NMR spectroscopy for Structural Biology NS

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Lecture: 08
Pulse Excitation and FT-NMR

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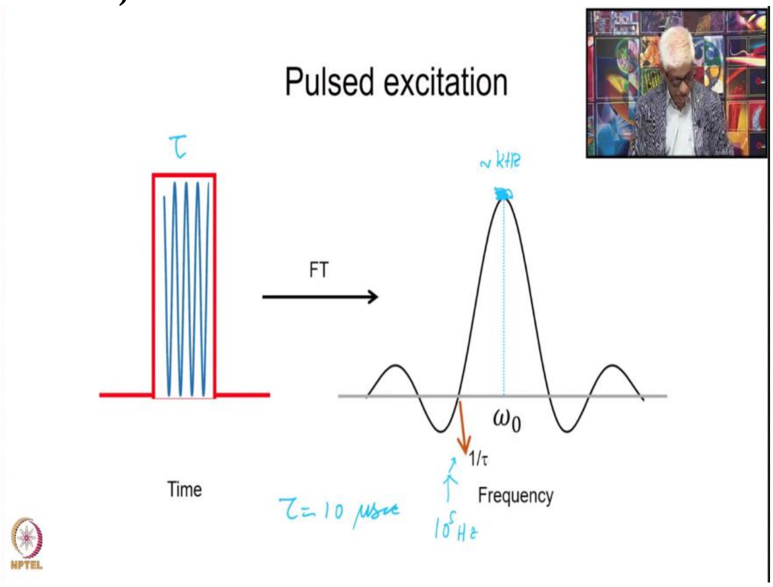

Pulsed excitation

So, we talked about the pulse excitation last time this slide which is there it was already shown to you last time. So, RF applied for a short time τ is called as an RF pulse. So, when we apply the RF pulse then it will result in an excitation of a larger number of frequencies as indicated in this side here and this is because there is a Fourier relationship between the time domain function here and the frequency domain distribution of the excitations there.

So, you have if you have the RF is at a frequency ω_0 it produces various frequencies with different amplitudes like this and this is a very huge range. So, if you are a pulse is of the 10^5 microseconds for example then you generate about at this point it is 10^5 hertz this point is again 10^5 hertz. So, up to the left and right of the ω_0.

And it has of course different amplitudes and we select only a small region here on the top which is the region which has which is more or less flat. So, that with the excitation efficiency is the same for all those frequencies this can be easily of the order of few kilohertz. And that is what we need and that is sufficient also for the range of spectrum for the frequencies we have in our NMR spectra. So, now we will see what is the effect of this pulse on the magnetization?

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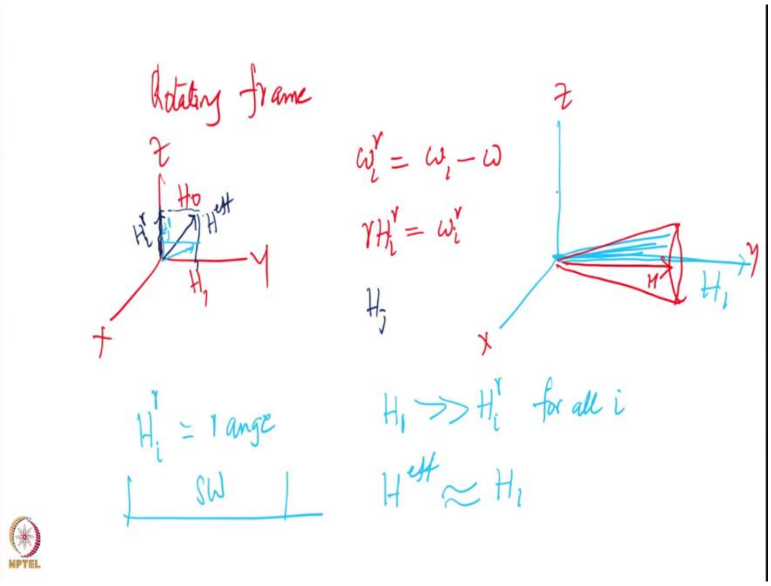

So, to do that let us go back into the rotating frame go to the rotating frame H_0 field applied like this and then we have the H_1 which is applied along the transverse plane along the X axis for example or the Y axis. So, here you have the x y and z if you say like that consider a frequency ω_i. So, in the rotating frame the frequency is $\omega_i^r = \omega_i - \omega$ which is the RF frequency omega is RF frequency and ω is this frequency of the particular nucleus and this when you convert it into the into the field.

So, then this will be $\gamma H_i^r = \omega_i^r$. So, what we will do is we will represent this field by this arrow here if this is H_i^r and I have the H_1 here. So, what will be the effective field. So, the spin sees is an effective field and the effective field is given by let us say the H 1 is here. So, the effective field will be along this axis here this is H effective ($H_{effective}$). So, when this happens what will happen to the magnetization.

Now similarly the effective field will be different for different frequencies because omega i is going to be different suppose I consider another nucleus let us say H_j. So, H_j let us say this frequency is here I let me use a different colour for that. So, suppose I have H_j here H_j^r then the effective field for that will be here right. So, therefore different nuclei will have different effective field.

So, now what we will do is if I have H_1 very large suppose I have a range of H_i^r certain range is a certain range because of the spectrometer frequency or the spectral range what I have here I have the spectral range here this much. So, correspondingly there will be H_i^r also will go to a certain range H_1 much larger than H_i^r for all i what will be the effective field. So, effective field will be approximately equal to H_1 one only because it will keep going horizontally down.

So, as i increase the field. So, it will keep going like this right $H_{effective}$ will keep coming down like that as I increase the magnetic field it will count more and more towards the H_1 axis therefore and your H_1 if it is here. So, this will be H_1 it effective field for all the nuclei will be H_1 that is along the xy plane. So, what is the consequence of this the consequences the individual magnetizations will tend to align themselves with respect to the H_1.

So, because they are now because that is only a magnetic field they see therefore the spins will have to be quantized along H_1 they will start precessing around that if I draw the individual things they have to come like this they have to orient like this the magnetization being the red is the magnetization if this is the magnetization it will have to orient itself with respect to this but how it will come like this.

So, it will come because this is that so far as this H_1 is concerned this is the transverse plane this is xyz that this is the transverse plane. So, therefore there will be precision in the exact plane and eventually they will come to that. So, let me show that in the next slide.

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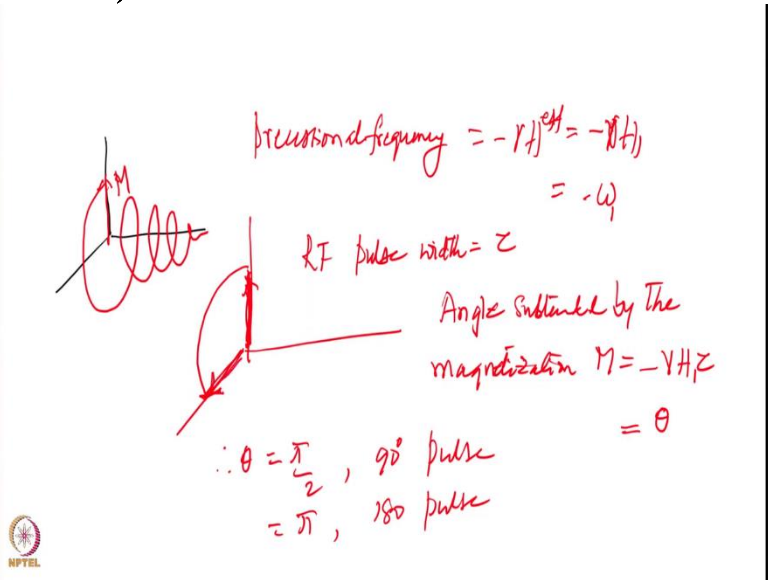

So, the magnetization which was here initially this was my m. So, the magnetization will go like this and eventually it will come here. So, this is the way it will move right. So, in the long run when you give enough time for the field the magnetization to adjust itself it will come to the axis of the H_1 in this manner. Now but we are not applying the RF for a very long time we are applying for RF for a very small time.

So, when this is precessing like this; what is the precision frequency precision frequency is equal to minus gamma H effective this is equal to minus gamma H 1 because H 1 is my effective field. So, therefore let me call this as minus omega gamma H 1 is equal to omega 1 let me call it and this is omega is the precessional frequency. So, now since I apply this RF only for a short period tau what will happen it will cover only a small range.

So, therefore the magnetization will rotate let us say from here and we stop suppose when it comes here we stop here RF is taken out. So, this time if I say RF is applied pulse is equal to pulse width pulse width is equal to τ. So, the angle subtended by the magnetization angle is subtended by the magnetization by $M = -\gamma H_1 \tau$. Let me call $M = -\gamma H_1 \tau = \theta$. So, therefore it covers because we have the frequency $-\gamma H_1$ is the frequency, frequency time τ is the angle covered right.

So, of course in radians so, you can multiply by 2π to get it in degrees. So, therefore this is you can this therefore we can move the magnetization by 90° or 180° and. So, so forth therefore if $\theta = \frac{\pi}{2}$ then we have a 90° pulse is equal to π then you have 180° pulse and so on. So, this is the language we use for describing the rotation of the magnetization.

Now the magnetization has come on to the transverse plane as a result of this the magnetization which was here initially has now moved over to this place right. So, this was the initial magnetization and this is M and now it has moved over to this place what will happen after this.

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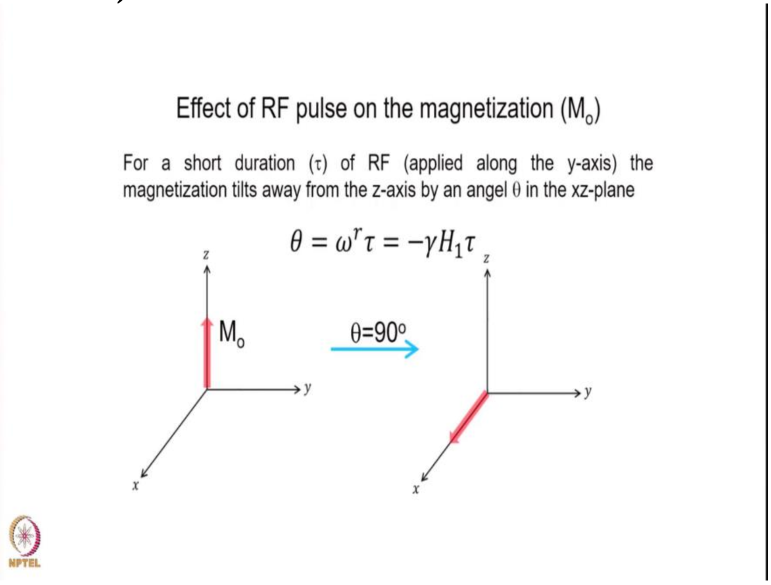

Effect of RF pulse on the magnetization (M_o)

For a short duration (τ) of RF (applied along the y-axis) the magnetization tilts away from the z-axis by an angel θ in the xz-plane

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

So, therefore this is what is described here if I apply the RF on the magnetization for a short time tau then the magnetization covers an angle of theta and that is called as the flip angle. So, this theta is called as the flip angle and if you have θ = 90° then you have a 90° pulse. So, now the magnetization is in the transverse plane. So, in this case of course the pulse is applied along the Y axis and therefore the magnetization is rotated in the along the X axis.

So, now what happens after this. Now the pulse is removed. So, therefore there is no more rotating frame the magnetization will have to go back to the equilibrium and this is what will happen.

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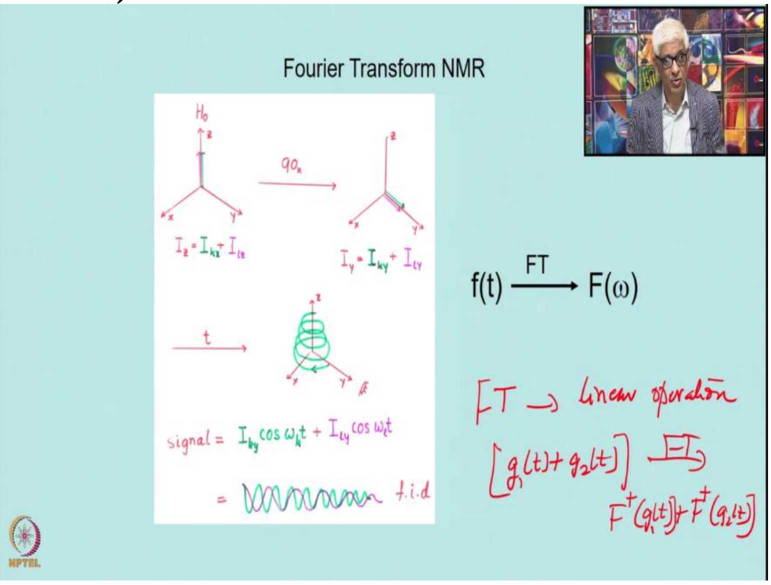

Fourier Transform NMR

$$f(t) \xrightarrow{FT} F(\omega)$$

FT → linear operation

$$[g_1(t) + g_2(t)] \xrightarrow{FT} F^+(g_1(t)) + F^+(g_2(t))$$

signal = $I_{ky} \cos \omega_k t + I_{ly} \cos \omega_l t$

= f.i.d

Sometimes I put the magnetization on the x and sometimes on the y but does not matter they are all in the transverse plane. So, in this case then we applied a 90x pulse the magnetization has come in the y and the Y axis here in the previous slide it was here but it does not matter if I apply a pulse along x it will come on to the y. So, this is the sum of the components from k spin and the l spin because you remember this m magnetization is the superposition of all the frequencies all the spins components contributions.

Therefore I taken here only 2 of them to indicate the general phenomenon. So, here you have the 2 ones which are along the Y axis. Now during the next time tau what will happen after the pulse is removed as a function of time this magnetization will try to recover back to the Z axis. So, it will start precessing in the transverse plane like this and then it will go on to the Z axis here. So, while it is precessing here.

So, suppose I make the detection here because the rotating magnetization will induce a signal in my detector that is a voltage it will induce a voltage and that is my signal. So, the various components which are present here both will produce their own inductive voltage in this in this detector therefore they will produce a signal and this will be time dependent. And that is what is written here the signal is equal to the sum of the contributions from the k spin cosine omega k t and from the l spin this is cosine omega l t both these frequencies are present here and what we will see is the superposition of the 2 frequencies here.

Now you see one more thing that happens that is the there is the signal decays as a function of time the signal is decaying as a function of time why does this happen this is because of the spin-spin relaxation or the transverse relaxation. We have discussed about transverse relaxation when we discuss the block equations when the magnetization in the transverse plane it will decay with a characteristic time which is called as T_2.

Therefore this signal as it is recovering back to equilibrium it will also decay because of the transverse relaxation time T_2 and therefore this superposition of the signal which will be a total time dependent function which is a decaying function. Therefore this is called as free induction decay FID. Free induction decay is a superposition of the individual frequencies decaying with their characteristic time constants or the transverse relaxation times.

So, this is the signal you will get. Now obviously if it is a superposition what it means if a Fourier transform this signal I will get the individual frequencies f of omega because this superposition means it is a Fourier transform the spectrum. Therefore I do a Fourier transform of the time dependent signal I will get the frequency domain spectrum. Now you see initially I excited a larger number of frequencies but the detection process picks up only those ones which correspond to the lines in your NMR spectrum. Therefore this is called as Fourier Transform NMR.

Now you see what we have achieved in this in this case the FID lasts for how long? The FID lasts for the depending on the transverse relaxation time if the transverse relaxation time is of the order of 100 milliseconds or 200 milliseconds or even say maximum one second this FID will last only that long. So, it would 2 times T_2 or 3 times T_2 then it will after that it will only be zero there will be no signal therefore you collect the signal for that much of time which is where the signal is present.

And how long it took for us to excite the spin system? 10 microseconds, 10 microseconds excitation and we collect the signal for one second maximum one second. So, therefore the Fourier transformation is a mathematical operation which can be done outside the spectrometer. And so therefore your data collection is reduced to one second or approximately 1.1 second compared to what it was of 16, 17 minutes in the case of a CW spectrometer.

This continuous wave where you are sweeping the field through the spectrum collecting the signals one by one compared to that you have got the full spectrum in about a second. And therefore obviously there is a quite substantial gain in your spectrometer time and what does that mean that means that you can actually collect these scans several times and Fourier transform once more. And that is an important factor here that the Fourier transform is an operation which is linear.

In the sense that a Fourier transform is a linear operation that means if I collect 2 FIDs g (t) then another one $g_1(t)$and $g_2(t)$ both are the same that means I collect the FIDs again and again I collect the FID then again apply a pulse collect the FID apply the pulse collect the FID all the 3 times I can do I keep on doing as many times as I want then if I take the Fourier transform of this then it is the Fourier transform of $F^+ (g_1 (t)) + F^+ (g_2 (t))$ F^+ is the Fourier transform operator.

So, therefore what I can do is I can keep collecting the signals FIDs and Fourier transformation once. I have to keep collecting the fids several times and only at the end of collecting let us say I collect hundred times. So, after collecting hundred times I do a Fourier transformation that is equivalent to doing individual Fourier transformations and adding them. So, this is the quite a substantial saving in time because the Fourier transformation itself may take some time.

So, therefore if you have to do it only once instead of 100 times so getting the same result then it is quite saving in time.

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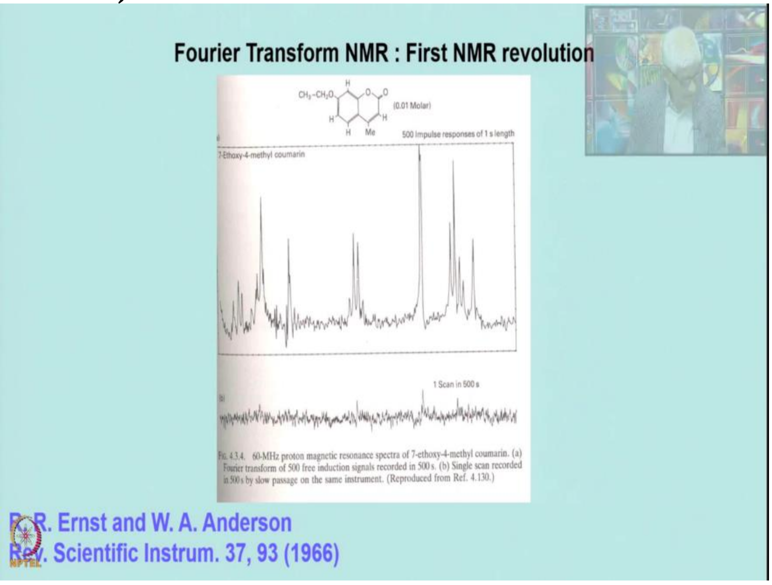

Fourier Transform NMR : First NMR revolution

Fig. 4.3.4. 60-MHz proton magnetic resonance spectra of 7-ethoxy-4-methyl coumarin. (a) Fourier transform of 500 free induction signals recorded in 500 s. (b) Single scan recorded in 500 s by slow passage on the same instrument. (Reproduced from Ref. 4.130.)

R. R. Ernst and W. A. Anderson
Rev. Scientific Instrum. 37, 93 (1966)

Therefore the result of this is what you can see in the next slide here. So, this is a comparison of a spectrum which is a CW spectrum here recorded in 500 seconds and it was only one scan because you have to do it very slowly this scheme has to be done very sweeping has to be done very slowly. As we discussed before and the same molecule spectrum recorded in the same time using the Fourier transform approach you get this kind of a signal to noise ratio.

You see how much of enhancement is there in the signal to noise. So, 500 impulse responses that is the 500 fids of one second length and you do a Fourier transformation at the end. So, therefore you gain substantially with regard to the signal to noise ratio because the signal to noise ratio as I said is proportional to the square root of the number of scans the number of scans you add.

So, therefore you can collect 500 scans and then you take the square root of those and that gives you the signal to noise ratio. So, this is the significant gain with regard to the FT NMR. So, what are the benefits here the benefits are. So, let me write here the benefits (1) gain in signal to noise as a result of (2) the gain in signal to noise you can collect signals of rare nuclei such as ^{13}C, ^{15}N etc can be collected easily.

(3)low concentrations can be used low concentrations can be used and then say the (4) kinetic phenomena can be study kinetic phenomena because since you are able to collect the data very rapidly you can do it as a function of time. Kinetic phenomena can be studied and then let me write the (5) here unstable molecules can be studied in other words some species are very short-lived and they decay after about a second or 2 seconds or a minute or ten minutes or something like that.

So, your CW is impossible to study such kind of things here you can do it very rapidly. So, even if it is short-lived for about a minute or. So, you can still collect the data with one with one scan with sufficient concentration if it is. So, these are the specific advantages of the Fourier transform NMR spectrometer.

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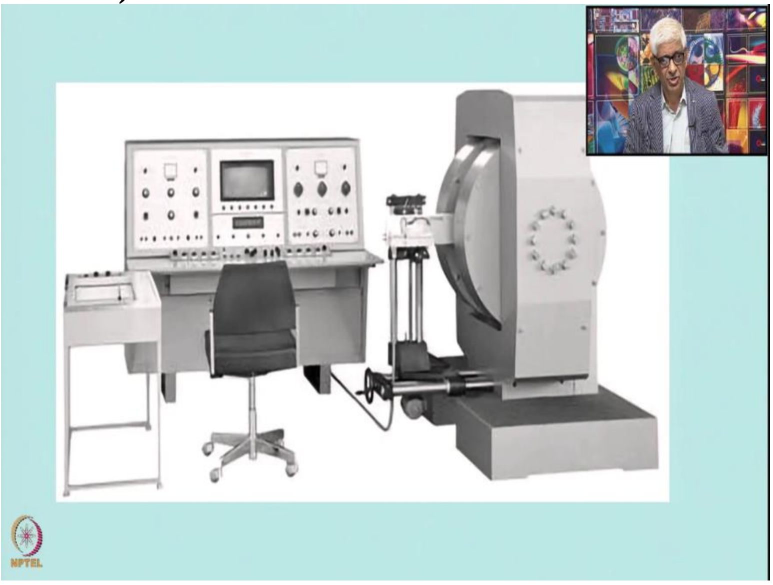

So, this was one of the conventional electromagnet. Now here with the electromagnet you have the FT NMR spectrometer but with the electromagnet there is a certain limit as to how much you can go with respect to the field. So, that is because the large amount of current has to be passed and that will result in substantial heating of the system and huge amount of space will be required you have to cool the magnet all of that thing will be required.

And therefore there is a limit on how much you can go with respect to the magnetic field why do you want to increase the magnetic field. Because if you increase the magnetic field you increase the sensitivity you increase the dispersion of the signals therefore you would like to go as high as possible with respect to the magnetic field.

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How to increase the strength of the magnetic field?

Superconducting magnets

So, what do we do how do we increase the strength of the magnetic field. So, this is where actually the superconducting magnets came into existence and these are extremely important later development this came in the 1970s later 70s and subsequently the thing has been growing. So, what are the benefits of superconducting magnets? Superconducting magnets are very stable and in a not energy dissipating. So, the benefits are superconducting magnets.

So, let me write here very stable then you have very large fields can be achieved. So, all these are applying to this superconducting magnets and very high homogeneity can be achieved. So, how what are the superconducting magnets?

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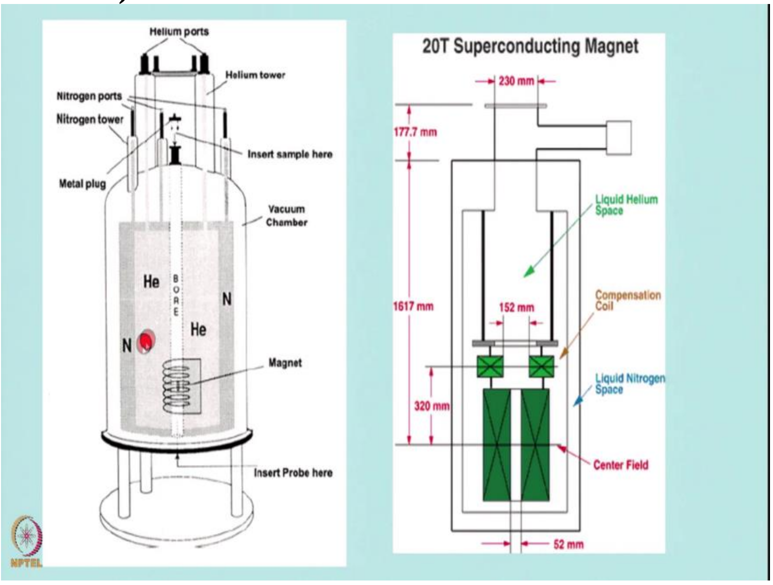

The superconducting magnet has the following components here. So, this is the magnet this is the main magnet here. So, this is the superconducting coil and this is inserted in a tank which is liquid helium which contains liquid helium liquid helium is at 4.2 degree Kelvin. So, superconductivity is a phenomenon which is observed at very low temperature like 20, 18 degree Kelvin 20 degree Kelvin and things like that.

And what is superconductivity if the current is going in the coil in a superconducting coil there is no resistance for it there is zero resistance the superconducting coil is zero resistance therefore the current does not decay at all. So, current does not decay means it keeps going once you induce current in a coil like this it will keep going without any decay. Therefore it can last for a long time only thing is you should keep it in liquid helium, liquid helium may have to keep refilling.

But the liquid Helium can evaporate very rapidly therefore you will have to produce a nice insulation for it. So, this is the liquid helium tank and then outside the liquid helium tank you have a liquid nitrogen tank and the liquid nitrogen tank provides additional insulation. So, that the dissipation of heat is not possible and therefore and then after the liquid nitrogen tank there is a vacuum here.

The vacuum chamber see the huge vacuum like 1 in 10^9 that sort of a vacuum you have therefore no heat can come from outside to inside into this liquid helium tank. Therefore liquid helium can sustain for a very long time today you have magnets which can hold liquid helium for almost one year. So, therefore this is extremely stable. So, if that is the case then the perturbations also will not reach there the current cannot be changed unless of course you bring a magnetic material outside.

And then of course it can influence it therefore you should not bring magnetic materials near the super counting magnets. Now there is a hole in the middle if you see there is interesting design here your sample which sits in the middle here that is a white bore white bore that remains at room temperature. So, therefore you look at the kind of insulation they should have this bore is at room temperature your sample sits at room temperature and it is covered by this liquid helium by this coil which is at liquid helium temperature is ok.

And then of course you have all other kinds of accessories which are required to maintain all of these things the coils and evaporators and measurements and things like that. So, your sample remains these are the dimensions because we do not need to go into that details.

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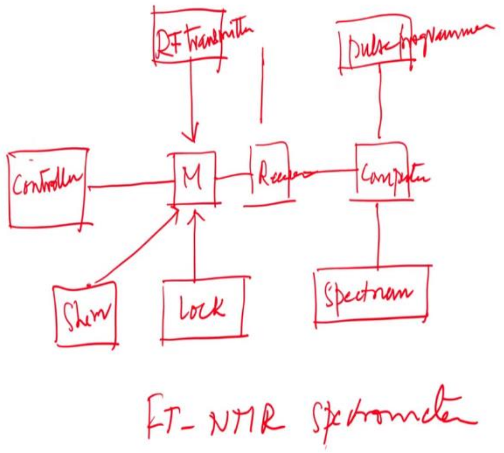

We have talked about the advantages of the Fourier transform and the FT NMR spectrometer how does it look. So, let us draw a few of those aspects here FT NMR spectrometer consists of this is the magnet. Then you have the receiver here then of course this is connected to a computer why do you need the computer because now we require extremely high time controlling and then this is connected to a pulse programmer because you have to apply 10 micro second pulse or 2 micro second pulse and so on and so forth.

Then you have a RF transmitter here RF transmitter this is the one which actually supplies the RF and then we have here a controller this is RF controller and then of course in addition you will have to have certain other things which are called as the field frequency lock and then you have the so called shim system. What is the field frequency lock? The lock is it controls the magnetic field.

And the shim system the stream system adjust for a variations in the magnetic field if there is any perturbation from outside then you have a variation in the magnetic field. So, therefore the shims are certain kinds of coils which correct for the variations in the magnetic field and therefore they help to bring in very high homogeneity. And then of course from this computer you have the spectrum. So, this is the typically the configuration of the FT NMR spectrometer.

This is a very rough picture because there are more greater details which actually one buys it or works with the spectrometer and of course you will discover all of those things. So, these are the essential elements. Now having obtained this FT NMR results in some very special features in other words compared to the CW NMR there are very special aspects of Fourier transform NMR spectrum and this is because of the way we are detecting the signal.

The way we are exciting this spin system and the way we are detecting the signal accordingly we have different kinds of features in the NMR spectra and these are very important because of the new thing these are exclusive to the FT NMR acquisition and this we will discuss in the next class. So, we can stop here and we can go with the details of the practical aspects of Fourier transform NMR in the next class.