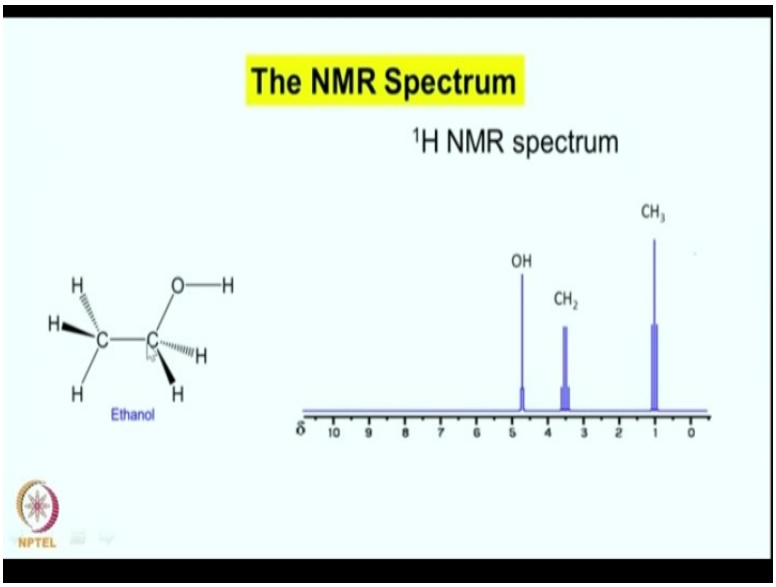
NMR Spectroscopy for Chemists and Biologists
Professor. Ramkrishna V. Hosur
Department of Bioscience & Engineering,
Indian Institute of Technology, Bombay.
Lecture 13
Fourier Transform NMR

Today we are going to start a new chapter namely Fourier Transform NMR Spectroscopy. This has to do with how we actually record the NMR spectra. You have already seen in the previous lectures some details about the characteristics of the NMR Spectra. There are parameters called as chemical shifts, coupling constants.

(Refer Slide Time: 0:45)

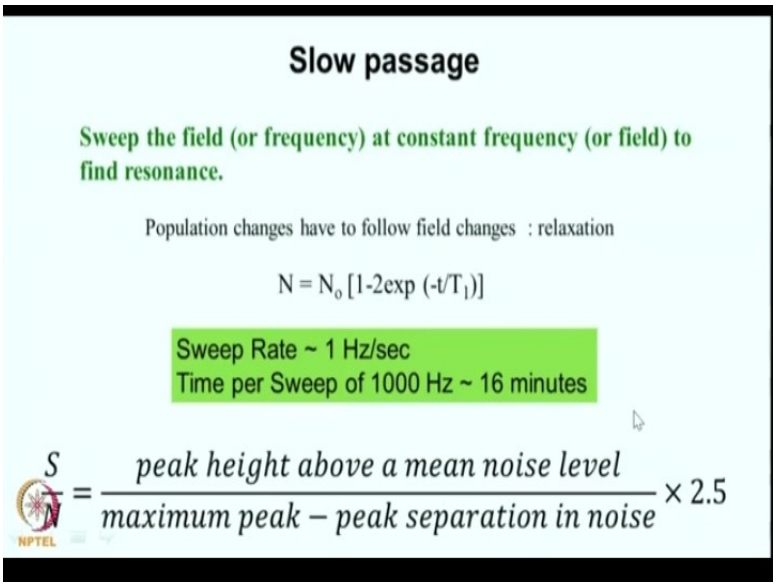


For example, if you have a molecule such as this which is ethanol, one of the simplest molecule on which actually the chemical shift was discovered by Dr Dharmati when he was in Felix Bloc's lab. Look at this molecule. This has 3 protons here, 2 protons here and 1 proton here. All these 3 protons have different absorption frequencies. This CH_3 group absorbs signal at this chemical shift. The CH_2 group, this absorb energy at this chemical shift, this frequency and the OH group appears somewhere here.

Notice there is also fine structure in each of these lines. This happens because of the spin-spin couplings between the protons of the different types. This has been discussed previously. Now the question is how do we measure this, how do we know what are the frequencies present in your NMR Spectrum.

In the early days, the method was called slow passage experiment that means you have a particular magnetic field you sweep the frequency, reach the resonance condition here, you get the signal here. Reach resonance condition here, you get the signal here. Then you reach the resonance condition here, you get the signal here. So this is sweeping the field constant you sweep the frequency. Alternately what you can also do is, keep the frequency constant and sweep the field.

(Refer Slide Time: 2:25)



So one by one you will reach the resonance condition for the individual lines. It was more convenient to sweep the field rather than sweeping the frequency in those days because the magnets were electromagnets and you can simply change the current through the electromagnet and that will change that field. Now, how fast we can sweep the field? We recall through a discussion with regard to the relaxation time. Whenever we change the field there will be changes in the energy levels. There will be changes in the populations.

Therefore, the population changes have to follow the field changes in order that you represent your equilibrium situation appropriately. The system has to be in equilibrium. You notice here that the population changes follow this kind of an equation where population difference

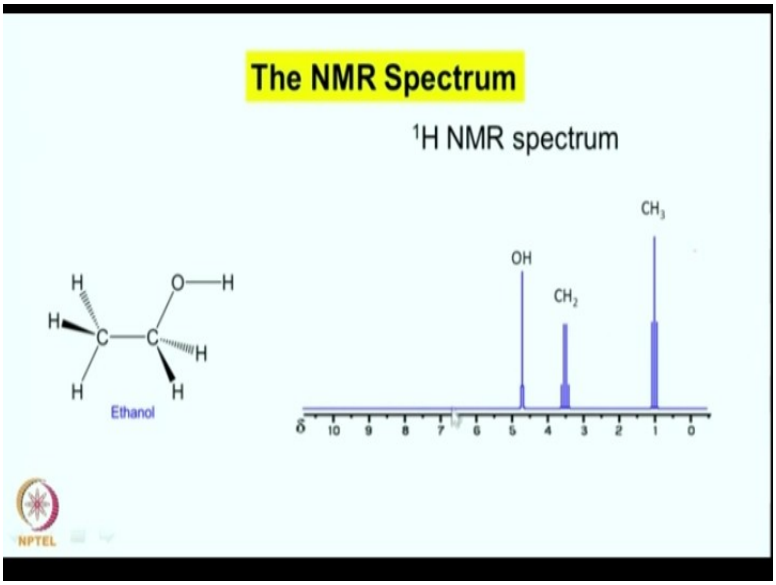
$$N = N_0\left[1 - 2\exp\left(\frac{-t}{T}\right)\right]$$

Where, T_1 is called the spin lattice relaxation time.

Therefore, if the T_1s are of the order of seconds, then you cannot be sweeping this field too fast. Otherwise, you will not be reaching an equilibrium situation at every point you try to measure the resonance. So typically if I have to sweep the field at the rate of 1 Hertz per second, then the time per sweep of the spectrum of width 1000 Hertz. What is the meaning of 1000 Hertz here? If I am looking at the proton spectrum, typically the proton spectrum has a range of 10 ppm. The 10 ppm on 100 megahertz spectrometer corresponds to 1000 Hertz.

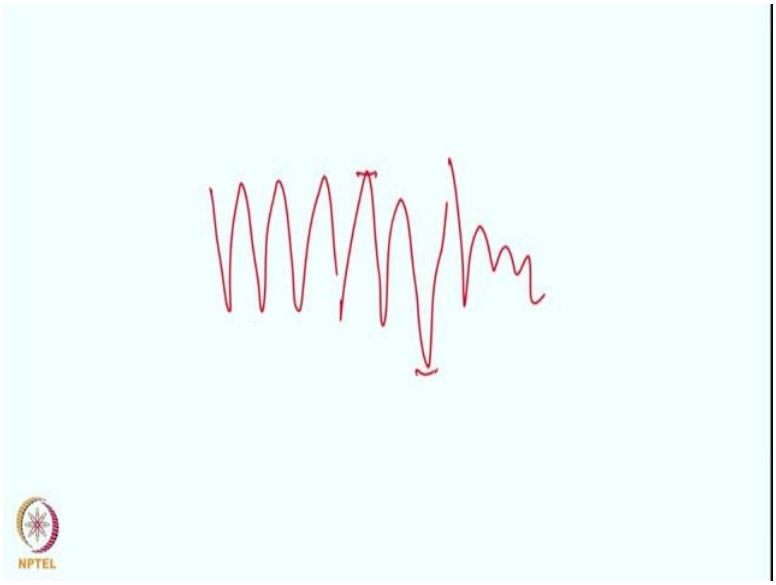
If we do a 500 Megahertz it will be 5000 Megahertz and considering just now the 100 Megahertz, which is 1000 Hertz. If we do it at this rate, this will take me 16 minutes approximately for 1 sweep. Now when you actually look at the spectrum, the signal to noise ratio. What is signal to noise ratio?

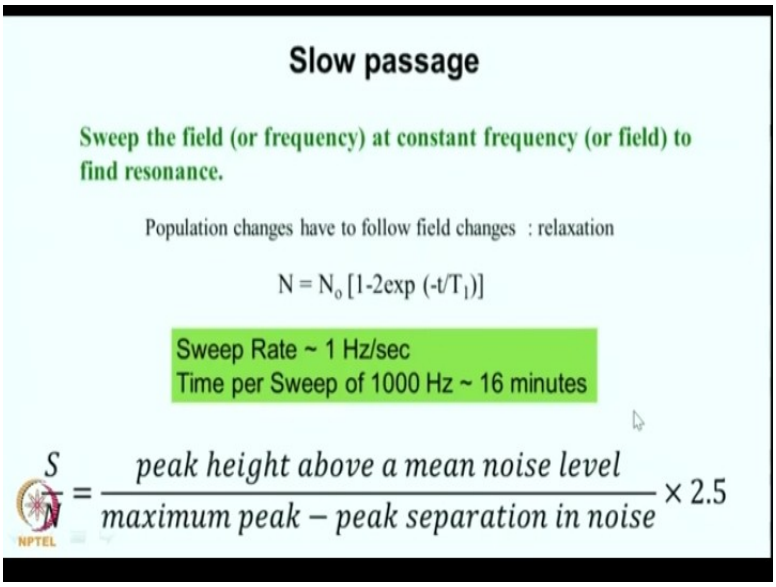
(Refer Slide Time: 4:30)



That is if you were to take the peak height here. Take the signal and measure the peak height and you take somewhere here with there is no signal there is only noise. When you have the noise then you have a peak to peak separation between the noise.

(Refer Slide Time: 4:47)



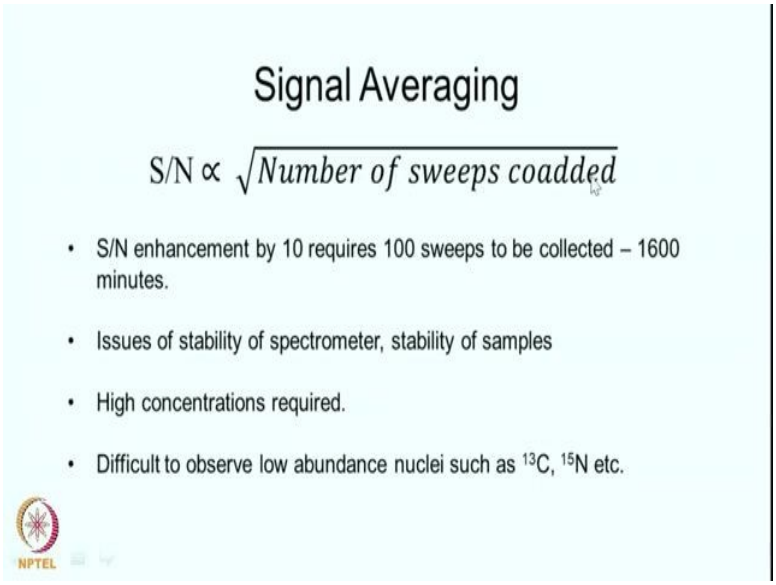


So you have noise like this. So you take the peak to peak separation from here to here. That is a separation for the noise.

$$\frac{S}{N} = \frac{peak\ height\ above\ a\ mean\ noise\ level}{maximum\ peak - peak\ separation \in noise} \times 2.5$$

This happen because of the stochastic reasons and that is called as a signal to noise and typically this is very small because the as I mentioned in the previous classes. Thus NMR spectrum sensitivity is actually quite low. Now if I want to increase the sensitivity signal to noise ratio so that i can actually measure it with confidence what should I do?

(Refer Slide Time: 5:37)



This is a technique called the signal averaging. What you do is you collect sweep the frequency several times sweep the spectrum several times and add them together and this is an additive process. Therefore, once you add them, the signal adds as the number of scans whereas the noise adds by square root of the number of scans.

Therefore,

$$\frac{S}{N} \propto \sqrt{Number\ of\ sweeps\ coadded}$$

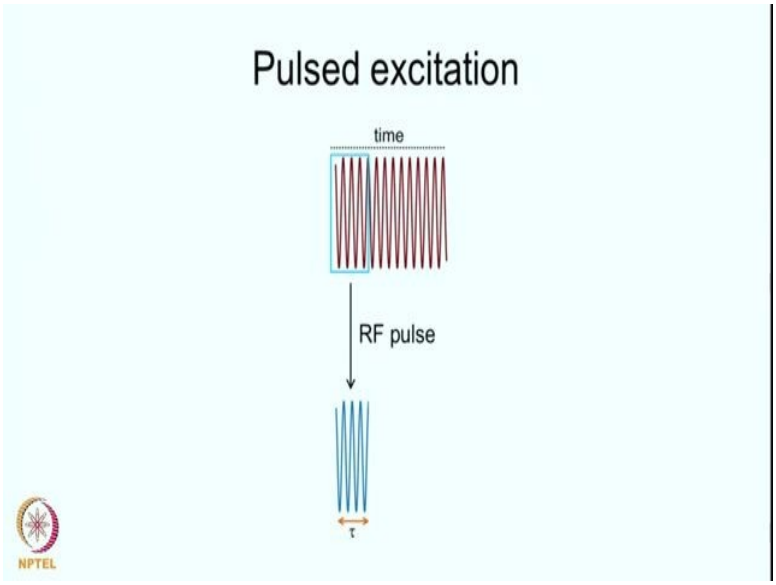
So for example, if I want to enhance my signal to noise ratio by a factor of 10, then I have to sweep a 100 times to get this enhancement by a factor of 10, which means if it were 16 minutes per scan then I would need 1600 minutes and this is a huge amount of time.

It puts a lot of pressure on your spectrometer conditions. This spectrometer has to be stable for that long period of time, the current should not vary, the field should not vary and that too of the stability of the samples. The samples have to be stable for a long period. If your signal to noise is very poor alternatively one would then require a use very high concentrations of samples

But high concentrated sample may require that the sample is properly soluble in your solution. If the solubility is very low, then high concentrations cannot be achieved. Then it is such a situation, it is difficult to observe low abundance nuclei such as carbon 13 and

nitrogen 15. Carbon 13 you remember in 1.1 percent natural abundant, N 15 is 0.37 percent natural abundance. Such nuclei it is almost very difficult to observe. So what do we do?

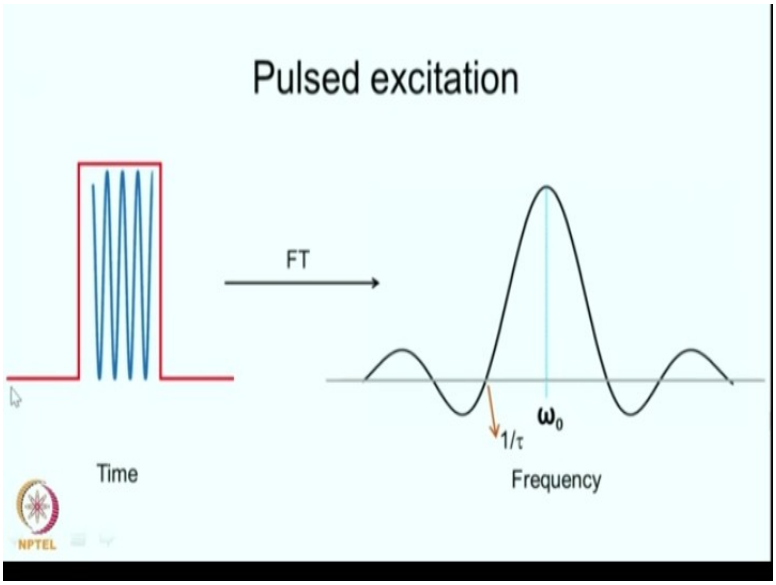
(Refer Slide Time: 7:28)



So there is a new strategy here instead of sweeping the field as we did earlier can we do the following. Keep the field constant; apply all the frequencies in one go. Generate so many frequencies in one go so that one or the other of the frequency matches the resonance condition and you will have excitation of all the spins in one go. This is termed as pulse excitation.

How is it achieved? You have the *RF* frequency going. Let us say this is the time here. This is the time axis and the *RF* is a sine wave, or the cosine wave whatever you want to call it. It is going like this continuously. But what we do now is, you apply this *RF* only for a short period of time. You just start here and cut it off here. So therefore we apply the *RF* only for a short period and we call this as the pulse. You applied for a period τ and what is the consequence of this?

(Refer Slide Time: 8:27)



The consequence of this now your time axis is time profile of your *RF* looks like this. You have no *RF* here, then suddenly you applied an *RF* for certain time. Then you put it off again. Then you again 0 here. So your time profile is like this as indicated. What does it imply? How do I get this sort of a profile now? This amounts to applying a large number of frequencies in one go and what is the distribution of these frequencies and what are the amplitudes of these frequencies which you apply when generate in this manner and that is indicated by this figure.

It shows that this is the main frequency ω_0 and in addition to this, it generates a large number of other frequencies with different amplitudes. Some are higher than ω_0, some are smaller than $\omega_0$and at this time this at this frequency the excitation is 0, this is 0 then it becomes negative here and so on.

So you generate large number of frequencies in the consequence of such a kind of a treatment and all of them have different amplitudes. In other words, if I wait to take individually all of these frequencies with the respective amplitudes all of these and superimpose all of them, co-add all of them.

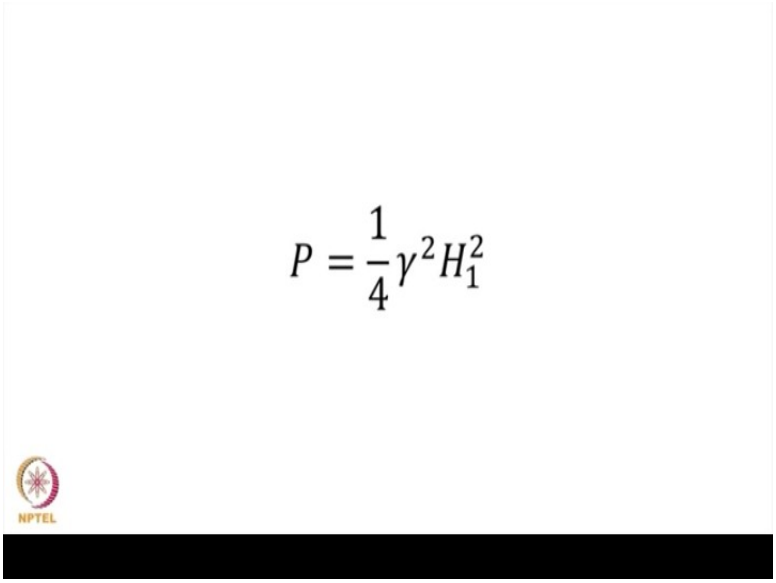
Then you will generate a time domain profile which is like this. This is called as the Fourier Transform of the pulse. So there is a relationship between this time domain and this frequency domain and that relationship is the Fourier Transform. So by doing this applying for a short period of time you generated a larger number of other frequencies. Now how long

this should be? If you look here, the tau of the period for which it is pulse was applied the the red of frequency was applied and this comes to 0 the excitation comes to 0 at 1 by tau.

So suppose τ is 1 microsecond, then this will be like $10^6 . \frac{1}{\tau} = 10^6$. So compared to this you have 10^6 Hertz. That means 1 Megahertz range is covered here. 1 Megahertz range is covered here, 1 Megahertz range is covered here. That is the kind of an excitation we have. Although they have difference between different amplitude at different frequency values.

Now what do we do with those many frequencies? We do not need so many frequencies. We have another condition that is if I want to have a proper excitation of the spin system I must have the same power for all of these. Why do I say this?

(Refer Slide Time: 11:23)

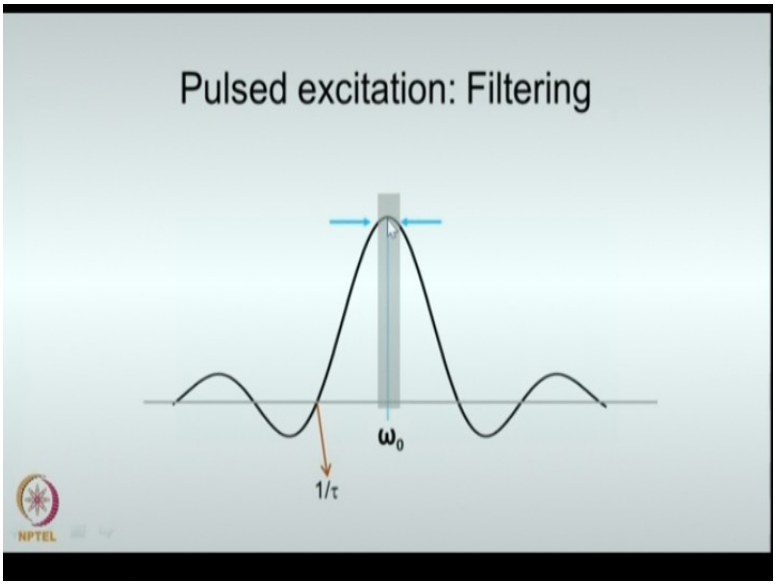


Let us look at recall the discussion from the first chapter. You remember the transition probability induced by the *RF* was written as

$$P = \frac{1}{4}\gamma^2 H_1^2$$

H_1 is amplitude of the *RF* that is applied and Υ is the gyromagnetic ratio and the transition probability $P \propto \sqrt{H_1}$. If I want to excite all these things similarly then I must have the same power applied to all them. So how do I achieve that?

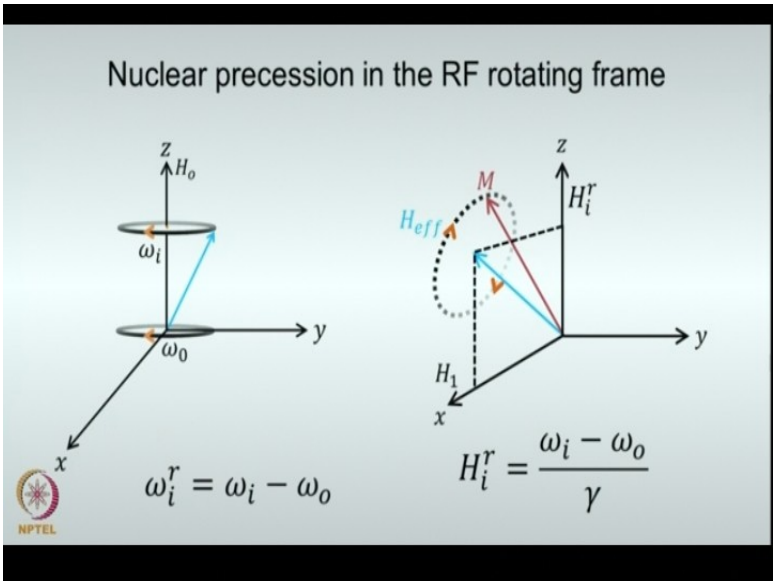
(Refer Slide Time: 12:03)



So what do I do? I select only a small portion here. This small portion which has nearly similar amplitudes and therefore their transition probability induced at these frequencies will be the same. Then I can compare the intensities of my signals in the NMR spectrum. So this is a requirement. Therefore, I have to do what is called as filtering. I filter the frequency response to keep only these many frequencies and throw the rest.

Now I notice this is quite sufficient because if this is Megahertz this will be observed of Kilohertz roughly of few Kilohertz. A few Kilohertz a few 1000 Hertz and remember in 100 Mega the proton spectrum was 1000 Hertz. So even if you go 5000 Hertz I will be able to excite this with a reasonable uniformity if I filter out this kind of thing. So depending upon the tau value I choose I will have a distribution of powers and accordingly I can choose how much should be the spectral width.

(Refer Slide Time: 13:06)



Now what is the effect of this kind of an *RF* on this spin system? Let us go back and look at the nuclear precession in the *RF* rotating frame. You remember the nuclei are precessing around the H_0 field in this manner with the frequency ω_i and ω_0 is the RF frequency we have applied.

Now if I were to sit on this omega naught, RF frequency and look at the spins, then the spins will be precessing with a frequency

$$\omega_i^r = \omega_i - \omega_0$$

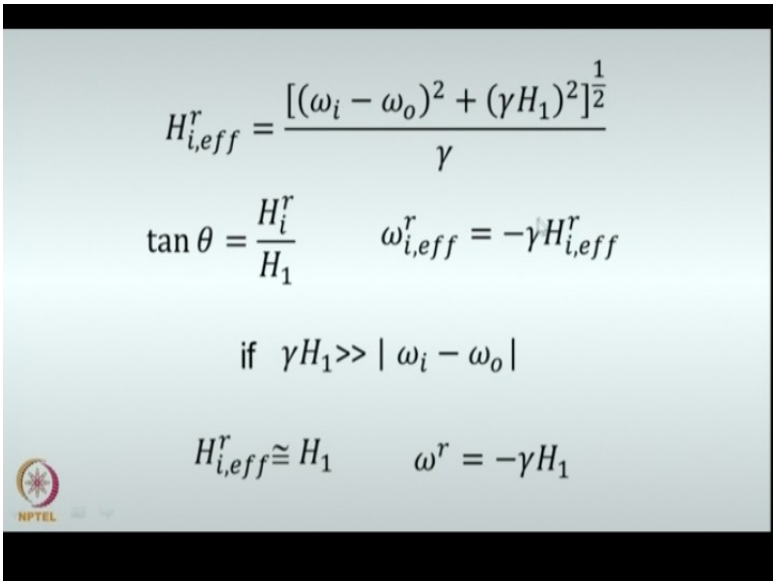
Now we consider the H_1 field as well. If I am sitting on this $\omega_0$then the H_1 field is stationary in that one. Now I have 2 frequencies, one is the $\omega_i - \omega_0$a not which is on the rotating frequency. $\omega_0$is the precisional frequency, ω_i is precisional the frequency of the spins and ω_0 is the RF frequency.

Now, if I want to convert this frequency into magnetic field, then what I do?

$$H_i^r = \frac{\omega_i - \omega_0}{\gamma}$$

If I have the H_1 as well, now we all have a new effective field which will be the vector addition of H_i^r and H_1 and that will be this H_{eff}. The magnetization now persists tilts away from this Z-axis and we will have to orient itself with respect to the H_{eff} and it will start precessing around this H_{eff}.in this manner. So what is the consequence of this?

(Refer Slide Time: 14:58)



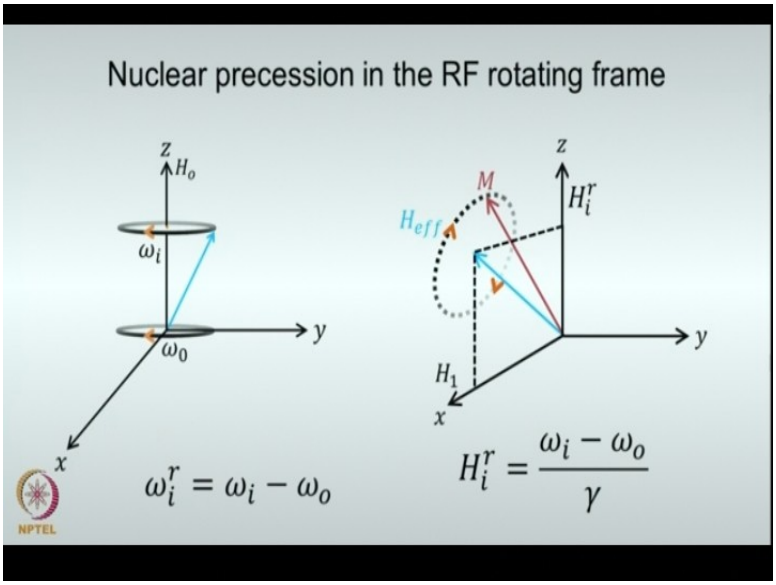
We calculate here what is an effective field in a more quantitative terms here.

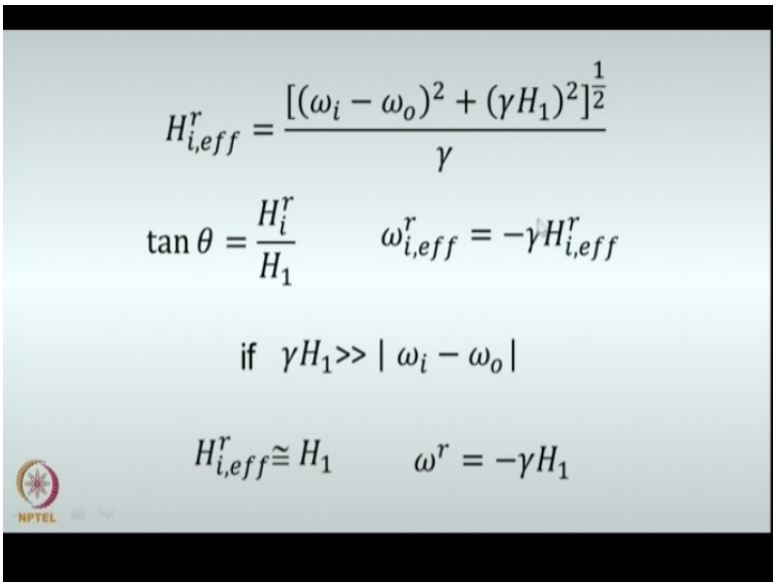
$$H^r_{i,eff} = \frac{[(\omega ¿ ¿ i - \omega_0) ¿ ¿ 2 + (\gamma H_1)^2]^{\frac{1}{2}}}{\gamma} ¿ ¿$$

This is basically a vector addition of the H^r_i and the H_1 field vector addition of this. Now this will be oriented with respect to the Z- axis and that is your base of this angle,

$$\tan\theta = \frac{H^r_i}{H_1}$$

(Refer Slide Time: 15:34)





Now the precessional frequency here. What will be precisional frequency?

$\omega_{i,eff}^{r} = -\gamma H_{i,eff}^{r}$

Now suppose I choose

$\gamma H_1 \gg \vee \omega_i - \omega_0 \vee ¿$

How do I do this? Because H_1 one is in my control. H_1 is the amplitude of the *RF* which I am applying therefore I apply with a very high power, very high amplitude here. Suppose this is much much larger than this $\omega_i - \omega_0$. That means this term is much larger than this term for the

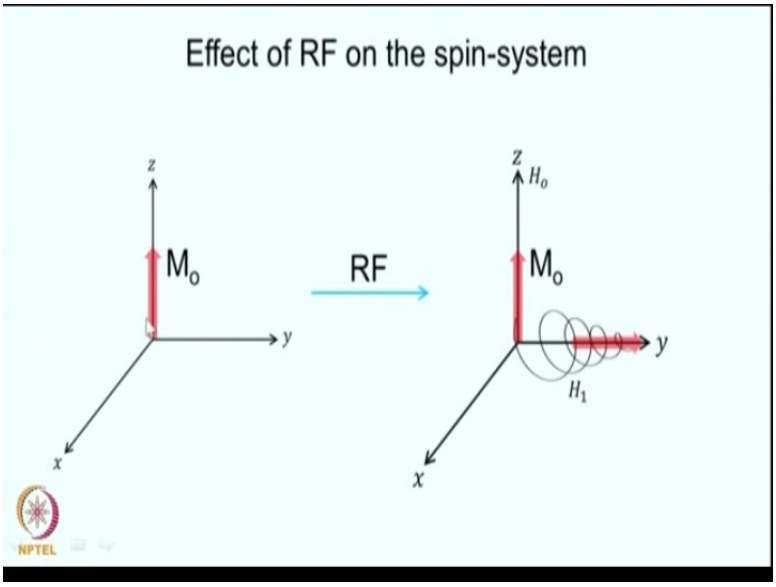
whole range of frequencies that are present in your spectrum. Then I can ignore this compared to this.

Therefore,

$H^{r}_{i,eff} \cong H_1$

$\omega^{r} = -\gamma H_1$

(Refer Slide Time: 16:56)

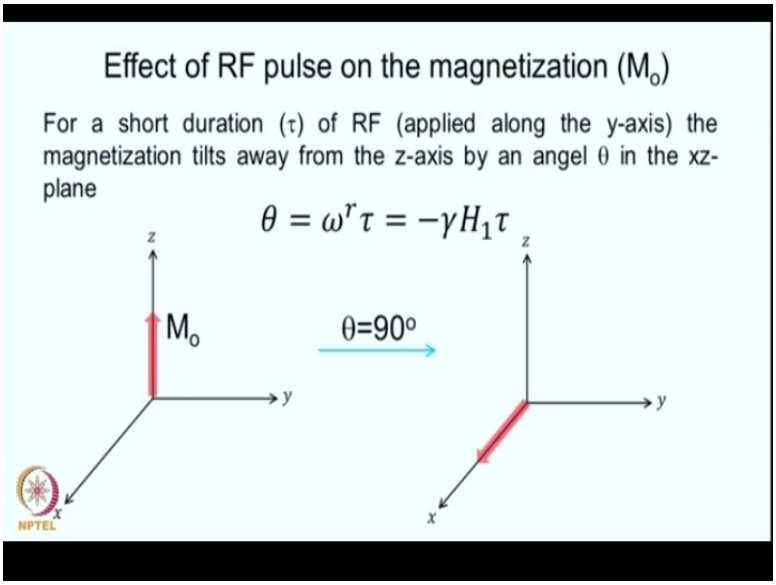


What is the consequence of this? Remember, my equilibrium magnetization was M_0 in the absence of any other perturbation for a 2 spin system or whatever. We have the M_0 which is along the Z-axis. When I apply the *RF* suppose I apply it along the Y-axis then I said the H_1 is here. The magnetization will have to orient itself with respect to this axis. Now this is the field. This is the effective field. So the magnetization will eventually have to rotate here.

How does it do it? It goes out like this, moves like this and eventually after a long time it will come back and orient itself with respect to the H_1 field, parallel to the H_1 field. This will take a long time because this depends upon the relaxation phenomena. How fast the system can move and this will eventually come down to reach this Y' axis. This is the effect of the *RF*.

Notice here that the *RF* has produced transverse magnetization. Earlier the magnetization was along the Z-axis. The magnetization will get reoriented itself with respect to the *RF* field.

(Refer Slide Time: 18:09)

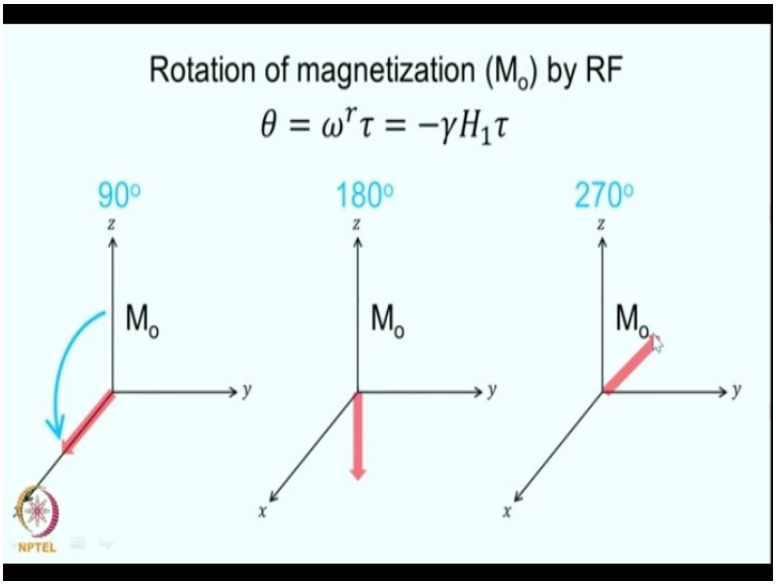


Now suppose I do not give enough time for the system to move from here to here. I stop somewhere in between. I stop before it while it is moving I suddenly stop the *RF* because I know I am applying a pulse and I suddenly stop the *RF*. Then it would stop there, the magnetization whatever it has moved it will stop there and that is indicated by this how much angle it has covered when applied for a short time tau.

$\theta = \omega^r \tau = -\gamma H_1 \tau$

If this is arranged in such a way that $\theta = 90^\circ$,then the magnetization would simply move from here to here. So this is the effect of that *RF* pulse. So let us continue looking at the effect of the *RF* on the magnetization.

(Refer Slide Time: 19:27)



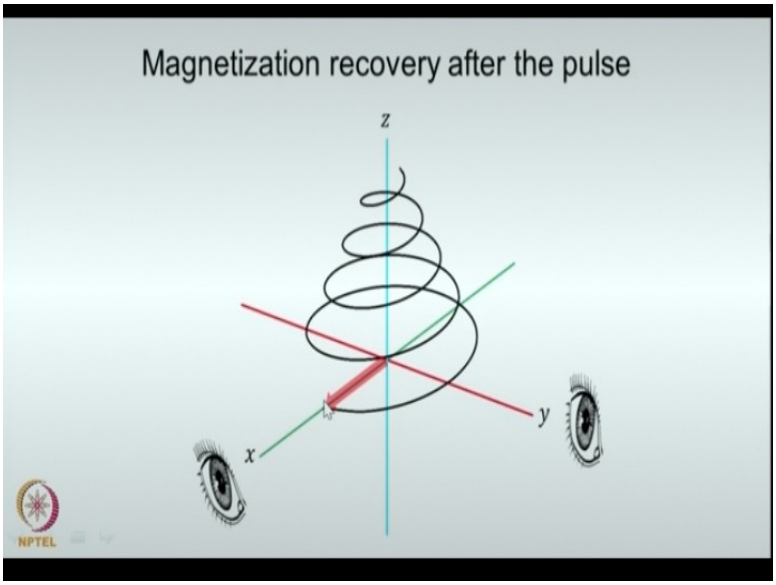
We said that the *RF* rotates the magnetization away from the Z-axis and the rotation is given by this equation here. $\theta = \omega^r \tau$, ω^r is the precessional frequency in the rotating frame and τ is the pulse rate that is for which the time for which you apply the RF and this is given by $-\gamma H_1 \tau = 90^\circ$, $\theta = 90^\circ$.

See this is a 90˚pulse. We can look at it in the particular sense of rotation. We maintain the same sort of our rotation while describing the various pulses. Now if $\theta = 180^\circ$, I produce put the magnetization along the minus Z-axis. This is also called as inversion.

If $\theta = 270^\circ$,then the magnetization goes here. This is along the minus x-axis. Remember the RF was applied along the y-axis 90˚ pulse rotate in the magnetization here, 180˚ pulse rotated here and the 270˚takes all the way like this and brings it down to this axis.

So in principle you can apply a pulse of any angle. You do not need to be only 90, 180 or 270. You can also apply 45˚pulse or a 10˚pulse or a 20˚ pulse or whatever and you simply have to adjust the time tau for this and that is the one parameter which typically one has to adjust when you record during in your NMR spectra. Now what happens after the pulse? After the pulse there is no perturbation.

(Refer Slide Time: 21:00)

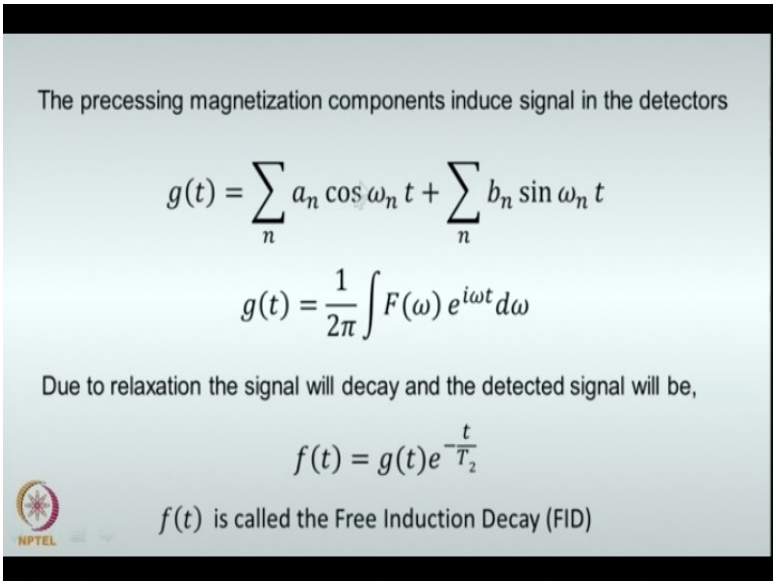


The magnet assume that you have put the magnetization along the X-axis here. You apply a 90˚ pulse and the magnetization has come along the X-axis. Now if you have to recover back to equilibrium, it has to go back to the Z-axis because now there is no *RF*. Therefore, the only field which is at present is H_0 field which is along the Z-axis. So therefore the magnetization has to go back here. This will now this is now with the transverse plane.

So it has to recover. As it recovers, it starts precessing here and precesses and starts recovering along the Z-axis. Both T_2 relaxation and the T_1 relaxation will be operative here. So the magnetization takes a spiral pathway here, it goes like this, this, this and then eventually it will come back along the Z-axis. So this is indicated here and eventually it will align itself along the Z-axis. As they are rotating magnetization here, there are components along the X and the Y-axis.

These are fluctuating magnetization components. Therefore, if we are to look at these components since they are fluctuating, they actually induce a certain kind of a voltage, if you put a receiver here, it will in a coil it will induce a voltage likewise here. So therefore rotating magnetization components will induce a signal in your detectors if we keep them along the X and the Y-axis and that constitutes to a signal.

(Refer Slide Time: 22:27)



The processing magnetization components induce a signal in the detectors and if there are many many frequency components which are present, each one of them will introduce a signal in your detectors and this will be the *y* component detector and this will be the *x* component detector. You have therefore the signal total signal which is induced in your detectors will if I want to represent as

$$g(t) = \sum_n a_n \cos \omega_n t + \sum_n b_n \sin \omega_n t$$

Now put it in the kind of a continuous form,

$$g(t) = \frac{1}{2\pi} \int F(\omega) e^{i\omega t} d\omega$$

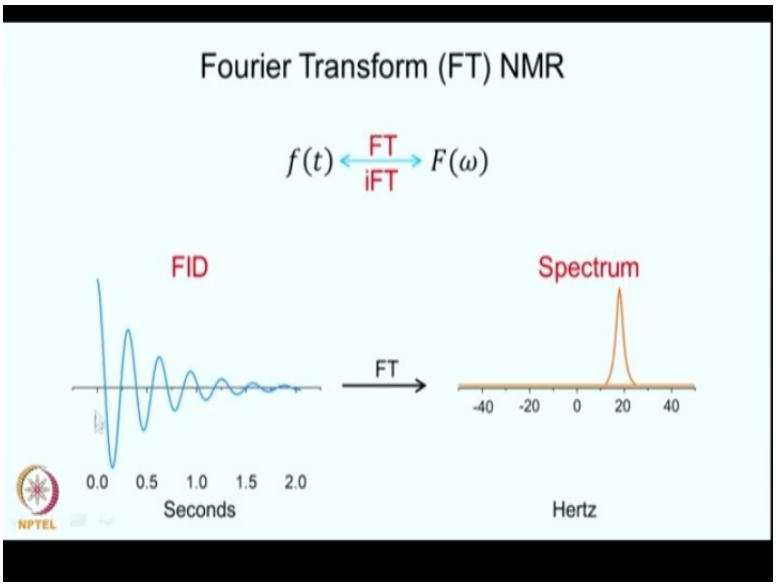
Therefore, this is the Fourier transform relationship between this time domain function and the frequency domain function. However, there is one thing we have not included here. That is the relaxation. The frequency components are processing in the *xy* plane. At the same time as I said, they are relaxing.

Therefore, the transverse components which are in the along the X or the Y-axis the decay with the relaxation time T_2.

$$f(t) = g(t) e^{-t/T_2}$$

And, f(t) is called as the free induction decay. Why it is called free induction decay? Because the result of free precession there is no perturbation. It is induction because rotating magnetizations induced voltage in your detectors and it is decay because the signal decays because of the transverse relaxation.

(Refer Slide Time: 24:10)

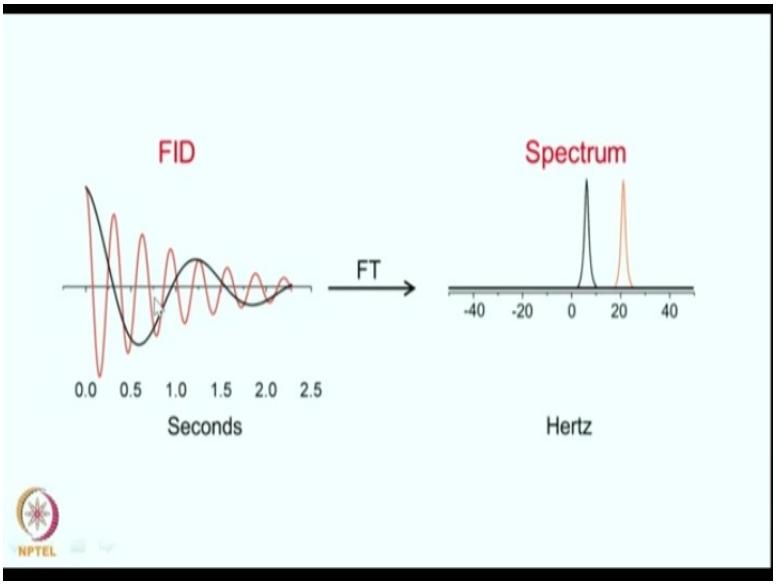


Now therefore the f of t and the f of omega are the Fourier pace. If I do a Fourier transformation along this, I got call it as FT, gives me a frequency domain spectrum. I can also do an inverse Fourier transform which is called as iFT. Often one does this as well from the frequency domain spectrum to go to the time domain signal and that is called as inverse FT(iFT).

So this is indicated in more explicit manner here. You have the FID which is a superposition of various frequencies. In this case considering only one and you have the exponential decay factor. This is e to the minus t by T_2. You remember we multiplied by this factor and that is to take care of the relaxation. So when I multiply it by this factor then I generate a FID which is like this. Otherwise, it would have been simply going like this when I multiply with e^{-t/T_2} because of the decay it goes like this.

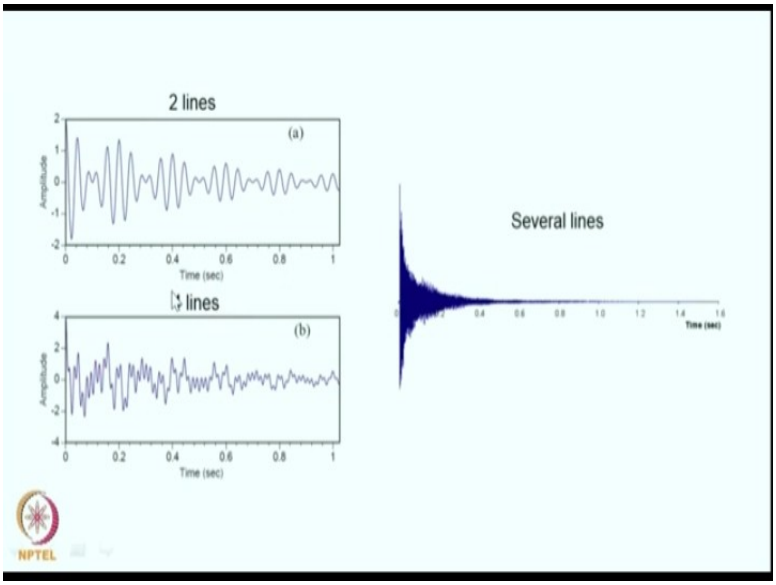
Now if I do a Fourier transform of this time domain function, I get this signal in frequency domain spectrum. This is a time axis in seconds. Now, this is the frequency axis if represent this as Hertz.

(Refer Slide Time: 25:23)



So now if I have earlier I showed you one frequency decay. Now we have another frequency, which is a black curve. I have 2 frequencies here. I have a red frequency and the black frequency both are present at the same time and therefore of course, what we will observe will be the superposition of this and if you do your Fourier transformation of this I get 2 frequencies here. This is how you unravel frequencies that are present in your FID.

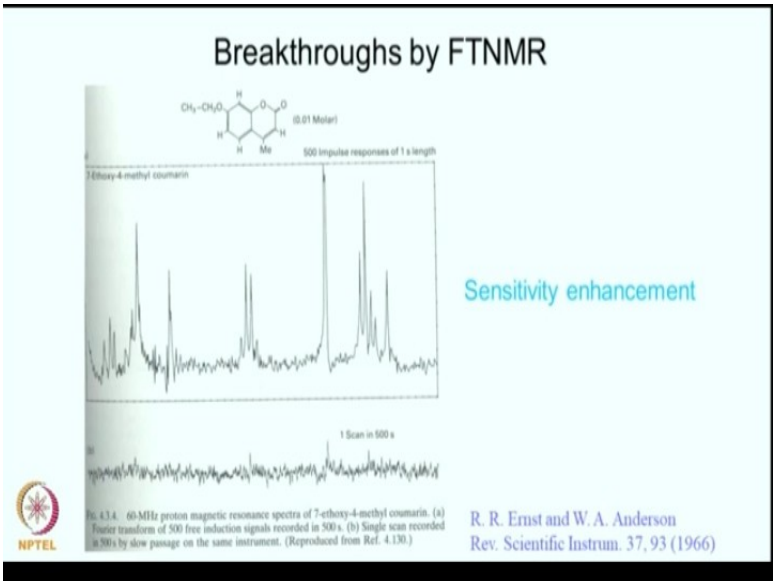
(Refer Slide Time: 25:52)

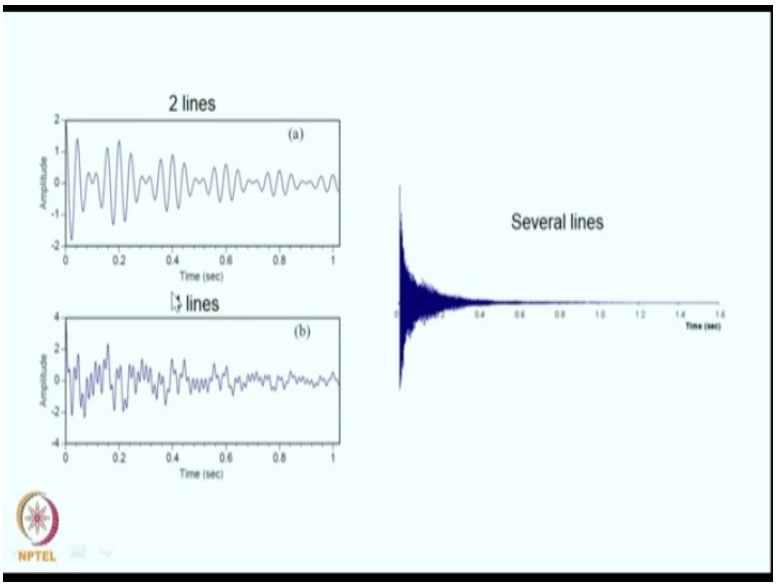


I explicitly show you here. This is how they FID even look like if you have 2 lines separated by certain number. If I have 4 lines, I will have the FID which is looking like this. Looking like this of course, you cannot figure out what the frequencies that are present here. What if a 100 lines, a1000 lines, then you have several lines which are present. Then your FID may look like this. Now if your Fourier Transform this one, then you will get your frequency domain spectrum.

Notice what we have got here. How long is this? This will be obviously determined by your $e^{-t/T_2}$is of the order of what? That is of the order of seconds. It will never go beyond a few seconds. In most cases it will be few hundreds of milliseconds and notice here this in this particular case is spectrum of some large molecule. This goes up to over by about 400 milliseconds. It has already come down to 0 nearly close to 0. So depending upon the T_2 the length of the FID is determined.

(Refer Slide Time: 26:59)





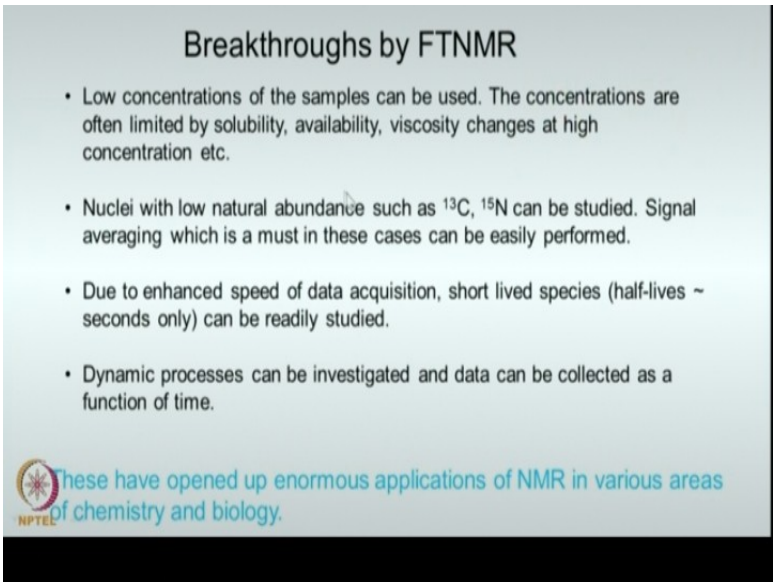
This was the major breakthrough. Why is it a breakthrough? Now you collected the entire spectrum in few 100 of milliseconds. How much time did it take for you to excite it? You applied the pulse for about a few microseconds, maybe 1 microsecond and immediately after that you collect the signal for about a few 100 milliseconds and you do Fourier transformation, which is done off the line. You can do it on your computer and then you generate a frequency domain spectrum, which is complete representation of your NMR sample and that is the sensitivity enhancement you achieve.

Here is a comparison of the Fourier transform spectrum, which the CW spectrum. Here is a CW spectrum that is a slow passage spectrum recorded in 1 scan in 500 seconds you scan

through the whole spectral region and it took 500 seconds. Now this is an experiment which is signal averaged 500 times because you took only 1 second to record 1 FID. Now you add 500 FIDs. That is equivalent to doing 500 scans. You add 500 FIDs and then you take a Fourier transform.

Then you get the spectrum which is looking like this. Look at the signal to noise enhancement. That is a major breakthrough. In fact, initially many people did not believe. This was achieved by Richard Ernst, W. Anderson and people initially did not believe this and therefore obviously did not go to high profile journal so to say and I remember Richard Ernst telling me that this was rejected 3 times. First of the so-called high-profile journals, never mind, but eventually it was published in review of scientific instruments and you can see the signal to noise enhancement per unit time.

(Refer Slide Time: 29:05)



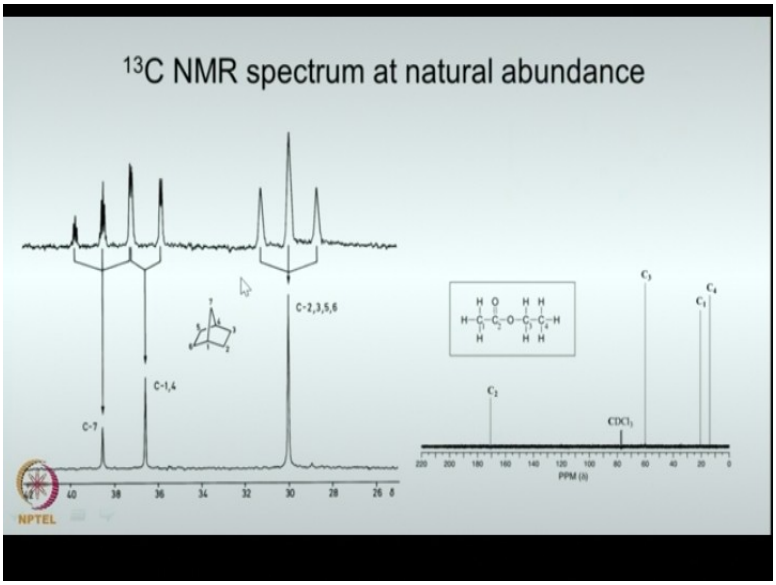
And therefore what it entails. It entails that you can use low concentration of the sample low concentration of the samples can be used. The concentrations are often limited by solubility, availability, viscosity, changes at high concentration, etc. A nuclei with low natural abundance such as carbon 13, nitrogen 15 can be studied. Signal averaging which is a must in these cases can be easily performed. You can add as many signals as you want before you Fourier transform.

Collect the FIDs as many FIDs as you want and then you do put it on formation in the end once. We will see it in more detail as you look at the theorems of Fourier transform. Now due to enhanced speed of data acquisition short-lived species, which have half-life of seconds

only can be readily studied. In earlier case you could not have studied this kind of species. If we do it would take 16 to 20 minutes for you to record 1 spectrum. Then short-lived species would have decayed or disintegrated by then and you would never get a proper spectrum of such kind of molecules.

The dynamic processes can be investigated and data can be collected as a function of time. Dynamic processes meaning there are various kinds of exchange phenomena that happen in the molecules or there rotational processes that happen in your molecules and all of these can be studied because you can actually record data as a function of time. These have opened up enormous applications of NMR in various areas of chemistry and biology. These of course will be discussed at a later stage.

(Refer Slide Time: 30:42)



Now here is an example. This is the carbon 13 NMR spectrum at natural abundance recorded by the Fourier Transform NMR. Here is the spectrum which is of this molecule and this has a coupling constants indicated here. There are carbons. There are 3 carbons, 3 types of carbons and these are split because of the couplings between the various carbons. Protons carbon proton coupling and this is the triplet and because of the carbon couplings the carbon proton couplings.

There is a process called a spin decoupling which you can do. If you, we will discuss that at the later stages, and if you do that, all the couplings will be removed and then we will get only the chemical shift sides of the 3 carbons of the carbons that are present here. All these

carbons appear at one place and these 2 carbons appear at one place. This 1 carbon appears at 1 place. How do we figure this out?

You figure this out by looking at the intensities of this signal. Now these intensities are proportional to the number of nuclear presented that particular chemical shift. These 4 carbons are equivalent and therefore it has an important information about the chemical structure of the molecule. These 2 carbons are equivalent and whereas this carbon is a single carbon and therefore this intensity is twice that intensity. This intensity is 4 times this intensity. Therefore, this is enormous information about the structure of your molecule, which you are studying.

Likewise, if you took this molecule, you have the CH_3 group here and the carbonyl here and you have the CH_2 here and the CH_3 here. So the different kinds of carbons. Now all of these carbons are now separated. They appear at different with chemical shift notice in the carbon frequency, it goes all the way from 0 to 200 PPM. The carbonyl appears at 180 PPM close to the area between 160 to 180. This chemical shift is very characteristic of carbonyl carbons and these are aliphatic carbons. Here the CH_3, CH_2 and the CH_3. All the 3 carbons are separated here and each one of them is 1.

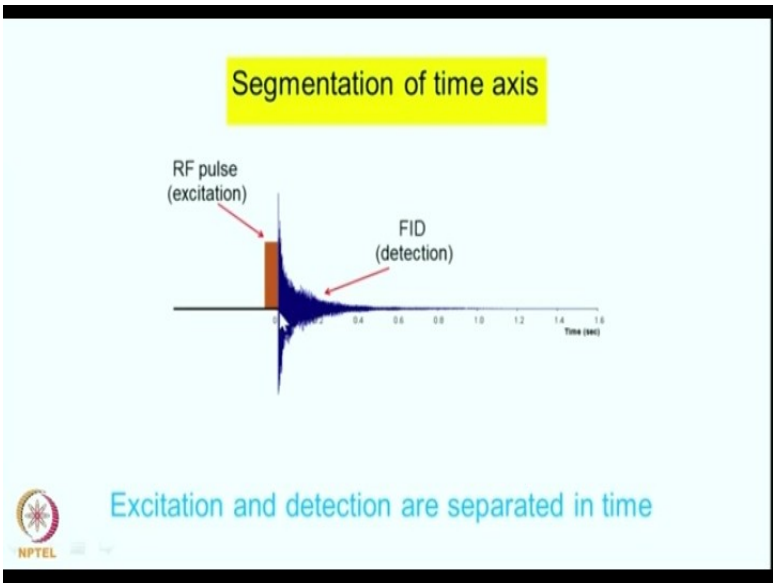
This is appearing with a shorter of intensity because of some relaxation at innovations which happen when you are not optimizing for the intensity measurements in your carbon spectrum. How much time you give between 2 scans will determine how much the intensities can be calibrated. So nonetheless this gives you the number of carbons present in your molecule and obviously these ones that can be considered to have nearly similar intensities and these one one each and that of course is indicating to you on the basis of the chemical shift, which carbon is what.

So thus even in natural abundance, you are able to acquire data which is of great value for structure characterization. This is how you enter the realm of chemistry in a big way. You could record carbon 13 spectra which will tell you how many carbons are present in your molecule. Then look at the coupling constants. Then we can say what kind of a pattern it is.

So for example, this one will tell you that this is a proton, that this is a CH_2 carbon because it has a coupling to 2 protons and therefore it appears as a triplet. This of course you would see in your have seen in your analysis of spectra. If you have a CH_2 group it produces a triplet in 1 is to 2 is to 1 ratio.

That is the great application of Fourier transform NMR. There is one other important consequence of Fourier transform NMR that is often not talked about but this is the most important in some sense.

(Refer Slide Time: 34:39)



Recall again what is Fourier transform NMR, how you perform the experiment. You apply an RF pulse here RF pulse, which is an excitation pulse, which is applied for a short period of time tau which may be of the order of 1 microsecond to microsecond or 5 microseconds or whatever and then recollecting the FID here when there is no RF when there is no perturbation. If this is representing excitation, this is representing detection along the time axis, you have separated the excitation in the detection. This is called a segmentation of time axis.

Excitation and detection of separated in time, and this has important implications for variety of further developments in multi-dimensional NMR spectroscopy or various kinds of pulse techniques, which have been developed subsequently. These ones we will describe in greater detail with that I think we will stop here. Thank you.