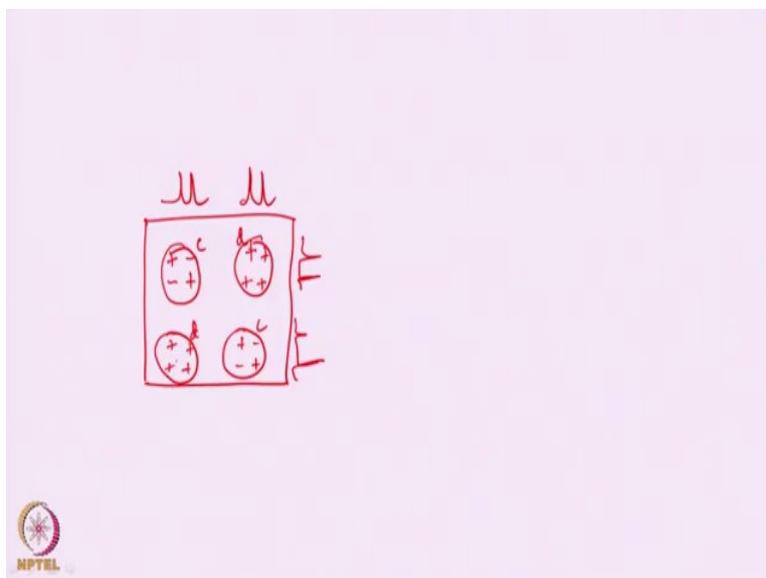


**NMR Spectroscopy for Chemists and Biologists**  
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**Lecture 48:**  
**Double Quantum Filtered COSY (DQF-COSY)**

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In the last class we discussed the details of the two dimensional COSY experiment and we will just have recap of that one here so two dimensional COSY experiment looks like this, so for a two spins systems so you will have a so called diagonal peak for each of the spins and this will appear with a  $\cos$  sinusoidal component so there are two signals of this spin in the one dimensional case so here also we have two line here, and here two lines here for the one dimensional experiment and this produces the two diagonal peak.

And then here you will have via plus minus, minus plus and then you have plus minus, minus plus so this is the structure of the two dimensional COSY experiment we called this one as the cross peak this is the diagonal peak this is the diagonal this is the cross and this is the diagonal this is the cross and the cross peaks has all absorptive line shape and the diagonal peaks have all dispersive line shape because of this it has lower resolution in the spectrum.

So we discussed the consequences of this we also calculated the spectra for 3 spins different kinds of 3 spins in every case the same situation appears the diagonal will have dispersive line shapes with the fine structure of the individual spins and the cross peaks will have this plus minus, minus plus characters with absorptive line shapes now the diagonal having the dispersive line shape is the disadvantage here.

And therefore to circumvent this problem a new idea emerged and that is what we are going to discuss today.

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**Two-dimensional correlation experiments**

**The DQF-COSY experiment**

Scan no	$\phi$	Receiver
1	$x$	$+$
2	$y$	$-$
3	$-x$	$+$
4	$-y$	$-$

So and that is called a two dimensional double quantum filtered COSY experiment which is given in this manner it is a new pulse sequence this first of all it overcomes difficulties of the COSY experiment which we will see soon, it also introduced is say new concept called as phase cycling which is much more general than what is indicated here for this particular experiment however this will illustrate the use of phase cycling for obtaining a kind of information you may want have in your two dimensional spectrum.

The pulse sequence for the double quantum filtered COSY is given here so we have here a 90-degree pulse now we have written here for the phase as  $\phi$  in the case of COSY we had written this as  $x$  and this also as a COSY was the two pulse experiment it has 90- $t_1$ -90 and then the rate hours collected immediately after here we introduce small time period here, this is extremely small time period.

So this is about the of total few microseconds so this time period is extremely small. This is few microsecond and that is given to allow for changes in the phases as we will see soon. And then following that you apply a final 90 degree pulse and then you have the data collected as a function of time  $t_2$ .

Now here this  $\phi$  is a phase which is change from one experiment to another experiment. You see here it is indicated scan number 1, 2, 3, 4 for this 4 experiments they are all collected with the same value of  $t_1$  so for every value of  $t_1$  this is what you do, you collect 4

experiments and that together you make one FID, well each one is then FID this of course is collected in is the receiver as it is a plus sign may say it is, added and the minus sign means the FID that comes here is subtracted and the third experiment with this phases for the pulses this is added and here the for the fourth experiment with this phases for the pulse it is subtracted.

So collection of all of this is for 1 value of  $t_1$  so when we increment the  $t_1$ , again you do these 4 experiments this strategy is known as phase cycling. This allows us to filter out certain signals and selects certain signals and that is called as selection of coherence transfer pathways this is illustrated very well, and we will see how, it works. And so therefore let me repeat here.

For each value of  $t_1$  you record 4 FIDs with the phase of this 90 degree pulses changing as indicated here. For the first one it is  $x$  then it is  $y$  then it is  $-x$  then it is  $-y$  and the data in the receiver is added here the subtracted here, added here and subtracted here.

So this whole strategy is called as phase cycling. So you may have multiples of this 4, does not means that you can only have 4, suppose you decide to have 8 scan this is called as a scan number so suppose you want to have 8 scans to improve a signal to noise then what you do is, you repeat this 4 once more. So you have 1, 2, 3, 4 with this then you have the 5, 6, 7, 8 again with this and the data is repeat in this manner so in can blocks of 4 you can collect the data in this particular manner.

You can use 4 scans, 8 scans, 12 scans, 16 scans so on and so forth depending upon how much single averaging you want to do, and that is characteristic of this particular experiment. After you do all of this you have a small time period here, which is the few microseconds and this allows us to convert something what we have created here. Into a observation by application of this last 90 degree pulse which is done with a fixed phase. This is done with  $x$  or it can be done with  $y$  also it does not matter. And then the data is collected here.

As the data is collected here, remember whatever comes out is stored in the receiver in this manner so this strategy is called as phase cycling which is a very general strategy which can be used for various other purposes at all as we will demonstrate the particular use in this particular double quantum filtered COSY experiment.

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### Product operator calculations

When the first two pulses are considered to be applied along the x axis ( $\phi=x$ ).

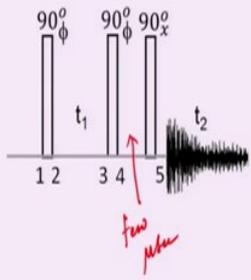
$$\rho_4(\phi = x) = [-I_{kz} \cos \pi J_{kl} t_1 - 2I_{kx} I_{ly} \sin \pi J_{kl} t_1] \cos \omega_k t_1 + [I_{kx} \cos \pi J_{kl} t_1 - 2I_{kz} I_{ly} \sin \pi J_{kl} t_1] \sin \omega_k t_1 \rightarrow (1)$$

Consider when the two pulses are applied along the y axis ( $\phi=y$ ).

$$\rho_4(\phi = y) = [-I_{kz} \cos \pi J_{kl} t_1 + 2I_{ky} I_{lx} \sin \pi J_{kl} t_1] \cos \omega_k t_1 + [I_{ky} \cos \pi J_{kl} t_1 + 2I_{kz} I_{lx} \sin \pi J_{kl} t_1] \sin \omega_k t_1 \rightarrow (2)$$


### Two-dimensional correlation experiments

#### The DQF-COSY experiment



Phase cycling		
Scan no	$\phi$	Receiver
1	x	+
2	y	-
3	-x	+
4	-y	-



Now let us try and understand this how this works and what is a consequence of this doing 4 different scans and adding and subtracting them as we indicated here, If you remember the first 2 pulses are similar as in the case of the COSY so the two 90 degree pulses but, the important thing is the two 90 degree pulses have the same phase of course in the COSY also we consider 90x 90x, so in this case also we do this 90x 90x and this calculation for the density operator remains the same as in this case what we do here is, we directly write the value of density operator at this point starting from the  $k$  spin magnetization here.

Here the initial magnetization is it will be  $z$  magnetization of  $k$  spin and  $l$  spin for a two spin system  $kl$  system and we illustrate the calculation starting from the  $k$  spin and with the  $k$  spin here these are the  $kz$  magnetization you apply this 90 degree pulse and continue through the

evolution as we did in the case of the COSY and arrive at this point. When you arrive at this point what is the density operator so this is

$$\rho_4(\phi = x) = [-I_{kz} \cos \pi J_{kl} t_1 - 2I_{kx} I_{ly} \sin \pi J_{kl} t_1] \cos \omega_k t_1 + [I_{kx} \cos \pi J_{kl} t_1 - 2I_{kz} I_{ly} \sin \pi J_{kl} t_1] \sin \omega_k t_1 \quad \rightarrow (1)$$

Like the of COSY what we did was, we said, this portion of the density operator is not observable so therefore we discard.

We did not want to consider this any further of course afterwards when you collect the data immediately this does not contribute to observable magnetization therefore we discarded it and started continuing with the calculation further using only these two terms and this we said this  $kx$  terms leads to the diagonal term and this is the  $2I_{kz}I_{ly}$  and this is the  $y$  magnetization of the  $l$  spin and this leads to the cross peak.

This leads to the diagonal peak this leads to the cross peak because in the  $t_1$  dimension we have the frequency  $\omega_k$  so  $\omega_k$  and this will evolve with  $\omega_k$  in  $t_2$  therefore this will give the diagonal peak and this will evolve with  $\omega_l$  in  $t_2$  therefore this will provide produce may the cross peak.

And notice here, here is the  $y$  magnetization for the cross peak at this point and here it is  $x$  magnetization of the  $k$  spin at this point. So this is the important difference which you must remember because you are going to come back to it at the later stage now.

So when we repeat this experiment with phase of the pulse incremented by 90 degrees instead of  $\phi = x$  now we put  $\phi = y$  so what happens then you do the same calculation once more then you what you arrive is, is given here.

So individual steps I am not going to give here again because you done that in great detail when we did the COSY experiment so therefore we can simply take this result when

$$\rho_4(\phi = y) = [-I_{kz} \cos \pi J_{kl} t_1 + 2I_{ky} I_{lx} \sin \pi J_{kl} t_1] \cos \omega_k t_1 + [I_{ky} \cos \pi J_{kl} t_1 + 2I_{kz} I_{lx} \sin \pi J_{kl} t_1] \sin \omega_k t_1 \quad \rightarrow (2)$$

So, according to the changes in phase here, we can notice the changes in the various density operator components. Here you have  $kx$   $ly$  and here you have  $ky$   $lx$  here you have  $kx$  now we

have  $k_y$  so here at  $k_z$   $l_y$  now we have  $k_z$   $l_x$ , so these are the consequences of changing the phase or  $\phi$ . So now we are discarding anything at the stage we are keeping all of these.

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Repeat the calculation when the two pulses are applied along the -x axis ( $\phi=-x$ ).

$$\rho_4(\phi = -x) = [-I_{kz} \cos \pi J_{kl} t_1 - 2I_{kx} I_{ly} \sin \pi J_{kl} t_1] \cos \omega_k t_1 + [-I_{kx} \cos \pi J_{kl} t_1 + 2I_{kz} I_{ly} \sin \pi J_{kl} t_1] \sin \omega_k t_1 \quad \rightarrow (3)$$

Similarly, when the two pulses are applied along the -y axis ( $\phi=-y$ ).

$$\rho_4(\phi = -y) = [-I_{kz} \cos \pi J_{kl} t_1 + 2I_{ky} I_{lx} \sin \pi J_{kl} t_1] \cos \omega_k t_1 + [-I_{ky} \cos \pi J_{kl} t_1 - 2I_{kz} I_{lx} \sin \pi J_{kl} t_1] \sin \omega_k t_1 \quad \rightarrow (4)$$


Now let us look at the third experiment the third experiment is

$$\rho_4(\phi = -x) = [-I_{kz} \cos \pi J_{kl} t_1 - 2I_{kx} I_{ly} \sin \pi J_{kl} t_1] \cos \omega_k t_1 + [-I_{kx} \cos \pi J_{kl} t_1 + 2I_{kz} I_{ly} \sin \pi J_{kl} t_1] \sin \omega_k t_1 \quad \rightarrow (3)$$

I numbered this equation as 1, 2, 3, 4 this is the equation number 3. And similarly when the two pulses are applied along -y axis that is now  $\phi = -y$  then I have here

$$\rho_4(\phi = -y) = [-I_{kz} \cos \pi J_{kl} t_1 + 2I_{ky} I_{lx} \sin \pi J_{kl} t_1] \cos \omega_k t_1 + [-I_{ky} \cos \pi J_{kl} t_1 - 2I_{kz} I_{lx} \sin \pi J_{kl} t_1] \sin \omega_k t_1 \quad \rightarrow (4)$$

So these are the 4 equations I get and these represent the density operator at the end of the COSY sequence.

So, now what we since the last pulse is a constant phase pulse so it does not going to make any difference with respect to whether we consider the addition or subtraction of the results of all of these, before or after the evolution after the 90 degree pulse but, what we will do here is.

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We now add/ subtract these four equations as indicated for the receiver phase in the table. This leads to the resultant density operator,

$$\begin{aligned}\rho_4 &= \rho_4(\phi = x) - \rho_4(\phi = y) + \rho_4(\phi = -x) - \rho_4(\phi = -y) \\ &= -4(I_{ky}I_{lx} + I_{kx}I_{ly}) \sin \pi J_{kl}t_1 \cos \omega_k t_1\end{aligned}$$

Pure double-quantum operator

The final  $90_x^\circ$  pulse transforms double quantum operator to single quantum operator.



Let us now try and see what is a consequence of the phase cycling that means if I consider the operations of this 4 FIDs and say I take here the plus sign and the subtract the second result add the third result and subtract the fourth result. If I do all of these because any way this is the phase cycling so in the data is added or subtracted I can add those results here itself and see what is the final density operator at  $\rho_4$ .

So the results of the 4 experiments which we have done here if I did a addition, subtraction, addition, subtraction if I take these 4 density operators and do this operation what do I get, and this algebra of course one can do it very easily and then we will find here that I get

$$= -4(I_{ky}I_{lx} + I_{kx}I_{ly}) \sin \pi J_{kl}t_1 \cos \omega_k t_1$$

So you see this is very interesting, and we have here this  $I_{ky} I_{lx} I_{kx} I_{ly}$  terms, these once were neglected in the COSY we said these once are not observable therefore we are not going to consider that. Here what we have done is, we actually selecting only those we have eliminated all the other terms. We eliminated the  $I_{kz}$  term we eliminated the  $I_{kx}$  term and also the  $I_{kx}$  or  $I_{kz} I_{ly}$  or  $I_{kz} I_{lx}$  whatever that was, we have eliminated all those single quantum does.

Now, this if we recall from the product operated descriptions this is pure double quantum operator. Therefore, by doing this operation at the end of 4 experiments the  $\rho_4$  contains a pure double quantum operator. The  $t_1$  dependent term of course remains this  $\sin \pi J_{kl}t_1 \cos \omega_k t_1$ . This is single quantum and this here is, at this point of course we do not decide this is not, does not decide whether it is, single quantum double quantum these are the coefficients.

The nature of this coherence is decided by these operators so this is pure double quantum operator. Now, these are double quantum operator is not observable, so therefore, in order to observe these what we do is, we apply the third 90 degree pulse the final 90 degree pulse transforms the double quantum operator to single quantum operator because it is only the single quantum which we can detect. Which leads to observable magnetization in the detection period.

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The corresponding density operator  $\rho_5$  will be

$$\rho_5 = -4 (I_{kz}I_{lx} + I_{kx}I_{lz}) \sin \pi J_{kl}t_1 \cos \omega_k t_1$$

This now consists of anti-phase magnetizations of both k, and l spins with the same phase. They will evolve during the  $t_2$  period into in-phase magnetizations of k and l spins, whereby it becomes observable.

$$\rho_5 = -4 (I_{kz}I_{lx} + I_{kx}I_{lz}) f(t_1)$$

$$f(t_1) = \sin \pi J_{kl}t_1 \cos \omega_k t_1$$


So, therefore when we apply the last 90 degree pulse then I write the density operator  $\rho_5$  as

$$\rho_5 = -4 (I_{kz}I_{lx} + I_{kx}I_{lz}) \sin \pi J_{kl}t_1 \cos \omega_k t_1$$

Now we see this is an important part in the earlier case when we are considering just before the detection the diagonal peak had one particular kind of a phase the cross peak had a different kind of phase.

Now we see both this one leads to the cross peak this leads to the diagonal peak why do I say it is, because here we have  $\cos \omega_k t_1$  and this term evolves now with  $k$  spin frequencies and this term evolves with the  $l$  spin frequencies and therefore this is the diagonal term and this is the cross term.

Now we notice both of them have the same kind of a pattern this is also the  $kx$  and this is  $lx$  is anti-phase with respect to  $l$  for the diagonal anti phase with respect to  $k$  for the cross peak and therefore the patterns of these are similar. So therefore, is expect in end that the patterns of the peaks in the diagonal and the cross spins would be similar.

Along the  $t_1$  axis with these coefficients and this will result in the frequency modulation according to the evolutions here. Now, this consists of anti-phase magnetization of both  $k$  and  $l$  spins with the same phase so they will evolve during the  $t_2$  period into in phase magnetization of  $k$  and  $l$  spins where by it becomes observable. As such these terms are not observable, remember earlier also when these terms are presented after the evolution of the  $t_2$ , we had ignored this because these once do not lead to observable magnetization as they are unless they evolve further into  $I_{ly}$  and  $I_{ky}$  when they evolve into  $I_{ky}$  and  $I_{ly}$  then they lead to in phase magnetization which has a non-zero trace with the magnetization operators  $I_{kx}$  or  $I_{lx}$ .

So therefore, this will evolve in the  $t_2$  period to lead to observable magnetization which is in-phase magnetization of  $k$  and  $l$  spins where by it becomes observable. Therefore, we are now going to consider the evolution of these let us rewrite this  $\rho_5$  operator as in the simplified notation we have seen this part and for the this coefficient I written as  $f(t_1)$ ,

$$f(t_1) = \sin \pi J_{kl} t_1 \cos \omega_k t_1$$

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Evolve  $\rho_5$  under  $\mathcal{H}_J$  and  $\mathcal{H}_z$  sequentially

$$\rho_5 \xrightarrow{\mathcal{H}_J} -2 \{ [2I_{kx}I_{lx} \cos(\pi J_{kl} t_2) + I_{ly} \sin(\pi J_{kl} t_2)] + [2I_{kx}I_{lx} \cos(\pi J_{kl} t_2) + I_{ky} \sin(\pi J_{kl} t_2)] \} f(t_1)$$

$$\xrightarrow{\mathcal{H}_z} -2 \{ [I_{ly} \cos \omega_l t_2 - I_{lx} \sin \omega_l t_2] \sin(\pi J_{kl} t_2) + [I_{ky} \cos \omega_k t_2 - I_{kx} \sin \omega_k t_2] \sin(\pi J_{kl} t_2) \} f(t_1)$$

Assuming y-detection, we have the following signal

$$\text{Signal} = -2 [\cos \omega_l t_2 \sin(\pi J_{kl} t_2) + \cos \omega_k t_2 \sin(\pi J_{kl} t_2)] \sin \pi J_{kl} t_1 \cos \omega_k t_1$$


Now, let us consider this evolution of  $\rho_5$  in the  $t_2$  period now the  $t_2$  period both to the Hamiltonian are operative the  $J$  coupling Hamiltonian as well as the shift evolution Hamiltonian they are both operative. Now we can consider that evolution independent of the sequence let us first consider the  $J$  evolution. So if I do the  $J$  evolution, I take 1 2 factor inside so I remain keep the second one here minus 2 and then this gives me

$$\rho_5 \xrightarrow{\mathcal{H}_J} -2 \{ [\cancel{2I_{kz}I_{lx}} \cos(\pi J_{kl}t_2) + I_{ly} \sin(\pi J_{kl}t_2)] + [\cancel{2I_{kx}I_{lz}} \cos(\pi J_{kl}t_2) + I_{ky} \sin(\pi J_{kl}t_2)] \} f(t_1)$$

Now this is after the  $t_2$  evolution, so after the  $t_2$  evolution in  $J$  this term as the anti-phase this term has remain as it is and we have generated in phase terms yet with the coefficient  $\sin \pi J_{kl}t_2$  but, there is residual this anti phase terms with the coefficient  $\cos \pi J_{kl}t_2$  this once will not be observable.

Because if I take the trace of these operators, with  $I_{lx}$  or  $I_{kx}$  this will be 0, and therefore these terms we need not consider and we will consider only these terms which will be observable magnetization.

So next we consider therefore, the evolution of this under the shift Hamiltonian so this gives

$$\rho_5 \xrightarrow{\mathcal{H}_Z} -2 \{ [I_{ly} \cos \omega_l t_2 - I_{lx} \sin \omega_l t_2] \sin(\pi J_{kl}t_2) + [I_{ky} \cos \omega_k t_2 - I_{kx} \sin \omega_k t_2] \sin(\pi J_{kl}t_2) \} f(t_1)$$

So now, we consider  $y$  detection as has been convention we have always assumed this and therefore let us continue to assume that cannot so detect at magnetization of course when they does not matter so for without the loss of generality we will assume this particular detection here, and then we have the following signals so at that point we will ignore this  $x$  terms so I will only have this

$$\text{Signal} = -2 [\cos \omega_l t_2 \sin(\pi J_{kl}t_2) + \cos \omega_k t_2 \sin(\pi J_{kl}t_2)] \sin \pi J_{kl}t_1 \cos \omega_k t_1$$

Therefore, what I have got here, this part this gives me the diagonal peak after two dimensional Fourier Transformation because this the  $\cos \omega_k$  here and here this term gives me the cross peak because the I have  $\cos \omega_l$  here, and  $\cos \omega_k$  here. Therefore, along therefore this leads to the cross peak and this leads to the diagonal peak let us look at this in more explicit forms.

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$$\text{Signal (S)} = -2[\underbrace{\cos \omega_l t_2 \sin(\pi J_{kl} t_2)}_{\text{cross-peak}} + \underbrace{\cos \omega_k t_2 \sin(\pi J_{kl} t_2)}_{\text{diagonal-peak}}] \sin \pi J_{kl} t_1 \cos \omega_k t_1$$

(a) cross-peak

$$\cos \omega_l t_2 \sin(\pi J_{kl} t_2) \sin \pi J_{kl} t_1 \cos \omega_k t_1$$

$$= \frac{1}{4} [\sin(\omega_k + \pi J_{kl}) t_1 - \sin(\omega_k - \pi J_{kl}) t_1] \cdot [\sin(\omega_l + \pi J_{kl}) t_2 - \sin(\omega_l - \pi J_{kl}) t_2]$$

$$+ \sin(\omega_k + \pi J_{kl}) t_1 \sin(\omega_l + \pi J_{kl}) t_2$$

$$- \sin(\omega_k + \pi J_{kl}) t_1 \sin(\omega_l - \pi J_{kl}) t_2$$

$$- \sin(\omega_k - \pi J_{kl}) t_1 \sin(\omega_l + \pi J_{kl}) t_2$$

$$+ \sin(\omega_k - \pi J_{kl}) t_1 \sin(\omega_l - \pi J_{kl}) t_2$$


So, the signal that I am going to collect will be of this type because in the operator part has the removed because we already take in the trace with  $I_{ly}$  and  $I_{ky}$ , so therefore I only written the coefficients which are the once which are responsible for the frequencies. The cross peak is

$$\cos \omega_l t_2 \sin(\pi J_{kl} t_2) \sin \pi J_{kl} t_1 \cos \omega_k t_1$$

Let us analyze the cross peak, for the cross peak I take this and this product and that is written here.  $\cos \omega_l t_2 \sin \pi J_{kl} t_2 \cos \omega_k t_1$ . So therefore, the  $t_1$  part can be split into 2 as indicated here so this is a sum of 2 sine terms similarly, this  $t_2$  dependent part is also sum of 2 sine terms here. And this is indicated both are sine terms this  $\sin(\omega_k + \pi J_{kl}) t_1, -\sin(\omega_k - \pi J_{kl}) t_1$  and the likewise the  $t_2$  part also gives me 2 sine terms  $\sin(\omega_l + \pi J_{kl}) t_2, -\sin(\omega_l - \pi J_{kl}) t_2$ . So those ones are written explicitly here the 4 terms are separated out from this product.

So what this gives me, now if we see this first term the first term has one  $t_1$  part and one  $t_2$  part so, after two dimensional for Fourier Transformations this will give me a frequency at  $\omega_k + \pi J_{kl}$  and this give along the  $F_1$  dimension this is gives me a peak at  $(\omega_l - \pi J_{kl}) t_2$  and along the  $F_2$  dimension. This is one component of the cross peak this has a plus sign. The second component is  $\sin(\omega_k + \pi J_{kl}) t_1$  as the same as this and  $\sin(\omega_l + \pi J_{kl}) t_2$  and this gives me a frequency at  $\omega_k + \pi J_{kl}$  along  $F_1$ ,  $\omega_l - \pi J_{kl}$  and  $F_2$  and this has a minus sign.

So similarly there will be another minus sign minus peak here negative peak with  $\omega_k$  at  $-\pi J_{kl} t_1$  and  $\omega_l - \pi J_{kl} t_2 / J_{kl}$ . This is the along the  $F_2$  this is along  $F_1$  so the fourth one will be with the positive sign  $\omega$  at frequency  $\omega_l - \pi J_{kl}$  along  $F_1$ , and  $\omega_l - \pi J_{kl}$  along  $F_2$ . So therefore this 4 cross peaks have 4 components with the cross peak have  $\hat{i}$  structure.

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(a) cross-peak

This leads to four peaks at the following coordinates.

	90° phase shift
$(F_1, F_2) = \left( \nu_k + \frac{J_{kl}}{2}, \nu_l + \frac{J_{kl}}{2} \right)$ ; positive, dispersive	→ +, Absorptive
$\left( \nu_k + \frac{J_{kl}}{2}, \nu_l - \frac{J_{kl}}{2} \right)$ ; negative, dispersive	→ -, Absorptive
$\left( \nu_k - \frac{J_{kl}}{2}, \nu_l + \frac{J_{kl}}{2} \right)$ ; negative, dispersive	→ -, Absorptive
$\left( \nu_k - \frac{J_{kl}}{2}, \nu_l - \frac{J_{kl}}{2} \right)$ ; positive, dispersive	→ +, Absorptive



So this is what is indicated here, now notice also that all of them were sine terms and we said earlier that this sine terms will actually lead to a dispersive line shape each one of them was a sin term therefore it would dispersive line shape but we want an absorptive line shape, and absorptive line shape, what we get it is possible to get an absorptive line shape, by doing a 90 degree as a phase shift on the signal. A 90 degrees phase shift on the signal we can do in which case we will generate a absorptive line shape.

Of course one could have argued that we could have done the same thing is the COSY as well. Yes indeed but, then of course when you do that you see the COSY the diagonal peak and the cross peak had different line shapes and therefore if you convert one of them into one shape the other one will be changed to another shape. therefore if you make the diagonal peak dispersive and the cross peak absorptive as it was but if you convert that COSY diagonal peak into absorptive line shape, then it will have a dispersive line shape in the cross peak which is not desirable.

So now, we want to have a absorptive line shape in the cross peak and therefore we did not do anything with regard to cross peak in the COSY spectrum but, here we have a dispersive line shape as it is coming therefore we convert this into absorptive line shape by doing a 90

degrees phase shift what is the consequence of this in the diagonal we will see in the next few minutes, so when we do this 90 degrees phase shift the sign does not change, so I get the plus absorptive minus absorptive minus absorptive and plus absorptive.

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$$\text{Signal (S)} = -2[\underbrace{\cos \omega_l t_2 \sin(\pi J_{kl} t_2)}_{\text{cross-peak}} + \underbrace{\cos \omega_k t_2 \sin(\pi J_{kl} t_2)}_{\text{diagonal-peak}}] \sin \pi J_{kl} t_1 \cos \omega_k t_1$$

(b) diagonal-peak

$$\begin{aligned} & \cos \omega_k t_2 \sin(\pi J_{kl} t_2) \sin \pi J_{kl} t_1 \cos \omega_k t_1 \\ &= \frac{1}{4} [\sin(\omega_k + \pi J_{kl}) t_1 - \sin(\omega_k - \pi J_{kl}) t_1] \cdot [\sin(\omega_k + \pi J_{kl}) t_2 - \sin(\omega_k - \pi J_{kl}) t_2] \\ & \quad + \sin(\omega_k + \pi J_{kl}) t_1 \sin(\omega_k + \pi J_{kl}) t_2 \\ & \quad - \sin(\omega_k + \pi J_{kl}) t_1 \sin(\omega_k - \pi J_{kl}) t_2 \\ & \quad - \sin(\omega_k - \pi J_{kl}) t_1 \sin(\omega_k + \pi J_{kl}) t_2 \\ & \quad + \sin(\omega_k - \pi J_{kl}) t_1 \sin(\omega_k - \pi J_{kl}) t_2 \end{aligned}$$


Therefore, the 4 components will have the  $\delta$  structure with the absorptive line shapes now, let us look at the diagonal peak, the diagonal peak has

$$\cos \omega_k t_2 \sin(\pi J_{kl} t_2) \sin \pi J_{kl} t_1 \cos \omega_k t_1$$

Notice it is same kind of structure as it was in the cross peak. Except that we have omega k frequency here.

Now you write the split them out in the same manner as before, then I get the same 4 terms here at frequencies is  $\omega_k + \pi J_{kl}$  along  $F_1$   $\omega_k + \pi J_{kl}$  along  $F_2$  and  $\omega_k + \pi J_{kl}$  along  $F_1$   $\omega_k - \pi J_{kl}$  along  $F_2$   $\omega_k - \pi J_{kl}$  along  $F_1$   $\omega_k + \pi J_{kl}$  along  $F_2$ , notice these are all diagonal component therefore I have omega k here in all of them.

And  $\omega_k - \pi J_{kl}$  along  $F_1$ ,  $\omega_k - \pi J_{kl}$  along  $F_2$ . Now once again all of them are sine terms.

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(b) diagonal-peak

This leads to four peaks at the following coordinates.

(F1, F2) =	$[(\nu_k + \frac{J_{kl}}{2}), (\nu_k + \frac{J_{kl}}{2})]$	positive, dispersive	$\rightarrow$	<sup>90° phase shift</sup> +, Absorptive
	$[(\nu_k + \frac{J_{kl}}{2}), (\nu_k - \frac{J_{kl}}{2})]$	negative, dispersive	$\rightarrow$	-, Absorptive
	$[(\nu_k - \frac{J_{kl}}{2}), (\nu_k + \frac{J_{kl}}{2})]$	negative, dispersive	$\rightarrow$	-, Absorptive
	$[(\nu_k - \frac{J_{kl}}{2}), (\nu_k - \frac{J_{kl}}{2})]$	positive, dispersive	$\rightarrow$	+, Absorptive

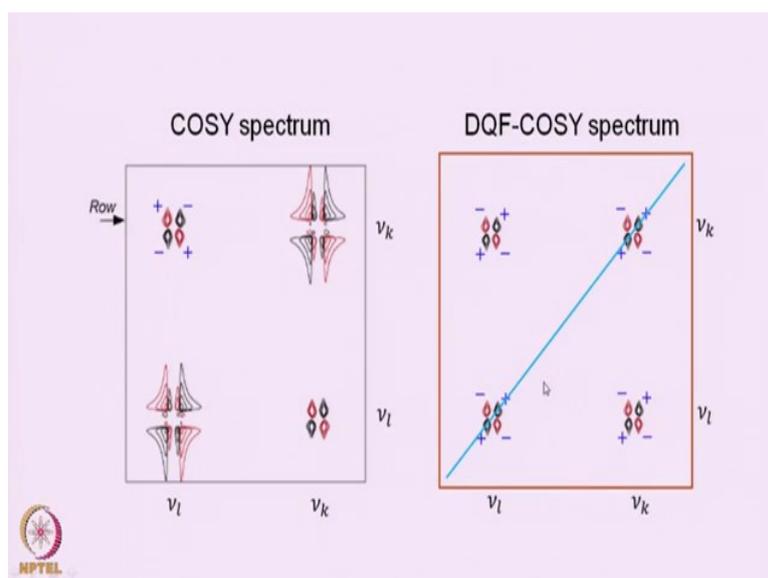


So therefore, I get here in the diagonal peak, the 4 peaks at the following coordinates as indicated here  $\nu_k + \frac{J_{kl}}{2}, \nu_k + \frac{J_{kl}}{2}$ ;  $\nu_k + \frac{J_{kl}}{2}, \nu_k - \frac{J_{kl}}{2}$  and  $\nu_k - \frac{J_{kl}}{2}, \nu_k + \frac{J_{kl}}{2}$ ;  $\nu_k - \frac{J_{kl}}{2}, \nu_k - \frac{J_{kl}}{2}$  and all of them have this kind of a sign this is positive negative, negative positive and this also has dispersive line shapes as they are sine terms but, now therefore I can do a phase shift so that is the important difference.

So in either we have dispersive line shapes in both the cross peak and the diagonal peak, therefore, if I apply 90 degrees phase shift at here also I will get absorptive line shapes. Therefore I get plus absorptive minus absorptive minus absorptive plus absorptive. This is the important difference with respect to the COSY.

That those to the both of the diagonal now and the cross peak have the same line shapes and also a positive negative component structure and therefore if I do a phase correction on the entire spectrum then I get absorptive line shapes in both of the diagonal and the cross peak at the same time. And additionally I will have plus minus structure in the diagonal as well.

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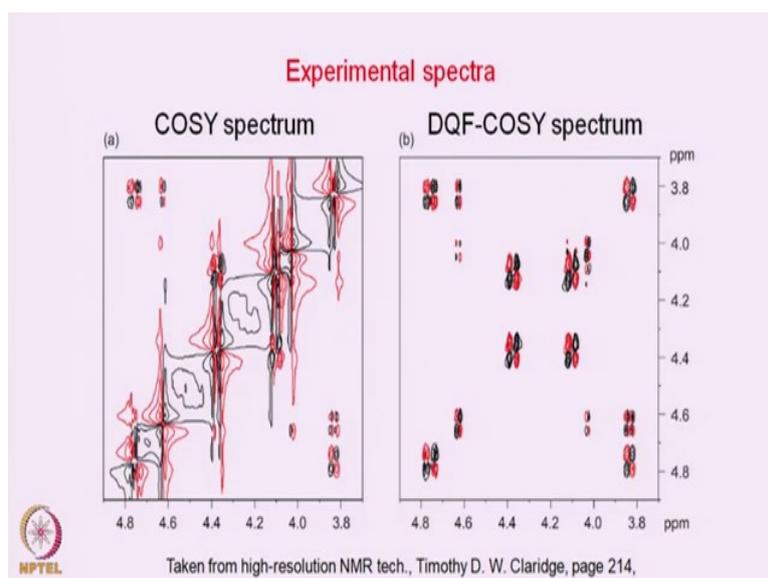
So what is the consequence the consequence is this, so this was the COSY and this is the double quantum filtered COSY so here I have the dispersive line shapes in the COSY and absorptive line shapes in the COSY in the cross peak and the diagonal has dispersive line shapes. If I were to convert this into absorptive line shape this would become dispersive line shape and vice versa.

And that is why we do not want to do that we want have a good sign shape here because these are the information carriers we do not want these wants to be masked and resolution to be loss so, it is not possible for us to do that and we keep the dispersive line shape in the diagonal peak and absorptive line shape in this. This of course was a disadvantage.

Now in the double quantum filtered COSY we get over this problem I get absorptive line shapes in the diagonal as well as in the cross peak and I also have plus minus, plus minus character here. The result of this will be any tails which are present on this line shapes will get cancelled and therefore I will have much higher resolution in the diagonal area as well.

The cross peak dispersion remains the same as in this case. The cross peak the fine structure and therefore the resolution in this remains the same as it is here. But, the game is in the diagonal peak you have the diagonals are absorptive line shapes and plus minus dispersion. The same thing happens for the  $l$  spin we started the calculation with the  $k$  spin so we went through the double quantum filtered and came to the observable single quantum magnetization so it resulted in this structure here, if I where to start from the  $l$  spin then I will also get diagonal peak here and the cross peak here, for with the similar kind of structure so this is the big advantage.

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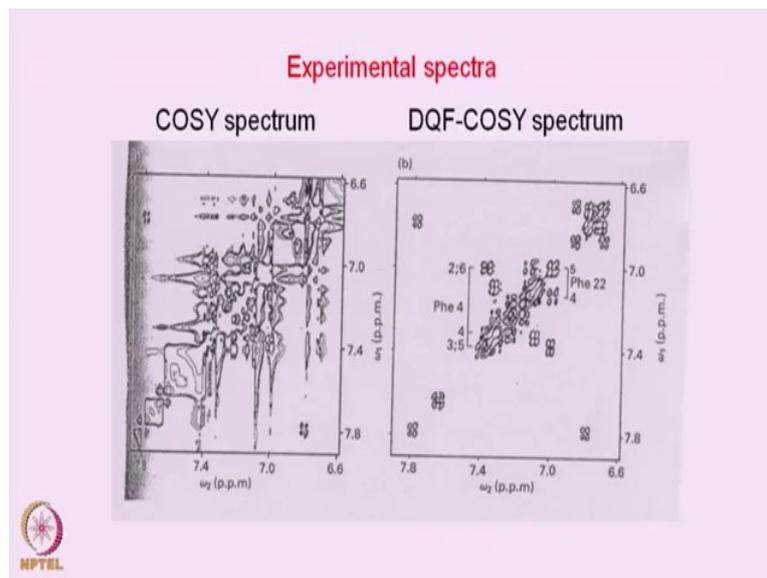
You see the experimental spectrum the experimental spectrum you see this comparison here for a simple small molecule this is taken from this book here and at the page shown 214 we can see the phenomenal improvement in the resolution around the diagonal so, you see all those things which are very close with diagonal you cannot see them here at all.

And these ones are very clear in this spectrum. And that very well resolved. See a consequence of that is because of the cancellations from the tails this cross peak is very poorly seen here and this is seen very clearly in this. And therefore the double quantum filtered COSY says a lot of trouble and the resolution improvement allows us to determine the structure much better.

Notice, also here that these ones are not simple to spin systems in the experimental spectra these also a further fine structures you have so called in-phase splitting as well. And this is a consequence of multi spin systems which we discussed in the COSY experiment earlier. So same thing is happening here there is a further splitting here in this in phases splitting plus plus minus minus and the same thing is retained here as well.

So therefore, you have for all the multiple spin systems you have an improvement in the diagonal peak and the cross pre fine structure remains more or less the same and this is the big advantage in your two dimensional spectrum.

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And here is another example of a much larger system there was a smaller system this is actually on a protein system so one of the region from the protein phenylalanine residue here. And this is actually completely merged so it is impossible to anything in the COSY spectrum here you cannot identify any cross peak here in this COSY spectrum whereas those ones have become very clear here in this as well as in this area so this cross peaks are coming so closed to the diagonal these are very well resolved in this double quantum filtered COSY.

So, this was a big boost for resolution enhancement double in the correlation spectrum. So therefore, the double quantum filtered COSY on the one hand improved the resolution and allowed you to analyze complex spectra and on the other hand it also demonstrated a strategy how to select particular path ways on magnetization so we chose here a double quantum signal and converted the double quantum signal into observable single quantum by applying a third pulse and therefore your pulse sequence was slightly modified.

So with the very general phenomenal one can do this for various kind of filters and also use this strategy for selecting different kinds of path ways for example if you want to select the  $z$  magnetization then also it is possible, you also use the different kinds of the phase cycling the 5 pulses which we I had mentioned there. Use a particular kind of phase cycling for addition subtraction and changing in the phase  $xy$   $-x$   $-y$  and addition subtraction etc.

But, you can use the different combination of this phi's and the receiver phases addition subtraction etc. to choose another kind of magnetization for example the  $z$  magnetization, which case you generate a different kind of correlations in your spectrum. So therefore that

also will be used and we will see therefore this is the good illustration of the strategy of selecting the coherence transfer path ways in your spectrum.

So with that I think I will stop here and we will continue with further methods of improving the spectra in the next class.