period, and collect the FID in the t2 period for a constant time; this is what is happening.

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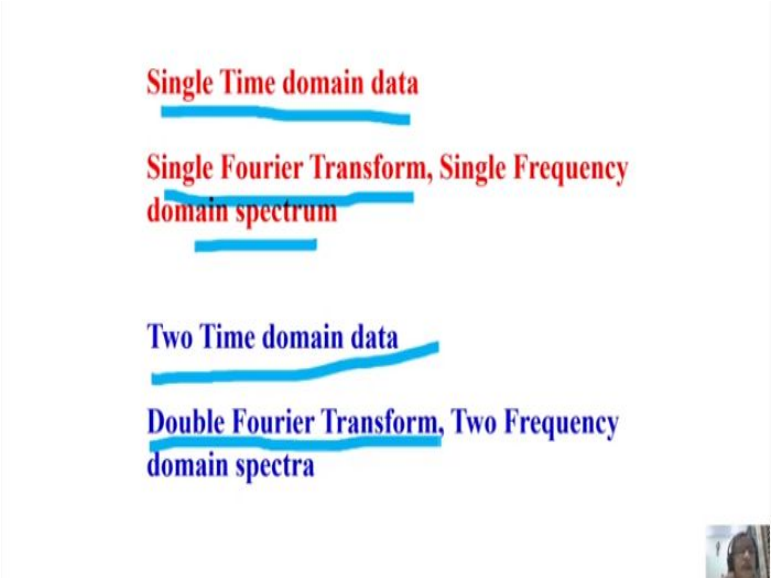

Now simple logic you extend. in this 1-dimensional NMR we had a single time domain data. We did single Fourier transform and get a single frequency domain spectrum that is all; one-time domain data one Fourier transform you get single frequency domain. Now we have a two-time domain data, data acquired in the time domain oscillatory; dampened oscillatory decaying signal in the t2 period and also the t1 period.

So, we have two-time domain data, make double Fourier transformation in both the dimensions. Do the Fourier transformation in this dimension; and the Fourier transformation in this dimension. In other words, Fourier transformation in the t1 dimension and also do the Fourier transformation in the t2 dimension; you can do double Fourier transformation. What you are going to get is two frequency domain spectra. So, you are going to get frequency in both the dimensions.

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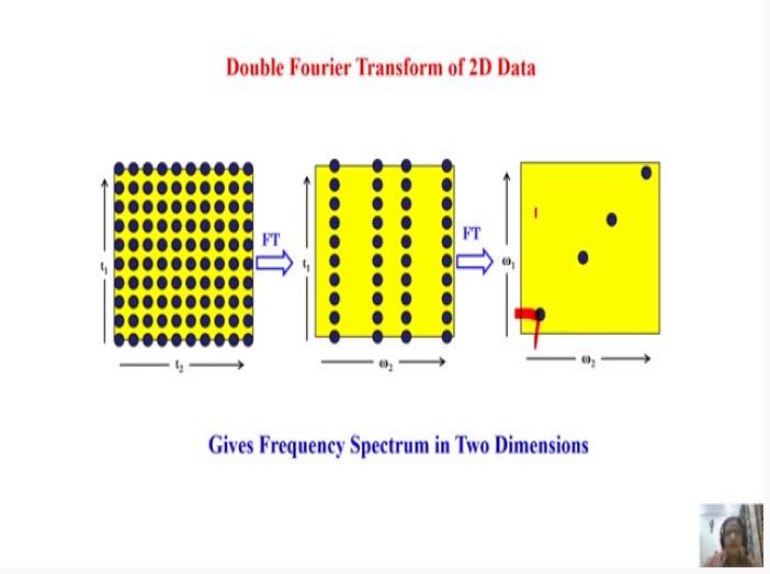

Let us see how we can look at the time domain data. This is what I showed you. We keep on incrementing and finally acquired a data right. This is a time domain data acquired by incrementing systematically t1 time period. I increment the time during t1 and collected the FID. Now what we do is we can individually do the Fourier transformation; convert time domain data of t2 domain or t1 domain into frequency.

First let us say you are doing the Fourier transformation in this dimension in the t2 dimension. So this is remaining; let us say we have 4 frequencies present here. In the t2 dimension you have 4 peaks. But in the t1 dimension it is still a data point like this. It is the time domain signal, it is varying signal okay. Now we do the Fourier transformation also simultaneously or immediately after this we do Fourier transformation in the t1 dimension also. Now we are going to see only the frequencies. In this dimension you get frequency, and in this dimension also you get frequency. Now if I look at this peak here, I can say in this dimension; in f2 dimension or t2 dimension. See dimensions can be mentioned t2, t1, f1, f2, omega1, omega2, direct dimension, indirect dimension. These are the nomenclatures people use. For example, t2 dimension it is a direct dimension if you detect the signal.

After Fourier transformation it is a omega 2, frequency or f1 and f2 are the two frequencies dimension, it is called f2 dimension. Similarly, this is called F1 dimension, t1 dimension or omega1 dimension. Since it is a direct dimension where you detect the signal it is called direct dimension; in this indirectly you are acquiring the signal, it is called indirect dimension.

These are the nomenclatures which are adapted and are used. Most often you come across in the textbooks or literature. Please do not get confused everything is same; it is only the way we use it. So, now we read the spectrum. If I come here just exactly at omega2 dimension, I have a frequency here; and the same peak you go in this direction, in the omega1 dimension it has frequency. So, that means this peak has a particular frequency here in the omega1 dimension and it has peak frequency here , in the omega2 dimension.

This can be said as, we have two frequencies; one in this dimension it is one frequency; in this dimension we have another frequency. So, we can know looking at the where the peak is coming, I can say what is its frequency here. This is the way you can represent the two-dimensional data after Fourier transformation. So, we have got frequencies in both the dimensions.

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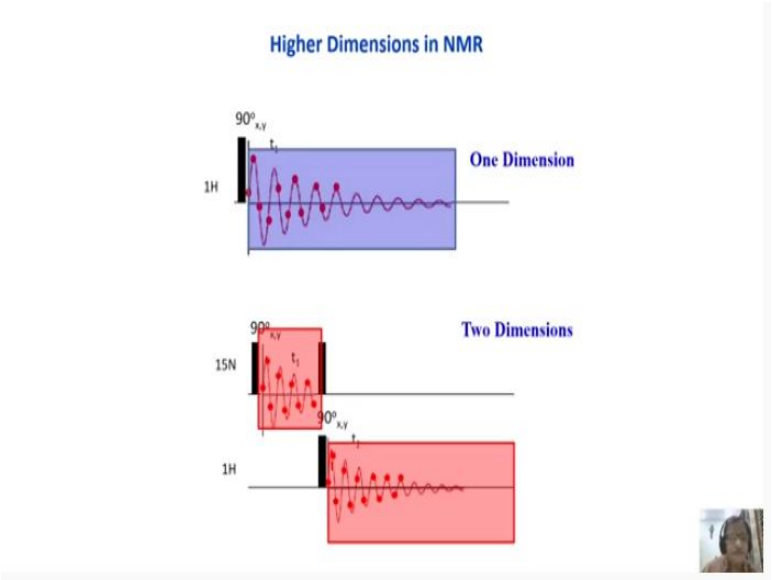

Now we will generalise dimensions; higher dimensions in NMR. First these is the basic thing I said; 90-degree pulse collect the signal as a function of time. One-time domain and one frequency domain it is a one-dimensional data; it is called one dimension.

Now we have 2 time periods, I can do double Fourier transformation; I get two dimensions; two-dimensional sequence and we get two-dimensional data. There is no reason why we have to stop here. depending upon relaxation time and how long the free induction will last, we can design more number of experiments. More time dimensions we can add.

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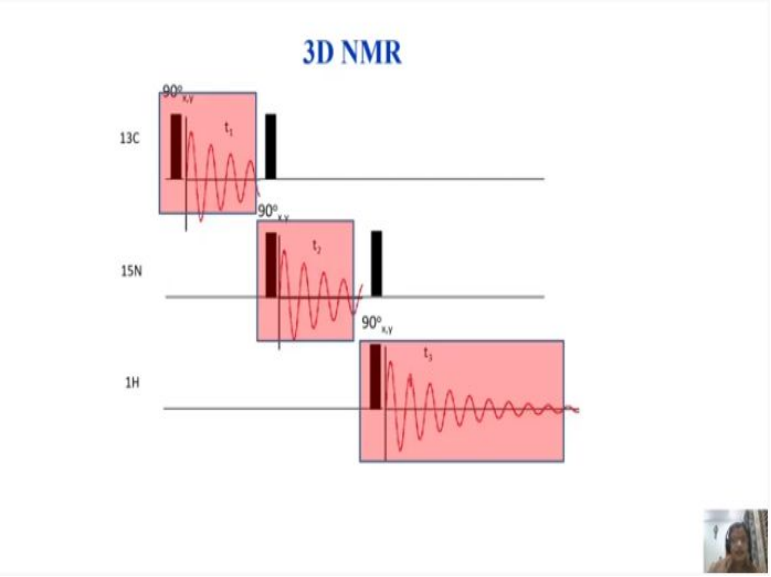

For example, I can have a three time periods like this, t1 period, t2 period and t3 period. These are three dimensions; three dimensions I can do Fourier transformation and then three-dimensional spectrum I am going to get. See two dimensions is like; the three dimensions you can imagine; a cube, a box it is like a box. You can have one dimension here, one dimension here and one dimension here. So, three dimensions are there; we can have three-dimensional spectrum, with three-time periods. So, this how we get.

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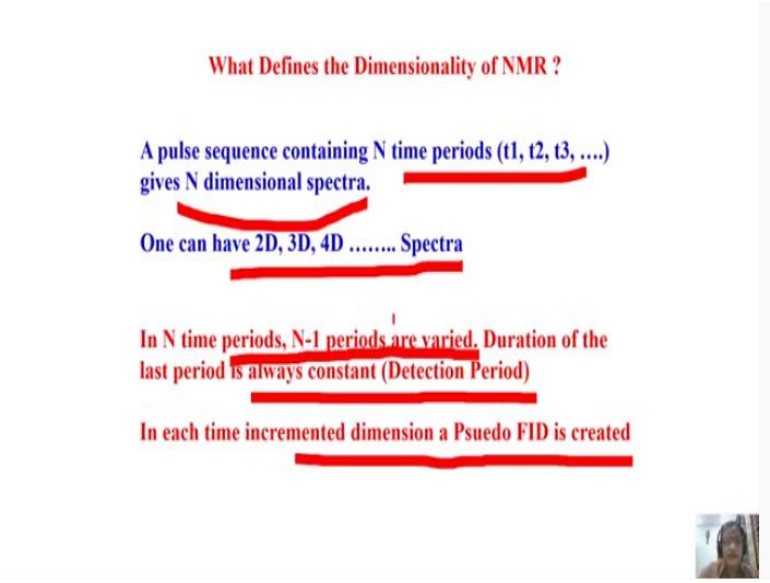

Now the question is, with this we can generalize and say, what is that which defines the dimensionality of NMR? Simple logic; a pulse sequence containing N time periods gives N dimensional spectra. Remember, you can have pulse sequence containing N time periods that t1, t2, t3 are the nomenclature we use, and gives N dimensional spectra. So, we have 2D, 3D, 4D, etc spectra. Important point to remember in the N time periods, N-1 periods are varied

Remember in the two-dimensional data, t1 was varied, t2 was kept constant, okay. I showed you the example t1 systematically was varied, but the t2 period has kept constant.

Similarly, in N time periods N-1 periods are varied and the duration of the last period that is called a detection period is always constant. Always the last detection period is kept constant. Each time period we have to increment data to create a pseudo FID; exactly what we did I showed you the free induction decay was created by systematically incrementing the t1 points that is pseudo Fid. So, we created a pseudo FID in time domain, and the t2 domain detection period we have a regular FID. Same way if we have N time periods, N dimensional spectra and we can have N-1 pseudo FID created; and last one is the real FID collected in the detection period. Of course it is a general sequence, you can keep some of the periods constant depending upon what type of experiment you want to do. There are various possibilities we can think of; may be one of them deliberately you can keep constant for getting some information or you can play around. But generally see in the N dimensional data N-1 periods are systematically varied.

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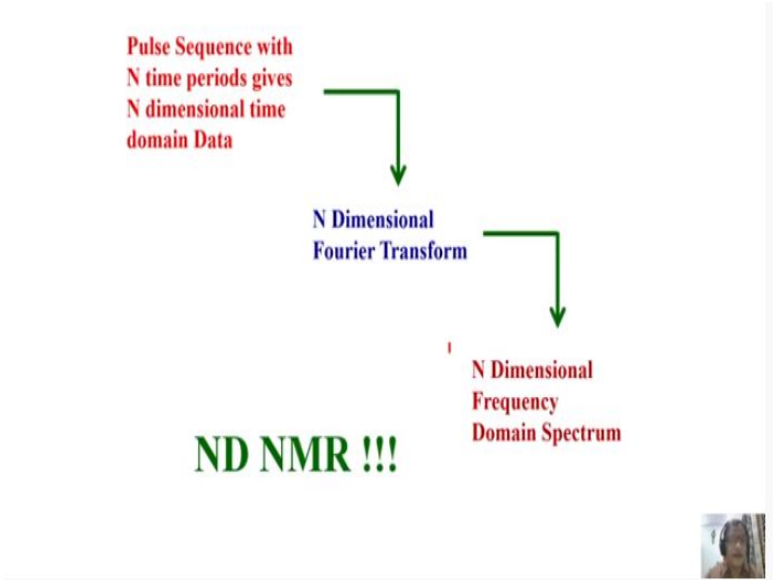

Now you see the logic. The pulse sequence with N time periods gives N time dimensional data, you do N dimensional Fourier transform you get N dimensional frequency spectrum; this is called N dimensional NMR. Do you understand? Pulse sequence with N time periods gives N dimensional time domain data, do the N dimensional Fourier transform, you get N dimensional frequency spectrum it is called the ND NMR.

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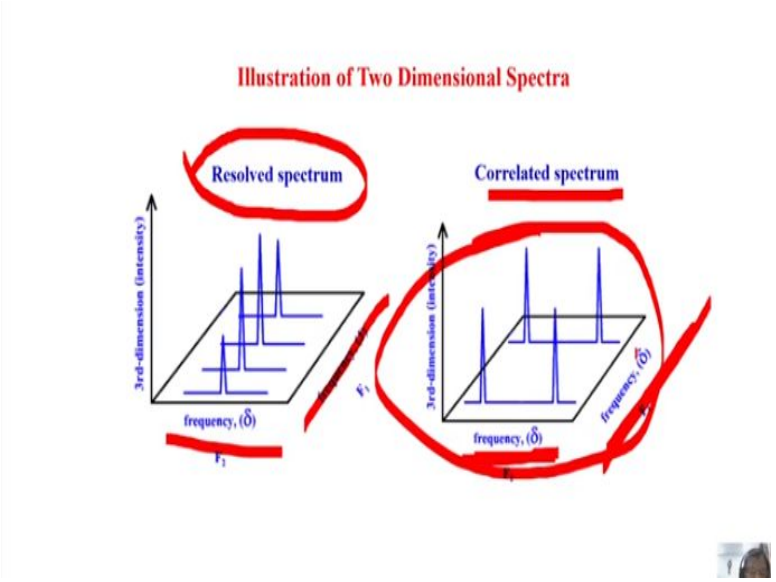

Now we can see how this spectrum looks in the two dimensional way. You can have a spectrum like this, okay. If this is the frequency domain here; also here a frequency domain. This is a resolved type experiment; where the chemical shift information is here, J coupling information is here. They are all resolved; they are separated.

This is a correlated spectrum where frequency of one nucleus is correlated with the frequency of other nuclei here. Or the frequency of the same nuclei, or between two different nuclei spins can be correlated. It could be chemical shift of one nuclei here; chemical shift of other coupled nuclei here. That is also possible. I gave you two examples; this is the way we represent two dimensional spectra. This is called stack plot; when we plot like this, with an intensity pattern like this; the number of stacks depend upon number of t1 points you have. You can represent like this, it is a stack plot of the 2D spectra; understand.

It is a stack part of the 2D spectrum; you can define the number of t1 points, or incremental points and have a stack plot of the 2D or 3D spectra. But remember this is okay for 2D you can have a stack plot; how do you want to have a 3D? very difficult to see and imagine, okay.

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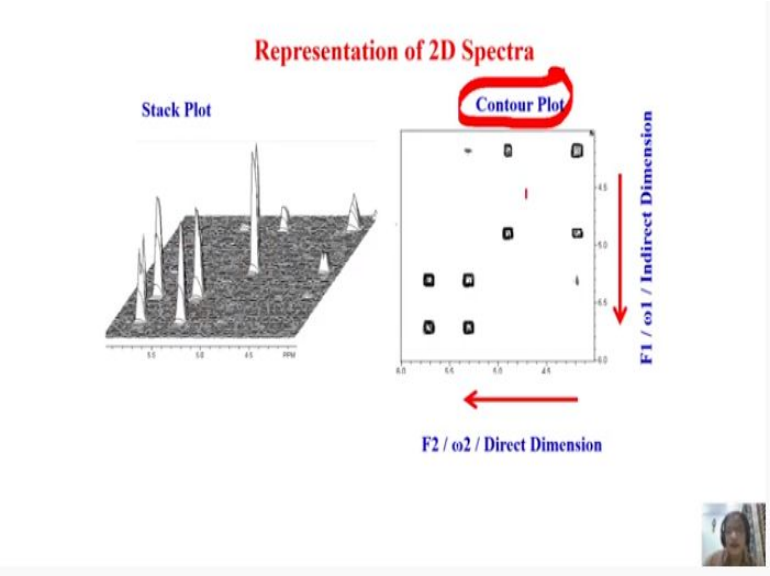

As a consequence, it has been modified. The stack plot like this can be modified to give a spectrum like this. This is a stack plot, this is called a contour plot. See each peak here for example 1, 2, 3, 4 peaks are there, they represent a contours here; and each here 2D spectrum. This is spectrum like this, exactly similar to this; but what you got the vertical peak with some line width what you saw in 1D spectrum; 1 dimensional spectrum, the same thing has been converted into a sort of contours like this. Contours are like circles, there are several circles are there, one within the other. Okay so these are called contours. There are different contours one within the other. There are several such contours may be 1 to 10 or 20 number of contours will be there. Mind you, area of this contour exactly matches with the area of the corresponding peak of stack plot. For example, I consider this peak which is highly intense. Okay and are of that peak, perfectly agrees. Okay, the just translation of the stack plot; the peak which is in the normal one dimensional pattern, will be transferred to the contour like this, so that it is that easily represented. This way we can represent much easily.

So, always this is the way which is represented, this is called a direct dimension, that is the detection dimension F2, omega 2, etc. This is F1 omega1, indirect dimension, etc, okay? This is the way we represent 2D data, but this type of stack plot is outdated, and nobody will do it. You always require only contour plot. Remember all the time we use only contour plot for representation of any 2D spectrum; 3D spectrum or 4D spectrum, does not matter; any Multi-dimensional spectrum nowadays are represented only by contour plots. Now you may ask me a question how did this peak stack plot was converted into a contour?

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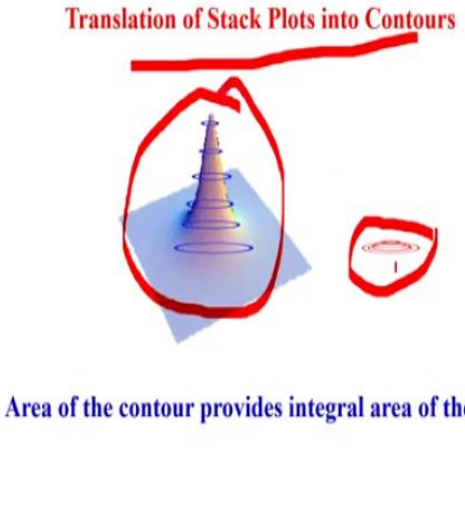

How do I translate this peak, into the circles, like contours. I am trying to give this picture for you; look at it very carefully. Imagine this is a mountain, a steep mountain. Now what we can do; we start from here; go round, I cannot go back in the figure here, Imagine you have to go behind this drawing; But imagine you are going around the hill like this. From the bottom go down the hill like this; find the area. Okay, and then what you do is you know this is the area of this mountain at this point, at the bottom, then put a circle here; corresponding to this area this is the area of the circle, which is the area you got by going around this mountain in a circular manner like this. Now I go slightly above, again go round the mountain, find out the area or circumference come back and put it here. Now again go up, measure the area come back and put another one; another one, like this. Keep on going high, above and above the hill, go round the circumference, measure the area come and put here. So, it is like areas of circles at different places, you understand. Now I am going through a circular path here; when I go to a circular path here this is area of a circular path here, here and here and each time what you are going to get is translated into the contour like this. This is the how stack plots have been converted or translated into the contours.

Now this representation is much easier is it not? compared to this, this is very clumsy. One peak is fine, if you have 100 peaks or 1000 peaks you cannot have like this, it is very clumsy to see. That is why the stack plots are nowadays represented as contours; this is the way you undergo translation of that. Area of the contour I said now; we have done one thing the area of the entire peak here as I said with an example of different mountain at different places is taken and represented here. So, what does it mean the area of the entire peak correspond to

the area of this contour, that is all. The integral area of contour is nothing but the integral area of the stack plot.

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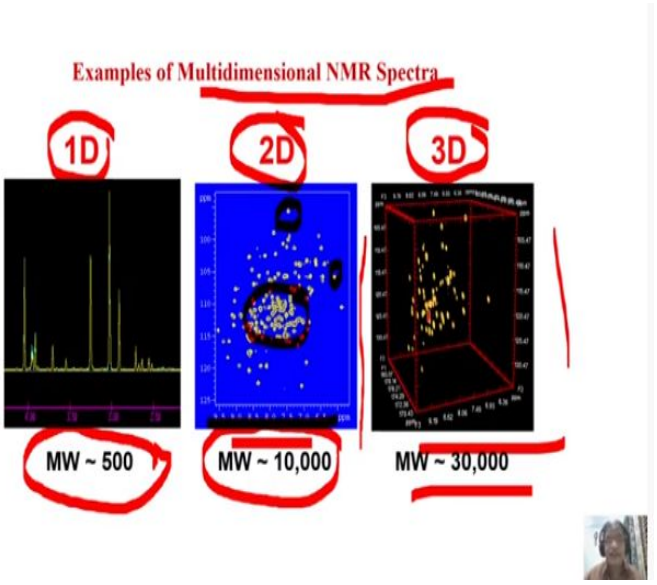

This is what I wanted to show you. Now there are many examples as we go ahead and see multi-dimensional spectra. Just to give you, how we represent contour plot of the different multi-dimensional NMR spectra; this is 1D, this is 2D, and this is 3D. In the one dimensional spectrum we have a frequency domain here, frequency in this axis are only chemical shifts. Here in the two dimension you can have one frequency domain here and frequency domain here; In 3 dimension one here, one here and one in this direction. And you see something is written here.

The molecular weight up to 500. By 1D it is very easy to handle up to molecular weight of 500; very easily you will get it. Let us say I go to molecular weight of 10000; still not bad, you can manage with 2D; you see still you get well resolved peaks. Peaks are very well resolved here, compared to this and this. Okay, imagine in this region if there was a stack plot how clumsy it would have been; so that is why very easily we can represent this way and we can see these types of things. We can still get better resolution up to molecular weight of 10000. We can still manage 10000 Dalton we can work with 2D spectra. Still it is enough to give you the information. You can get complete information from 2D.

But when it exceeds that, go to molecular weight 30000 or even more; 2D is not sufficient then we need to go to higher dimensions, to get better resolution, to resolve the information in

different directions; to corelate the information in different directions; so that the analysis become more simpler.

So, if molecular weight is even higher, we will see, we may have to even higher dimension. How you get dimensionality of the spectrum, where do you represent the contour plot is like this. The 1D is simple; it is a peak height, frequency and the peak here. 2D and 3D onwards everything will be in the contour fashion; okay, and molecular size also defines type of experiment you want.

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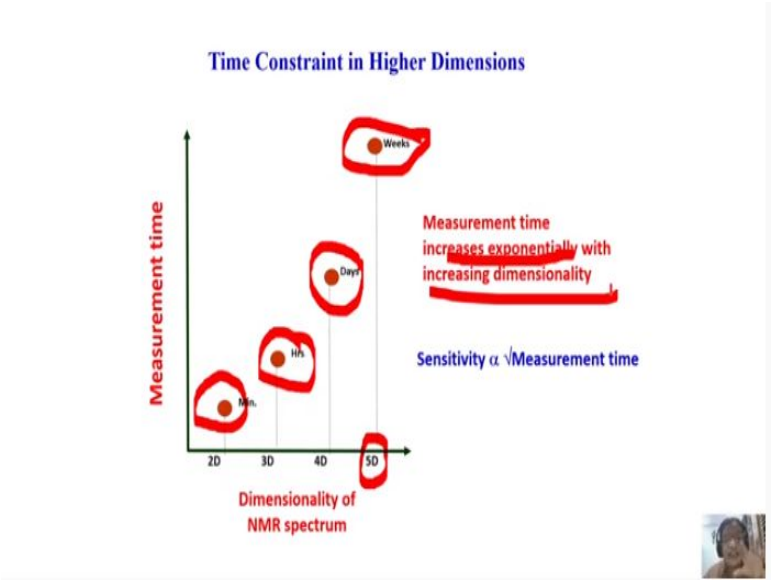

Now what is it time constraint? There is a time constraint you cannot just like that start doing the higher dimension experiment. Remember if I take a 1D experiment, nowadays in the present-day sophisticated spectrometers, thank to many vendors who developed and designed very sophisticated experimental probes and NMR spectrometers, magnet, compared to about 2 to 3 decades back. The time which is required to get NMR spectra is really fast. It does not take much time, very less time.

In fact, 1D NMR you can do in a fraction of a minute, now in about less than a minute you can get spectrum; because sensitivity is much better in present day spectrometers compared to olden days. 2D you can do in few minutes let us say in 15, 20 or 30 minutes you can get a very good quality 2D spectrum. The same molecule if you want to do 3-D it will take hours. Now I have this molecule, let us say very big molecule, I want to do 4D, it takes days.

If I want to do 5D experiment on that molecule, it will take weeks. Remember it will take weeks of the instrument time. Thus the measurement time exponentially increases with the increasing dimensionality. So, we have to be very, very choosy in finding out what type of 2D experiment or 3D or 1D we require for the sample, because measurement time goes exponentially with increasing dimensionality. Look here, it is almost exponentially going like this, okay.

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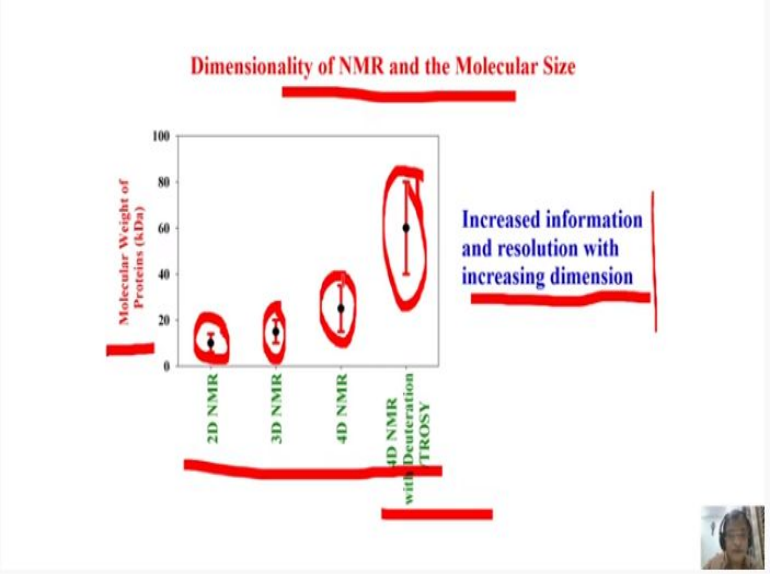

Also, it depends upon the molecule. For example look at it in the 2D NMR up to 20 kilo Dalton is manageable; 3D it is okay 20 to 30 is still okay, that we can manage. Above that we need 4D NMR; and even bigger molecule, the 4D NMR plus and additional NMR techniques, like TROSY, etc; Which you do not know; I do not know whether I will have time to talk about TROSY. They are really sophisticated experments, which I do not think for your routine analysis you require this. I do not think, I have time to cover in this course.

But basically, you remember depending upon the molecular weight you have to choose the dimensionality; okay. And increased information and resolution will be there with increase in dimension. From here to here if I come, I get better resolution and better information, here to here even better information and better resolution like that. Dimensionality has to be chosen based on the size of the molecule, based on molecular weight.

As you go from one dimension to the next higher dimension you have more information and more resolution. But of course you pay a penalty, you take more time; instrument time goes

exponentially. All these factors you have to decide before doing an experiment. So now the question is, how do we choose the dimensionality?

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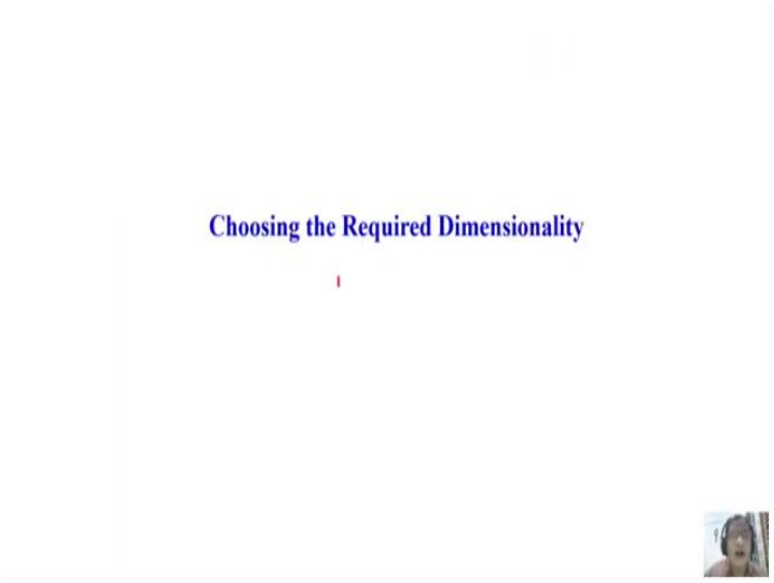

I stop here today we will come back and tomorrow discuss about how we choose a required dimensionality for a given molecule of your interest.