NMR Spectroscopy for Chemists and Biologists
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Lecture No. 42
Two Dimensional NMR - part II

So we have been discussing 2D spectra; two dimensional Fourier transformation. Let us do a quick recap of what we did in the last class so we said if your time domain data.

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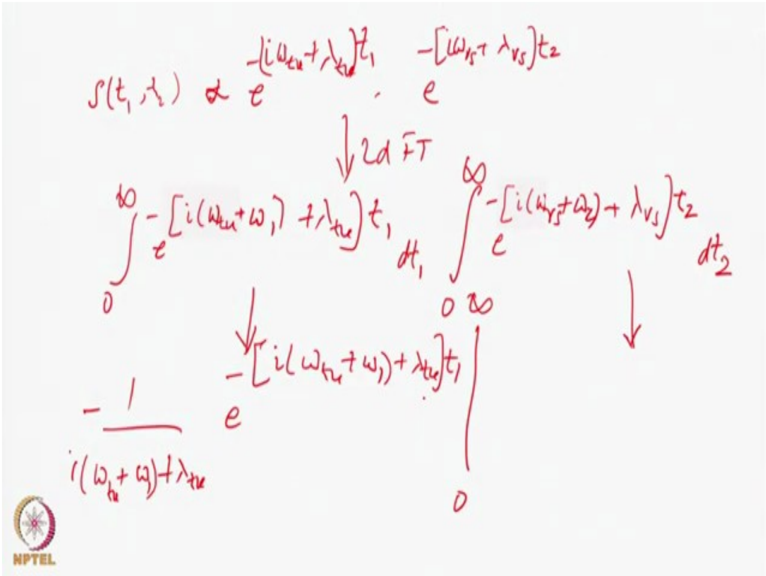

If we write it as

$$S(t_1,t_2) \propto e^{-(i\omega_{tu}+\lambda_{tu})t_1} e^{-(i\omega_{rs}+\lambda_{rs})t_2}$$

This comes from the evolution during the t_1 period and that during the detection we have also this $e^{-(i\omega_{rs}+\lambda_{rs})t_2}$. If this is the kind of a FID what is it we are going to get? The first term comes as a result of evolution during the t1 period and we see that this is the complex data which is collected e to the $e^{-(i\omega_{tu}+\lambda_{tu})t_1}$ is a complex data.

And $e^{-(i\omega_{tu}+\lambda_{tu})t_1}$represents the decay of the signal due to relaxation. Similarly $e^{-(i\omega_{rs}+\lambda_{rs})t_2}$ is the complex signal collected during the t_2 period and $e^{-(i\omega_{rs}+\lambda_{rs})t_2}$ is the decay of the signal due to the relaxation in the t_2 period. Now if we were to do a two dimensional Fourier transformation of this then what we get? We have

$$\int_0^\infty e^{-[i(\omega_{tu}+\omega_1)+\lambda_{tu}]t_1} dt_1$$

ω_1 is the Fourier transformation variable $+\lambda_{tu}$ t_1. So this is the ω_1 is the thing which is coming from the Fourier transformation variable. And for the second Fourier transformation similarly, we will have

$$\int_0^\infty e^{-[i(\omega_{rs}+\omega_2)+\lambda_{rs}]t_2}\,dt_2$$

So let us consider the just transformation of this one and the integral of this is given by

$$\frac{-1}{i(\omega_{tu}+\omega_1)+\lambda_{tu}}$$

$$e^{-[i(\omega_{tu}+\omega_1)+\lambda_{tu}]t_1}$$

And this is taken the limit from 0 to infinity and you can write a similar expression for this as well. Now what happens here? If you see this one $e^{-\lambda t_1}$ at the limit is infinity. This actually decays to 0 and we will only have this one is oscillating function so therefore we only have contribution coming from the when $t_1=0$.

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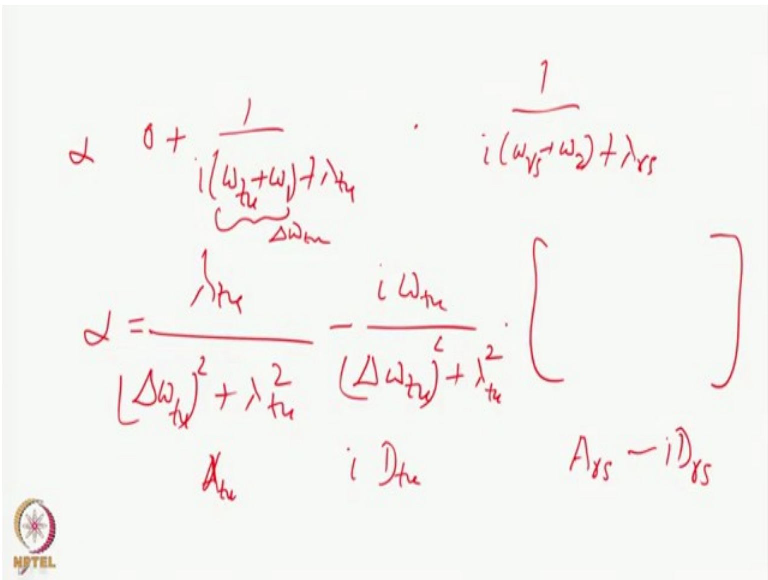

So therefore, so this the first term will be proportional to we have 0; that first integral will be

$$0+\frac{1}{i\left(\omega_{tu}+\omega_{1}\right)+\lambda_{tu}}$$

and this 0 is because that when the $t_1 = 0 \rightarrow \infty$.

And you will have similarly from the t_2 transformation, you will have

$$\frac{1}{i\left(\omega_{rs}+\omega_{2}\right)+\lambda_{rs}}$$

So this is the product of these two will be the result of the two dimensional Fourier transformation. Now so therefore you can write this as a proportional to λ_{tu} we multiply bottom and top by the minus of this component say λ_{tu}minus $i\left(\omega_{tu}\right)$ multiplied by that. You get here

$$¿\frac{\lambda_{tu}}{(\Delta\omega_{tu})^2+\lambda_{tu}^{\;2}}-\frac{i\omega_{tu}}{(\Delta\omega_{tu})^2+\lambda_{tu}^{\;2}}$$

So this is the first term multiply this by a similar term coming from this.

Now this is now a and what is $\Delta\omega_{tu}$? $\Delta\omega_{tu}$is this. This is $\Delta\omega_{tu}$where ω_1 is the running variable, so if you were to plot this as a function of frequency you will see that this is an absorptive signal λ A_{tu} and this is the dispersive signal D_{tu}. And similarly he will have an absorptive signal and a dispersive signal, this is i and it is dispersive signal the *rs*.

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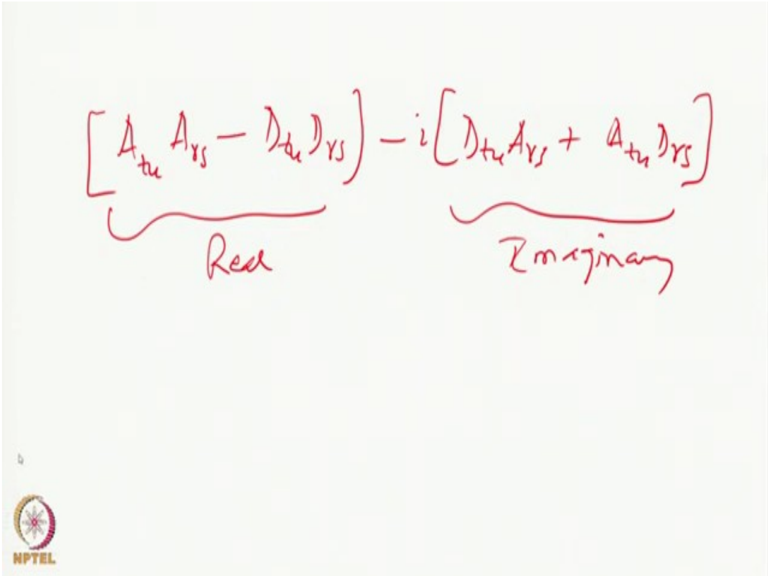

So now let us say you when you take the product, so you will get

$[A_{tu} A_{rs} - D_{tu} D_{rs}] - i[D_{tu} A_{rs} + A_{tu} D_{rs}]$

So you have absorptive line shape absorptive line shape and this is the whole thing is the real part and this whole thing is the imaginary part. So we are going to collect the real data, if you collect the real data therefore you will see it is a mixture of $[A_{tu} A_{rs} - D_{tu} D_{rs}]$ and this results in a mixed line shape.

So when you have a complex signal as $e^{-i\omega_1 t_1 \omega_{tu} t_1}$ and $e^{-i\omega_{tu} t_1}$ this is what we are going to get. We get mixed line shapes and that is what we demonstrated by peak shapes and this is one I am going to repeat that to you.

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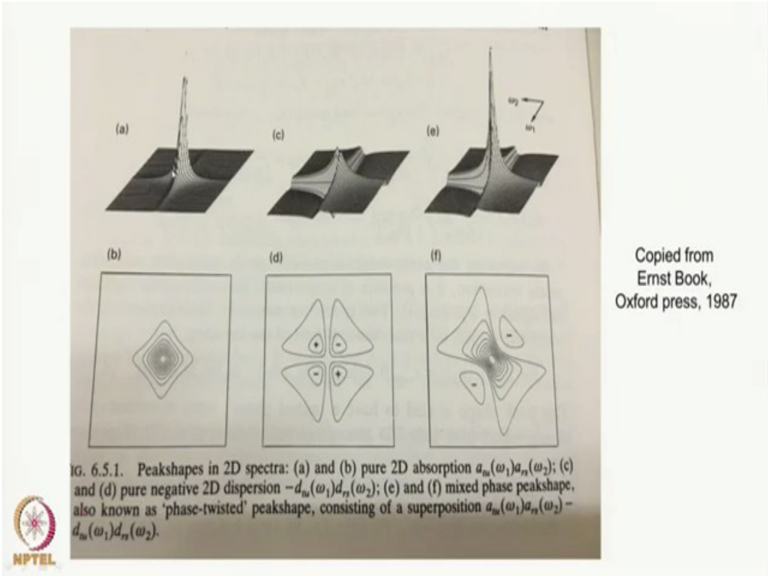

Now, so here you have the line shapes indicated here. This is the first one is the A tu of A rs that is you have absorptive line absorptive line shape along both of the frequency axis. And this is pure dispersed two line shapes along both to the frequency axis because if you take a cross section here, so it is a dispersive line shape whichever section you go with this is ω_1 and ω_2 directions are indicated here.

And if you take this one this is a mixture of this and this, so that is $A_{rs}\omega_1$ and $A_{rs}\omega_2 - \omega_1 D_{rs}\omega_2$. That is the real part the total real part is represented by this and this is a mixed phase or is also called as the phase twisted line shape. And these sorts of line shapes are not or desirable and we would like to have line shapes of this type and this is what we would like to achieve.

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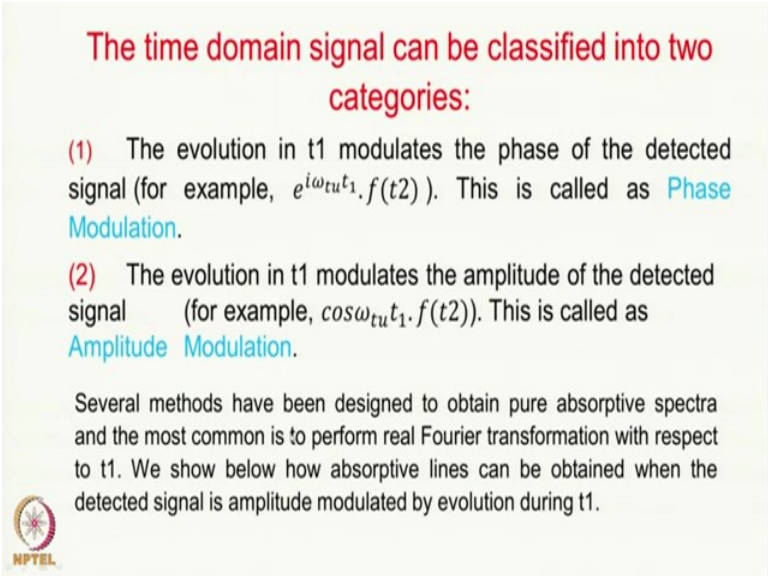

So now see how one can achieve so typically in order to do this we must analyze there were time dependent data in a little bit more formal way the evolution we seen here they will in the earlier case, we can considered the evolutionary $e^{-i\omega_1 t_1 \omega_{tu} t_1}$, so such a kind of a thing this is actually a phase factor.

The phase of the signal is getting modulated by evolution during the t_1 , $e^{i\omega_{tu} t_1}$and the detected signal is modulated by this term which is the evolution in the t_1 period and we call this as the phase modulation of the detected signal.

So and the evolution you can have the other another type of modulation where the evolution in t_1 modulates the amplitude of the detected signal. For example, if you detected signal is of this type $\cos\omega_{tu}t_1$ instead of this then this actually contributes to the amplitude of the detected signal $f(t_2)$ and this is called as amplitude modulation. So we will see that if we have an amplitude modulated signal you will get a pure absorptive spectra.

Now, several methods have been designed to obtain pure absorptive spectra and the most common is to perform real Fourier transformation with respect to t_1 and then it will also depend upon what sort of way but data you will have.

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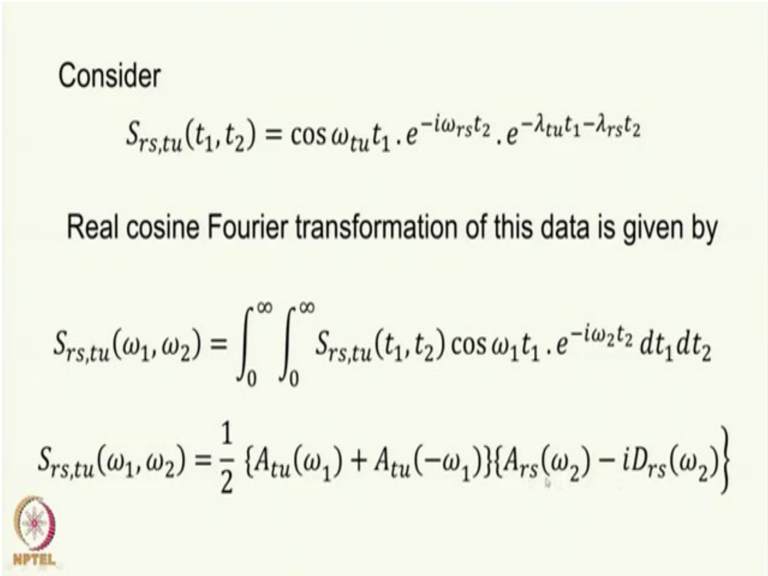

So therefore what we will do? Will consider the following here, we consider an amplitude modulated signal. So this is my amplitude modulation this is the amplitude modulation

$$S_{rs,tu}(t_1,t_2) = \cos\omega_{tu}t_1 \,.\, e^{-i\omega_{rs}t_2} \,.\, e^{-\lambda_{tu}t_1-\lambda_{rs}t_2}$$

Therefore the detected signal its amplitude is modulated by evolution during the t_1. It is not the phase remember with respect to the previous case where it was $e^{-i\omega_{tu}t_1}$ which was a phase modulation and that resulted in mixed line shapes.

So now we do it take this sort of an FID and do a real cosine Fourier transformation of this data and that is given by these. So you have double integral here

$$S_{rs,tu}(\omega_1,\omega_2) = \int_0^\infty \int_0^\infty S_{rs,tu}(t_1,t_2)\cos\omega_1 t_1 \,.\, e^{-i\omega_2 t_2}\, dt_1 dt_2$$

When you do this, I already shown you how to do the integration and things like that we will not do that again here. So I will just write here the finally what we will get as result after the 2 dimensional Fourier transformation.

So you will get here absorptive signal

$$S_{rs,tu}(\omega_1,\omega_2) = \frac{1}{2}\{A_{tu}(\omega_1) + A_{tu}(-\omega_1)\}\{A_{rs}(\omega_2) - iD_{rs}(\omega_2)\}$$

This is the absorptive to signal and this is a dispersive signal. So on this side we have the purely absorptive signal and here I have a mixture of absorptive and dispersive components and they are in the real and the imaginary parts of the spectrum.

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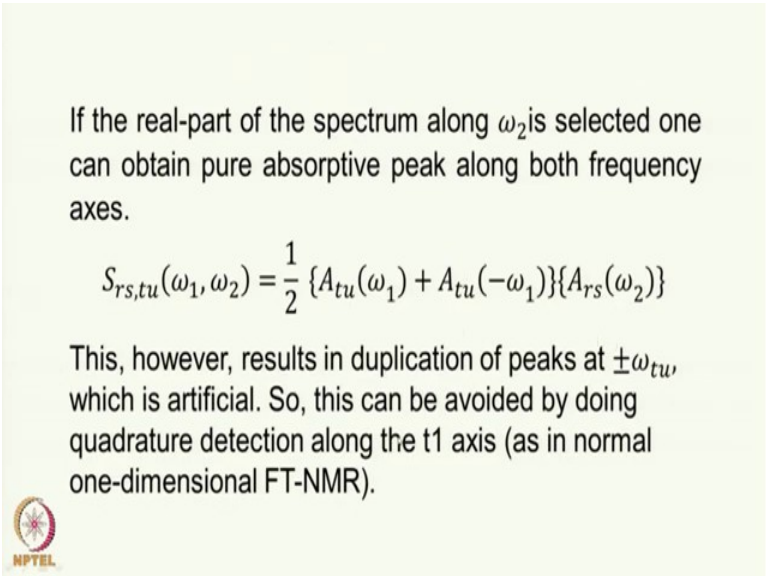

So therefore I now if you are detecting only the real part of the spectrum you are recording only the real part of the spectrum then of course what I will have here that is because these things can be stored separately on your computer.

The real and the imaginary components can be stored separately in the computer and you can pick up only the real part. So if we pick the real part what I have here is

$$S_{rs,tu}(\omega_1,\omega_2) = \frac{1}{2}\{A_{tu}(\omega_1) + A_{tu}(-\omega_1)\}\{A_{rs}(\omega_2)\}$$

So therefore I will have a pure absorptive but now we see I will have two frequencies, one will be at ω_{tu} and the other will be at $-\omega_{tu}$. And that is because of the minus sign here and this however is artificial we have actually only one frequency and that is ω_{tu}.

But I get two frequencies, ω_{tu}+¿ and a $-\omega_{tu}$so this we do not want to do this as a, to do this and though we will have to find methods to get rate of this. So this can be avoided by doing quadrature detection along the t_1 axis.

So we have seen this in normal one-dimensional NMR when you take a normal cosine FID when the Fourier transform it you get two signals at $\pm\omega$ and in the same manner here also the same thing is happening. So therefore by doing quadrature detection now along the t_1 axis we can get rid of this and select only one of the frequencies that means we can discriminate between the positive and negative frequencies and cancel out one of those and keep only the one which is required for you. Now but since you are not actually collecting the data in the t_1 axis how do we achieve this?

We are actually collecting data only during the t_2 period how do you achieve this quadrant direction along this thing?

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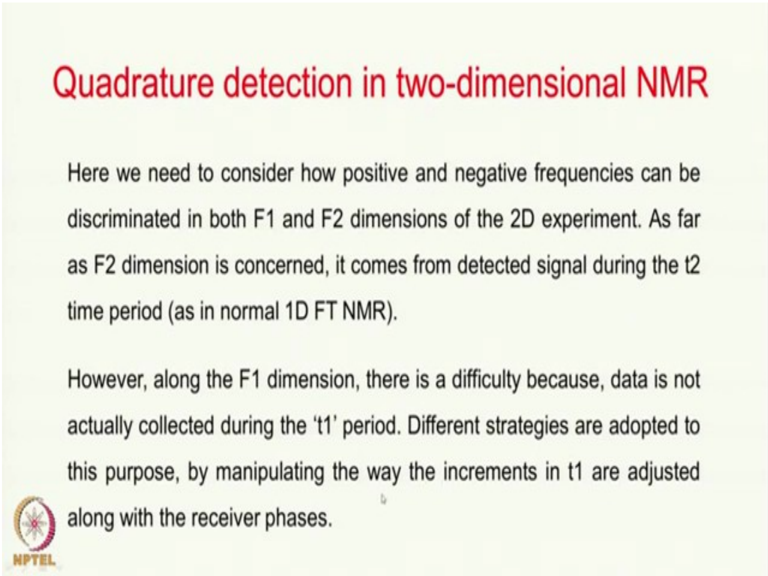

Now here there are many ways one can do it and that is along the F_1 dimension there is a difficulty because data is not collected actually during the t_1 period. For along the F_2 axis there is no problem we collected data as before in the normal one-dimensional NMR and we can do Fourier transformation.

Collect the two things two components separately and add and subtract as we saw in the case of normal Fourier transform NMR and we get the pure absorption signal and the discrimination of the positive and the negative frequencies.

So here we will have to use different strategies for achieving this and this is achieved by manipulating the way the increments in t_1 are given so they are adjusted along with the receiver phases in your experiment.

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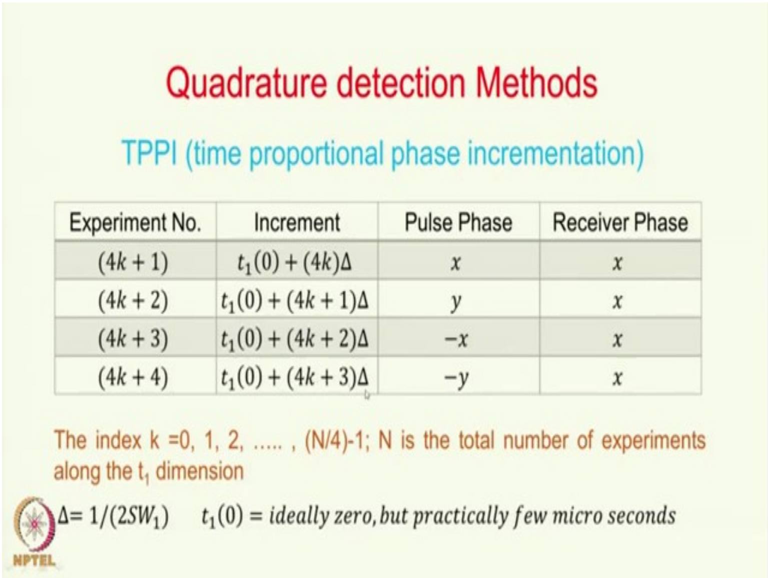

There are three different methods to do that, we are not going to go into the theory of this but we will only see the way these things are done. So here the first method the quadrature detection method, the first method here is called time proportional phase incrimination.

When you are collecting 2D data we are doing number of experiments by incremental value of the t_1. So the t_1 starts with a particular value at time $t=0$ we call that the first experiment and we will have that is called as the $t_1(0)$ point. And then after that we keep incrementing it systematically and we have suppose you are doing n experiments total.

Total number of experiments if it is N and we divide this into groups of four experiments. So in how we manipulate the increments in these individual 4 experiments. So if I totally collecting I am n experiments then I divide this into $\frac{N}{4}$ groups, so therefore this is indicated by the index k so the index k here goes from 0, 1, 2, 3 up to $\left(\frac{N}{4}\right)-1$ where n is the total number of experiments.

So when $k=0$, I will have the first four experiments 1, 2, 3, 4 when I will have the $k=1$ then I will have the next four experiments 5, 6, 7, 8 and $k=3$ I will have the next four experiments 9, 10, 11, 12 and so on. So we collect the data in that manner, so we divide this into groups of four experiments all this total increments which you are doing in the t1 dimension they are divided into groups of 4. That is why it is always said this is $4k+1$, so what when it is $k=0$, I will have this 1, 2, 3, 4.

When $k=1$ what do I get? I get 5, 6, 7, 8. When $k=3$, I will have here a $k=2$ I will have here 9, 10, 11, 12, $k=3$ I will have 13, 14, 15, 16 and so on so forth. That is the way the total number of experiments are done. Now, so when we are doing this how the increments are given? The increment is given in a way it is proportional to the experiment number itself.

So for the first experiment, if your t1 value is a particular value starting value if it is for the time $t=0, k=0$ will have a particular t_1 value. So this is ideally this is 0. This t_1 0 is typically 0, however sometimes for practical considerations a small delay has to be given because of the hardware considerations and things like that practically a few microseconds may be given there.

So and then when you have then when k is equal to 0 of course these things are all you have 4 experiments here. You have first experiment, there is no increment then the second experiment there is an increment of delta than the third experiment there is an increment of 2Δ. For the fourth experiment there is an increment of 3Δ. So you have as you are the 4 experiments, the increments are going on as $\Delta 2\ \Delta$ and 3Δ.

Now along with that we also change the phase when the increments are being given we change the phase. How are these increments? This is that is why it is called as time proportional phase incrementation. The phase of incrementation of the phase of what we changed the phase of the excitation pulse.

This is the pulse phase the pulse phase the one which creates the transverse magnetization that is a first pulse. During the preparation period you also create transverse magnetization and the pulse which creates the transverse magnetization.

We are talking about the phase of of that pulse and that pulse the first experiment if it is x. For the second experiment when there is increment is delta the phase is also increment by 90 degrees we get *y*. And for the third experiment the phase goes to *-x* and for the experiment it goes to *–y* and the receiver phase is always kept constant here *x, x, x, x*.

So this increment this incrementation of the phase is represented by this terminology here, time proportional phase incrementation. As the time is getting incremented, you also increment the phase of the pulse the excitation pulse. Now what is the value of Delta? The value of Δ here is

$$\Delta = 1/(2SW_1)$$

SW_1 is your total spectral width in the t_1 dimension, so you have 2 times this is the normal mixed criterion for representing the frequencies that are present in your spectrum the largest frequencies that are present in your spectrum.

So you always take this as next criterion,

$$\Delta = 1/(2SW_1)$$

So let me repeat here so for the purpose because otherwise this are very important concepts. So you do if you are doing n experiments here, you group them into groups of 4. And in each group, the increment is systematically done as 0Δ, 2Δ, 3Δ and the pulse phase is incremented along with that as *x y -x -y*. And the receiver phase is kept constant here it is not changed *x x x x* and this results in discriminate.

It will allow you in fact what it will do is it will will have only one kind of a sign in your is frequency spectrum and that is the way it achieves the problem of positive negative frequencies in the spectrum. So your offset your carrier is still placed in the center of the spectrum. You notice that once you put a carrier at a particular portion in the spectrum, the same thing is valid in the t_1 and the t_2 periods.

So if you are doing quadrature detection in the t_2 axis, we the same offset is available in the t_1 axis as well. So if you put the carrier in the middle of the spectrum you have this strategy to shift the artificially shift the carrier to one of the spectrum, so that becomes a single channel detection in the t_1 dimension.

And therefore the problem of positive negative frequencies does not arise there and that is of your circumvent this problem of positive negative frequencies in the time proportional phase incrementation method. So we will not go into those theoretical details here, we will simply take it that with this sort of a strategy we will get over the problem of this positive negative frequencies in the spectrum finally.

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Quadrature detection Methods

States

Experiment No.	Increment	Pulse Phase	Receiver Phase
$(4k+1)$	$t_1(0)+(4k)2\Delta$	x	x
$(4k+2)$	$t_1(0)+(4k)2\Delta$	y	x
$(4k+3)$	$t_1(0)+(4k+1)2\Delta$	x	x
$(4k+4)$	$t_1(0)+(4k+1)2\Delta$	y	x

The index k =0, 1, 2, ….. , (N/4)-1; N is the total number of experiments along the t_1 dimension

$\Delta = 1/(2SW_1)$ $\quad t_1(0) = \textit{ideally zero, but practically few micro seconds}$

This is the first method then you have the second method called as States method. A once again here also you group this number of experiments into groups of 4. So if you are doing n experiments yet divide them by four you have that many experiments $\left(\frac{N}{4}\right)-1$, k is the index which runs through these groups.

So what is the way this is done here? Now once again if $k=0$, this is the first group I will have here experiments 1, 2, 3, 4 and what is the increment? The $t_1(0)$ remains the same as before but now what is the increment here? The increment here is 2Δ, the this definition of Δ being the same but the increment that is given here is 2Δ.

And for the second experiment, the increment remains the same but the pulse phase is changed to y here if the pulse phase was x this is y and the receiver phase is not changed it remains x and x. Now for the third experiment $k=0$, what you do now is? You have 1 2 the third experiment here. For $k=0$ of course there is no this thing here the delta is not there, so you have $t_1(0)$ x and y. For the third experiment $k=0$then they will have the increment 2Δ.

So let me correct it say clearly once more, when $k=0$there is in the increment is not due 2Δ is not there. We only have the $t_1(0)$ you have $t_1(0)$ and this is 0. For the third experiment there is this first increment that the increment is now 2 Δ and the phase of the pulse is x and here the once again we do 2 experiments. For the fourth experiment the increment is the same 2Δbut the phase of the pulse is shifted to y. And receiver phase is the same, so let me repeat in these four experiments you have the first two experiments.

For $k=0$ you have the $t_1(0)$ is the increment and which is usually 0 but it can be small as I mentioned before. For the second, for the third and the fourth experiments your increment is 2Δ $t_1(0) + 2\Delta$.

And phase of the pulse is changed from x to y and that is here as well. So this is the difference between those from from TPPI. Notice here that in TPPI where the increment as $1/2SW_1$ and here the increment is to $\Delta 2 = \frac{1}{SW_1}$. So therefore the increment here is higher, this phase remains the same.

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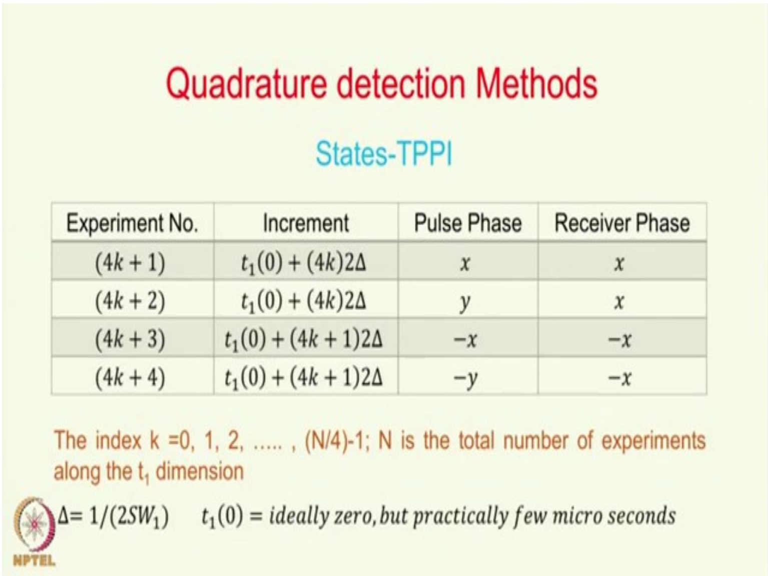

Now let us look at one more method which is called a states TPPI this is the combination of steps and TPPI methods. And it goes in the following manner, so once again groups of 4 so we start with let us say k is equal to 0, I will have the experiments 1, 2, 3, 4. And $k=1$, I will have the experiment 5, 6, 7, 8 and so on so forth as before.

So for here for the first 2 experiments what we do? This goes in the same manner as at the states that we have this t1 0 and no increment here. For the third and the fourth experiment, I will have the increment 2Δ 2Δ here.

Because $k=0$, I will have here 2Δ. So two experiments with the same increment but now the phase is one first and the second experiments it is x y. Now for the third and the fourth experiments it is $-x$ $-y$, so therefore you see a combined the phased incrementation.

Here with as in the TPPI method, so you have the incrementation here is 2Δ 2Δfirst two experiment the same increment. The third and the fourth experiments also the same increment but the phase is changed compared to what it was in the states method. So if I go from k is equal to 1 then I will have here experiments number 5, 6, 7, 8.

So therefore accordingly you will have different experiment numbers, so these other things remain the same. So therefore the States-TPPI and the States method are most commonly used and they eventually they achieve the same result of discrimination of the positive and the negative signals, So now having said this now we have to discuss the various kinds of truly experiments. We have generally covered the principles of obtaining 2D spectra, how to record 2D spectra? How to obtain pure phase experiments? And this will allow us to discuss the different experiments and there are various kinds of two-dimensional NMR experiments and we will discuss these things in the coming classes.

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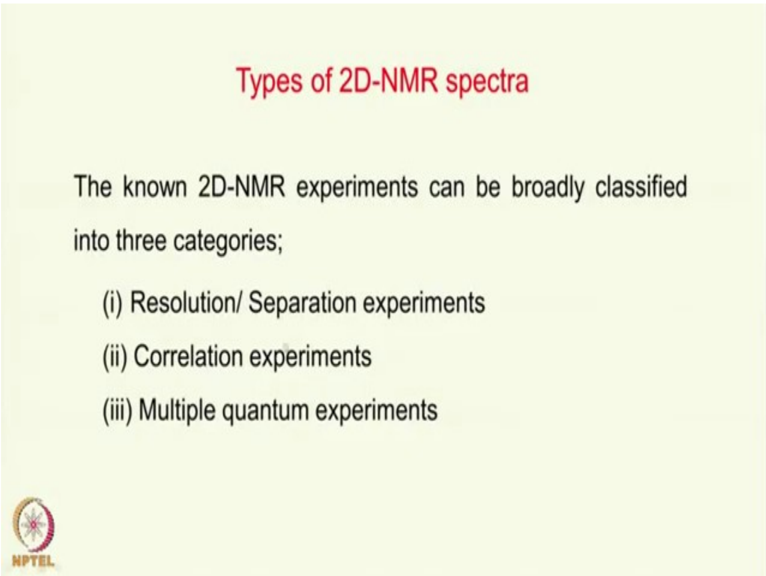

But briefly I would like to indicate here of course this is a topic which will go on for quite a long time. And the known 2D NMR experiments can be broadly classified into 3 categories these are called resolution and separation experiments, correlation experiments, multiple quantum experiments and so on so forth. So therefore this we will stop here and we are going to go into the individual experiments of these ones in greater detail one by one in the coming classes.