

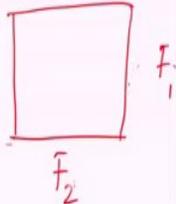
**NMR Spectroscopy for Chemists and Biologists**  
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**Lecture 43**  
**Types of 2D NMR Spectra**

(Refer Slide Time: 0:34)

**Types of 2D-NMR spectra**

The known 2D-NMR experiments can be broadly classified into three categories;

- (i) Resolution/ Separation experiments
- (ii) Correlation experiments
- (iii) Multiple quantum experiments



So, we are now going to discuss the various two dimensional NMR experiments that have been developed over the years. A large number of experiments have been developed and these are generally categorized into three groups. One we call them as resolution or separation experiments, or correlation experiments, multiple quantum experiments.

So, three categories of experiments are known and we put them as resolution or separation experiments or correlation experiments or multiple quantum experiments and this is a very unique feature. Multiple quantum experiment is a very unique feature of two dimensional NMR spectroscopy. This was not possible to observe in the conventional one dimensional NMR and that is I will just briefly tell you what it is all about.

And we know that in a two dimensional spectrum we have two frequency axis and we called this as  $F_2$  and  $F_1$ . So, whatever the information present in the  $t_1$  domain of the two dimensional data body, it appears along the  $F_1$  dimension, what is present during the detection periods appears

along the  $F_2$  dimensions. So, these experiments differ by the information content and what is present along the  $F_1$  axis or what is present in the map here in the two dimensional matrix.

So, that is how these experiments can be classified and grouped into three different types. You may have particular kind of information along the  $F_1$  axis. For example, you may only have the chemical shapes or you may have coupling constants along the  $F_1$  axis. And similarly, you may have coupling constants or the chemical shifts along the  $F_2$  axis then it will lead to one kind of experiments. And if we have multiple quantum frequencies along the  $F_1$  dimensions then you have what were called as multiple quantum experiments.

See, typically multiple quantum signals are not observable as we discussed in the product operator formalism, multiple quantum signals are not directly observable because the trace of these multiple quantum operators with the  $I_x$  or the  $I_y$  operator is 0. Therefore, they are not observable. But, in the  $F_1$  dimensions since you are not doing any direct detection, it is possible to represent this frequencies here and the evolution under the multiple quantum frequencies modulates the detected signal in the  $F_2$  dimension and eventually we detect only the single quantum frequencies in the  $F_2$  dimension, but the multiple quantum frequencies are obtained along the  $F_1$  dimension in an indirect manner.

In  $J$  resolved or resolution experiments  $F_2$  dimension will have the normal frequency information and  $F_1$  dimension may have the only the coupling information or the coupling plus chemical shift information so on and so forth. So, this is the way these various experiments have been designed and we are going to discuss this in somewhat greater detail in the following slides.

(Refer Slide Time: 3:42)

## 2D- resolution/ separation experiments

The primary aim in these experiments is to separate the different interactions in the Hamiltonian. In high resolution NMR, this amounts to separation of the Zeeman ( $\mathcal{H}_Z$ ) and the coupling Hamiltonians ( $\mathcal{H}_J$ ).

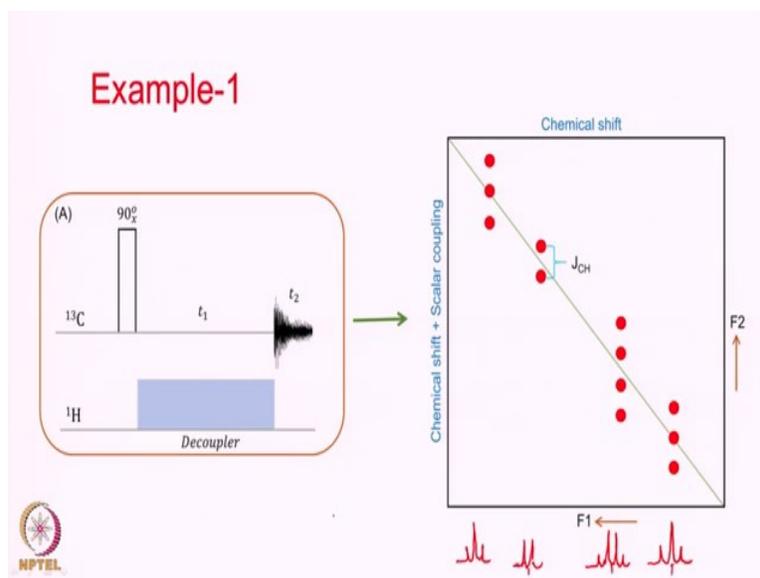
$$\mathcal{H} = \mathcal{H}_Z + \mathcal{H}_J$$

Different strategies can be defined depending upon the nature of the information required in the final spectrum.



So, let us first look at 2D resolution and separation experiment. So, the primary aim in these experiments is to separate the different interactions in the Hamiltonian. So, we are talking about high resolution NMR here. In high resolution NMR the Hamiltonian consist of two terms, one is the  $H_Z$  or the second one is  $H_J$  and this is a Zeeman Hamiltonian, and this is  $J$  coupling Hamiltonian. Zeeman Hamiltonian is responsible for the chemical shifts and the  $J$  coupling Hamiltonian is responsible for the couplings. Now, different strategies can be defined depending upon the nature of the information required in the final spectrum. Let us take a few examples and try and see how we can analyze that.

(Refer Slide Time: 4:27)



So, this is one example where in you do the experiment in the following manner. So, you can say that heteronuclear system carbon proton and we are talking about a natural abundance carbon 13. So, in a molecule which has only 1 percent carbon 13 and we are talking about those kinds of things. And of course the proton is 100 percent abundant. And there will be different kinds of carbons in a molecule, some of them may be attached to one proton, some may be attached to two protons, some may be attached to three protons and so and so forth. CH groups or CH<sub>2</sub> groups or CH<sub>3</sub> groups.

The experiment is performed in the following manner. You first apply 90 degree pulse to the carbon magnetization. Remember we are talking about the <sup>13</sup>C so that is at the natural abundance. So, and when we apply 90 degree pulse to this carbon which is initially magnetization here is along the Z axis and it will after the 90 degree pulse, this magnetization comes into the Y axis. Depending upon where you apply the pulse whether it is to the Y axis or the X axis depending upon the phase of this pulse, when you apply x pulse it will come to the minus Y axis and if you apply y pulse it goes on the X axis and so and so forth.

Now, during the  $t_1$  period this is your indirect detection period or the evolution period, we apply proton decoupler broadband decoupler here which means, so during this period the carbons will not evolve under the carbon proton coupling. They will only evolve under the carbon chemical shift. So, the different kinds of carbons which are present they will evolve under the carbon

chemical shift. So, what is information present here? Only the carbon chemical shift information is present here, ok.

So, at the end of the  $t_1$  period here we start the deduction period and there we turned off the proton decoupler. Therefore, what is the information present in the  $t_2$  period? We have both the carbon chemical shifts and the carbon proton couplings, right. So therefore, when we do a two dimensional experiment and two dimensional Fourier transformation the spectrum will look like this.

So, this is my  $F_1$  dimension here, here of course we are represented  $F_1$  in the different way and conventionally we had written this as the  $F_2$  dimension, this as the  $F_1$  dimension, but that does not matter you can choose whichever way you want. So, we have put here as  $F_1$  and I put here as a  $F_2$ . So now, if you see what is present along the  $F_2$  dimension?  $F_2$  dimension have this chemical shift. This is the chemical shift of this particular triplet here and this center is a chemical shift of quartet here and this is the doublet chemical shift here and this is again a triplet of this.

So, this one therefore this triplet here, this represents a  $\text{CH}_2$  group and this is the quartet which represents a  $\text{CH}_3$  group and this is a doublet which represents a  $\text{CH}$  group and this is again a triplet which represents a  $\text{CH}_2$  group. And this information, this coupling information represents only along the  $F_2$  dimensions here. Therefore, you have a triplet here, a quartet here, a doublet here and again a triplet there.

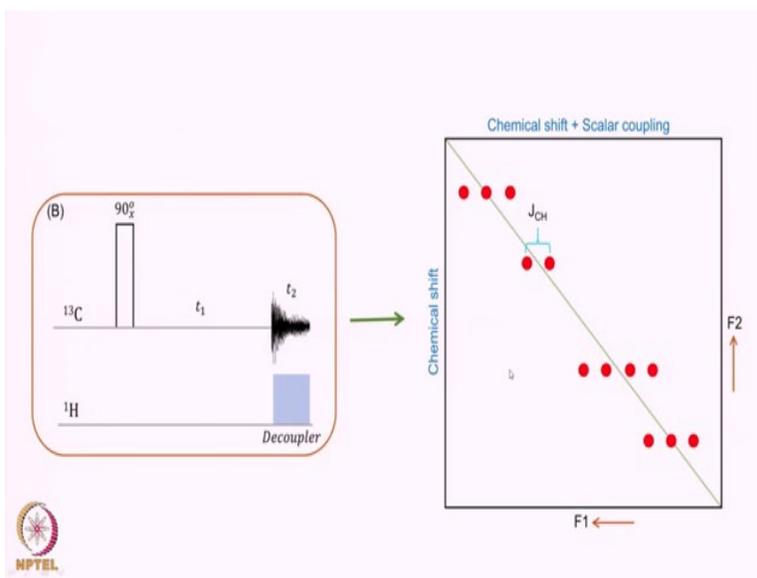
Now, along the  $F_1$  dimension what we have? If you take the projection you only have one line, you take the projection here you have only one line, similarly here. And this is at the chemical shift of the particular carbon. And see the line which has been drawn here this is to indicate the so called diagonal. You see this chemical shift along this axis or this axis is the same, this point is same on this axis or this axis.

Therefore, here I have the chemical shift information and here also I have chemical shift information at this position and if I come here I have a chemical shift information corresponding to this. And similarly, the chemical shift information is here. This is the center of the multiplet, this is the quartet and here the doublet and doublet is of course the center of the doublet is here, the chemical shift position is in the middle here and the two lines in the doublet are separated out from here.

So, if we were to take cross section here like this, then it will be a quartet of CH<sub>3</sub> group. The quartet of a CH<sub>3</sub> group how does it look? So, let us just try and draw it here. So, a triplet if I were to take a cross section here, so that will look so this will be the cross section here and if I were to take cross section here it will look like, it will be a quartet so this will be a quartet, ok. And the center is here, this is the chemical shift position, this is the chemical shift position.

So, likewise this one will be a doublet the chemical shift position is in the middle and thus last one be a triplet again and this is due to a carbon proton coupling ok, carbon proton one bond coupling these are. Carbon proton one bond coupling is like 140, 150 hertz so therefore these are the pretty well resolved and the center is in this empty position here, ok. So, this is one way to separate out the interactions. Notice here we have both chemical shift and coupling information along the  $F_2$  dimension and along the  $F_1$  dimension we have only the chemical shift information. Let us see we do the other way around.

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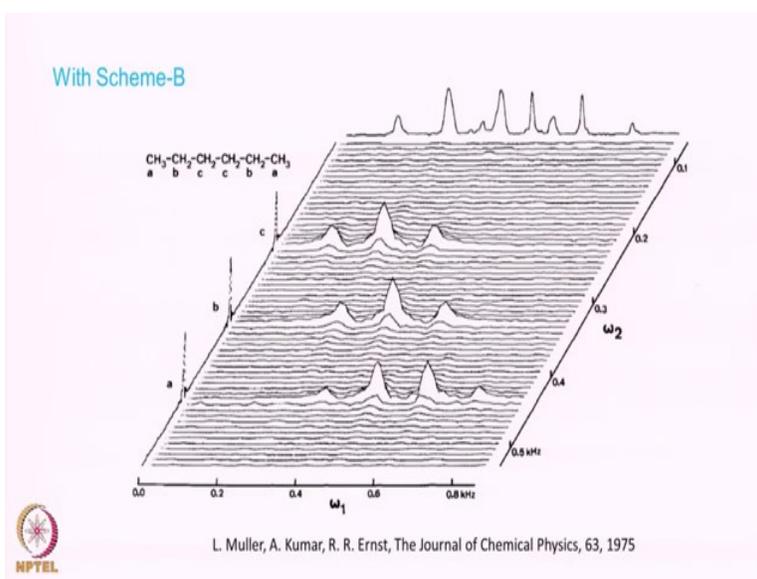
Yes. So, here we have the pulse sequence which is different compared to that one, you apply a 90 degree pulse along the on carbon as before, now during the  $t_1$  period now we do not apply decoupler, we apply the decoupler along the  $t_2$  axis. So, during the  $t_1$  period therefore we have chemical shift plus the coupling constant of the carbon and proton and therefore the appearance of this spectrum will now look different, ok. Here once again now if you see the diagonal is running, is running like this, you have this is the center which is the chemical shift of the

multiplet, and here again is a chemical shift of a multiplet, this is a chemical shift of a multiplet, this is a chemical shift of a multiplet.

Now, if you have to take cross section here, then this will be a triplet as before, and this will be a doublet, this will be a quartet with four lines, ok. And this will be a triplet. So, on this axis we have only chemical shift information if I were to take a projection here along this axis I only have one line and that will be a chemical shift so, similarly here. So, the appearance of the spectrum looks different.

Now, if we were to record an NMR spectrum experimental, why it is useful to have this? If the chemical shifts are not very well separated, sometimes this you may not able to see is in the 1D spectrum, one may wonder whether these quartets if they are very clear in the 1D spectrum do we really need to do it? But, you will see that sometimes it is not very clear.

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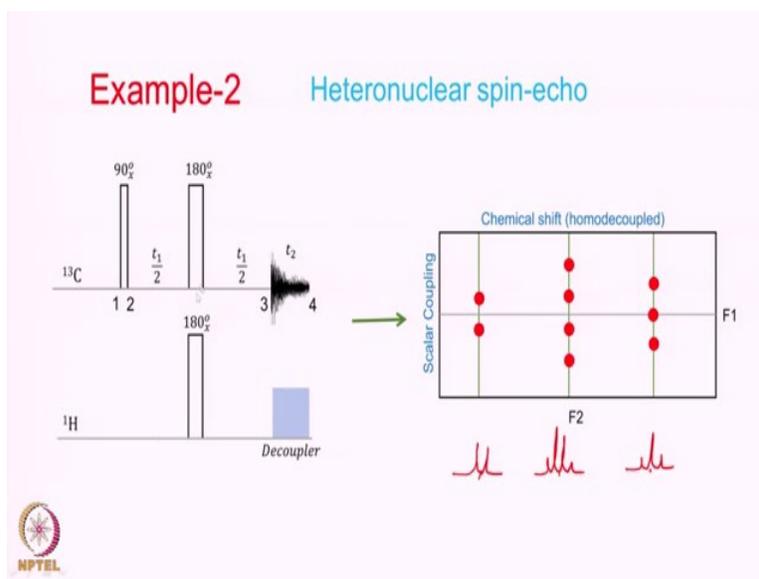
And I will show you an example here. So, here is an example which corresponds to the situation is the second pulse which I showed you. And this were the first actually two dimensional spectrum record, among the all 2D experiments recorded, this was the first experiment that was recorded way back in 1975.

So, here the  $F_1$  axis, this has the chemical shift and the coupling information, this is the  $F_2$  axis which has only the chemical shift information. That means we have applied decoupler along the

$F_2$  dimension, proton decoupler. So, now you see this one is a quartet, this is a triplet and this is a triplet. This is of what molecule? This is molecule here an hexane and you have  $\text{CH}_3$   $\text{CH}_2$   $\text{CH}_2$   $\text{CH}_2$   $\text{CH}_2$   $\text{CH}_3$  these the two end carbons are equivalent the two again these two central carbons are equivalent and these two carbons are equivalent, ok.

So, there are three types of carbons. The  $\text{CH}_2$ 's are triplets and  $\text{CH}_3$  is a quartet. Now, if we were to take a projection, this is what the one dimensional spectrum we will get, ok. Now suppose we did not do a 2D experiment this is a kind of a one dimensional spectrum we will get. See, here it is impossible to figure out what sort of multiplets you have in your molecule, ok. But, by this separation you are able to see clearly that there are two triplets and one quartet, ok. So, this is the benefit of separating out the interactions in the two dimensional plane.

(Refer Slide Time: 13:51)



Now, you can do even more that is we want to completely separate out the chemical shifts in the coupling constants. So, in the earlier cases we have one axis the chemical shift and other axis both chemical shifts and coupling constant. But can we do something better than that? So, let us consider a sequence which is like this. So, you have now a  $t_1$  period going from here to here but we apply 180 degree pulses in the middle of the  $t_1$  period on both carbon and proton.

Now, you recall this is like a spin echo sequence. This is a spin echo sequence from here to here it is a spin echo. So therefore, when I apply these 180 pulses on both carbon and proton what I am retaining is the coupling information all the way from here to here, coupling is not refocused

but this 180 degree pulses on the carbon channel refocused with the carbon chemical shifts. Because you have created carbon magnetization at this point and it will refocused the carbon chemical shifts. But the coupling information to the proton is retain and during the  $t_2$  period the chemical shifts are evolving but I do at the coupling of the proton.

So therefore, along the  $t_2$  axis I have only the chemical shift information. If you see now I am represent the  $F_2$  axis here and the  $F_1$  axis here so, what is it we have? We have on the  $F_2$  axis I have only the chemical shift information and along the  $F_1$  axis I do not have the chemical shift information, but only the coupling information. Therefore, it will spectrum will look much more simpler here as compare to the previous ones. And this you see is the scalar coupling so, the doublet will appear like this and the quartet will appear like this and the triplet will appear like this.

So, if you were to take cross sections here of this then they will all be as indicted before, let me just show that this is a see if I were to take a cross section here so, this will be a doublet with the chemical shift in the middle and this will be a quartet with the chemical shift in the middle again and this will be a triplet. Now the chemical shift will be on the middle again which will be it will fall on the line in the center.

So, this is the way you separate out the interactions. This is as though we have rotated this the whole multiplet along the orthogonal axis so you have put the multiplet structure along the orthogonal  $F_1$  axis and the chemical shifts are kept along the  $F_2$  axis. And this is extremely a useful technique and to do a little bit more rigorous analysis we can do a product operator calculation of this which is pretty simple as we have discussed in the previous classes.

So, in order to do that what we will do is a consider the time points explicitly here so, this is the initial magnetization therefore, I call this as the time point 1, then I have here time point 2, then the  $t_1$  by 2 evolution and  $t_1$  by 2 evolution this is the spin echo sequence. So therefore, here I will directly evolve the coupling information therefore I can directly go to this point here, level this as 3 and I level to this time point as 4. So therefore, we will calculate the density operator using the product operator formalism as to how the spin system will evolve through this pulse sequence. This is pretty straight forward.

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### Product operator calculation: for a CH system

$$\rho_1 = C_z$$

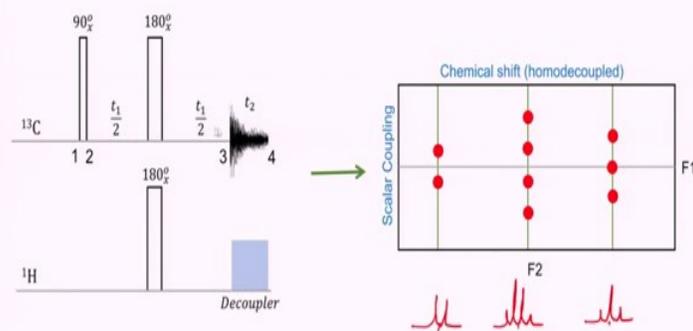
$$\rho_2 = -C_y$$

$$\rho_3 = -[C_y \cos \pi J_{HC} t_1 - 2C_x H_z \sin \pi J_{HC} t_1]$$

The second term does not lead to observable magnetization in  $t_2$  because of proton decoupling. The first term evolves under chemical shift only during  $t_2$ .



### Example-2 Heteronuclear spin-echo



And let us see what is  $\rho_1$ ,  $\rho_1$  is the  $Z$  magnetization of the carbon. I have we applied the 90 degree pulse to the carbon right. So therefore, initially I have the  $Z$  magnetization of the carbon, this is for a single carbon proton system. Of course we will now do the calculations for the  $\text{CH}_2$ 's and the  $\text{CH}_3$ 's with that we will easily doable but we will demonstrate it for a simple CH system, how it will a doublet will appear, how doublet will come from here.

Now, when I apply  $90^\circ$  pulse I create  $y$  magnetization which will be  $-C_y$ . Now, what I do during this whole period from here to here it is a spin echo sequence there is a no chemical shift evolution of carbon, there is only carbon proton evolution, coupling information, right. So therefore, I evolve this under carbon proton coupling. So therefore,

$$\rho_3 = -[C_y \cos \pi J_{Hc} t_1 - 2C_x H_z \sin \pi J_{Hc} t_1]$$

The second term does not lead observable magnetization in  $t_2$  because of proton decoupling, right. So, this is anti-phase magnetization, when you decouple the two anti-phase terms will simply collapse and therefore you will not have any signal coming from this  $2C_x H_z$  terms. So, what will remain is only  $C_y$  and we need to consider the evolution of this  $C_y$  term only.

(Refer Slide Time: 19:21)

Therefore,

$$C_y \xrightarrow{\mathcal{H}_Z} [C_y \cos \omega_c t_2 - C_x \sin \omega_c t_2]$$

Thus, assuming y-detection,

$$\rho_4 = C_y \cos \omega_c t_2 \cos \pi J_{Hc} t_1$$

Therefore, this leads to exclusively coupling information along  $t_1$  and chemical shift information along  $t_2$



So, now we consider the  $C_y$  evolution during the  $t_2$  under the influence of the chemical shift or the Zeeman Hamiltonian only. So, what does it give me?

$$C_y \xrightarrow{\mathcal{H}_Z} [C_y \cos \omega_c t_2 - C_x \sin \omega_c t_2]$$

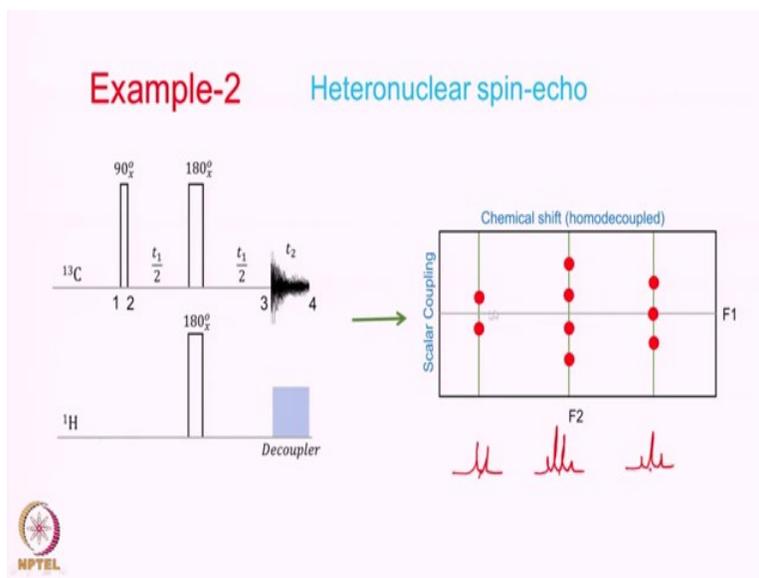
Now we can detect only the  $y$  magnetization and if you do so then of course I will have here the  $\rho_4$  is

$$\rho_4 = C_y \cos \omega_c t_2 \cos \pi J_{Hc} t_1$$

Now you see in the  $t_2$  domain I have only the frequency  $\omega_c$  the chemical shift. Therefore, if I put it transform along the  $t_2$  axis, I will get only the frequency information. Now, along the  $t_1$

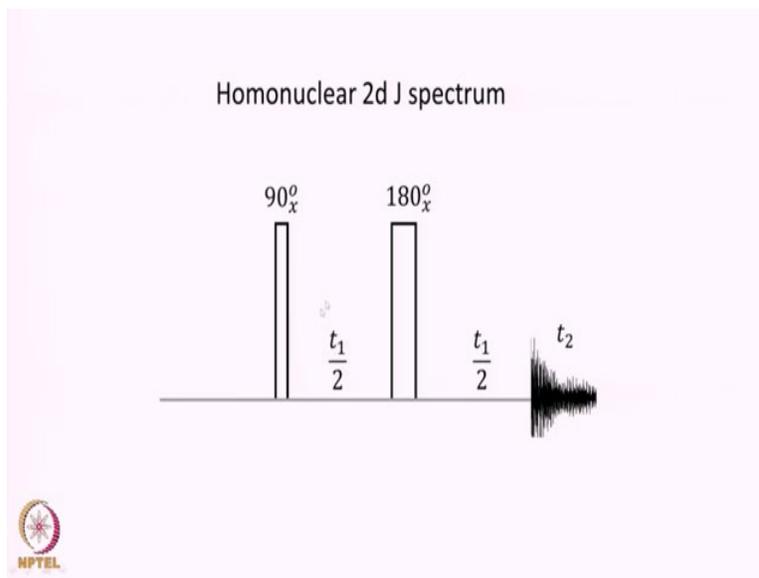
dimension I have only the coupling information this is  $\pi J_{HC} t_1$  therefore when I put it transform along the  $t_1$  dimension I only have the coupling information, ok. So therefore, this results in exclusively coupling information along the  $t_1$  dimension and chemical shift information along the  $t_2$  dimension.

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And that is indicated here in this schematic drawing here we have exclusively coupling information along the  $F_1$  dimension and exclusively chemical shift information along the  $F_2$  dimension. So, in all of these experiments we had considered carbon proton coupling, right. And we are talking about experiments at natural abundance of carbon 13. Therefore, there was no question of carbon-carbon couplings evolution there. But, if we were to do this experiments for protons then of course it can lead to more difficulties because there are proton-proton coupling which will be present in you experiments. And then you will have to deal with what is called as homo nuclear 2D  $J$  spectrum and this will results in more complications.

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The pulse sequence of course remains the same, except that you do not have thing on the another channel you only have the proton channel here and if it were of course also if you have carbon 13 labeled molecules, then also you can do the same thing except you can apply the decoupler along the proton channels, if you want to eliminate carbon proton couplings you want to measure only the homo nuclear couplings. Therefore, for a homo nuclear 2D experiments we define the

experimental sequence like this so  $\frac{t_1}{2}$ ,  $\frac{t_1}{2}$  and  $t_2$ , a detail theoretical analysis of this we will postponed to the next class. So, this will take time, ok. We stop here.