NMR Spectroscopy for Chemists and Biologists
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Lecture -45
Two Dimensional Correlation Experiments

We are now going to go were to second category of Two Dimensional Experiments, namely Two Dimensional Correlation Experiments, these have been by far the most useful experiments for studying a variety of systems large systems small systems extra, and has been extremely useful in characterising molecules. Understanding the fine structures in the molecules understanding the connectivities of the individual carbons in molecules and so and so forth.

And the typically it started with homo nuclear proton correlation experiments but today we also various kinds of correlation experiments which deal with proton or carbon 13 and proton with nitrogen 15 and so on so forth.

So, first we will look at the proton-proton correlation experiments and this because in the initial years it was all homo nuclear proton experiments only which we have design and they we use for variety of purposes. So, this experiment is called as the COSY, a correlated spectroscopy, so the abbreviation is COSY and it is very commonly used for all organic chemistry and biochemistry and biological chemistry and what not.

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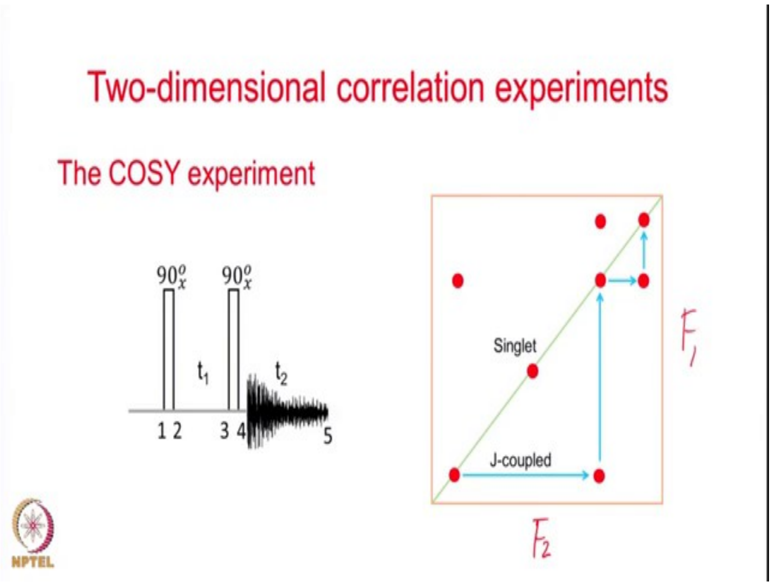

Two-dimensional correlation experiments

The COSY experiment

So, this experiment consist of simple pulse sequence it is a an experiment with the 2 pulses you start with a 90 degree pulse it can be *x* or *y* or whatever but conventionally we are simply sticking to *x* all the time and then you followed by t_1 evolution period then you have a another 90 degree *x* pulse and followed by the detection during the period t_2.

So, in this period of course you may have this what we call as a preparation this includes the 90*x* pulse in the preparation then you have the evolution this pulse 90*x* pulse here itself acts as the mixing pulse in the generalise context of 2D spectroscopy and this is a mixing pulse and then you have the detection here of the signal during the t_2 period.

Now, we will see that this kind of an experiment produces spectrum of this time, this is a schematic experiment. So, if I call this as F_2 axis which is where we actually detect the signal and this is the indirect detection axis we call it as F_1 and that come as a result of Fourier transformation along the t_1 dimension.

And now let us see what is the information here, if you generate peaks like this so I will have a so called a diagonal here the diagonal will have the same frequency along the F_2 and the F_1 axis so therefore this all this peaks which are here they have the same frequencies whether it is along the F_1 or F_2 axis.

Now, then it displays correlation here it displays these are called cross peak and the cross peak displays a correlation between this particular spin and this particular spin, call this as protons is this proton and this proton there is a correlation between these two, where does this correlation arise from? This arises from the coupling from the *J* coupling.

In this experiment, the correlation arises the result of a *J* coupling, these 2 protons are coupled to each other and therefore it produces a cross peak here and this is called as a cross peak or the off diagonal peak.

And now we notice that this peak is also attach to another proton and that is this proton is also coupled to this proton, therefore we generate a cross peak here as well between these 2 protons. So, therefore this forms a network, so you have a network of spins here these 3 protons are coupled to each other this is coupled to this and this is coupled to this and we produce peaks on both sides, so this will be symmetrical spectrum here and no matter which side of the diagonal you use the information is the same.

Now, if there is a proton in your molecule which is not coupled to anything it will produce what is called as a singlet and it will not have any correlations along any of the axis there, therefore it will be easy to identify which of your protons of singular which are not coupled and which of your protons are coupled and what sort of coupling pattern spins system exist in your molecule. So, this is an extremely useful information for characterising your molecular structures.

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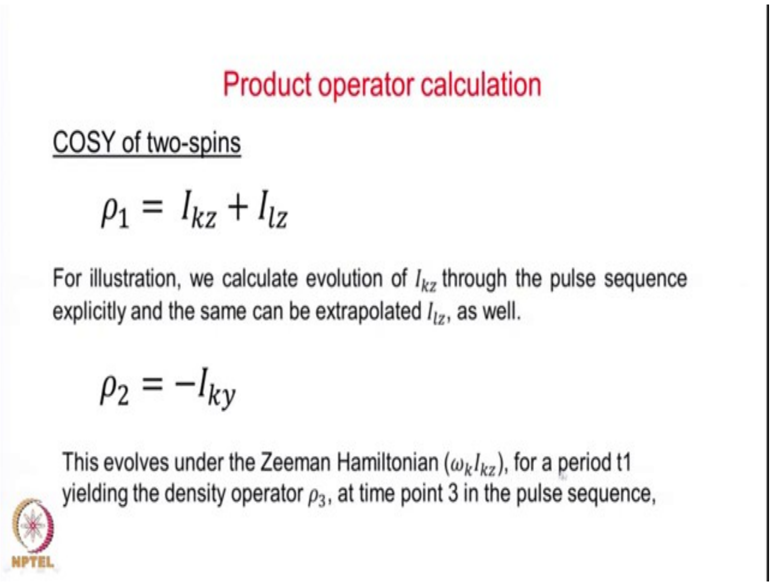


Product operator calculation

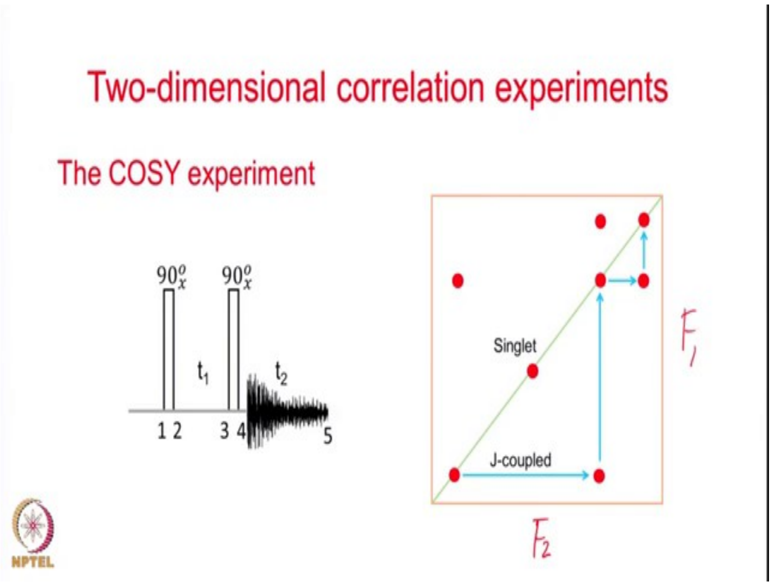
COSY of two-spins

$$\rho_1 = I_{kz} + I_{lz}$$

For illustration, we calculate evolution of I_{kz} through the pulse sequence explicitly and the same can be extrapolated I_{lz}, as well.

$$\rho_2 = -I_{ky}$$

This evolves under the Zeeman Hamiltonian ($\omega_k I_{kz}$), for a period t1 yielding the density operator ρ_3, at time point 3 in the pulse sequence,



Two-dimensional correlation experiments

The COSY experiment

Lets, try an understand this using our the standard method of product operator calculation. So, this is the cosy of two spins, once again which use two spins *k* and *l*, so at the time point 1, so I have written here time point 1 2 3 and 4 we will explicitly calculate the density operator at this individual time points, so that we know what is the information content here and how the information is flowing through the pulse sequence.

So, at time point 1, so I have the equilibrium magnetization, so the density operate is

$$\rho_1 = I_{kz} + I_{lz}$$

Considering the 2 spins. Now, individually we can calculate the evaluations of this through the pulse sequence but later form demonstration we will consider only the *k* spin and whatever result we generate similar calculations can be done for the *l* spin as well and therefore we do not want to repeat that for the 2 thing.

So, we consider the calculation for the k spin only in the further discussion. Now, so if I apply 90*x* pulse to the *k* magnetization I generate

$$\rho_2 = -I_{ky}$$

As before. Now, this evolves under the Zeeman Hamiltonian which is $\omega_k I_{kz}$ for a period t_1 yielding the density operator ρ_3 at time point 3 in the pulse sequence.

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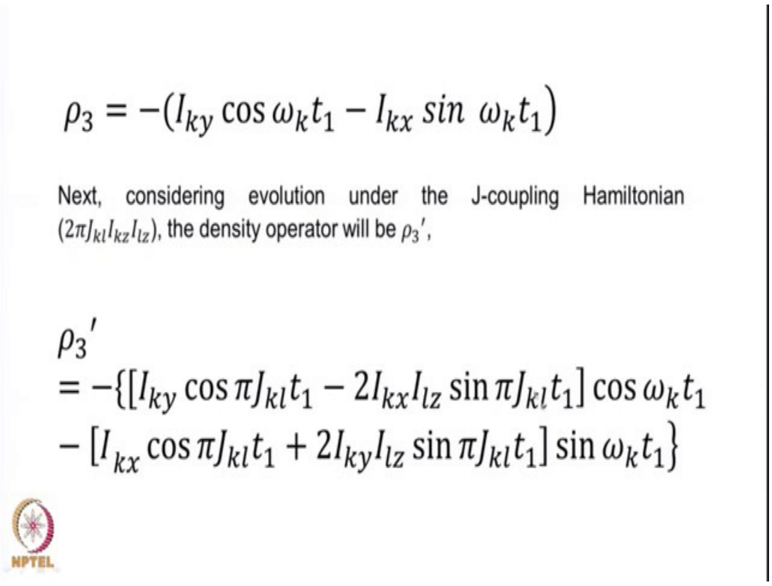

$$\rho_3 = -(I_{ky} \cos \omega_k t_1 - I_{kx} \sin \omega_k t_1)$$

Next, considering evolution under the J-coupling Hamiltonian ($2\pi J_{kl} I_{kz} I_{lz}$), the density operator will be $\rho_3{}'$,

$$\rho_3{}' = -\{[I_{ky} \cos \pi J_{kl} t_1 - 2 I_{kx} I_{lz} \sin \pi J_{kl} t_1] \cos \omega_k t_1 - [I_{kx} \cos \pi J_{kl} t_1 + 2 I_{ky} I_{lz} \sin \pi J_{kl} t_1] \sin \omega_k t_1\}$$

So, now considering the chemical shift evolution the I_{ky} terms who all in this manner the minus sign is kept out here, so I have

$$\rho_3 = -(I_{ky} \cos \omega_k t_1 - I_{kx} \sin \ \omega_k t_1)$$

Now, we have to consider this further for evolution under the coupling next considering evolution under J coupling Hamiltonian which is $2\pi J_{kl} I_{kz} I_{lz}$ so the density operator will be let say $\rho_3{}'$ prime.

So, the $\rho_3{}'$ will be even by keep this minus sign as before and now we individual terms this operators we have to evolve under the coupling. So, I_{ky} evolution gives you this

$$\rho_3{}' \\ = -\{[I_{ky} \cos \pi J_{kl} t_1 - 2 I_{kx} I_{lz} \sin \pi J_{kl} t_1] \cos \omega_k t_1 \\ - [I_{kz} \cos \pi J_{kl} t_1 + 2 I_{ky} I_{lz} \sin \pi J_{kl} t_1] \sin \omega_k t_1\}$$

Now, so this is the density operator at the end of the t_1 period.

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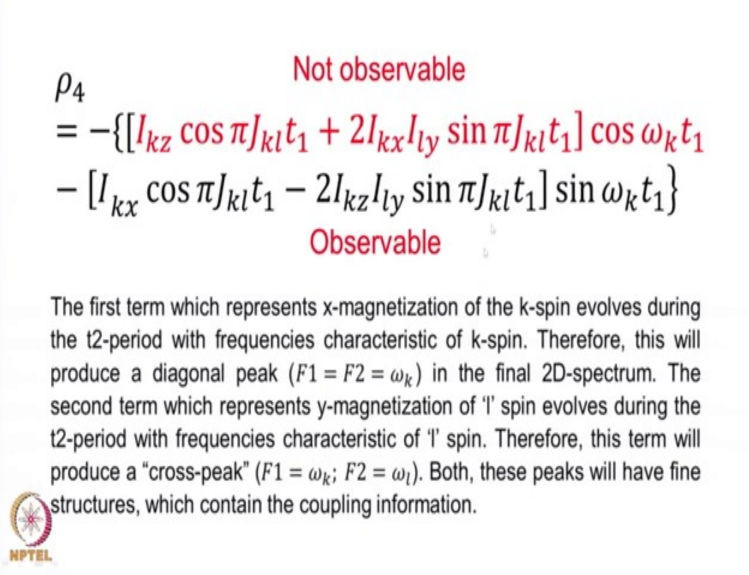

Not observable

$$\rho_4 \\ = -\{[I_{kz} \cos \pi J_{kl} t_1 + 2 I_{kx} I_{ly} \sin \pi J_{kl} t_1] \cos \omega_k t_1 \\ - [I_{kx} \cos \pi J_{kl} t_1 - 2 I_{kz} I_{ly} \sin \pi J_{kl} t_1] \sin \omega_k t_1\}$$

Observable

The first term which represents x-magnetization of the k-spin evolves during the t2-period with frequencies characteristic of k-spin. Therefore, this will produce a diagonal peak ($F1 = F2 = \omega_k$) in the final 2D-spectrum. The second term which represents y-magnetization of 'l' spin evolves during the t2-period with frequencies characteristic of 'l' spin. Therefore, this term will produce a "cross-peak" ($F1 = \omega_k$; $F2 = \omega_l$). Both, these peaks will have fine structures, which contain the coupling information.

Now, what we are doing at the end of the t_1 period we are applying a 90 degree pulse sequence. So, here we notice that after we apply the 90 degree pulse these terms will get transform into the particular manner we get here at

$$\rho_4 \\ = -\{[I_{kz} \cos \pi J_{kl} t_1 + 2 I_{kx} I_{ly} \sin \pi J_{kl} t_1] \cos \omega_k t_1 \\ - [I_{kx} \cos \pi J_{kl} t_1 - 2 I_{kz} I_{ly} \sin \pi J_{kl} t_1] \sin \omega_k t_1\}$$

The second term remains like this the *kx* is not affected so it remains as $kx \cos \pi J_{kl} t_1$but here you see there is a change, so I get here $-2I_{kz}I_{ly} \sin \pi J_{kl} t_1 \sin \omega_k t_1$. Lets, look at this individual terms. So, you see this one is *z* magnetization of the case *k* spin, and this is a mixture of a double quantum and 0 quantum conversances and this is the *x* magnetization of the *k* spin now this one here is the *l* magnetization this is the y magnetization of the *l* spin which is anti-phase with respect to *k*.

Therefore you see the 90*x* pulse the second 90 degree has cause a coherence transfer from the *k* spin to the *l* spin. So, from the *k* magnetization we have generated *l* magnetization here, so this represents a coherence transfer, both this are single quantum terms although this is anti-phase this in phase whereas this one is *z* magnetization and this is multiple quantum transition here. You notice that this entire this operators do not lead to observable magnetizations during the t_2 period.

Because the next what we are going to have is evolution during the t_2 period. Whatever is observable there we are going to return and what is not observable we will ignore, because it is not going to lead to us any signal. Therefore, these two terms here we can ignore because though this once do not lead to observable magnetization.

And these once actual lead to observable magnetization both these are observable terms. Now, the first term which represent *x* magnetization of the *l* spin evolves during the t_2 period with frequencies characteristic of *k* spin. So, during the t_2 period this evolves with a frequency ω_k in the *t1* also we have ω_k in t_2 also it will be ω_k.

Therefore this will produce the so called a diagonal peak which have mention you earlier $F_1 = F_2 = \omega_k$ in the final 2D spectrum. The second term which represents *y* magnetization of *l* spin evolves under the t_2 during the t_2 period with frequencies characteristic of *l* spin.

Therefore, this will have t_2 during the t_2 period this will have frequencies of *l* spin but t_l it had frequency of *k* spin, so therefore this is will be $F_1 = \omega_k$ and $F_2 = \omega_l$ therefore this produces what is called as the cross peak in the final 2D spectrum. Both, these peaks will have fine structures, which contain the coupling information.

And this we will we can calculate we can see that immediately from here that the J_{kl} term is appearing here and therefore we will have coupling information in the in this individual peaks.

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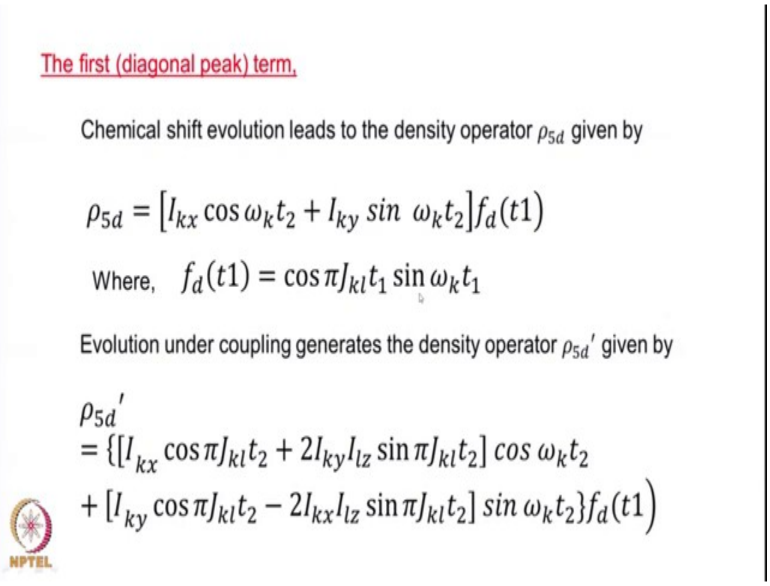


The first (diagonal peak) term,

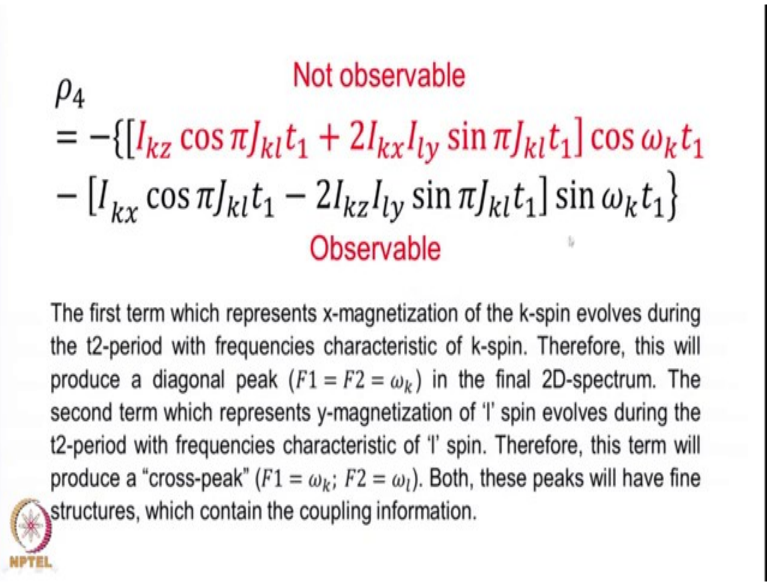
Chemical shift evolution leads to the density operator ρ_{5d} given by

$$\rho_{5d} = \left[I_{kx}\cos\omega_k t_2 + I_{ky}\,sin\;\omega_k t_2\right] f_d(t1)$$

Where, $f_d(t1) = \cos\pi J_{kl}t_1 \sin\omega_k t_1$

Evolution under coupling generates the density operator $\rho_{5d}{}'$ given by

$$\rho_{5d}{}' = \{[I_{kx}\cos\pi J_{kl}t_2 + 2I_{ky}I_{lz}\sin\pi J_{kl}t_2]\,cos\;\omega_k t_2 + [I_{ky}\cos\pi J_{kl}t_2 - 2I_{kx}I_{lz}\sin\pi J_{kl}t_2]\,sin\;\omega_k t_2\} f_d(t1)$$



Not observable

$$\rho_4 = -\{[I_{kz}\cos\pi J_{kl}t_1 + 2I_{kx}I_{ly}\sin\pi J_{kl}t_1]\cos\omega_k t_1 - [I_{kx}\cos\pi J_{kl}t_1 - 2I_{kz}I_{ly}\sin\pi J_{kl}t_1]\sin\omega_k t_1\}$$

Observable

The first term which represents x-magnetization of the k-spin evolves during the t2-period with frequencies characteristic of k-spin. Therefore, this will produce a diagonal peak ($F1 = F2 = \omega_k$) in the final 2D-spectrum. The second term which represents y-magnetization of 'l' spin evolves during the t2-period with frequencies characteristic of 'l' spin. Therefore, this term will produce a "cross-peak" ($F1 = \omega_k$; $F2 = \omega_l$). Both, these peaks will have fine structures, which contain the coupling information.

Let, us first consider first consider the diagonal peak term, the diagonal peak is arising from I_{kx} the first term in this observable part, so chemical shift evolution leads to the density operator ρ_{5d} this is during the t_2 period. So, I call this as ρ_{5d} and this is

$$\rho_{5d} = \left[I_{kx}\cos\omega_k t_2 + I_{ky}\,sin\;\omega_k t_2\right] f_d(t1)$$

So, you recall back and that is just this $\cos\pi J_{kl}t_1$ and $\sin\omega_k t_1$ so I call this as $f_d(t_1)$. And now we consider evolution of these terms under the coupling, evolution under coupling generates the density operator $\rho_{5d}{}'$ given by this formula here $\rho_{5d}{}'$ is now coming from the evolutions of the individual terms here.

$$\rho_{5d}{}' = \{[I_{kx}\cos\pi J_{kl}t_2 + 2I_{ky}I_{lz}\sin\pi J_{kl}t_2]\,cos\,\omega_k t_2 + [I_{ky}\cos\pi J_{kl}t_2 - 2I_{kx}I_{lz}\sin\pi J_{kl}t_2]\,sin\,\omega_k t_2\}f_d(t1)\Big)$$

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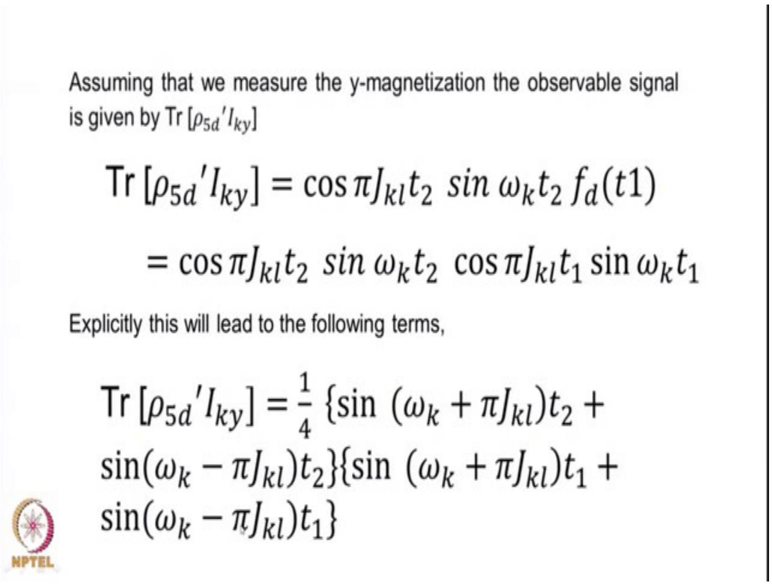

Assuming that we measure the y-magnetization the observable signal is given by Tr $[\rho_{5d}{}'I_{ky}]$

$$\text{Tr}\,[\rho_{5d}{}'I_{ky}] = \cos\pi J_{kl}t_2\ sin\,\omega_k t_2\ f_d(t1)$$

$$= \cos\pi J_{kl}t_2\ sin\,\omega_k t_2\ \cos\pi J_{kl}t_1 \sin\omega_k t_1$$

Explicitly this will lead to the following terms,

$$\text{Tr}\,[\rho_{5d}{}'I_{ky}] = \frac{1}{4}\ \{\sin\ (\omega_k + \pi J_{kl})t_2 + \sin(\omega_k - \pi J_{kl})t_2\}\{\sin\ (\omega_k + \pi J_{kl})t_1 + \sin(\omega_k - \pi J_{kl})t_1\}$$

Now, among this depends upon what signal we are going to measure and we will have to see what was the components which we have to retain. Let, us assume that we detect *y* magnetization, you will have to take the trace of the density operator with I_{ky}, so when we do that we will only have this one here this will represent our signal,

$$= \cos\pi J_{kl}t_2\ sin\,\omega_k t_2\ \cos\pi J_{kl}t_1 \sin\omega_k t_1$$

Not it is the pattern of this evolutions of the terms in the t_2 and the t_1 are the same, here is an sin cosine multiplication and the again here it is sin cosine multiplication. So, if we want to expand this further, so this will give me, so this the same thing which will written here

$$\text{Tr}\,[\rho_{5d}{}'I_{ky}] = \frac{1}{4}\ \{\sin\ (\omega_k + \pi J_{kl})t_2 + \sin(\omega_k - \pi J_{kl})t_2\}\{\sin\ (\omega_k + \pi J_{kl})t_1 + \sin(\omega_k - \pi J_{kl})t_1\}$$

So, what does this tell you that already indicates you there are going to be 2 frequencies along ω_2 that is $\omega_k+\pi J_{kl}$ or if you want to take out the to in terms of the hertz if you want to write frequencies then it will be $\nu_k+\frac{J_{kl}}{2}$ and this will be $\nu_k-\frac{J_{kl}}{2}$ because if you take away the 2π term here so this will be $\nu_k-\frac{J_{kl}}{2}, \nu_k+\frac{J_{kl}}{2}$.

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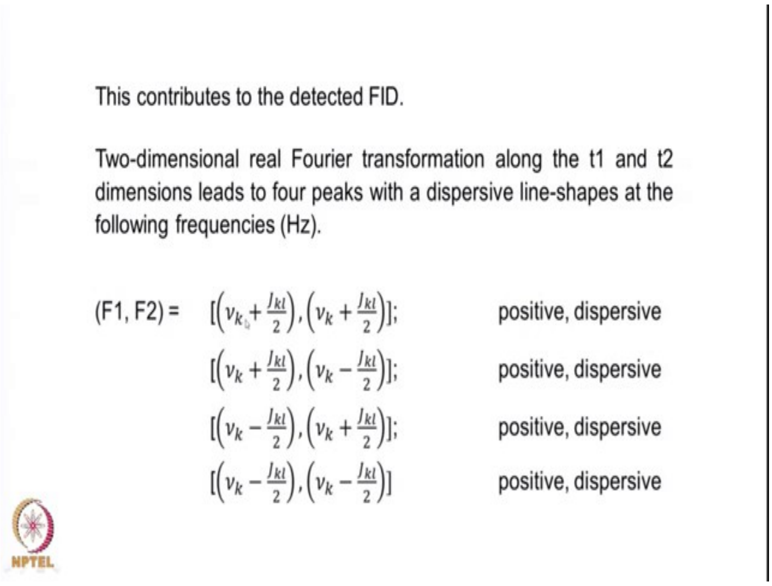
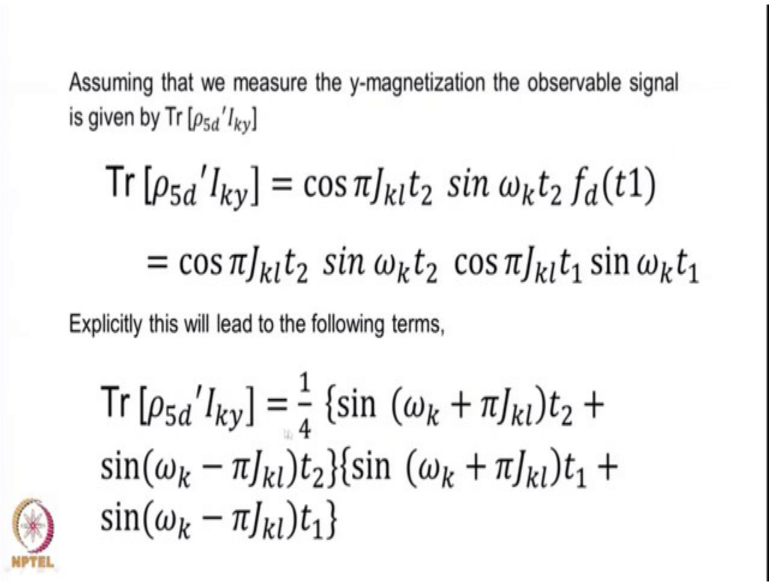


This contributes to the detected FID.

Two-dimensional real Fourier transformation along the t1 and t2 dimensions leads to four peaks with a dispersive line-shapes at the following frequencies (Hz).

(F1, F2) = $[(\nu_k+\frac{J_{kl}}{2}),(\nu_k+\frac{J_{kl}}{2})]$; positive, dispersive
$[(\nu_k+\frac{J_{kl}}{2}),(\nu_k-\frac{J_{kl}}{2})]$; positive, dispersive
$[(\nu_k-\frac{J_{kl}}{2}),(\nu_k+\frac{J_{kl}}{2})]$; positive, dispersive
$[(\nu_k-\frac{J_{kl}}{2}),(\nu_k-\frac{J_{kl}}{2})]$ positive, dispersive



Assuming that we measure the y-magnetization the observable signal is given by Tr $[\rho_{5d}' I_{ky}]$

$$\mathrm{Tr}\,[\rho_{5d}' I_{ky}] = \cos \pi J_{kl} t_2 \; sin\; \omega_k t_2 \; f_d(t1)$$

$$= \cos \pi J_{kl} t_2 \; sin\; \omega_k t_2 \; \cos \pi J_{kl} t_1 \sin \omega_k t_1$$

Explicitly this will lead to the following terms,

$$\mathrm{Tr}\,[\rho_{5d}' I_{ky}] = \frac{1}{4}\{\sin\,(\omega_k+\pi J_{kl})t_2 + \sin(\omega_k-\pi J_{kl})t_2\}\{\sin\,(\omega_k+\pi J_{kl})t_1 + \sin(\omega_k-\pi J_{kl})t_1\}$$

Similarly here along the F_1 dimension also I will have 2 terms the $v_k + \frac{J_{kl}}{2}$and $v_k - \frac{J_{kl}}{2}$, so therefore this entire diagonal peak will have 4 components 2 into 2 therefore this will produce 4 components.

And were this the signal that we detect were blue this one appears now I write in terms of the frequencies removing the pi part, so I will write in terms of the frequencies. 4 peaks with a dispersive line shape, why do we say dispersive line shape? Because this all have sign dependents and we have seen earlier that if there is a time domain signal which is sign dependent it will produce me dispersive signals.

So, we will have a frequency peak at $v_k + \frac{J_{kl}}{2}, v_k + \frac{J_{kl}}{2}$ along F_1 F_2 dimension then I will have here $v_k + \frac{J_{kl}}{2}$ and $v_k - \frac{J_{kl}}{2}$this is along the F_1 this is along the F_2 and this is again $v_k - \frac{J_{kl}}{2}$ and $v_k + \frac{J_{kl}}{2}$and $v_k - \frac{J_{kl}}{2}$and $v_k + \frac{J_{kl}}{2}$.

So, we will have 4 peaks this are centered around the v_k frequency, v_k is my frequency of the *k* spin so and all of this are positive because you see the pervious thing they all have positive components here this are plus plus plus all of the plus this is plus this is plus this is plus so all are plus. Therefore, I will have all positive peaks and they all have dispersive line shapes.

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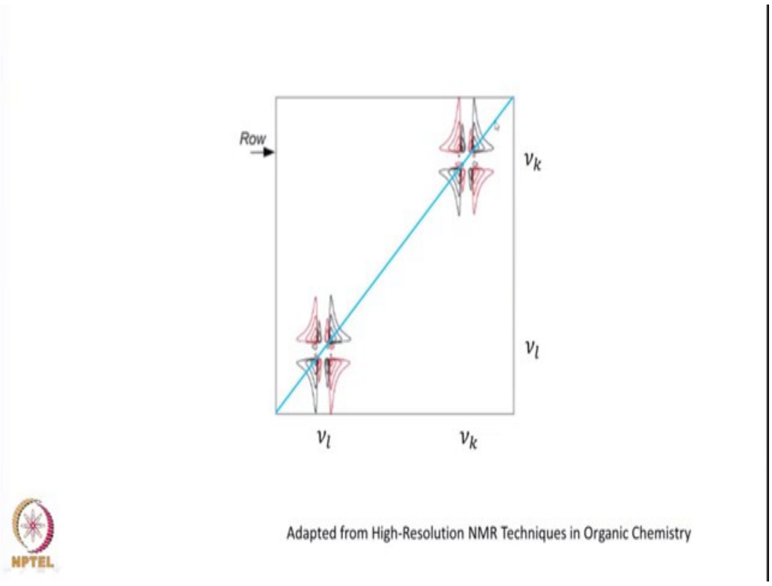
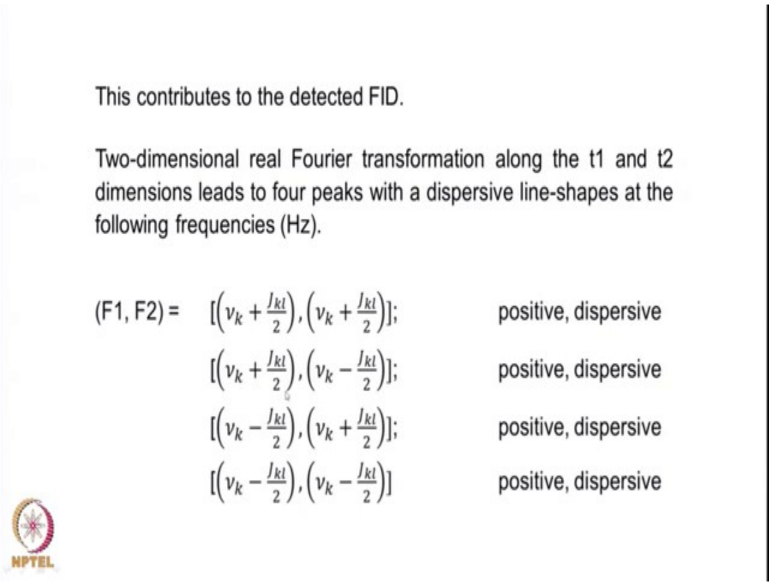


Adapted from High-Resolution NMR Techniques in Organic Chemistry



This contributes to the detected FID.

Two-dimensional real Fourier transformation along the t1 and t2 dimensions leads to four peaks with a dispersive line-shapes at the following frequencies (Hz).

$$(F1, F2) = \left[\left(\nu_k + \frac{J_{kl}}{2}\right), \left(\nu_k + \frac{J_{kl}}{2}\right)\right]; \quad \text{positive, dispersive}$$
$$\left[\left(\nu_k + \frac{J_{kl}}{2}\right), \left(\nu_k - \frac{J_{kl}}{2}\right)\right]; \quad \text{positive, dispersive}$$
$$\left[\left(\nu_k - \frac{J_{kl}}{2}\right), \left(\nu_k + \frac{J_{kl}}{2}\right)\right]; \quad \text{positive, dispersive}$$
$$\left[\left(\nu_k - \frac{J_{kl}}{2}\right), \left(\nu_k - \frac{J_{kl}}{2}\right)\right] \quad \text{positive, dispersive}$$

So, how does the spectrum look like? So, this is way the spectrum will look like I had drawn the diagonal here so which is running through, so the similar calculation for the l spin will produce may this 4 peaks, there are 4 peaks here, for the k spin when I will shown you the calculation for the *k* spin, so all of these are dispersive line shapes and the all have the same sign.

And it will produce many peak of this type. And like wise if you did for the *l* spin you will produce a spectrum of this type. And now this spectrum has been taken from one of the books NMR techniques in organic chemistry and spectacular things have been a drop and this will come in the next class when I will actually discuss as the cross peaks as well here.

And those peaks are also present here at this point, those will be the cross peaks. So, I think we discuss the diagonal peak here and in the next class we will look at the cross peaks how they appear, so you can look at this things once more at where this peaks appear and we have the peaks appearing at the $v_k+\frac{J_{kl}}{2}$ $v_k+\frac{J_{kl}}{2}$ $v_k+\frac{J_{kl}}{2}$ $v_k-\frac{J_{kl}}{2}$ $v_k-\frac{J_{kl}}{2}$ $v_k+\frac{J_{kl}}{2}$and here is $v_k-\frac{J_{kl}}{2}$ and $v_k-\frac{J_{kl}}{2}$.

So, therefore this produces a spectrum which is like this and we will see later that this sort of a dispersive line shape is not a very desirable thing and obviously one has to do something different there to get better line shapes in this point, otherwise this will mask your signals which are closed to the diagonal and that has been one of the problems of the normal cosy experiment and development switch happen to remove those.

And we will see of course in the next class how the peaks appear in the correlations which is the cross peak and we have consider one part of the density operator now we will had to consider second part of the density operator at time ρ_4 and which will then produces the signal which produce the cross peak. So, we will stop here and continue with cross peak calculation in the next class.