

**NMR Spectroscopy for Chemists and Biologists**  
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**Lecture 40**  
**Multidimensional NMR Spectroscopy**

So today we are going to start a new topic namely Multidimensional NMR Spectroscopy. This is basically an extension from the Fourier Transform NMR which came up in 1966 and that caused a revolution in NMR applications. Things which could not be handled earlier could be handled because of the sensitivity enhancement it allowed.

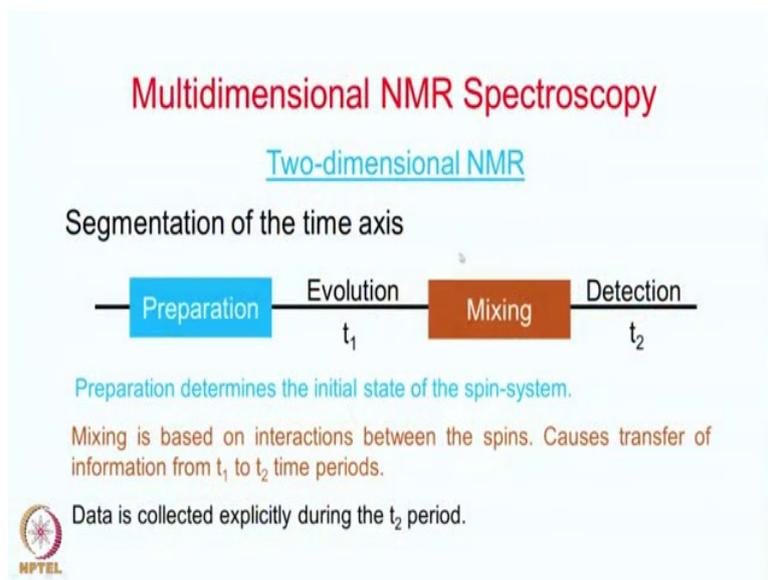
Fourier Transform NMR had another significant advantage which I mentioned earlier when we were discussing Fourier Transform NMR that is, it separated the excitation of the spin system from detection of the response to the excitation. So the time in-between was available for various kinds of manipulations and two-dimensional NMR and various other kinds of polarization transfer experiments which we discussed earlier were a consequence of this important concept.

So we will go through this development and this I consider as another major revolution in the application of NMR. Multidimensional NMR spectroscopy, it started with the two-dimensional NMR, then it went on to more dimensions, 3 dimensions, 4 dimensions etc, and the basic foundation was already laid when the schematic for the two-dimensional NMR was developed. The idea basically originated also quite early.

So, it originated somewhere in 1971 and this was put forward by a Belgian physicist called Jean Jenner in a Summer School where he proposed the idea of doing two-dimensional NMR spectroscopy. Since, then it has grown unparalleled and the kind of application which it has generated is quite mind-blowing. It allowed entry into new disciplines like biology. Structural biology became possible because of two-dimensional NMR in a big way and therefore, we are going to go through this exciting period, exciting development in a systematic manner.

All those who would like to pursue chemistry in big detail or structural biology as a profession, this becomes an important topic to learn. So we are going to discuss two-dimensional NMR to begin with and over then we go forward to the higher dimensions.

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The basic concept of two-dimensional NMR as I mentioned to you is in the segmentation of the time axis. This is called the segmentation of the time axis. Here we have the time axis and now you actually divide this time into 4 different periods. I will explain this to you what these one periods are and just to take note of this, this is the so-called one preparation period, then this is followed by an evolution period then a mixing period and then detection period.

Preparation period prepares the system in a suitable initial state. This may include the excitation, so when you do that you create transverse magnetization from the spin-system and then you allow this magnetization to evolve during the period  $t_1$  which is now a variable and therefore we call it as an evolution time, and this is a variable time.

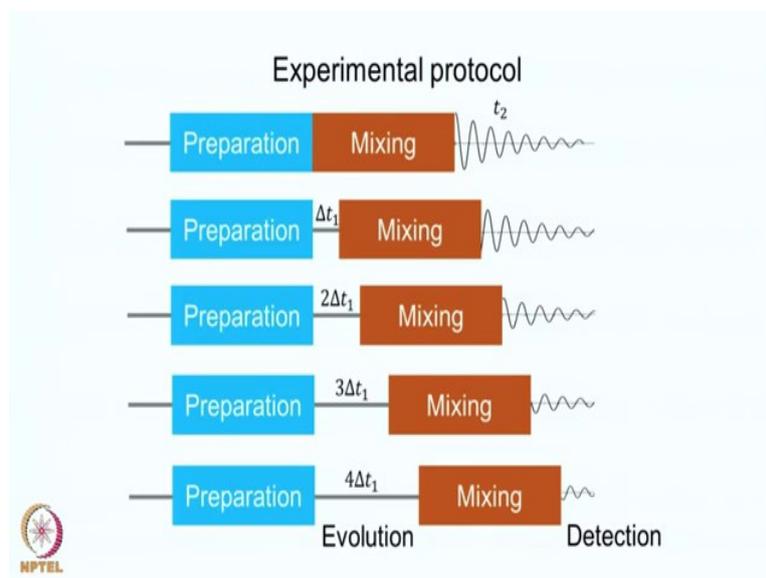
And the mixing is the period which allows mixing of spin systems so it can, it depends on various kinds of interactions between the spins and it will cause transfer of information from this state of the spin system to this state of the spin system. Eventually we collect the data only during the  $t_2$  period, but however it will have a modulation from the evolution of the spin system during the period  $t_1$ . Therefore, here this time period is often called as indirect detection period and this is the direct detection period.

The signal which is detected here will be modulated by what happens during this period and the mixing actually causes transfer of information from this period to this period and then you generate a two-dimensional spectrum, which displays the information present here and

present here and we will see this. Now each of these periods, preparation period, the mixing period or even the evolution period can have a variety of possibilities.

They may be simple delays or they can have pulses inbuilt which will help create a suitable initial state and here also it can help create a suitable information in your spin-system evolution and mixing depends upon what sort of interactions you want to reflect in your final spectrum and what sort of information transfer you want to achieve from here to here and that determines the so-called mixing. And data is collected explicitly during the  $t_2$  period only.

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Now, so how is the experiment actually performed? Experimental protocol is given here. So you do a series of experiments. In other words you collect series of FIDs. Typically the data you collect is in the form of FID and that happens in the  $t_2$  period. So, here you have the preparation period and the evolution time here, it is 0. So we call it as zero time period here, and is immediately followed by the mixing and you collect the data as a function of time  $t_2$ .

Now, you introduce a time period in-between. You call it as  $\Delta t_1$ . This is the evolution. This is the evolution time for the magnetization that has been created at this point in time and this one is then mixed by suitable interactions. The spin-system, the transfer of information happens from one spins to another spins and so on. And then the data is collected here as a function of time. Notice the initial condition of this FID is not the same as the initial condition of this FID and this is because of the mixing. And what has happened during the  $\Delta t_1$  period?

The magnetization has evolved if it is a transverse magnetization here, it evolved for a time  $\Delta t_1$ , and then you do a mixing and you collect the data here as a function of time  $t_2$ . Now, you increase this time to  $2\Delta t_1$ . Here it is an increment; let us say this may be about 100 microseconds. We will see what determines this time increment and this; you systematically increment this time increment. So, here you have  $\Delta t_1$  and you do this experiment once more with  $2\Delta t_1$ , this part remains the same, this remains the same. These do not change. Only this changes.

You increment this time, so it is  $2\Delta t_1$  and you collect an FID once more here. So, once again notice the initial time point here is not the same as the initial time point here and this is actually the crucial part of this experiment. And also, although the FIDs are looking the same here and here, the similarly, it may not be the case. It may have more complicated FIDs here, here and here and therefore, that also we will see as we go along.

Now, you do the third experiment,  $3\Delta t_1$ , do the same, collect the data here as  $t_2$ ,  $4\Delta t_1$ , do the same, collect the data as a function of  $t_2$ . Therefore, you repeat this several times. How many times you do it? And this will depend upon the resolution you require in your spectrum. Now, notice that, I mean, of course, you remember that this FID is connected as a function of time in a digitized manner. You collect various data points.

Although it is shown as analog signal here, but you actually collect it as a digital manner, In the Fourier Transform NMR this data is collected in a digital manner. So, here you will actually also generating time systematically incrementing the time. So, as the time is incremented here you are collecting data as a function of  $t_2$ . So, here you are actually generating the second time variable where you have first point at 0, the second point at  $\Delta t_1$ , the third point is at  $2\Delta t_1$ ,  $3\Delta t_1$ ,  $4\Delta t_1$ , these are systematically incremented.

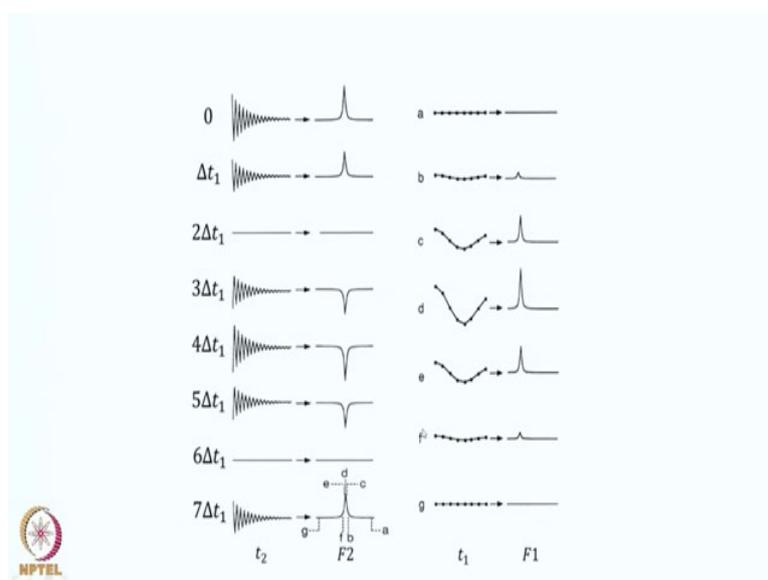
And you can collect as many FIDs here as many increments you want here. Typically how many you collect? And this will depend upon the spectral resolution you want in your spectrum. Here in the  $t_2$  period, often we know in 1D spectrum you collect something like about 2048 points or 4096 or 8192 and which is easy. So, this will go on for a short period of time which is just like about few hundred milliseconds. So, in few hundred milliseconds all this data collected.

Now, here if we increase, as you want to increase the number of points each data point is, is a separate experiment. So you will have to perform that many experiments, 2048 or 4096

experiments you will have to perform if you want to have that many data points here as a function of, in the time evolution here. So typically the number of data points here will be less than the number of data points here.

This is limited by the practical considerations, and if we need to have more, of course, you can collect more. And in each of these period, of course, you follow the same rules as you use in the case of Fourier Transform NMR. If you want to do signal averaging, for each of that time increment here, you must do the signal averaging, 8 scans, 16 scans etc whatever you have to do for each of these experiments and you collect a two-dimensional data body. Now, let us see what happens afterwards.

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So here is a actual demonstration. Here you have the first data point, time  $t=0$ . You have the first FID, and then  $\Delta t_1$  you have the second FID. The third  $2\Delta t_1$ , this is the third FID but there is no signal here. As, I said there will be modulation and depending upon what the initial point is, and how the system evolves, what the signal is present, you will have these different kinds of FIDs so each of these FIDs is different.

So,  $3\Delta t_1$  now it is actually starting from the negative value here, if you see this was positive here, reduced to positive value, 0 here, now it is negative and then it is further negative and then this starts decreasing again, therefore, the signal amplitude at the time  $t=0$  is getting modulated as you are going down with the increments. So you can keep collecting such data, of course, it will be repetitive when it is repetitive and you get a time dependent variation.

So, let us say you collect something about 256 data points or something like that so that much of time you will have to give. But let us look what happens afterwards. Now, you have collected the FID here and you Fourier transform this along this time axis, this is called as  $t_2$  time axis. This is  $t_2$ . You get a spectrum which is like this. Now the second one, you notice the first data point, intensity is reduced.

Therefore, what happens, this if it were a single line spectrum here it will be a single line spectrum and it will produce a line with a reduced intensity because the intensity is reduced here. If you remember correctly the first data points is a integral of the spectrum.  $F(0)$  is the integral of the spectrum. We have discussed this in Fourier transform NMR.

$$F(0) = \int Fw dw$$

So, therefore, that whatever is the initial point here and that is what you will get here in this one. Now for the third FID, for the fourth FID, the start is negative and the signal is now inverted.

So, it is going negative here and this is fourth point is also negative but with enhanced intensity because this data point, initial point is higher than this and  $5\Delta t_1$  again it reduces, sixth one is back to 0, seventh one it is again going positive, it is the positive signal and so on. So, therefore, this keeps getting modulated. The signal here is getting modulated by the evolution during the period  $t_1$ . Now, let us look at this in more detail.

This is the spectrum here. Now what you do, this is all also digital, so when you do Fourier transformation you get a digital spectrum. This is the, the spectrum is drawn as continuous analog signal but actually appears as a digital spectrum. This is each point here; there are points, 1, 2, 3, 4, 5 things like that various points are there. Now we will pick up a few of those points here.

So this is a, let us say this point is called a, these are the time axis, this is the frequency axis here. This frequency axis is on the  $F_2$ , after you did a fully transformation here you will get the  $F_2$  frequency axis, this is the transformation along  $t_2$  so you got the  $F_2$  frequency axis. Now this is a frequency a, and this is frequency b, then you have frequency c, frequency d then you have a frequency e, all these c, d, e they are all here. Then you have f and you have a point g.

You could have chosen any other points also, it does not matter. What is done here is you have chosen 2 points where there is no signal in the frequency spectrum and you have chosen at a few points which are in the signal, in this spectrum. Now, what you do is you take each, this point in each of these FIDs, each of this spectra and plot them here. So, let us look at what is point a? So point a, this here, this here, this here, this here, and you put all these points here. There is no variation here, because it is all the same.

Now, we come to point b. The point b, it is this one is here, and here it is 0. And then it is here, then you take this here, here, here, here and here and you plot that here. So initially therefore, this is going like down, see on this axis what is it now? This axis is  $t_l$  because what is varying here as you are going along is the  $t_l$  increment. Therefore, I want to call this as the  $t_l$  variable; this is the  $t_l$  time axis here. So this point b has got a small dip and there is an oscillation here. It is going at this point.

Now you take the third point which is well in your signal, in your line, so now we talk the third point, c, then you will see there is a greater dip here. So there is a greater intensity variation here in this point. So, and you take the fourth point; it is even greater so there is more variation here. Then you go to the e point then it is decreasing. Then you go to the f point further down and you go to g, once again it is very down.

So, what we have got here therefore? Therefore this is also an FID, and like here what you have the FID was going point by point, it was showing variation these ones are also FIDs. These FIDs are different depending upon which portion of the frequency spectrum here you are looking at. Therefore, I can do Fourier transformation along this time axis now. This is the time variable  $t_l$ .

So, if I do a Fourier transformation along this, against this time  $t_l$  there is no signal here therefore this is 0. If I do here there is a small signal. There is a small frequency component here. Therefore, you have a small signal with a particular intensity which is very small. Now if you come down further it is the same line, peaks at this point, now the intensity is more. So intensity is higher so this signal FID is increasing. The signal is higher here, Fourier transformation again decreases, decreases, goes to 0.

Therefore, you see here I have created a time variable which contains frequency information. So, this one now is called as the  $F_l$  axis. So, I got  $F_l$  frequency axis and I have  $F_2$  frequency axis. These 2 things are different. So I generate a 2 frequency axis by doing this sort of a

manipulation. So this is very important. This point must be very clear before we go forward. So we may be, I will try to repeat it here so that your things become absolutely clear.

So we do various experiments with increments along the  $t_1$  axis,  $0, \Delta t_1, 2\Delta t_1, 3\Delta t_1$  and each of these  $\Delta t_1$  values you connect an FID and FID has different behaviors because of the evolutionary during  $\Delta t_1$  and something may happen during the mixing also which can also affect this FID. So, therefore, you generate a whole series of FIDs here and if you Fourier transform this along the  $t_2$  dimension you generate a frequency domain spectrum and you can see the frequency domain spectrum has modulations here because the FID was modulated by the evolution period.

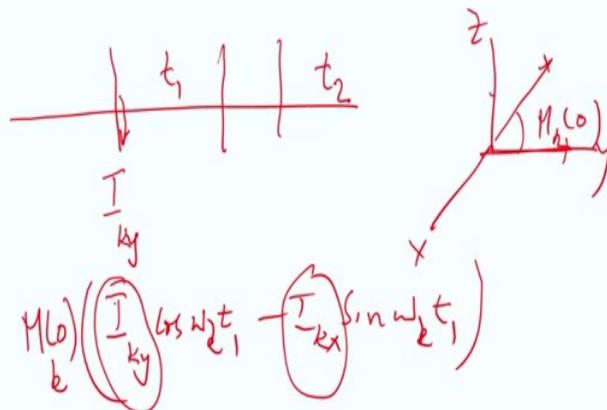
So this, you take all of these frequency points and join the individual frequency points and plot them here and generates a time axis, this variation is a representation of the variation due the time  $t_1$ . So this also has a frequency component. Therefore, if I Fourier transform each one of these FIDs here, these are not indirect FIDs here, these are not direct FIDs. These are not actually collect the signal during this. So these are all indirect points. After you have collected the signal and Fourier transformed it, by looking at these frequency points you generate a frequency dependent variation.

Therefore, this is a time variable which has a frequency component here, this displays a frequency and you Fourier transform here therefore you get a frequency component whatever is present here. So in this particular case, it is a single frequency. It is the same frequency which is present and it is, intensity is getting modulated by the evolution during the time  $t_1$ . So therefore these frequencies may be different, may be the same. So here we have just shown, we have not indicated what this frequency is. It may be the same frequency it can be different frequency, it can be combination of both.

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$$S(t_1, t_2) \xrightarrow{2d FT} S(F_1, F_2)$$

Consider a spin  $k$  whose  $xy$ -magnetization has been created by the preparation period and this magnetization evolves with a frequency  $\omega_k$  during the evolution period. At the end of the period  $t_1$  the magnetization has components,  $M_k(0) \cos \omega_k t_1$  and  $M_k(0) \sin \omega_k t_1$  along, say, the  $y$  and  $x$ -axis, respectively;  $M_k(0)$  is the magnetization at the beginning of the evolution period. Let us now assume that the mixing period transfers part of the magnetization to spin  $l$ , which has a characteristic frequency  $\omega_l$ .



So now let us put a little bit more formally. Here we have  $S(t_1, t_2)$ . We have generated a two-dimensional data body as a function of time variables  $t_1$  and  $t_2$ . Therefore, you can do a two-dimensional Fourier transformation and it will generate a two-dimensional frequency spectrum  $F_1, F_2$ . Whatever is information present in the  $t_1$  period will appear along  $F_1$  and whatever is the frequency information present during the  $t_2$  period will appear along the  $F_2$  axis.

So this is your two-dimensional frequency domain spectrum and this is the two-dimensional time domain signal. Now, consider a spin  $k$  whose  $x$ - $y$  magnetization has been created by the

preparation period. So, in the preparation period what you did is to create a transverse magnetization of the spin  $k$  like, for example, it may could have been  $I_{kx}$  or  $I_{ky}$  whatever.

So, and assume you created the transverse magnetization here and this magnetization you evolved with the frequency  $\omega_k$  during the evolution period because of the frequency evolution, the chemical shift evolution during the period  $t_1$ , it evolves with the frequency  $\omega_k$ , and of course there can be more than one frequencies as well here but let us assume one of those, there can be a frequency  $\omega_k$ .

It can be some other modulated frequency, chemical shift plus minus coupling if it is there and things like that but let us consider one particular evolution which is a  $\omega_k$  frequency. Now at the end of the period  $t_1$ , the magnetization has components  $M_k(0)\cos\omega_k t_1$  and  $M_k(0)\sin\omega_k t_1$ . So, let me explicitly state this to you. You have created the transverse magnetization.

After the preparation period, so here this is a preparation. You have created a transverse magnetization here. Let us say it is  $I_{ky}$ . And here during, this is the evolution period  $t_1$ , and this is our mixing period and here I have the  $t_2$  period,  $t_2$ . So during this period this magnetization evolves with this characteristic frequency  $I_{ky}\cos\omega_k t_1 - I_{kx}I_{ky}\sin\omega_k t_1$  and, of course, these are the operators here and I will put here a magnetization component.

This is the, to indicate the magnitude of the magnetization which is represented as  $M_k(0)$ . So this is the operated earlier. We ignore this. So the magnetization actually will be  $M_k(0)\cos\omega_k t_1$  and  $M_k(0)\sin\omega_k t_1$ . That is the detection during, you have this, so we have this, this is the  $y$ , and this is  $x$  and this is  $z$ , the magnetization which is here as  $M_k(0)$  and this as it is evolving so you get, magnetization moves here.

So this is  $\cos\omega_k t_1$  and this is the  $\sin\omega_k t_1$ , so these are the frequency components you will have. So we will have these components  $M_k(0)\cos\omega_k t_1$  and  $M_k(0)\sin\omega_k t_1$  along, let us say the  $Y$  and the  $X$  axis, this because these are the 2 components.  $M_k(0)$  is the magnetization at the beginning of the evolution period. Now, let us, now assume that the mixing period transfers part of the magnetization to spin  $l$ . I said what happens during the mixing is dependent on the interactions between the various spins.

Consider the spins  $k$  and  $l$ , and if there is an interaction between them of some sort, it may be the  $J$ -coupling interaction, or it could be dipolar coupling interaction, whatever it is but if this mixing allows transfer of magnetization from  $k$  to  $l$  then a part of this magnetization may get transferred to this spin  $l$ .

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The detected signal as a function of  $t_2$  will then have two contributions.

$$A = a M_k(0) \cos \omega_k t_1 \cdot \cos \omega_k t_2 \xrightarrow{2d FT} \text{Diagonal peak (F1=F2= } \omega_k)$$

$$B = b M_k(0) \cos \omega_k t_1 \cdot \cos \omega_l t_2 \xrightarrow{2d FT} \text{Cross peak (F1= } \omega_k, \text{ F2= } \omega_l)$$

Here it is assumed that only the y-component of the magnetization is detected during  $t_2$  period,  $a$  and  $b$  are some coefficients representing the relative contributions. These equations represent, of course over simplification made to convey the concepts.



In which case what happens during the period  $t_2$ ? So there will be some magnetization which remains on the spin  $k$  and some magnetization will be on spin  $l$ . So, and the component which is present there are represented by these coefficients here  $a$  and  $b$ .  $a$  is the component which remains on spin  $k$  itself and the  $b$  is the component which gets transferred to the spin  $l$ . Now, during the period  $t_2$  what happens? This magnetization continues to evolve with its own characteristic frequency  $\omega_k$ , so this is  $\omega_k$ , this is because of the  $k$  magnetization. So during  $t_2$  also it is goes with the same frequency  $\omega_k$ .

I mean I am giving a very simplistic picture here. The actual calculation will, may have to involve the Hamiltonian evolutions, consideration under the influence of the different Hamiltonians and here you are considering a very simplistic picture to bring out the concepts. So if it is going with the same frequency  $\omega_k$  then we have the, signal which you are going to detect, which you are going to detect here will be a  $M_k(0) \cos \omega_k t_1 \cos \omega_k t_2$

So this, and the one which is gone on to the  $l$ , that will evolve with the frequency  $\omega_l$  during the period  $t_2$ , So, therefore, in the  $t_1$  period this was  $\cos \omega_k t_1$ , that was the component

which had come as a result of evolution during the  $t_1$  period, and at the end of the  $t_1$  period this was partly transferred to  $b$  and some of it remained on a itself.

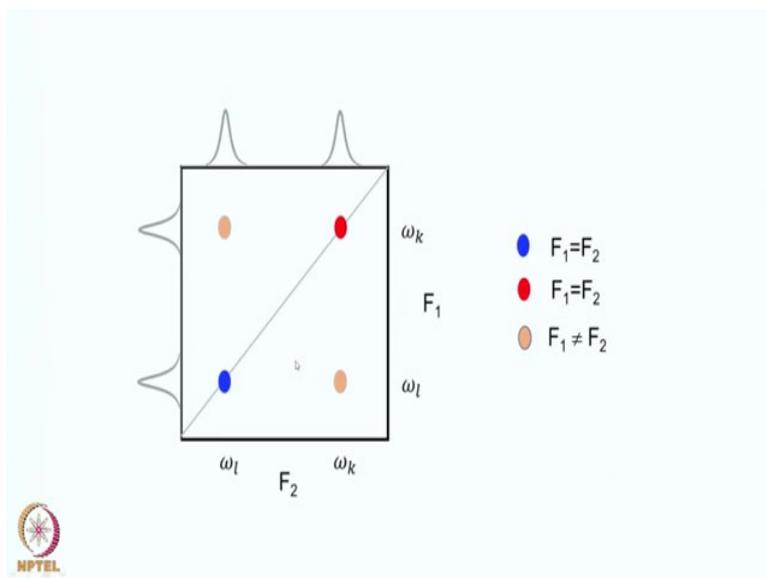
So therefore this portion is the, at the end of the  $t_1$  period and this is the result of evolution during the  $t_2$  period. Now we if I do a two-dimensional Fourier transformation of this signal, of course it will be combination, that won't be detected together. There will be both the components present in the signal what you will detect, in the FID what you will get as a function of  $t_2$ , it will be superposition of these two. Both these will be present.

Therefore, when you do Fourier transformation you have to consider Fourier transformation of both of these, both of these terms and when you consider this term what will it generate? It will generate a frequency  $\omega_k$  along the  $F_1$  dimension and the frequency  $\omega_k$  along the  $F_2$  dimension. Therefore, we call this as the diagonal, called as the diagonal in the frequency domains in the two dimension frequency domain spectrum this is  $F_1 = F_2 = \omega_k$ .

Now, in this particular case if we do a Fourier transformation then along the  $F_1$  dimension I will have this frequency  $\omega_k$  but along the  $F_2$  dimension I will have this frequency  $\omega_l$ . therefore this is  $F_1 = \omega_k, F_2 = \omega_l$ . Therefore this is called as the cross peak. Here it is assumed that only the y component of the magnetization is detected during  $t_2$  period. But this is very simplistic view.

As I mentioned you,  $a$  and  $b$  are some coefficients representing the relative contributions. These equations represent of course over-simplification as I mentioned to you already to bring out the concepts.

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Now schematically if you want to represent in a diagrammatic form this is the two-dimensional frequency spectrum and these are the two 1D signals here so one frequency  $\omega_k$ , other frequency  $\omega_l$ . We started the calculation with  $\omega_k$  frequency. We said  $\omega_k$  frequency, part of it remains at  $\omega_k$  and this produces the diagonal and part of this is transferred to the spin  $l$  and this becomes your cross peak. So, I generated from  $\omega_k$  this frequency and this frequency.

But there is no stopping if you are exciting both the spins at the same time then the same thing can happen for the spin  $l$  as well. So if you started with the spin  $l$  then during the  $t_1$  period it will evolve with the frequency  $\omega_l$  and during the mixing some of it will get transferred to  $\omega_k$ . Therefore this will produce a cross-peak here and diagonal peak here. So therefore each one of these will produce a diagonal peak and a cross peak. So this will depend upon the interactions between these two spins.

Whatever the interactions are, and that will be reflected in the two-dimensional plane. And this was a phenomenal effect because where there are lot of signals which are present underneath at various one particular place you could filter out those signals which are interacting. So we can figure out from the cross peaks which spins in this spectrum are interacting. If you notice this is essentially a one-dimensional spectrum.

So the diagonal essentially represents a one-dimensional spectrum. You take a projection here or here, it is the same frequency, here or here it is the same frequency. But when you

have lots of signals here present then you actually filter out those which are interacting. Therefore this was a major revolution because you could display the interactions between the spin in a two-dimensional plane and that allowed resolution enhancement in the spectrum, and extracting information which one could not do earlier.

So here, the, this one is  $F_1 = F_2$ , this is the diagonal and this is also diagonal and this color represents  $F_1 \neq F_2$ . And it could, where this peak will appear will depend upon what are the interactions between the spins and how the mixing transfers the magnetization between the spins. So we will stop here and we will continue with the theory of this in the next class.