NMR spectroscopy for Structural Biology NS

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Lecture: 16

General Concept of Multidimensional NMR - 1

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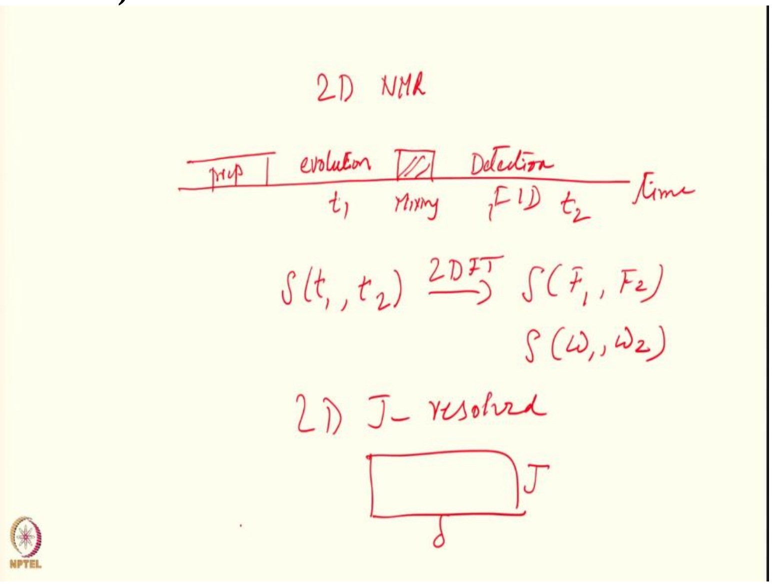

So, we started discussing about 2 dimensional NMR spectroscopy in the last class we introduced the general concept in a qualitative manner. We said this principle is like this that you have a time axis like this and you divide the time into multiple into 2 different segments and which in multiple segments we call the first 1 as the preparation and then you have the evolution we call it evolution and labelled it as t_1 period.

Then we had the so, called mixing here and then we had this detection and which is where we actually collect our FIDs and this is the time period t_2. So, we collect a series of FIDs incrementing the time t_1 from one experiment to another experiment therefore this will result in a 2 dimensional data body t_1 t_2 and you do a 2 dimensional Fourier transformation this will generate a 2 dimensional spectrum which we call as F_1, F_2.

Or sometimes people also use this as ω_1, ω_2 it does not matter either way it is the same thing you need a 2-dimensional Fourier transformation. So, the concept which you have to be clear is that this is segmentation of the time axis. So, the idea can be extended further to multiple dimensions as well you are introducing one evolution time t_1 you introduce other revolution times you can use 2 3 4 5 etcetera.

Then accordingly we will generate various kinds of evolution times. So, we took a particular examples of 2D spectra where we talked about the 2D J resolved ok. So, the J resolved spectroscopy and there the along the 2 frequency axis we had one axis we had the J other axis we had the δ. So, this became a truly separation experiment the 2 coupling constant is on one axis and the chemical shift is on the other axis.

And we are going to go into other kinds of 2D experiments and those are the correlation experiments. However so, correlation experiments before we actually embark onto the correlation experiments we need to clarify some of the other conception need to define certain terms some definitions we have to give because this will be required for all our future discussions for all your future decision what sort of a magnetization.

How do we generate various kinds of magnetizations? How do we generate different kind of information in different spectra. So, therefore here we will have to make a slight digression and define certain terms.

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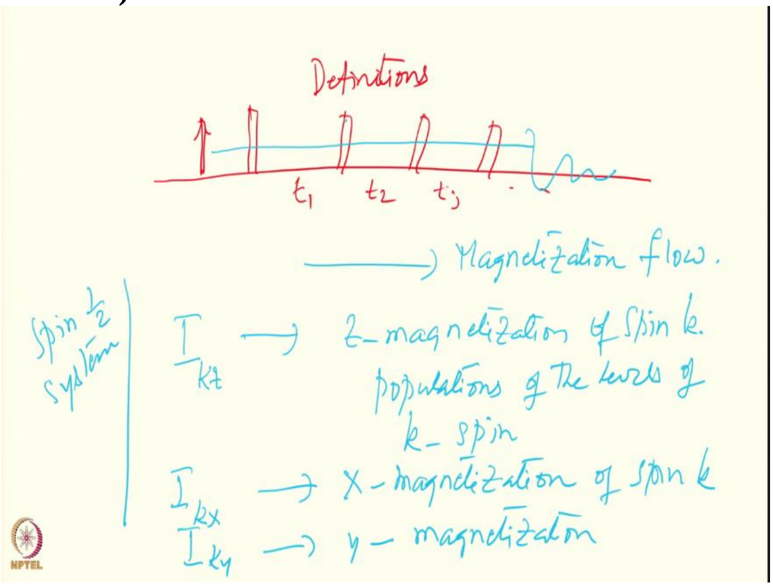

So, some definitions we will give here, some definitions because these are the terms which will be using all the time. So, in a generalized multiples experiment you have various kinds of evolution periods preparation periods as I mentioned here and there is what happens is you start from initial magnetization which is here and the magnetization flows through this various time periods this may be t_1 t_2 t_3 etcetera.

So, the magnetization flows if I want to use a different colour for the magnetization it will flow through this and ultimately we collect the data. So, this is called as the magnetization transfer magnetization transfer or magnetization flow . So, it goes from one state to another from one t_1 period to t_2 period to the t_3 period and so on so forth. So, here magnetization flow is something which one has to understand.

And that determines what magnetization is present in what time period data means what is the information in that time period when you Fourier transform it in a multi-dimensional way the information that is present in the t_1 will appear as F_1 what is present in t_2 will appear as F_2 what is present in t_3 will appear at F_3 and so on and so forth and this various time separations here which are there can be various kinds of mixing periods here which will allow transfer of magnetization.

That is why we are talking about magnetization flow magnetization terms will have to be defined here you have to define certain types of magnetizations that is what we will do here. So, let us keep those definitions here. Now let us say we have the term called I_{kz}, I_{kz} implies z magnetization of spin k and this has to do with the populations of the levels of k spin which will cause case spin transitions.

Then let us say I have then I have I_{kx} let us say this means x magnetization of the transverse magnetization x magnetization of spin k and we are talking about spin half systems here for all of these let us say we are talking about spin half systems all kl etcetera whatever label we use I have used is the label k that is to identify the spin k, I can use it l m p q r whatever they all indicate different spins and they are all spin half systems.

So, therefore and we need 2 different symbols here similarly if you have I_{ky} I_{ky} would imply y magnetization. Now among these, what is it that we observe it is a transverse magnetization right when you measure we measure the transverse magnetization.

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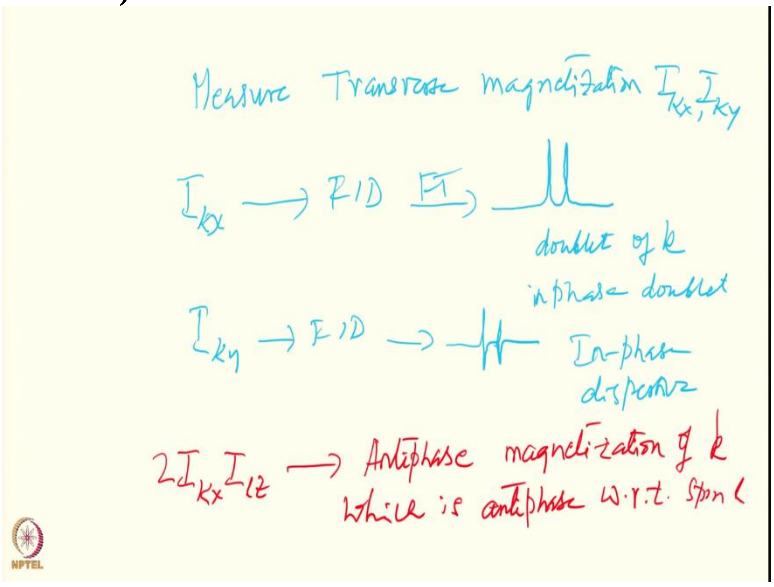

So, we measure transverse magnetization always measure transverse mechanization and that is I_{kx} or I_{ky}, I_{kz} is not observable because you have to convert this z magnetization into the transverse plane bring it to the transverse plane because your detector is in the transverse plane therefore you measure the transverse magnetization. Now if I measure the I_{kx} term if I measure the I_{kx} and after Fourier transformation etc.

You get the time domain signal you get the FID for this FID and if you do Fourier transformation you generate a suppose it is a spin which is coupled to another spin suppose it is coupled to another spin we will generate 2 components like this, this is a doublet of k if it is coupled to some other spin like l it will produce an in phase this is called as inphase doublet in phase signal.

Similarly you can write for I_{ky}, I_{ky} will give you antiphase terms I_{ky} FID and this may generate you a dispersive signal this is in phase dispersive you can choose either way I mean depends upon where my detector is if I choose I_{kx} to produce this absorptive phase then the I_{ky} produced me dispersive in phase and vice versa suppose I put the detector along the Y axis and I measure the y magnetization I get absorption signals for the I_{ky} magnetization and I_{kx} will give me dispersive signal.

So, these are interchangeable. So, this is one set of terms which we will always use I_{kx} I_{ky} I_{kz} and when we have other kinds of what are the other kinds of magnetization terms let me define few other types of magnetization terms. So, we also will come across terms something like this

I 2 I_{kx} I_{kz} and this is called as antiphase magnetization of of k which is antiphase with respect to spin l. What does it mean what does that mean.

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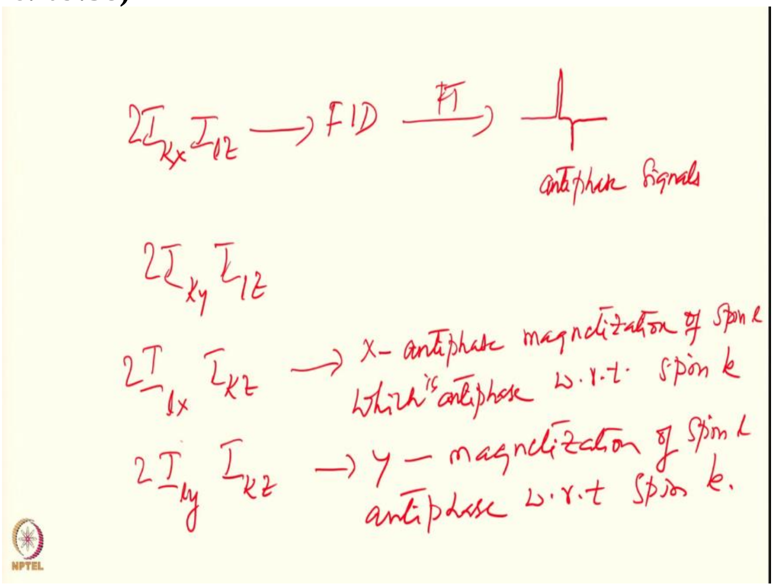

So, if I take term like this to I_{kx} I_{lz}. Now I do this is evolves during the generating FID from here because if I generate means this has to evolve various kinds of evolutions will happen and then of course after you do a FID then I do a Fourier transformation and collect the signal this will generate the same 2 lines but they will have appearance like this. So, this is called as antiphase magnetization.

So, I_{kx} I_{lz} this kind of a magnetization this is the magnetization term and which this evolves in the FID free induction decay because there is the J evolution that will happen there and after you do Fourier transformation of the resultant magnetization that is will produce you a spectrum which is like this. So, these are called as antiphase signals same will apply for 2 I_{ky} I_{lz} as well I_{ky} I_{lz} or suppose I take I_{lx} I_{kz}.

Suppose I do this to I_{kx} I_{lz} what does this mean this will mean the same sort of terminal we will get but this is antiphase magnetization of spin l this is spin l which is antiphase with respect to spin k see the kz indicates that it is with respect to spin k and lx means it is x magnetization this is x antiphase magnetization similarly I can also have 2I_{ly} I_{kz} this will be y magnetization of spin l antiphase with respect to spin k.

So, these are the kinds of terms which you will get when you are actually considering a series of transfers from one time period to another time period during the mixing periods we when you do mixing times during the mixing time we generate transfers from one spin to another spin see if I go from kx to let us say 2kx ly, 2kx lz to 2l x kz it would mean that I have transferred from the k spin to the l spin.

So, this sort of transverse will happen when you are doing this mixing periods during the mixing times this sort of transfers will happen that is why it is important to understand these terminologies how this will happen. So, and there will be other kinds of terms and all of these are. So, all of these are called single quantum transitions also.

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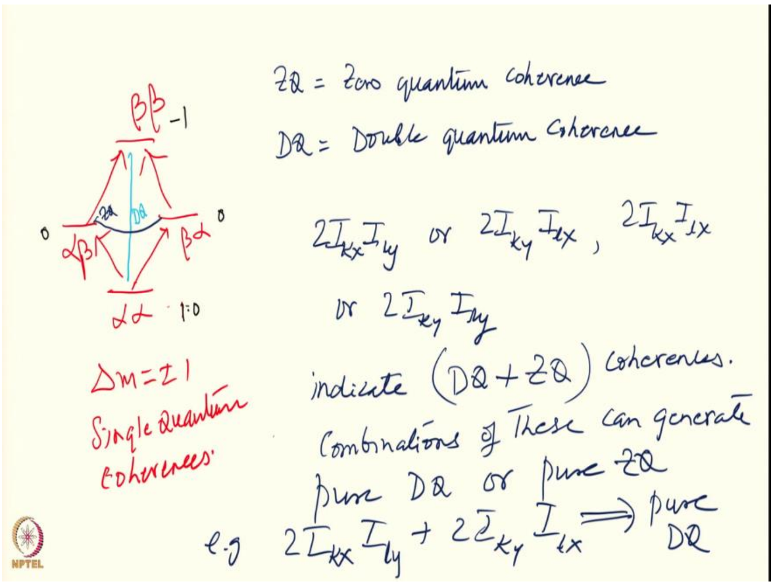

So, I will have to explain that in this context using energy level diagram there suppose I am taking a 2 spin system. So, then I will have here these are all let us say αα, αβ, βα, ββ for the two-spin system. So, I will have transitions here transitions here transitions here transitions here and all of these are delta these are $\Delta m = \pm 1$ therefore these are called as single quantum transitions or coherences.

And notice when I say transition coherences the transverse magnetization always represents the phase coherence between spins whereas the z magnetization represents populations as I mentioned earlier the transitions the transverse magnetization refers to single quantum coherences these are all single quantum because what is m value here m l here is 1.0, m is 1.0 here it is 0 here it is 0 here it is – 1.

Therefore the transitions which have indicated there these are all $\Delta m = \pm 1$ therefore these are single quantums. Now what are the other transitions possible there are other transitions possible you can have a transition here and this is DQ double quantum coherence because $\Delta m = 2$ these are not directly observable of course with selection rules when we considered the double quantums are not directly observable.

But these will be created in the course of your pathway magnetization flow this count of this kind of terms will also be created coherences will be created and then you also have a third kind of a thing possible let me see here and this is ZQ zero quantum. So, ZQ is called zero quantum coherence and DQ is called double quantum coherence. So, during the magnetization flow such kind of magnetization terms will be created. How do we know these ones what are the kinds of magnetization terms which represent this.

This is what we will see now here you can have things such as $2\ I_{kx}\ I_{ly}$ or $2\ I_{ky}\ I_{lx}$ or $2\ I_{kx}\ I_{lx}$ or $2\ I_{ky}\ I_{ly}$ this kind of terms indicate these indicate mixtures of DQ + ZQ a combination of these can create pure double quantum or pure zero quantum combinations of these can generate pure DQ or pure ZQ ok. So, for example $2\ I_{kx}\ I_{ly}$ for example $2\ I_{kx}\ I_{ly} + 2\ I_{ky}\ I_{lx}$ this represents pure w DQ this represents presence of pure DQ.

So, similarly I can also make I can also make $2\ I_{kx}\ I_{ly} - 2I_{ky}\ I_{lx}$ that will represent a zero quantum. So, such kind of combinations are possible you will generate pure double quantum or zero quantum or mixtures of double quantum zero quantums and double quantum or in general multiple quantums.

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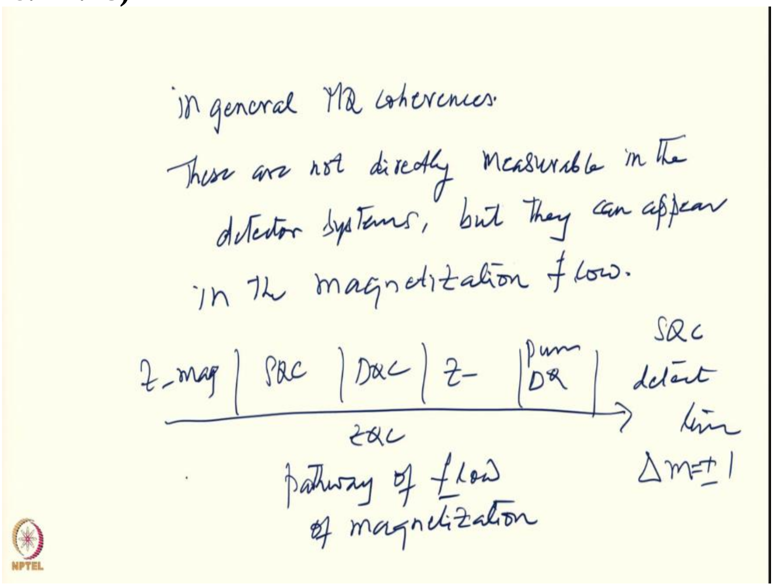

Or in general these are called multiple quantum coherences you can also have a triple quantum coherences but these are not directly observable when you put detector these are not directly observable in a detector and they have to be converted into single quantum coherences for detection but these can appear in their transfer pathway. So, these are not directly measurable directly measurable in the detector systems but they can appear in the magnetization flow.

So, therefore when you are having multiples experiments various kinds of transfers at various places all these kinds of terms will appear. So, and we are going to use this in all these multi-dimensional and NMR experiments which we will discuss various kinds of correlation experiments mentioned to you they will come across all of these kinds of terms and therefore it is important to understand what we are talking about here.

So, in the in the general scheme which you have various kinds of teams here you can have single quantums here you can have double quantums here or zero quantums there or you can have z magnetization. You can have pure double quantum I am just randomly writing here what all things can happen coherences and here you have z magnetization. So, these are the pathway this is the pathway along the time.

And when you detect when you detect here this will be pure this will be single quantum coherences only you can detect only single quantum coherences and here it becomes this is the selection rule $\Delta m = \pm 1$ only that thing can be detected but these ones can appear anywhere in the pathway. So, this actually represents the pathway of flow pathway of flow of magnetization.

So, therefore one can do design various kinds of experiments at what you want to have in a particular time period I may want to have single quantums here I may not want to have single quantums there I want to have double quantums there right they might want to have I can delete some of those ones how to manipulate those ones this is the thing which actually generates a whole lot of experimental schemes which are required for generating a specific type of information in your spectra.

And that is what is the power of this multi-dimensional spectroscopy you can use different kinds of nuclei generate different kinds of coherences in this experiment.

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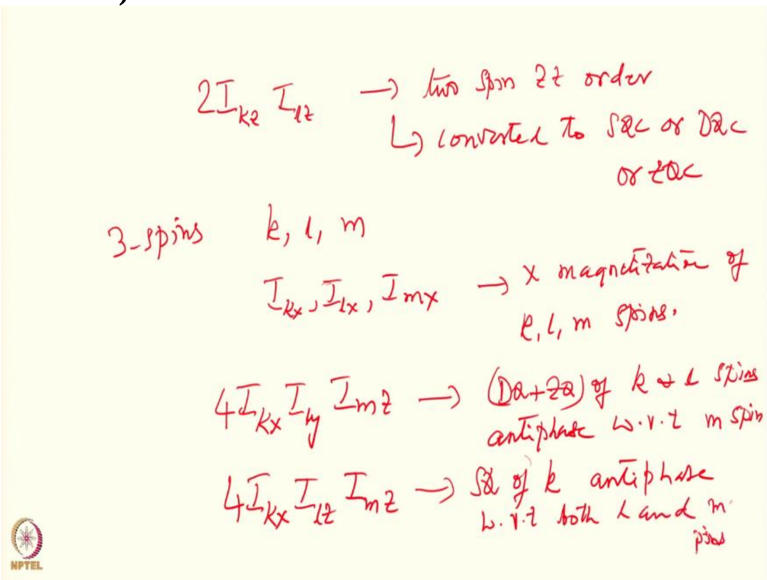

So, and then I will have terms such as 2 I_{kz} I_{lz} I will also have such kind of terms and this is called as 2 spin order 2 spin z order this can be converted into single quantum or double quantum this can be converted into can be converted to SQC or DQC or ZQC all such kind of terms can be there this is. So, far as two spins are concerned. Now let us consider what kind of things can happen for 3 spins.

3 spins again I can have let us say this spins are k l m suppose I have 3 spins then I can have terms like I_{kx} individual ones I_{lx} I_{mx} these are all m k l m spin magnetizations klm individually x magnetizations of these are all x magnetizations of klm spins alright. So, and the 2 spin terms will also be available you can make combinations of 2 I_{kx} I_{lz} or 2 I_{kx} I_{mz} and so on so forth.

So, there will be 2 spin terms also there. Now there can be 3 spin terms 3 spin terms are like suppose some I have things like that 2 4 I_{kx} I_{ly} I_{mz}. Suppose I have a term like this I get a term magnetization in the pathway I get things like that what does this imply? This will imply DQ plus ZQ of k and l spins antiphase with respect to m spin note whichever one is z it is antiphase with respect to m spin.

So, this will lead to a particular kind of a pattern in your final spectrum when you generate it either I can also have things like this 4 I_{kx} I_{lz} I_{mz}. So, now I have 2 z parts transverse is only one transverse is only with respect to the case pin but I will have the z with respect to l and m. So, this will be single quantum single quantum of k antiphase single quantum what transverse magnetization x spin this is x magnetization anti phase with respect to both l and m spins.

This will generate different kind of terms when you actually do a measurement. So, if you have such a kind of a term let us take for example I_{kx} I_{lz}.

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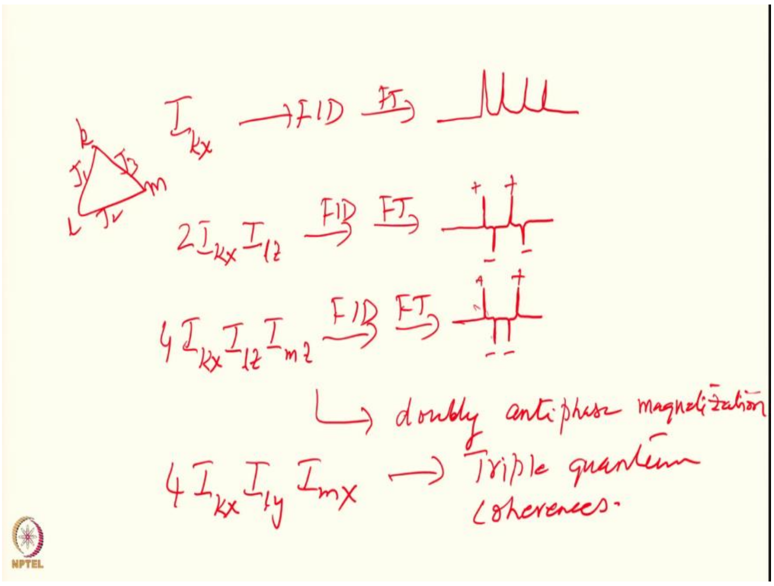

Now in the 3 spin system in the 3 spin system how will the I kx look like. So, then the 3 spin system is like this I have the k spin l spin m spin and there is a J for each one of those J_1 J_2 J_3 let us say if I have those how will the I kx look like after this generates a FID and then you measure they take the FT and this will give me for the k I am only writing for the k because this is the k spin.

So, the k spin will give me 4 lines which is a doublet of a doublet right because it is 2 couplings J_1 and J_3 couplings this will a doublet of a doublet. So, if this coupling constants are not equal then it will be doublet of a doublet. In the same one suppose I have a term which is like this 2 I_{kx} I_{lz}. So, what it will generate to me this will this term in the FID and then you do FT and this will produce spectrum like this in the spectrum it will appear like this 2 I_{kx} I_{lz}.

Notice here this repeats here positive negative positive negative. So, it repeats this is called as it is anti-phase with respect to the l spin. Now suppose I have this kind of a term 4 I_{kx} I_{lz} I_{mz} and this generates an FID and then I have the FT and this will produce a pattern like this after Fourier transformation. Now you see this is positive or negative this came from the anti-phase with respect to the l spin and then again now negative of this.

So, therefore positive negative negative positive . So, therefore for this is plus minus plus minus and here I have +-, -+. So, 2 negatives so, one negative coming from the k to l and the second negative coming from k to m. So, therefore you get this kind of a pattern. So, this is a doubly antiphase magnetization therefore this is called as doubly antiphase magnetization.

So, all right then let me also write one more turn which is to complete the story here I_{kx} I_{ly} I_{mx} or any combinations of those if I write like this these are called as triple quantum coherences. Once again this will not be directly observable these will have to be converted into single quantum coherences before actually you can observe and then we will generate the various kinds of spectral features as I indicated in different things like this.

So, we will be dealing with such kind of magnetization transfer pathways as we go into the multi-dimensional NMR experiments in fact this is when you want to talk about the COSY and the NOESY and things like that these are required that is why I introduced these ones beforehand. So that we; will have no difficulty in understanding this kind of situations so alright. So, then I think we can stop here and we go into the next phase into the next class.