NMR Spectroscopy for Chemists and Biologists

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Lecture 41

Two Dimensional NMR – Part I

So let us do a recap of the last lecture and this is the slide which kind of summarizes what we did in the last lecture.

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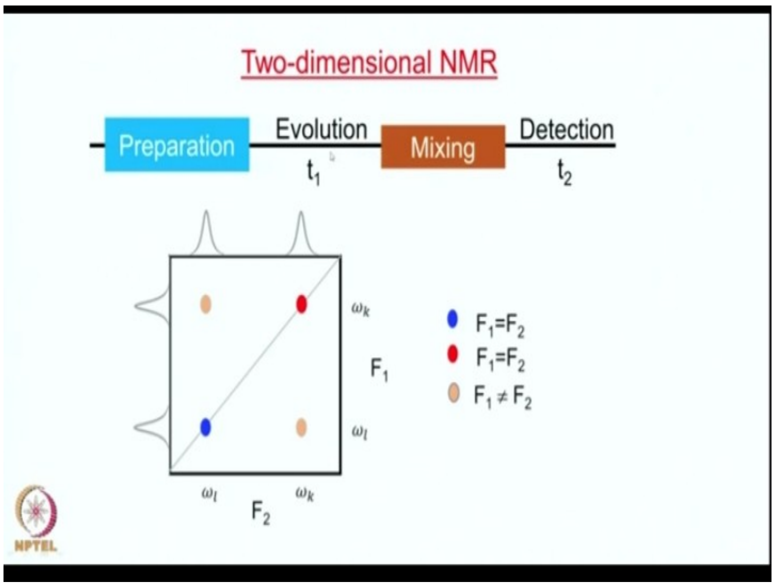

This is two dimensional NMR and this is based on the concept of segmentation of the time axis and the time axis is separated into 4 periods like this to the preparation period and then you have the evolution period t_1 and the mixing period, then you have the detection period t_2. These are time variables which means you will do lots of experiments, a series of experiments varying the values of t_2 and the data is collected as a function of time during the detection period t_2.

Therefore it generate a two dimensional data matrix. In of course in every case of the preparation and the mixing remain the same. They remain the same. So you systematically increment the value of t_1 and collect an FID. So if you have to do signal averaging then you have to do for each value of the t_1. So we will have to start with the 0 value of t_1, then you have $1\Delta t_1$, then you have $2\Delta t_1$, $3\Delta t_1$ and so on so forth. You collect a large number of FIDs so you generate a two dimensional matrix of FIDs.

So many FIDs as many increments you use in this evolution period and then we said that you have to do a Fourier transformation that you first do a Fourier transformation along the way of two dimension. Then you do a Fourier transformation along the one dimension. That means that against the t_1 period the first this will be for the t_2 time variable, other one will be for the t_1 variable and this generates a two dimensional spectrum which is indicated like this.

So that is if you have a frequency here, a particular frequency which is indicated by this particular line here, so during the evolution period then during the mixing part of this magnetization of the spin is retained and part of the thing is transferred to another spin well.

Suppose I take with the *k* spin then I transfer part of it to the *l* spin and that appears is a cross peak here on this and the whatever remains on the *k* itself, which evolves during the period t_2 appears as a peak here and this is then called the diagonal peak and this is the cross peak.

Similarly, if I have a frequency ω_l during the period t_1 evolving and then during the mixing period for the same interaction there will be transferred to the *k* spin. Then therefore here you will have part of the magnetization on the *k* spin and part will be on the *l* spin. So they evolve with the respective frequencies, so the data you collect here will have two frequencies. So it generates after the 2 dimensional Fourier transformation. A diagonal peak here and a cross peak here. So it reproduces a symmetrical spectrum like this.

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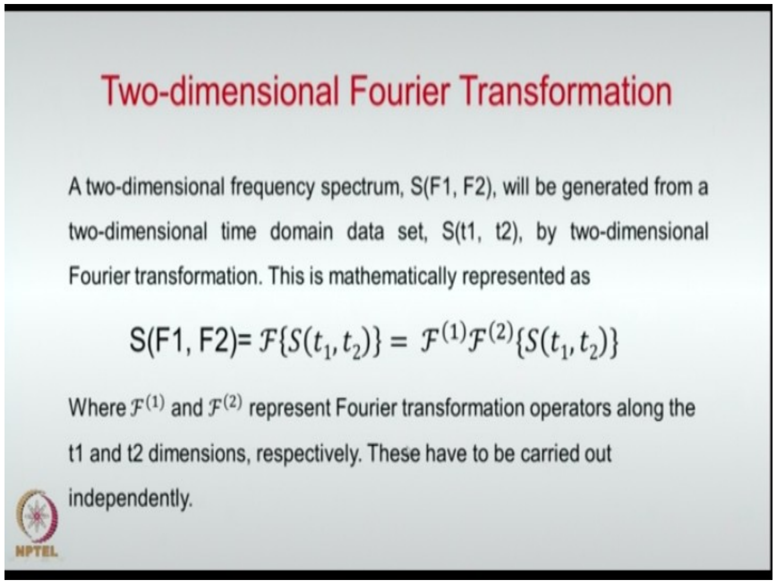

So, now let us go into more details with regard to the mathematical operations which is important to understand the phenomena in greater detail because there is going to be more and more experiments coming in and this will depend upon what sort of preparation you do and what sort of mixings you do and depending upon due to generate various kinds of data bodies and it is important to create a formalism or a general formal structure to analyze these kind of spectrum.

So let us first therefore look at two dimensional Fourier transformation. A two dimensional frequency spectrum, which will represent by the frequencies F_1 and F_2, the two axis are represented as F_1 F_2 will be generated from a two-dimensional time domain data set which is represented as $S(t_1,t_2)$ by two dimensional Fourier transformation. So this is mathematically represented in this manner.

$$S(F_1,F_2)=F\{S(t_1,t_2)\}=F^{(1)}F^{(2)}\{S(t_1,t_2)\}$$

and now these two Fourier transformations are separately written here.

This one along the t_1 axis other one along the t_2 axis so this is the operators for the 2 and here you have the time domain data $S(t_1,t_2)$. F_1 and F_2 represent Fourier transformation operators along the t_1 and t_2 dimensions respectively. These have to be carried out independently.

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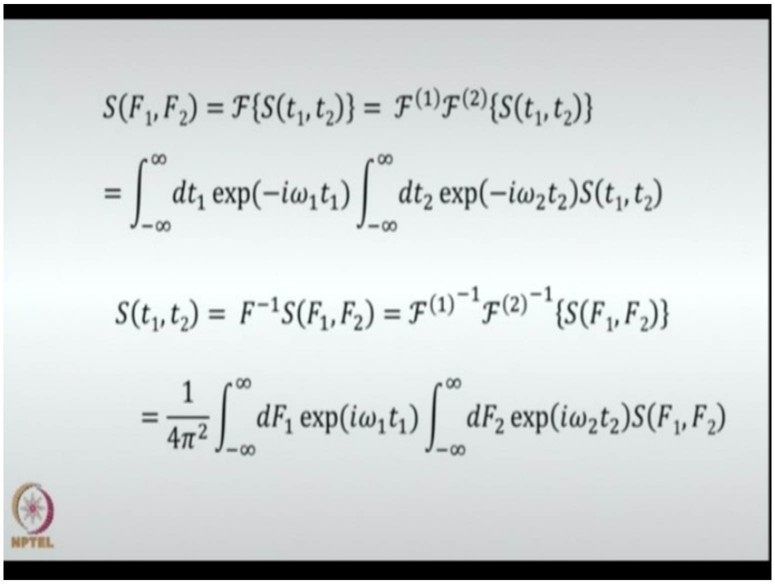

Let us look at that in somewhat more detail. So here you have the formula for this Fourier transformation.

$$S(F_1, F_2) = \mathcal{F}\{S(t_1, t_2)\} = \mathcal{F}^{(1)}\mathcal{F}^{(2)}\{S(t_1, t_2)\}$$

$$= \int_{-\infty}^{\infty} dt_1 \exp(-i\omega_1 t_1) \int_{-\infty}^{\infty} dt_2 \exp(-i\omega_2 t_2) S(t_1, t_2)$$

This ω_1 and ω_2 are the Fourier transformation frequency variables along the t_1 and the t_2 hence, and $S(t_1, t_2)$ appears here, of course at the end as a two dimensional data body. So these Fourier transformations integrals are calculated independently one after the other.

Conversely, so if you want to get the time domain data here it is an inverse Fourier transform of the frequency domain spectrum, $S(F_1, F_2)$. This is the inverse Fourier transform F inverse and this again can be split into the 2 individual inverse Fourier transform,

$$S(t_1, t_2) = F^{-1}S(F_1, F_2) = \mathcal{F}^{(1)^{-1}}\mathcal{F}^{(2)^{-1}}\{S(F_1, F_2)\}$$

So put it in more explicit terms you have this is explicitly given as

$$= \frac{1}{4\pi^2} \int_{-\infty}^{\infty} dF_1 \exp(i\omega_1 t_1) \int_{-\infty}^{\infty} dF_2 \exp(i\omega_2 t_2) S(F_1, F_2)$$

now this is the variable of the frequency spectrum and this is the Fourier transformation spectrum what in the.

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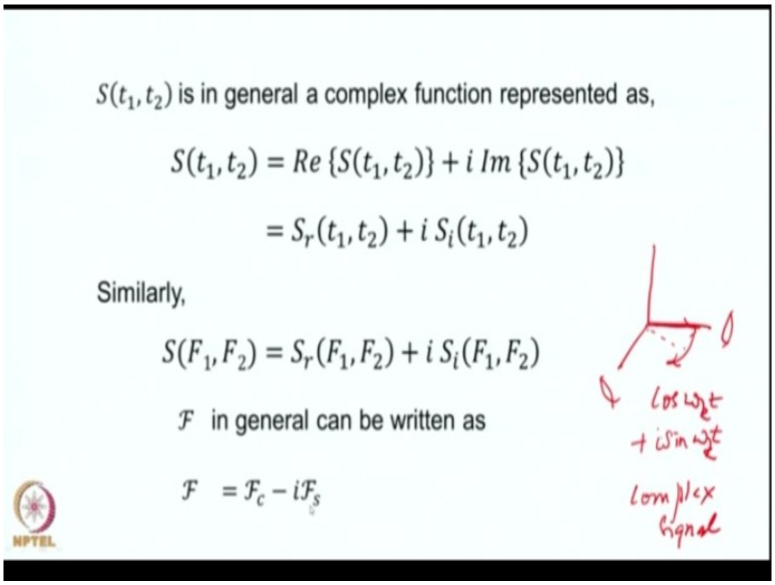

Now generally the time domain function is a complex function. Let me try explain this to you. We have seen when we do data collection in the one dimensional experiment you have this frequency axis and if the magnetization is here and if this is precessing along this during the detection period and the detection here and here possible, so this component of the magnetization as it comes here, it generates a $\cos\omega_k t$and this component produces the sine part and we write it as $i\sin\omega_k t$ for the k spin.

If it is going with the frequency ω_k then we will have the FID which we are going to collect by collecting both these components will have these 2 terms $\cos\omega_k t + i\sin\omega_k t$ and to represent their orthogonal say this i is coming here. So therefore this is a complex signal.

You do the same thing for t_1 and t_2 axes and therefore here in general this $S(t_1, t_2)$ will be a complex function and we will write that as a real part and an imaginary part. So here you see this is the real part and this is the imaginary part. So in two dimensional data matrix as well, we will have a real part and also an imaginary part will write in this two dimensional data body.

Explicitly

$$S(t_1, t_2) = Re\,\{S(t_1, t_2)\} + i\; Im\,\{S(t_1, t_2)\}$$

$$= S_r(t_1, t_2) + i\; S_i(t_1, t_2)$$

This is with regard to the spectrum.

Although I use the same symbol here but notice this actually will be the discriminating factor. The variables here are F_1 F_2. Variables here are t_1 t_2 therefore this is simply to indicate as the signal what you are going to measure then the two cases.

Now, this Fourier transformation in general can be written as a sum of 2 transformations. This we have already seen.

$$\mathcal{F} \quad = \mathcal{F}_c - i\mathcal{F}_s$$

a general Fourier transformation is written as a sum of these two. What are these? This is the cosine Fourier transform and this is the sin Fourier transform.

So when we write the general variable as $e^{-i\omega_1 t_1}$, so this is the can write it as $\cos\omega_1 t_1 - \sin\omega_1 t_1$ Therefore this actually will have two components

$$\mathcal{F}^{cc}\{S_r(t_1, t_2)\} = \int_{-\infty}^{+\infty} dt_1 \cos\omega_1\, t_1 \int_{-\infty}^{+\infty} dt_2 \cos\omega_2\, t_2\;\; S_r(t_1, t_2)$$

$$\mathcal{F}^{ss}\{S_r(t_1, t_2)\} = \int_{-\infty}^{+\infty} dt_1 \sin\omega_1\, t_1 \int_{-\infty}^{+\infty} dt_2 \sin\omega_2\, t_2\;\; S_r(t_1, t_2)$$

and that will have an *i* factor.

So therefore the first term will be the cosine transform and the second one will be the sine transform. Therefore we write here F the general Fourier transformation as

$$\mathcal{F} \quad = \mathcal{F}_c - i\mathcal{F}_s$$

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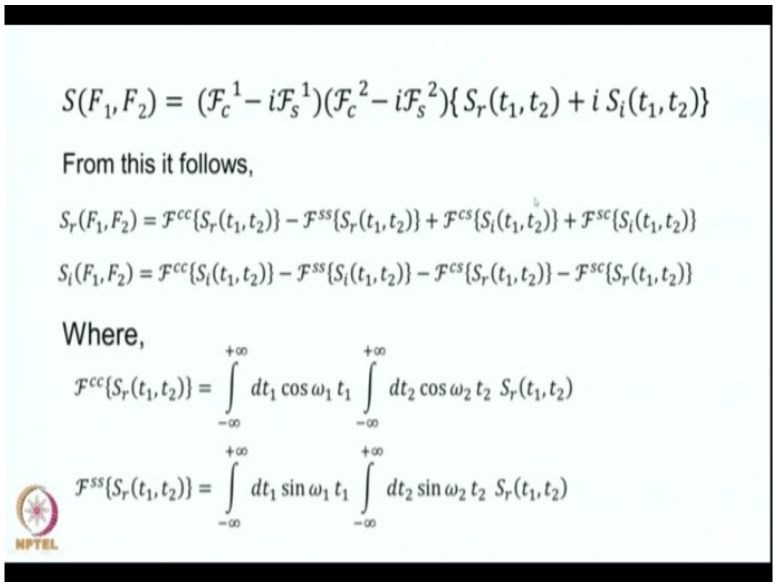

Now we apply this formulation to both the domains. So this is for the first domain which is the t_1 domain and this is for the t_2 domain. Time axis of

$$S(F_1, F_2) = \ (\mathcal{F}_c^{\ 1} - i\mathcal{F}_s^{\ 1})(\mathcal{F}_c^{\ 2} - i\mathcal{F}_s^{\ 2})\{ S_r(t_1, t_2) + i\ S_i(t_1, t_2)\}$$

So now what you do? You do these operations explicitly independently all of them. So you will have this operating F_C^2operating on this and on this, likewise F_S^2 operating on this and on this. Similarly after that you get this F_C^1 operating on the result of those two and likewise Fs1 operating on the results of those two.

Therefore, you can combine these two together say

$$S_r(F_1, F_2) = \mathcal{F}^{cc}\{S_r(t_1, t_2)\} - \mathcal{F}^{ss}\{S_r(t_1, t_2)\} + \mathcal{F}^{cs}\{S_i(t_1, t_2)\} + \mathcal{F}^{sc}\{S_i(t_1, t_2)\}$$

So as a result of this what you get, you get the real terms with do not have the *i* part and the imaginary terms as the frequency domain spectrum which has the *i* part.

So which are the ones which gives the *i* part? The real part F^{cc} this, this and this, this gives me a real part. Plus and multiplication of this, this and this.

$$S_i(F_1, F_2) = \mathcal{F}^{cc}\{S_i(t_1, t_2)\} - \mathcal{F}^{ss}\{S_i(t_1, t_2)\} - \mathcal{F}^{cs}\{S_r(t_1, t_2)\} - \mathcal{F}^{sc}\{S_r(t_1, t_2)\}$$

Likewise you also have F^{sc} that is this one, this one and this one, because you must have one i from here and one I from here. So this one, this one and this one.

So $F^{sc}\{S_i(t_1, t_2)\}$ all of these will be real and likewise if you see the F^{cc} operating on *i* so this, this and this will give you $F^{cc}\{S_i(t_1, t_2)\}$ because this will have the *i* component here and therefore this is imaginary and similarly $-F^{ss}$ that is this product and product with this.

So this will be i^2 then you have another *i* here and therefore you will get $-F^{ss}\{S_i(t_1, t_2)\}$ and then you will have $-F^{cs}$ that is this one and this one operating on this F^{cs}, $F_C^1 - i F_s^2$ operating on $S_r(t_1, t_2)$ that gives you this term.

So this is again imaginary because of this i. Then you have $-F^{sc}$and $S_r(t_1, t_2)$ *sc* that means it is this one, this one operating on $S_r(t_1, t_2)$. This will have the *-i* component therefore you get the minus sin. So $F^{sc}\{S_r(t_1, t_2)\}$.

Now let us write these terms explicitly

$$\mathcal{F}^{cc}\{S_r(t_1, t_2)\} = \int_{-\infty}^{+\infty} dt_1 \cos\omega_1 t_1 \int_{-\infty}^{+\infty} dt_2 \cos\omega_2 t_2 \; S_r(t_1, t_2)$$

and what about this F^{ss}. F^{ss} that is this one here. So it is sin transform along both the dimensions therefore here you have

$$\mathcal{F}^{ss}\{S_r(t_1, t_2)\} = \int_{-\infty}^{+\infty} dt_1 \sin\omega_1 t_1 \int_{-\infty}^{+\infty} dt_2 \sin\omega_2 t_2 \; S_r(t_1, t_2)$$

so this is also a real number.

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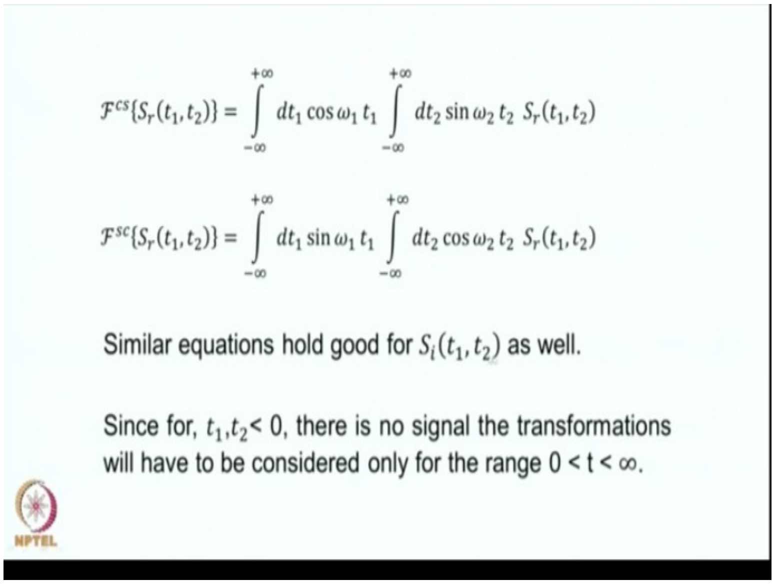

So likewise now if I took F^{cs} so along the t_1 dimension I have the cosine Fourier transform therefore this is

$$\mathcal{F}^{cs}\{S_r(t_1,t_2)\} = \int_{-\infty}^{+\infty} dt_1 \cos\omega_1 t_1 \int_{-\infty}^{+\infty} dt_2 \sin\omega_2 t_2 \; S_r(t_1,t_2)$$

Similarly this F^{sc} is along the t_1 dimension I have

$$\mathcal{F}^{sc}\{S_r(t_1,t_2)\} = \int_{-\infty}^{+\infty} dt_1 \sin\omega_1 t_1 \int_{-\infty}^{+\infty} dt_2 \cos\omega_2 t_2 \; S_r(t_1,t_2)$$

So I have written here all those explicitly for the real part of the frequency domain spectrum.

Now, you can do similar equations for the imaginary part of the Fourier transformations $S_i(t_1,t_2)$ as well, all of these you notice here our $S_r(t_1,t_2)$and so similarly you can write for the $S_i(t_1,t_2)$ what terms that will come. Now the FID is of course within transformations actually go from minus infinity to infinity but for time less than 0 there is no signal. Therefore these FIDs will have 0 signal. Therefore for $(t_1, t_2) \ll 0$ there is no signal. Transformations will have to be considered only for the range $0 < t < \infty$.

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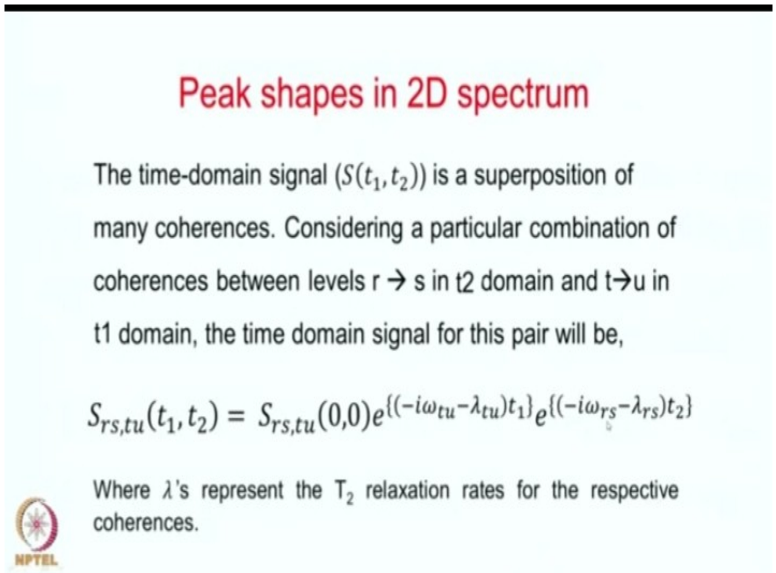

So that is so much the formalism for the Fourier transformation. Those are the definitions. Now let us look at the end of this what sort of spectra we will get, what sort of the peak shapes we will have. What does the Fourier transformation yield? So we recall the Fourier transformations in the normal case 1 dimensional Fourier Transformations. We will have real and imaginary components and we will had different peak shapes. The peak shapes will be absorptive peak shapes and dispersive peak shapes. So here also we can expect a similar thing. So what we will do is let us explicitly consider 2 particular transitions.

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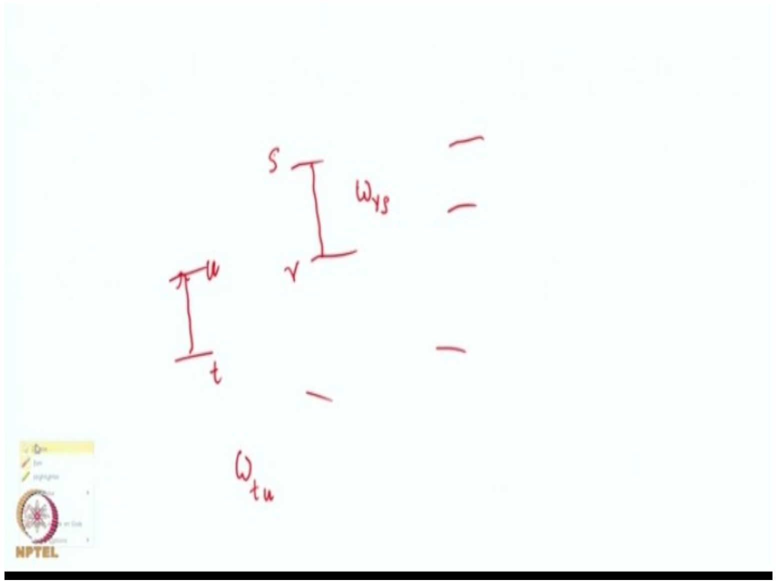

Let us say we have an energy level diagram something like this various energy levels at various places and let us represent this energy levels with particular symbols that is called this energy level as *t* and this energy level as *u* and let us call this energy level as *r* and this energy level as *s*. There will be transition from here to here and this will be represented as a *tu* and there can be a transition from here to here. This will be represented as *rs* transition. This is a *tu* transition and this is an *rs* transition.

Now, we assume that during the evolution period there is a particular transition. There is a particular frequency *tu* and this *tu* we write it as $\omega_{tu}$and this transition will therefore write it as ω_{rs}. So let us go back and see what we are going to get.

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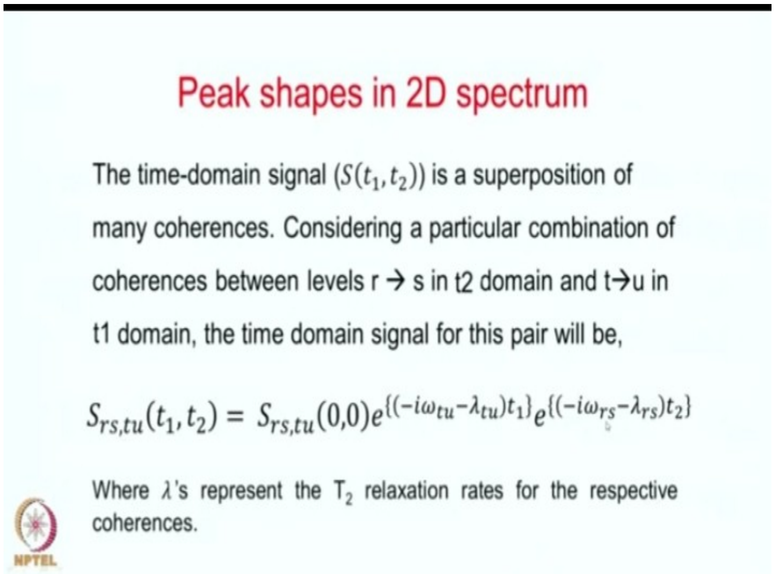

Considering a particular combination of coherences between levels *r* and *s*. Now *r* and *s* is taken in t_2 domain and *t* to *u* in the t_1 domain. The time domain signal for this pair will be $S_{rs,tu}(t_1, t_2)$ and we have here, well in fact *tu* is ω_{tu} is taken as in the t_1 dimension and ω_{rs} is taken as t_2 dimension. Complex signal is written as

$$S_{rs,tu}(t_1, t_2) = S_{rs,tu}(0,0)e^{\{(-i\omega_{tu}-\lambda_{tu})t_1\}}e^{\{(-i\omega_{rs}-\lambda_{rs})t_2\}}$$

Now this coherence, this is the coherence this is the coherence in the transverse plane. This coherence decays and these decays with the transverse relaxation rates, and these are the transverse relaxation rates.

λ_{tu} is the transverse relaxation rate for the transition *tu* and λ_{rs} is the transverse relaxation rate for the *rs* transition and so therefore this decay has to be included in the FID. This is a free induction decay along the t_1 axis and this is a free induction decay along the t_2 axis.

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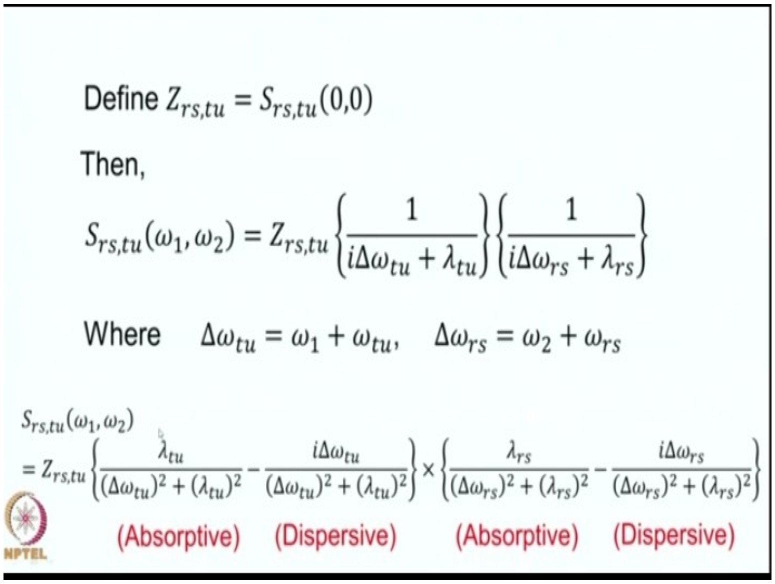

Now let us define this particular term $Z_{rs,tu} = S_{rs,tu}$ this is the amplitude. This is amplitude for the after the Fourier transformation what we get for the frequency domain spectrum. This is amplitude and the frequency domain spectrum is now written as $S_{rs,tu}(\omega_1, \omega_2)$ and this is given by this expression and that actually comes from this particular integral as I can show you here.

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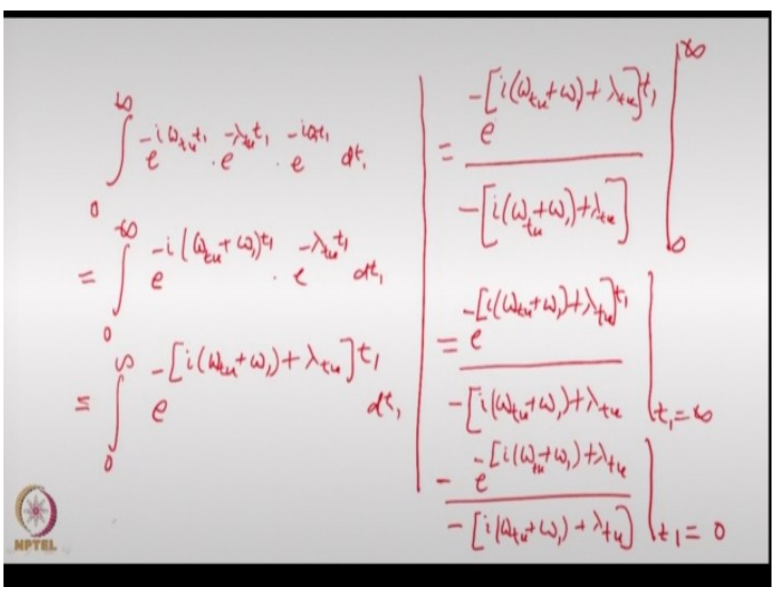

$$\int_0^\infty e^{-i\omega_{tu}t_1} e^{-\lambda_{tu}t_1} e^{-i\omega_1 t_1} dt$$

So pulling the terms. So this will be

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

So this is equal to I will write here. This one is next step. This is equal to

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

$$\frac{e^{-[i(\omega_{tu}+\omega_1)+\lambda_{tu}]t_1}}{-[i(\omega_{tu}+\omega_1)+\lambda_{tu}]} \text{¿}_0^\infty$$

So if you want to expand this and this will be given as the first at the value of the infinity and then minus the value at 0. So this is explicitly writing it as

$$\frac{e^{-[i(\omega_{tu}+\omega_1)+\lambda_{tu}]t_1}}{-[i(\omega_{tu}+\omega_1)+\lambda_{tu}]} \text{¿}_{t_1=\infty}$$

$$\frac{e^{-[i(\omega_{tu}+\omega_1)+\lambda_{tu}]t_1}}{-[i(\omega_{tu}+\omega_1)+\lambda_{tu}]} \text{¿}_{t_1=0}$$

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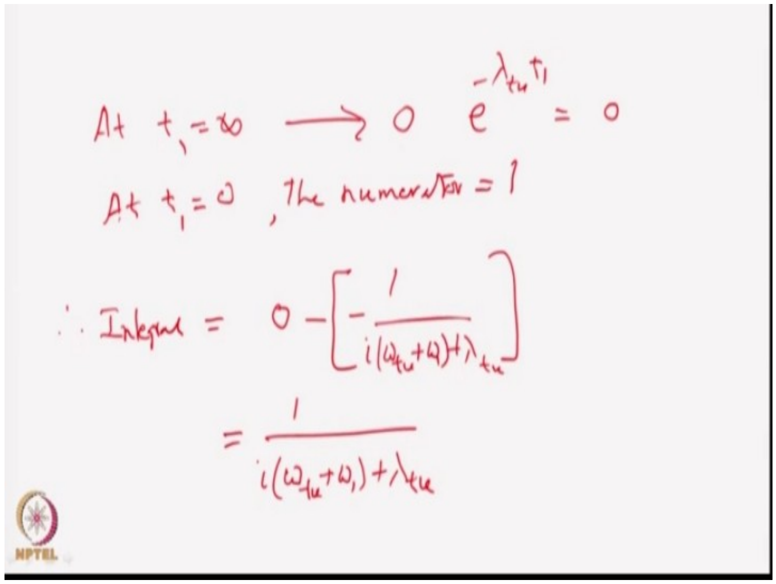

At $t_1 = \infty \rightarrow 0$, because $e^{-\lambda_{tu} t_1} = 0$. At $t_1 = 0$the numerator = 1. Therefore we get finally the integral, Integral is equal to

¿ $0 - \left[\frac{-1}{i(\omega_{tu}+\omega_1)+\lambda_{tu}} \right]$

Therefore,

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

So this is the calculation of the integral and subsequently, of course, you can multiply this by $i(\omega_{tu}+\omega_1)+\lambda_{tu}$ to the numerator as well as the denominator. Then you get rate of the i part and then you will get expression in two different terms as indicated here.

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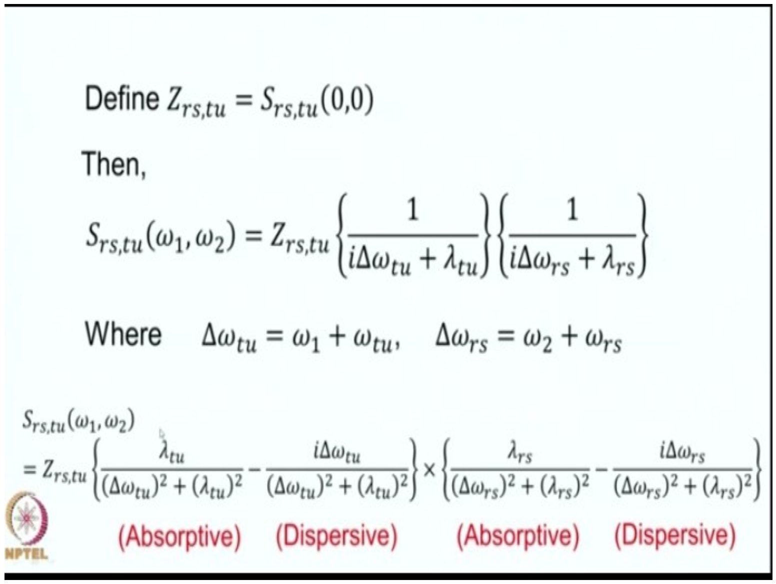

So, we have here this is the first term, this is the Fourier transformation with respect to the t_1 axis and this is the Fourier transformation with respect to the t_2 axis. Now here we have written here what is

$$\text{Where}\quad \Delta\omega_{tu} = \omega_1 + \omega_{tu},\quad \Delta\omega_{rs} = \omega_2 + \omega_{rs}$$

this relaxation factor comes in here as well and this is the amplitude. This is the amplitude after the Fourier transformation.

So

$$S_{rs,tu}(\omega_1,\omega_2) = Z_{rs,tu}\left\{\frac{\lambda_{tu}}{(\Delta\omega_{tu})^2+(\lambda_{tu})^2} - \frac{i\Delta\omega_{tu}}{(\Delta\omega_{tu})^2+(\lambda_{tu})^2}\right\}\times\left\{\frac{\lambda_{rs}}{(\Delta\omega_{rs})^2+(\lambda_{rs})^2} - \frac{i\Delta\omega_{rs}}{(\Delta\omega_{rs})^2+(\lambda_{rs})^2}\right\}$$

Now we recall from the discussions in the very first chapter that what are these lines shapes. So here you are plotting as a function of the frequency. If you plot this as a function of frequency what frequency ω_{tu}, these are the various frequencies which maybe be present in your spectrum and this ω_1 and ω_2 are the running variables of the Fourier transformation, So for the various frequencies that are present.

So what you get as a line shape in your spectrum so if you plot this if you plot this as a function of frequency, then you will see that this will actually generate a absorptive line shape and this is the same as what we had done earlier in the case of one dimensional Fourier transformation and this will generate a dispersive line shape because this is an i $(\Delta \omega ¿¿ tu)^2 + (\lambda ¿¿ tu)^2 ¿¿$ and similarly, this is a absorptive and dispersive components along the F_1 axis and now this is on the F_2 axis you have a absorptive component and a dispersive component present.

Now remember here we just put here ω_1 ω_2 that is because we use running variables ω_1 and ω_2 but in the frequency domain spectrum, finally, you may represent this as F_1 F_2 as well. There is a running variable along the frequency axis.

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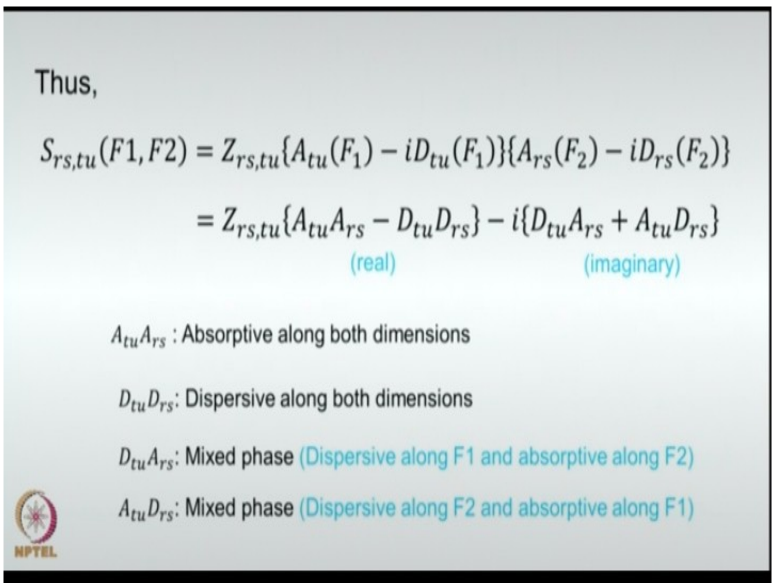

So now therefore I will return use these symbols F_1 and F_2 here so I have here absorptive spectrum for the transition A_{tu} for the coherence and a dispersive line shape for the along the F_1 axis for the same coherence *tu* and here I have A_{rs} F_2 and $-iD_{rs}$ F_2. This is absorptive component and this is a dispersive component after the Fourier transformation. Now if I multiply this, if I multiply this so what do I get? A_{tu} A_{rs} and this will be real because there is no i component there and similarly this product these two terms product this give me real component again. This is plus i square and therefore it is - D_{tu} D_{rs} and the cross terms, this is - i D_{tu} and A_{rs}.

This produces an imaginary term and this one again this will also produce an imaginary term imaginary term therefore this satisfies what we said earlier that the frequency domain spectrum also has real part and an imaginary part. Now A_{tu} A_{rs} this one will now if you plot this, it would have absorptive line shape along both dimensions and this will have a dispersive line shape along both dimensions.

Now, if you look at these two terms, this will have mixed line shapes. The first term here $D_{tu} A_{rs}$, this produces is a dispersive line shape along F_1 and an absorptive line shape along F_2 and this term A_{tu} D_{rs} again produces a mixed phase and this is dispersive along F_2 and absorptive along F_1 one. So, now if you look at the real overall real part this has both the absorptive and dispersal components.

So in principle, if you collect the entire real part, it will also have mixed line shapes. So therefore now we have to choose what we want to have. So how do we choose it and what are the criteria? How do you choose it.

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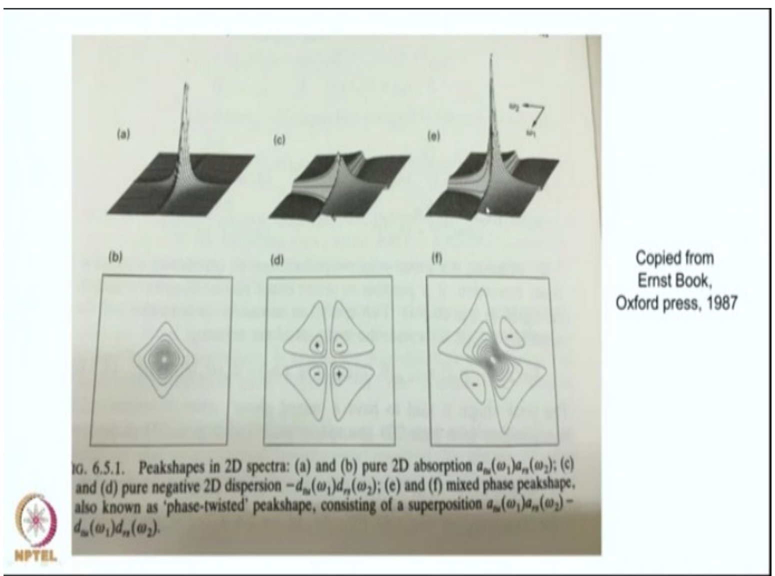

Now this is clear when you make a plot of this various lines shapes. So this is a line shape, which is absorptive along both dimensions. This is the first term that A_{tu} A_{rs} and here you have the dispersive line shape along the both the dimensions that is the D_{tu} and D_{rs} that is this one here. These both contributed the real part of the spectrum. If you collected both of these then of course you will have a mixture of the both the line shapes and so it will be mixed phase.

Now here it is a more a complicated situation that you have absorptive line shape along one axis and the dispersive line shape along the other axis. Now, therefore now if I were to take a individually these line shapes and take their cross sections heights at various places across the general plot, the contours this peak will look like this and if we were to take the cross sections here at various levels, we will have a peak shape which is like this.

It has 0 at the center and it has loops going out like this. That is the characteristic of the dispersive line shapes, Along both axes the dispersive line shape is of this type, it has 0 at the center and it has lobes on both the other sides and therefore this has a very broad signal and the [+,-] indicate the positive and the negative signals and here it is a combination of the two and you have [-,-] here and this is a very ugly line shape.

So typically we would like to have this. So typically we would like to collect only the absorptive component of the line shape so that we have much better spectrum, much better resolution in your two dimensional spectrum. So therefore we have to play around with the data acquisition and Fourier transformations, so that in the end we generate a spectrum of this type which has absorptive line shapes along both the dimensions F_1 and F_2.

So we stop here and quick recap that we have done today is the two dimensional Fourier transformation the theory of that one and we have seen how it generates various kinds of lines shapes and how to optimize what we should do. What we need is an absorptive line shape along both dimensions and we have to optimize your experiments so that we collect data in the appropriate manner and do a processing also in that manner so that we generate absorptive line shapes along both to the frequency axis. So we stop here and continue with the same in the future classes.