Principles and Applications of NMR spectroscopy
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Module 1
Lecture No 04

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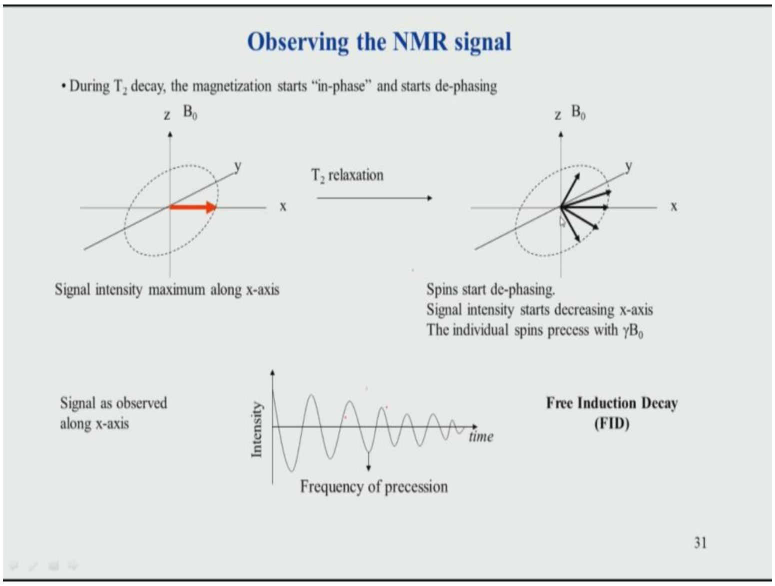

Observing the NMR signal

- During T_2 decay, the magnetization starts "in-phase" and starts de-phasing

Signal intensity maximum along x-axis

Spins start de-phasing.
Signal intensity starts decreasing x-axis
The individual spins precess with γB_0

Signal as observed along x-axis

Free Induction Decay (FID)

Welcome back, so in the last class we saw what is a free induction decay. This is basically the signal which is physically detected when the NMR hardware… Ah let us have a look at it once more to make it Ah more clear. So what is a free induction decay? So this is what is shown here. A free induction decay, essentially is EMF which is induced in the coil when the nuclear spin starts moving around.

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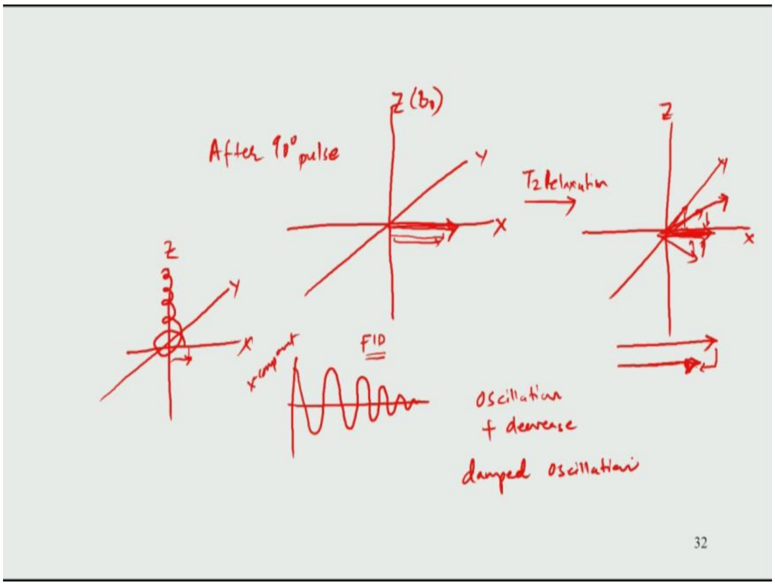

So let me explain this more in a, in a pictorial form. So let us look at how we started from. So this is the net magnetisation which is now along the x-axis, this is our Z axis, this is where we apply the original B0 magnetic field and this is Y axis. So this is the scenario after application of a pulse. So after 90 degree pulse. So after a 90 degrees pulse, the magnetisation is lying along the XY plane, even the x-axis. Now what happens is during this T2 relaxation, during T2 relaxation, molecules, the Spins now start dephasing.

Okay, so this is again X axis, this is Z axis and this is Y. Now if you look carefully, at this particular vector, what has happened is this vector is now the sum of many small vectors which are all bunched together. But when they start moving away from each other, during T2 relaxation, if you now take the components of all these vectors along the X axis, the components will now not add up perfectly. So the initial magnetisation is somewhat this much, after sometime the magnetisation has become this much.

This is because it has reduced because of T2 relaxation. So this is reduction happening because the Spins are now moving away in all directions in the, in this XY plane. And because of this movement, the total component, the vector addition of this screen is actually now reduced. But this magnetised vector is also rotating. So if you recollect the last lecture, I mentioned that we have the spins which are moving in the spiral manner. That means the net magnetisation is actually moving in a spiral manner.

So if you now visualize, it has reduced in intensity but it is also the net vector has moved away from this axis. It has come some very and it has come to this, this is basically showing this particular motion. So if you are again go back to this picture where it is spiralling, you can see what is happening is that the spins are oscillating in the XY plane. So when this is the X plane and this is the Y direction. So in the XY plane, if you visualise in the XY plane, the spins are actually oscillating and slowly decreasing.

So if you look at, let us say only the X component, suppose you look at only the X component, the X component starts like this, then it goes negative - X, then again come so positive + X, the negative - X and so on. So this X component if you plot the X component, it is basically showing an oscillatory behaviour. But remember its amplitude is also slowly decreasing and that decrease is coming basically from this picture here. You see the smaller, this shrinking along the x-axis and finally it is 0.

So basically this is what is shown in this. So our FID is nothing but a projection of this magnetisation along the X plane, along the X axis. So this is what is FID which, which I showed in the previous class, previous slide. And you see what is happening here. This is basically an oscillation, oscillation + decrease. So we use the word damped oscillation, damped oscillation. So let us look at this now with this in mind, how does the, **so** Ah so the FID is now shown in the next slide.

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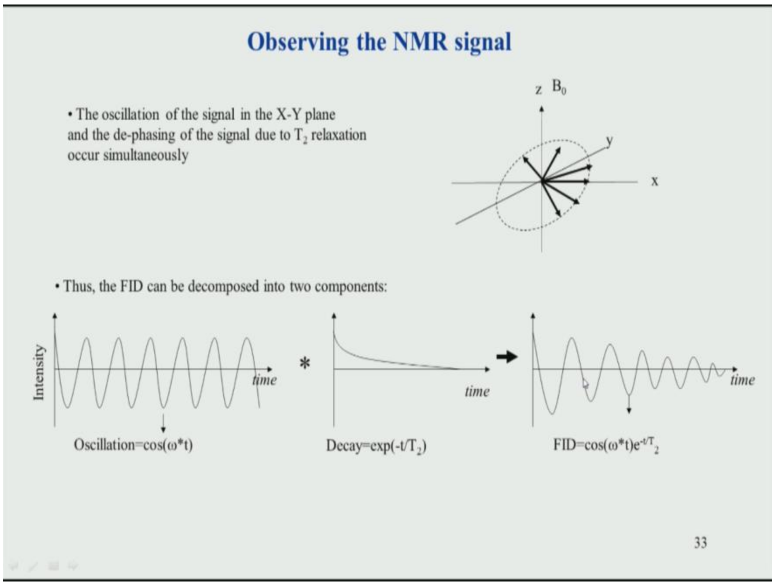

Observing the NMR signal

- The oscillation of the signal in the X-Y plane and the de-phasing of the signal due to T_2 relaxation occur simultaneously

- Thus, the FID can be decomposed into two components:

Oscillation=cos(ω*t)

Decay=exp(-t/T_2)

FID=cos(ω*t)e^{-t/T_2}

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So this is shown here, so we can see this is what the FID which I should earlier, you have an oscillation which is going around. Now at the same time there is a decrease in the amplitude and this decrease is because of the spiralling or the T2 relaxation which I showed in the previous slide. The spins are actually spiralling along the Z axis, so the X and Y component is actually decreasing in time. So that is, that is shown exponential. So this is typically an exponential decay which is depicted like this.

So this is the formula, the decay is exponential in nature and it has a time constant, means every exponential function has a time constant, is a time constant and that constant is T1 is T2 here. So that means in 1 times T2, the signal would have come by about 63 percent and so on. So this is how the exponential decay is depicted and now this, this is the decay but the oscillation is captured here. So the oscillation of the FID is because of the rotation of the spins along the XY plane because they are strictly precessing around the Z axis, so that precession which is the Larmer precession is what is shown here.

So this is basically nothing but the Larmer precession shown in a simple sinusoidal manner, the oscillating manner and this is nothing but a simple sinusoid function, it is cosine of Omega T. So what essentially is FID, FID is nothing but a multiplication of the oscillation with the decay. And these 2 together is what is shown here, that is, the index, the EMF, that is FID is the free induction decay. So remember the word induction is because EMF is induced in the coil because of the magnets which are moving and this F is free because spins are rotating freely in the XY plane and going along the equilibrium to the equilibrium state.

And decay because of this decay factor. That is why we use the free induction decay. And this free induction decay mathematically can be now written in this form. What is this form saying that it is basically a multiplication of a cosine function which is Ah oscillation which is shown here with an exponential decay function. So this is what why we use the word damped oscillation. So now this is the frequency, now how do we expect the frequency from the FID? So remember FID is simple depiction of an oscillation with a particular frequency and decaying with time.

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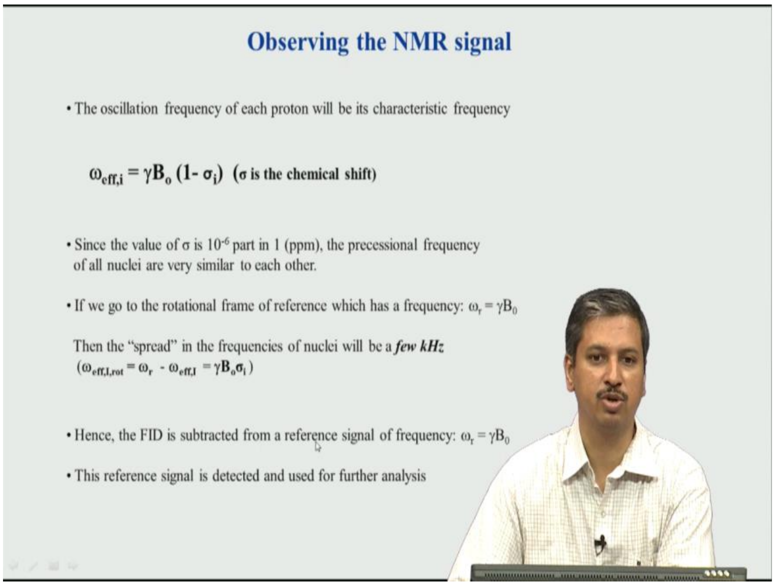

Observing the NMR signal

- The oscillation frequency of each proton will be its characteristic frequency

$$\omega_{eff,i} = \gamma B_o (1- \sigma_i) \text{ (}\sigma\text{ is the chemical shift)}$$

- Since the value of σ is 10^{-6} part in 1 (ppm), the precessional frequency of all nuclei are very similar to each other.
- If we go to the rotational frame of reference which has a frequency: $\omega_r = \gamma B_0$

  Then the "spread" in the frequencies of nuclei will be a ***few kHz***
  $(\omega_{eff,I,rot} = \omega_r - \omega_{eff,I} = \gamma B_o \sigma_i)$
- Hence, the FID is subtracted from a reference signal of frequency: $\omega_r = \gamma B_0$
- This reference signal is detected and used for further analysis

So to extract this frequency out of this oscillation, we now, we have to use the mathematical technique called Fourier transform. So see, now let us see what is this oscillation coming from. If you look at this formula, so what is happening is your omega is equal to gamma into B0. So remember this is a very basic concept which we saw earlier that if the rotation, the precessional frequency of a spin is basically related to the Gyro magnetic ratio B0. Now what happens is, this is where the concept of chemical shift will come into picture.

We will see more detail, in more detail manner chemical shift later but right now what is important here is that the chemical shift is essentially a parameter which is now deal Ah Ah which is making the spins have a frequently different from gamma B0. So suppose, let us say Sigma is 0, so if Sigma is 0, then omega is equal to gamma into B0. But many spins do not have, they have concept of chemical shift which is parameter, which deviates the effective magnetic field from the B0.

That means the spins do not experience all the spins. Let us say you take a molecule, we will come to the more detailed torture later. Let us say you are taking a molecule like ethanol. So ethanol has CH3CH2 and 0H. So each of these hydrogens, CH3CH2OH will have a slightly different chemical shift from each other and that because of that all of them do not see the same B0 field. They experience is slightly different B0 field and that is what is captured here. It says, that the effective field experienced by some nuclei I is basically gamma B0 into 1 - Sigma I.

So this is what is called a shielding factor or chemical shift Ah Ah in different ways. We will look at it in more detail but for now that is what is important to understand is that this is a small deviation from the main magnetic field, typically days of the order of 10 to the power -6 compared to. So that is why we use the word PPM but we will, we will make it more strong in the later slides. But what is important to understand is this the oscillation which I showed in the previous slide is basically the oscillation which is coming because of this factor.

So what is happening is that every hydrogen atom in a molecule will not have the same oscillation frequency, oscillation frequency is nothing but chemical shift. So the oscillation frequencies are not the same and because of that we get different frequencies in the different atoms of the same molecules. So typically what happens is we use this rotating frame concept here.

So now let us look at the rotating frame concept here again, what is happening is you, in the rotating frame, we are in this frequency, remember I said when we synchronise the motion of the spins with the B1 field, what essentially we are doing is the B1 field is applied at this frequency omega equals to gamma B0. So in this frequency all the Spins are now static because they are all synchronised and this is. But all the spins do not have the same frequency, they have slightly differing frequency because of this factor. So this factor makes them different from each other.

So therefore you can never be synchronised with one all the spins at the same time. So you choose a particular frequency as a reference frequency, let us say omega B0, gamma B0 and that reference frequency is basically your, rotating frame of reference frequency. In that in that frame of reference, now different spins will have a slightly different frequencies. So let us take an example analogy again of a train. Let us say it is moving at 100 kilometre per hour or let us say we have 3 trains, one is moving at 100 kilometres per hour, one is moving at 105 kilometres per hour and another one moving at 95 kilometres per hour.

Now let us say we go into a frame of reference of the train which is moving at 100 kilometre per hour. So in that particular train, the reference frame, the frame of reference, you will see that the train which is moving at 95 kilometres per hour is actually moving at - 5, 100 - 95, it is moving in the negative -5 because you are faster than that particular train. The other train which is moving at 105 kilometres per hour is now +5 kilometres per hour.

So you see now with respect to you, with you are at 0 kilometres per hour because your train is essentially fixing, you are on resonance with your train’s speed, so you do not experience the movement of your train but the train which is moving next you with 95 moves at -5 and the train which moves next to you moves at 105 moves at +5. So this is called the rotating frame of reference where you keep your frequency aligned with one particular frequency region, one source peak let us say and that peak becomes 0 frequency because it is no longer having any frequency, it is static.

But with respect to that frequency, all the other frequencies are now no longer 0, they are either positive or negative. So this is the concept of chemical shift offset and different terminologies which we will use later. But what is important from an FID point of view is that now this FID, as I showed in the last slide consists of oscillations. And what is this oscillations they are coming from? Oscillations are coming because different atoms in the same molecule are having different frequencies, so therefore they will have different oscillations.

And all these oscillations are captured together in a single FID. So that is what is written here, that the FID, what we are doing essentially is we are taking a reference signal which is some frequency omega B0, gamma B0 and that reference frequency is subtracted, is subtracted from all the frequencies and what you essentially get is finally the subtracted frequency. This is again the same analogy as a train that if I have a train moving at 100 kilometre per hour, I subtract the speed from 100, so that it becomes 0.

But the train which is moving at 95 kilometres per hour becomes -5 and train which is moving at 105 will become +5. So with respect to the reference I get negative, positive 0. So that is what happens here, same in NMR. That you take a reference signal, you subtract that frequency from the FID and what remains in the FID is the remaining these oscillations which is because of the chemical shift. So if you have a positive deviation or negative deviation or it can be 0.

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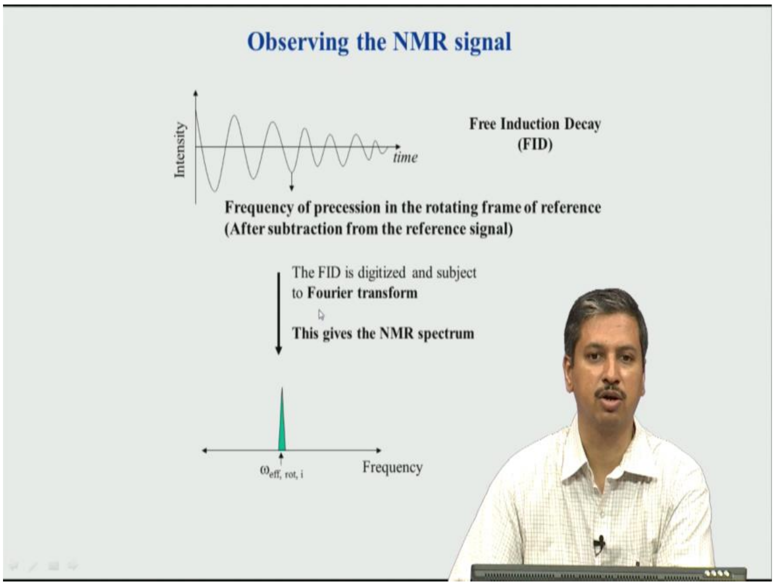

Observing the NMR signal

Free Induction Decay (FID)

Frequency of precession in the rotating frame of reference (After subtraction from the reference signal)

The FID is digitized and subject to **Fourier transform**

This gives the NMR spectrum

Zero means the signal, reference signal is exactly the same frequency as your spin and therefore the spin does not experience any oscillation in that frame. Remember again, it is all frame dependent, so we are always using what is called rotational frame, so NMR, the whole NMR technique is built around this concept of rotating frame of reference. So now let us come back to this FID now. So once I subtract, so this is what is written here.

The frequency of precession been rotating frame of reference is, signal is subtracted from the reference signal. After subtracting you will get the remaining oscillation. So this oscillation is now, after subtraction because it is because of this chemical shift and it is slightly different from the reference angle. If it is same as reference signal, it would have been 0, no frequency. But the fact that it is lightly different is because it is many spins may not have exactly the same as reference and they will have an oscillation.

So this is the free induction decay after subtraction from the reference. Now this FID is now further digitised. How do you digitise this? Digitisation is what again the components we will see during when we come to the hardware part of it in the course. But what is happening essentially is that you take every equal intervals of time, you take the point. You get physically points, instead of recording this, I mean collecting the signal as a continuous wave, it is recorded in a discreet manner by collecting points, but discreetizing at different points.

So by doing that we use the word digitisation, we are digitising the FID but for the mathematical perspective, you do not have to worry about the digitisation. We can think of it as a continuous wave. Now if I do a mathematical technique called Fourier transform will give me the frequency of this peak. So Fourier transform is something which we will not be able to go into detail in this particular course but it is recommended that you can refer to the books, many of the NMR textbooks have very interesting, very basics introduction to Fourier transform and they also give a different ways to do Fourier transformation and what is the affect and so on.

So for this particular course what is important to know is that you have a signal which has a frequency, so this is a time domain signal. So we use the word time domain because we are capturing the signal as a function of time. This is what is done by the hardware electronics which is part of the whole instrument and that basically captures the signal is a function of time and this signal is now Fourier transformed to get the frequency and that is called a spectrum.

So NMR spectrum is now a frequency domain spectrum. So you see the frequency is written here. So what basically we are getting now is a frequency. So this frequency, means there is F, we can say that this FID contains a frequency omega in the rotating frame. So again remember the concept of rotating frame is very important, we are not looking at the signal as such, we are after, we subtracted from the reference signal and then do a Fourier transform and in the rotating frame, that is the spectrum which you would normally in all your day-to-day life or during the analysis, these are all in a rotating frame.

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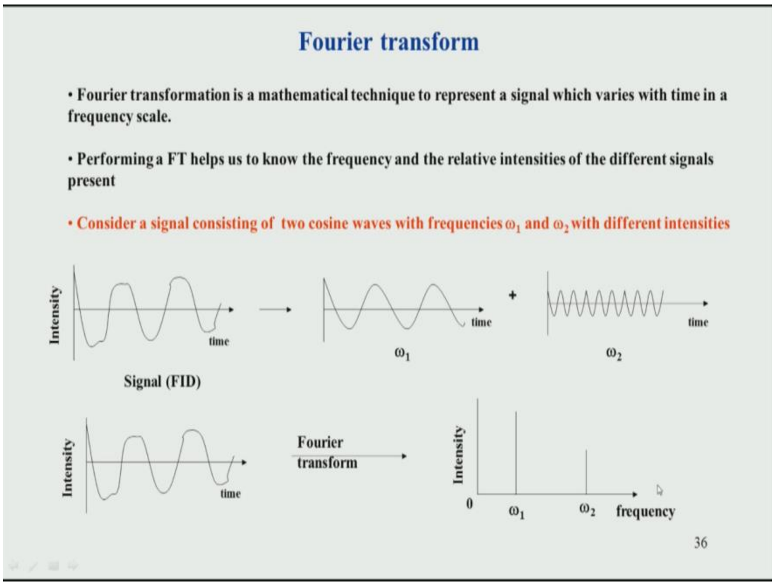

Fourier transform

- Fourier transformation is a mathematical technique to represent a signal which varies with time in a frequency scale.
- Performing a FT helps us to know the frequency and the relative intensities of the different signals present
- Consider a signal consisting of two cosine waves with frequencies ω_1 and ω_2 with different intensities

And then the frequency is basically is the frequency of oscillation. So this is fine as far as one frequency is present. But remember I give you an example of a molecule, let us say we have a molecule which has 2 frequencies present. So example is methanol, a methanol is CH3OH, so it has to types of hydrogen is, one is CH 3, H of the CH 3 and the H of the OH, so there 2 frequencies. Now in the reference frame, both will, let us say we keep the reference at the centre of the 2. So I keep, let us say CH3 is one frequency Omega 1 and omega 2, I keep at the Centre, so therefore the CH 3 will have a particular oscillation with respect to the reference signal and the OH will have another oscillation with respect to the reference.

So you can see the frequencies are not same now because they are now 2 different atoms and 2 different atoms in the same molecule. And therefore they have 2 different frequencies and these 2 different frequencies also have different amplitudes. You see the height, the height is also not the same here and here, that is because this particular frequency may let us say come from 3 hydrogen atoms, so three-times the signal of this, this may come from one hydrogen atom.

So you see that is the difference in NMR, everything, all the signals, all the items contribute to the FID but their contribution depends on 2 parameters. The conservation defence number-one on the frequency of the oscillation and 2nd thing they depend on the number of hydrogen. So in case of a methanol, again the CH3OH, your H3 has 3 hydrogens, so this particular frequency of CH3 will be 3 times stronger compared in terms of amplitude, in terms of this idea will be 3 times bigger than compared to OH peak which has only one hydrogen, but frequencies are different, 2 different frequencies.

So what you actually capture, the hardware which captures the signal, it captures the total of these 2. If you combine these 2 as a simple addition, you will end up something like this, which is just a schematic diagram showing the combination of these 2 signals. So this will, so this is what is actually given, captured by the hardware and now how do we, from here find out the 2 frequencies which are present.

So this is what we know later but in the initial stage we only have this FID with us which has a mix of 2 signals or it may be many signals, we may never know, we have to do a Fourier transform. So when you do a Fourier transform of this particular FID, this will now decompose, it will give us immediately 2 different frequencies. So this is the beauty of Fourier transform that it captures the frequencies which are present in this time domain FID and that is represented in the frequency domain spectrum.

So if you see here now the intensity of this peak is now directly proportional to the number of hydrogens here. So in the case of methanol where we discussed that there are 3 hydrogens, so this could be 3 times stronger. So this signal will be 3 times stronger compared to this. Of course in this picture it does not look like so but as the qualitatively you can think of it that this particular thing is three-time this, basically it depends on the number of hydrogen. So if we have 4 or 5 or N number of hydrogens, this signal will directly be related to the number of hydrogens.

Whereas this is also similar. So this is how a spectrum is recorded, basically you record a time domain FID and then you then combine, the FIDs are automatically combined because that is how the whole signal will be detected. And upon Fourier transformation which is typically done by the computer Ah automatically and that will give you a spectrum.

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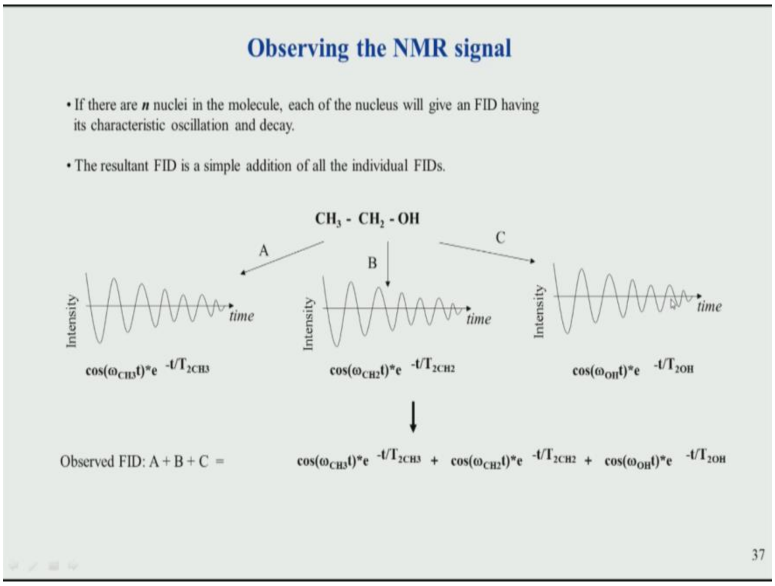

Observing the NMR signal

- If there are *n* nuclei in the molecule, each of the nucleus will give an FID having its characteristic oscillation and decay.
- The resultant FID is a simple addition of all the individual FIDs.

$CH_3 - CH_2 - OH$

$\cos(\omega_{CH3}t)*e^{-t/T_{2CH3}}$ $\cos(\omega_{CH2}t)*e^{-t/T_{2CH2}}$ $\cos(\omega_{OH}t)*e^{-t/T_{2OH}}$

Observed FID: A + B + C = $\cos(\omega_{CH3}t)*e^{-t/T_{2CH3}} + \cos(\omega_{CH2}t)*e^{-t/T_{2CH2}} + \cos(\omega_{OH}t)*e^{-t/T_{2OH}}$

So you can look at this in the same molecule. Let us take an example of ethanol, in ethanol you have CH3CH2 and OH. So you can see here there are different hydrogens this type of, this is one hydrogen, this is another kind of hydrogen and this is another. And why do we say that 3 different hydrogens, we will look at it in a later time Ah that is based on the chemical shifts. But the point here to take home from here is that there are 3 different hydrogens here. And each of these hydrogens has a particular oscillation because this will have a frequency, this will have a different frequency of oscillation, this will have a different frequency and when you subtract from the reference, you will have this particular frequency shown schematically here.

Now this frequency which is shown here is nothing but a multiplication of the cosine function of that is oscillation of the CH 3 group with the exponential decay of the CH 3 group. So this is what is I showed in the previous slides (())(21:52) FID. Similarly here, this will be now a multiplication of the frequency of B, that is this hydrogen H2 with its decay which is shown in this FID and that the case now depending on the CH 2 T2 relaxation, relaxation. So T2 relaxation of this hydrogen need not be same as the T2 of this which need not be same as the T2 of this.

So generally we can assign, assume a different T2 for each of this. Now for OH, similarly you have a frequency of OH oscillation multiplied with the, the time domain, that is the decay of the OH signal which is captured here as a T2 of OH. So these 3 together will combine and we can see the total FID now, whatever you captured by the hardware is now a combination of all the 3. Because the hardware does not distinguish between different oscillations. All it can see can see is oscillations and oscillations can come from anywhere, from all sources, all of these together are added together and what is given to the computer is essentially these 3, these 3 FIDs are added together.

So you see this is what the signal comes and this will look a very strange signal because it depends on this factor, this factor and all the different factors and not only that, one factor which is not shown here is the number of hydrogens. So number of hydrogens is 3 here, so this signal will be 3 times stronger compared to the signal which is let us say 2 times compared to this signal. So 1 is to 2 is to 3, so that particular thing is not shown here but you have to keep in mind that the FID is not only simple addition, it is addition of again the amplitudes.

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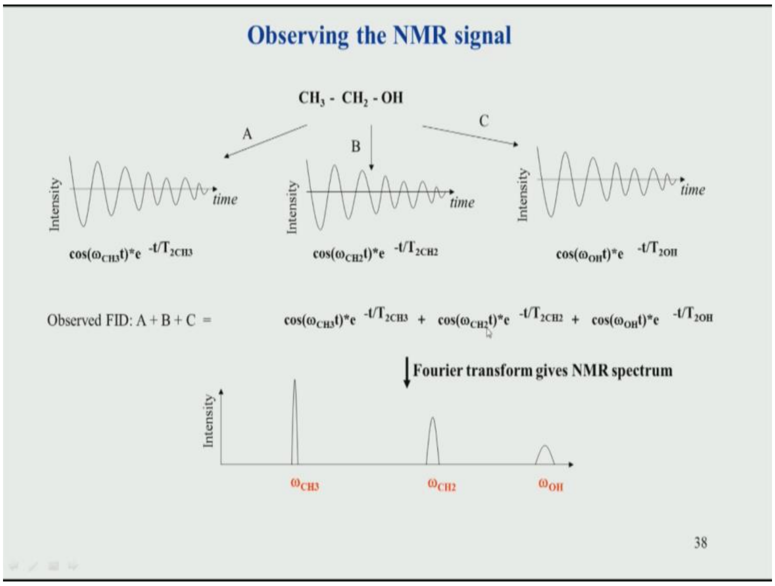

Observing the NMR signal

$CH_3 - CH_2 - OH$

$\cos(\omega_{CH3}t)*e^{-t/T_{2CH3}}$ $\cos(\omega_{CH2}t)*e^{-t/T_{2CH2}}$ $\cos(\omega_{OH}t)*e^{-t/T_{2OH}}$

Observed FID: A + B + C = $\cos(\omega_{CH3}t)*e^{-t/T_{2CH3}} + \cos(\omega_{CH2}t)*e^{-t/T_{2CH2}} + \cos(\omega_{OH}t)*e^{-t/T_{2OH}}$

Fourier transform gives NMR spectrum

In the sense, it is a weighted addition of the amplitude because this will be 3 times stronger compared to this, compared to that. So this is how the FID is captured and when you do a Fourier transform, will give you an NMR spectrum. So this is what is shown here, these 3 frequencies are basically the 3 different oscillations which we saw in the previous slides coming from CH 3, CH 2… This is what is shown here, if you go back in this manner, we can see that this is mathematically coming because there are 3 terms here, each term contributes to the difference peaks. So these are, this is the spectrum, what is shown on the bottom is basically a spectrum.

A spectrum consists of a frequency axis which is the x-axis and Y axis is the intensity. Now remember the intensity is basically proportional to the number of hydrogens. So there are 3 hydrogens, so this will be 3 times. But again one thing is, before we look at the height of intensity, when we will talk about intensity in NMR, it means the area. So suppose I take a area of this week here, this is like a triangle. In fact this is actually in reality is not a triangle but let us say it is like a triangle.

If I take the area of the peak here, that area will be 3 times the area of this peak here. So this is 3 is to 1 ratio. About this one which is CH2 will be 2 times the peak of the area of this peak. So you see the area is what is important in NMR, not just the peak height. The peak height as a different connotation, what is important is the area of the peak. So the peak area is basically proportional to the number of hydrogens. So there are 3 hydrogens, this peak will be 3 times, let us say 3X, this is 2, this is 2X and this is X, so 1 is to 2.

So area is very important in NMR as we will see later. This is where the quantification aspect of NMR plays, comes into picture, the whole quantification depends on the area of the peak. So we look at area to decide how many hydrogens are there and what is the concentration. For example if I take a mixture of ethanol and methanol, so I take a mixture of 2 different molecules and if these are 2 different concentrations, the areas of the peaks of ethanol will be proportional to its concentration, area of concentration, area of peak of methanol, which is also in the same sample will be proportional to its concentration.

So therefore directly by comparing the peak areas in NMR, we can actually figure out or find out the concentration, of course provided we have reference concentration which we will see that later how the reference etc. is done. So summarising this whole aspect, essentially we have Ah we will repeat this that they have, there is a molecule, let us say ethanol which has 3 different hydrogen atoms and 3 therefore 3 different oscillations or 3 different frequencies or 3 different chemical shifts.

So a chemical shift was briefly introduced but we will come back to chemical shift at a later point. So chemical shift is nothing but the Ah Ah what is the frequency of a particular hydrogen atom. So you see is there are 3 different frequencies which are 3 different shown here and each one has its own decay constant which is given by T2 relaxation and this decay constant now is, all multiplied with the frequency. So these are 3 different signals which are added together, added in the sense we do not physically add them, it is automatically captured by this Ah hardware because all 3 come at the same time and they are represented by a simple addition.

And this addition upon Fourier transform gives you different peaks where the height of, the area of the peak is proportional to the number of hydrogen. So this is how a basic Fourier transformation is done in NMR (())(27:21) spectrum. Ah What we will do now is we will move onto the NMR hardware in the next class. We will see how all this hardware is built, how the spectrum is recorded, how does the spectrometer look, what does the magnet look like and then after that we will come back to analyse how all the spectrum can be interpreted.