One and Two Dimensional NMR Spectroscopy for Chemists

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Lecture – 57
NMR Data Acquisition

Welcome back. In the last few classes we discussed two-dimensional NMR extensively. Especially we also attempted to interpret COSY, TOCSY, coupled and decoupled HSQC, HMBC spectra, and also we looked at homonuclear J-resolved spectra. Of course, there is no difference between homonuclear and heteronuclear J-resolved spectra, except that the pulse sequence is slightly different.

The information content what you derive is exactly same, because you are going to get the chemical shift information in the direct detection dimension and the coupling information in the indirect dimension. Apart from that there is no difference between homonuclear J-resolved and heteronuclear J-resolved spectra.

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Today, let us discuss something about some of the practical things like whatever you are required to know for practical consideration, as far as the NMR data acquisition and data processing is concerned. That is what we will discuss today.

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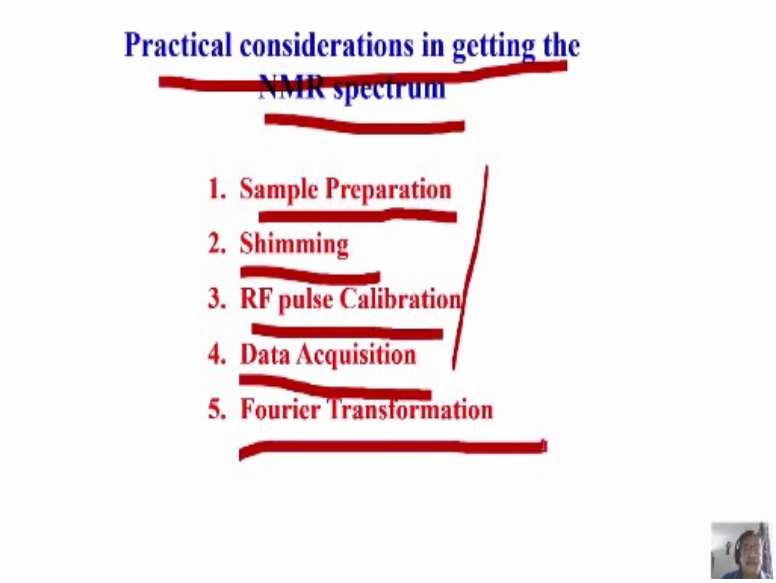

As far as the practical considerations in getting the NMR spectra are concerned; first we should know about sample preparation, shimming of the magnetic field to get very good homogeneity, calibration of pulses for appropriate degrees for example I need 90 degree pulse, 180 degree pulse or any angle and also the pulse phases. And I should know about data acquisition like choosing the appropriate spectral width, and deciding the number of time domain points, size of the memory for acquisition of the data.

These small considerations which are essential we need to know that. and of course we have to do Fourier transformation of the data, so we should know how to go ahead in doing these things. These are all essential things; essential practical important points, we should know in acquiring the spectrum. I am talking about one dimensional spectrum today; we are not discussing about two-dimensional spectra yet. These are all the considerations for acquisition of one-dimensional spectra.

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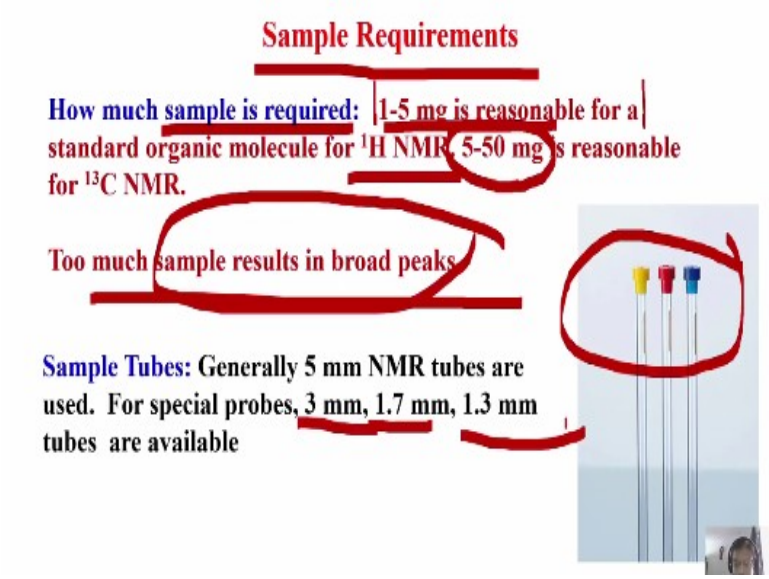

First thing what are the requirements for the samples? We need to know how to prepare the sample, what is the quantity of the sample we require etcetera. First, as far as the sample quantity requirement is concerned we require approximately 1 to 5 milligrams is reasonable for a small organic molecule, especially for proton NMR. If you want to look for carbon 13 NMR sample quantities of little bit higher is required, because you know the sensitivity of detection of carbon is much less compared to that of proton. So that is what the requirement. Of course you may say I have synthesized, , lot of compound is there, quantity is very high. I can take more samples, so that I can do the experiment faster; no that is not allowed. Too much of samples sometimes results in broad peaks. You have to take appropriate quantity. Optimum quantity of the sample is required. You cannot take too less also because it takes more time for you to get the signal. At the same time just because you have lot of sample you cannot take too much of sample also, that gives broad signal. So, you have to see the optimum quantity, what I have mentioned here. It is only a guideline, of course, you can decide the way you want.

And you have to choose the proper NMR sample tubes. Normally what is used is 5 millimeter NMR tube. Remember, these are all the things which are shown here. These are 5 mm NMR tubes. If you have special probes you can use 3 millimeter, 1.7 millimeter, and also 1.3 mm tubes; they are available for special probes.

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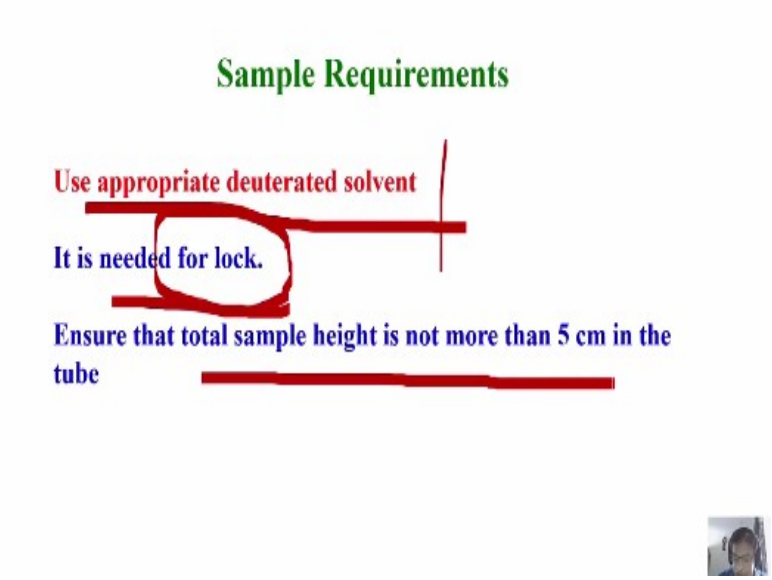

Now with the sample requirement if you continue, you need to choose appropriate deuterated solvent. What is appropriate deuterated solvent? If your sample is soluble in chloroform, you cannot take CHCl3 as a solvent. You are always required to take deuterated solvents in NMR; like you need CDCl3, dimethyl sulfoxide, CD3CN. If it is a water soluble sample, it is D2O. These are the things you need. Why we required deuterated solvents? It is because I have to use lock.

What is lock? The lock is something which I am going to tell you in the next couple of slides. It is to ensure that field is not drifting. You know what happens if the field starts drifting? Inhomogeneity will come in, because every time you send one pulse the samples or nuclear spins see different magnetic field. Now if you want the data you acquire the signal again and again for their coaddition. By then if the field has drifted, it has changed a bit; your resonating frequencies are different. So, you will get broad peaks or distorted spectra. You will not get high resolution spectra, lines are terribly broad and distorted. So you need to have a lock. For that you require deuterated solvent, because the lock frequency in all the spectrometers is deuterium frequency. For example, if you have a 300 mega spectrometer. The deuterium resonates at 46 megahertz approximately; then your lock frequency will be at that frequency of nearly 46 megahertz, you can see the precise value. I am approximately telling you, what I remember. That is very important and you have to take this sample in the NMR tube which I have showed you, such that the total sample volume or the height of the sample should not exceed 4 or 5 centimeter.

Just because you have sample you cannot fill the entire NMR tube, that is also not good, too less quantity is also not good. You are to have optimum quantity we should cover the RF coil area taking highest amount of sample or less amount of sample both are disastrous. It gives you distorted peak shapes. So this is very important the sample height is very important remember that.

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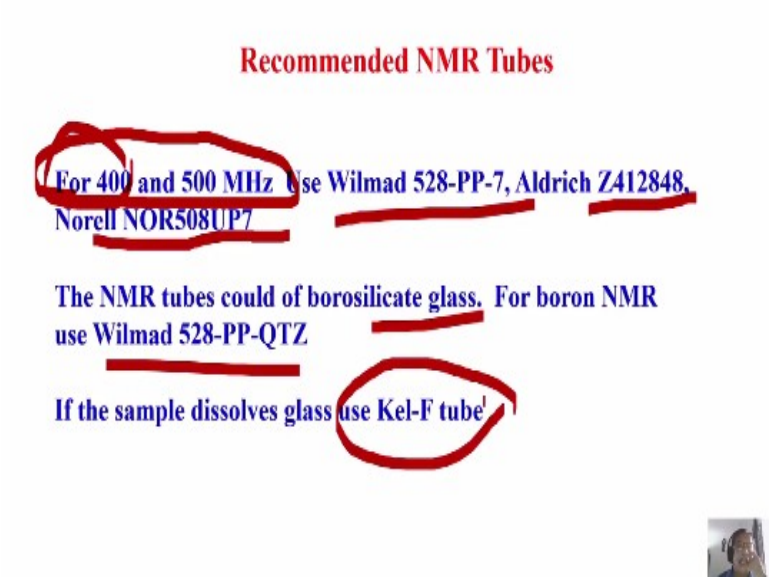

And of course, NMR tube also has to be properly chosen. We cannot take any NMR tube of our choice. This has been already worked out. Many pioneers in this area have reported what is the optimum choice of the NMR tubes for the particular frequency of the spectrometer. For example, if you have a 400 and 500 Megahertz spectrometers, and if you are recording the spectrum in either of them you have to use Wilmad NMR tubes whose numbers are 528-PP-7 and I will read this number and Norell NOR508UP7. These are the tubes which you have to use because they are time tested. And the NMR tube could be of borosilicate glass, you also know that, it is a borosilicate glass. Suppose, you want to see boron NMR then you cannot use this tube. You have to use different types of tube that is called quartz tube. And because sample dissolves glass, sometime you have to use Kel-F tube. See these are some of practical difficulties you will know that later, when you know when you start using the NMR, start using the spectrometer and start recording the spectra, you will have to understand these things. So the proper choice of NMR tube is essential. You have to use only the identified or those which are prescribed NMR tubes for a particular frequency of the spectrometer. For a particular Larmor frequency you need to use particular NMR tubes which are prescribed.

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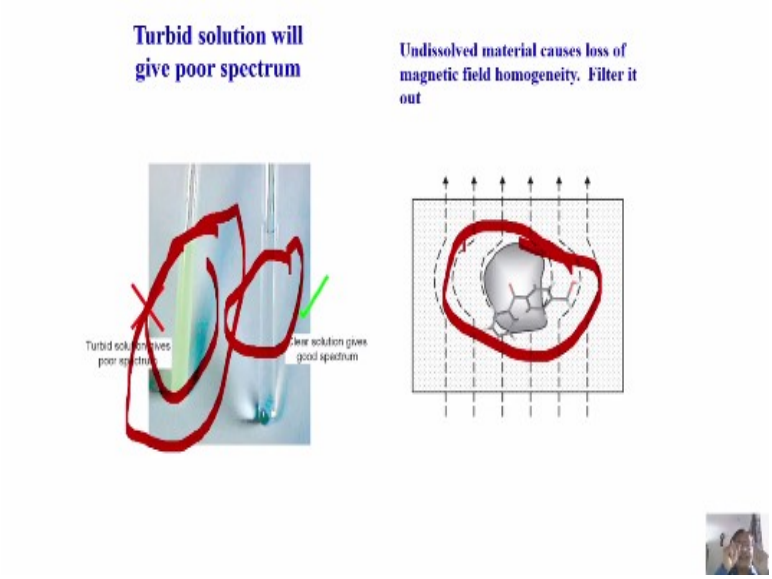

When you are making the sample, I am sorry when you are making the solution by dissolving the sample in the solvent, please ensure there is no turbidity. A turbidity like this will give you bad line shapes, homogeneity will be destroyed. The B0 field homogeneity will not be very good. This nuclear, spins will not see homogenous magnetic field. So your line shape will be distorted.

You must have a clear solution like this, very important. You must have a clear solution. And if there are suspended particles which are un-dissolved, small microscopic particles which are suspended, please filter them out. Otherwise that will give rise to magnetic field inhomogeneity. It will destroy the field homogeneity at the site of the nuclear spins. Please remember these are the important points you have to take into a consideration.

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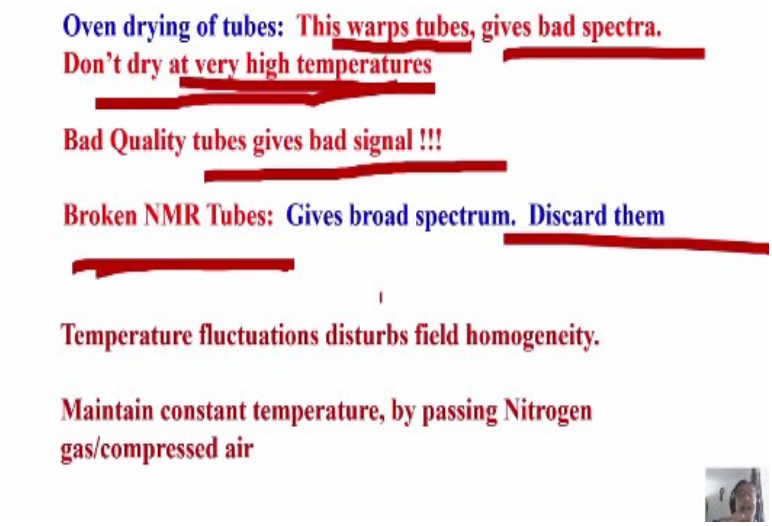

After doing the experiment, you clean the tube. Do not do this at a very high temperature, do not use microwave oven or oven and then dry the sample at very high temperature. Sometimes these will warps the tubes. When the tubes are warped it give bad spectra. So, bad quality NMR tubes give bad quality signal. So you do not broken NMR tubes, sometimes we are used to that. Some tip of the NMR tube, let us say, is broken. We say okay, no problem, use the same tube. Sometimes it gives very broad spectrum. If there are broken NMR tubes please discard them. And temperature fluctuations will be there near the sample in the probe. You have to maintain uniform temperature. If there are temperature fluctuations that will disturb field homogeneity. So please maintain uniform temperature. For which we have to pass nitrogen gas or compressed air always continuously, and maintain uniform temperature. That is essential. The temperature fluctuations or gradients in the temperature will create inhomogeneity and your spectra get distorted.

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For doing the experiments, these things will be supplied for you by the vendors. This is called a spinner; this is called a depth gauge. See here marks are given, markings like this.

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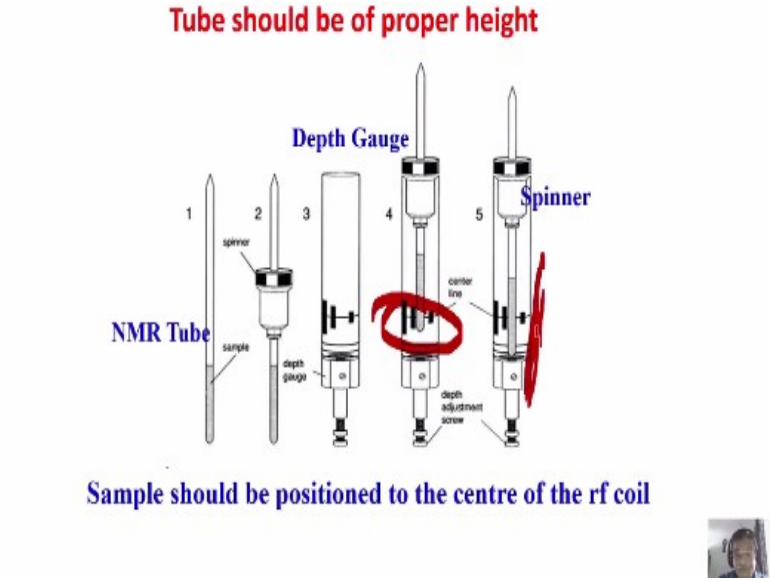

You have to put your NMR tubes into this spinner, inside this spinner. The spinner should be put into the depth gauge and make sure the sample is exactly of this height, so that your sample is at the center of the RF coil. The markings are given like this. Suppose you put your sample like this, if your NMR tube height is little above. See it is not exactly like the center of the receiver coil, RF coil. As a consequence, you will get bad signal. Your lines shapes get distorted. It should be properly positioned like this. You have to adjust the height of the spinner so that the NMR tube is properly positioned, take it out put it in the magnet along with the spinner, then you will get a very good spectrum; only after making sure it is properly positioned. That is very important.

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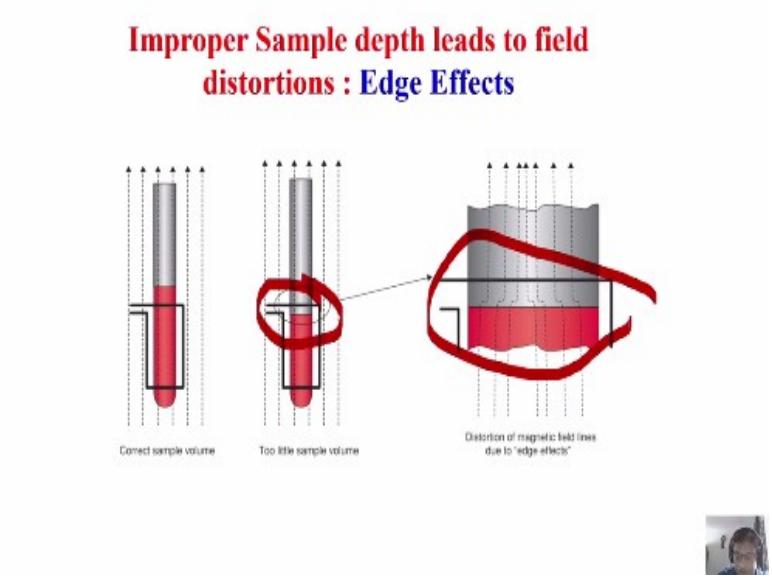

See improper depth leads to some edge effects. It causes field distortions because of edge effect. If it is instead of completely within this RF coil, if let us say, the edge of the sample is

at the center, at the edge, or middle of the RF coil, you will get edge effect like this. That gives bad line shapes. Remember, that gives very bad line shapes.

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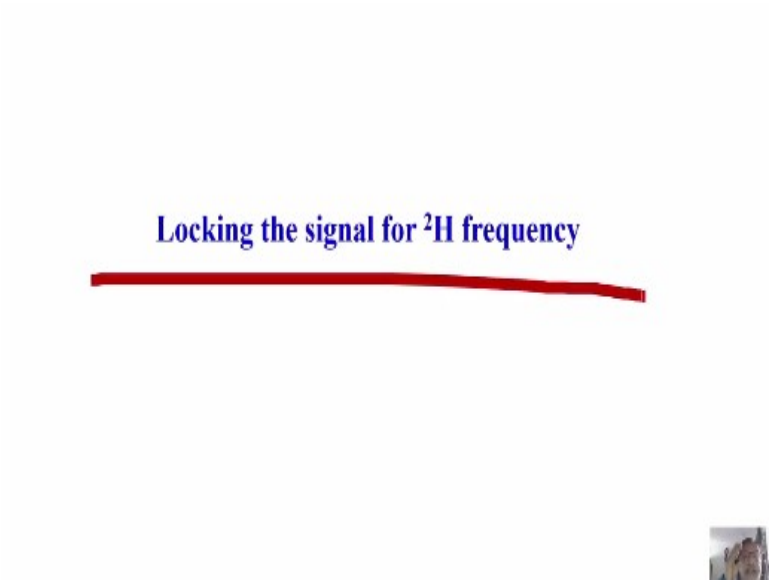

So these precautions you must take. Afterwards, you let us say you have prepare the sample in a deuterated solvent, put it in the magnetic field. What is the next job? you have to lock the signal.

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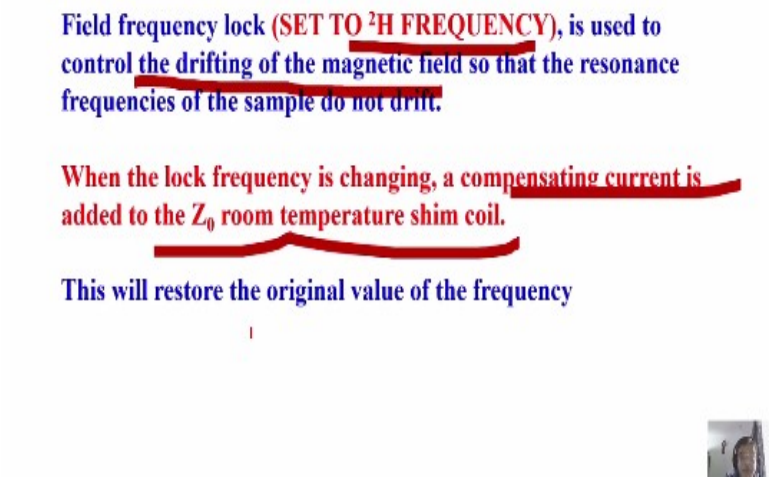

I told you locking of the signal is to prevent the drifting of the field, set it to 2H frequency of your spectrometer to control the drift. What happens when the lock frequency is getting changed because of some reason, then there is a compensating current added to the Z0 room temperature shim coil; so that it will restore the frequency to the original value. If any field drift is there, compensating field is always supplied by the lock circuit.

So that the lock will maintain the frequency; that means you will maintain the field and prevent it from drifting. So you will have uniform magnetic field all across the sample volume; so that all the nuclear spins will feel the same magnetic field, will sense the same magnetic field. Thus there is no distortion of the line shapes, no additional broadening because of field drift.

If the field enormously drifts, remember, if the field is drifting more; then what happens is different times the nuclear spins see different magnetic fields, so the resonating frequency will be different at different times. Microscopically, it may be for a small duration of time of the order of microseconds or milliseconds. But does not matter, that will be a devastating effect on your spectrum. So you have to prevent drifting of the field by locking your sample at the Deuterium frequency. Very important one please remember this.

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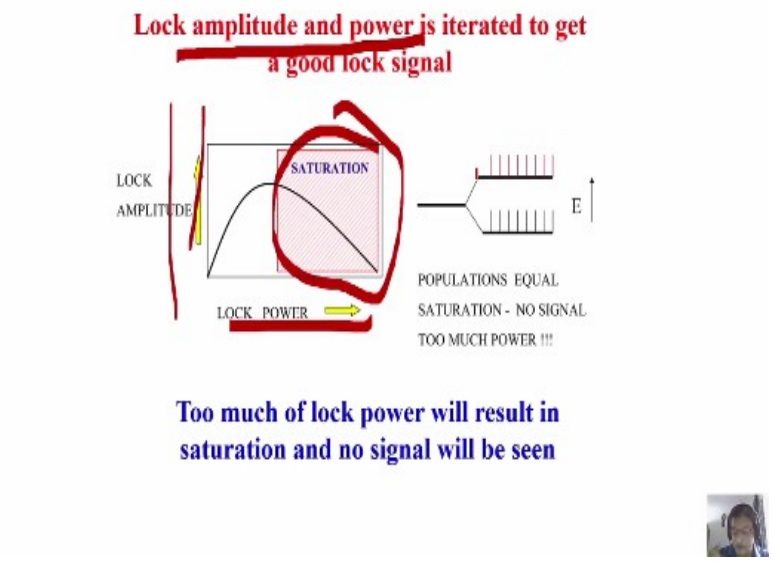

Now let us continue further. Remember, when we are locking the signal, lock amplitude and lock power are very important. These two parameters have to be iterated so that you will get a good lock signal and thereafter locking you have to ensure the signal will not get saturated. Let us say, if you have more lock power, higher lock amplitude or higher lock power it may so happen in this region you are saturating.

That means your spin population of this Deuterium signals are equal you will not see the signal. So any amount of power if you give or any amount of lock power you give after sometime you will not see signal at all, it get saturated. So you do not use too much of lock

power or do not use too much of lock amplitude, else signal get saturated, that is very important please remember this.

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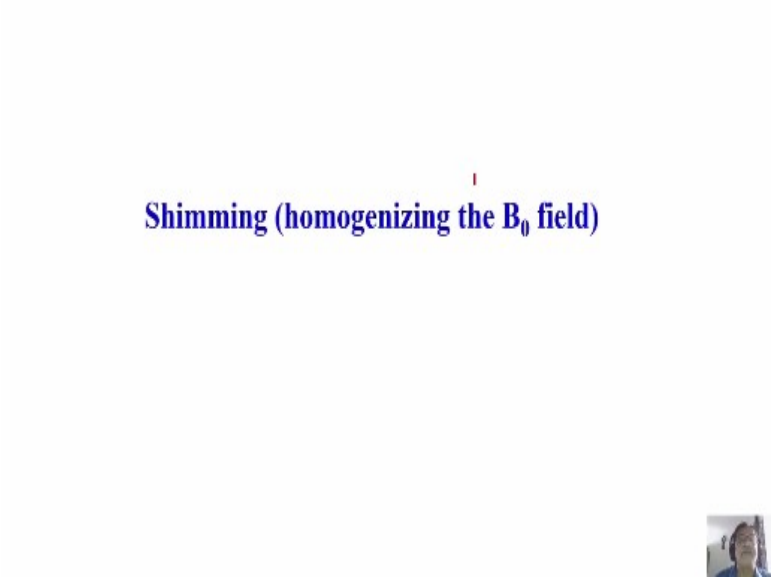

Next, after locking, you have to homogenize the B0 field. This is done by what is called shimming.

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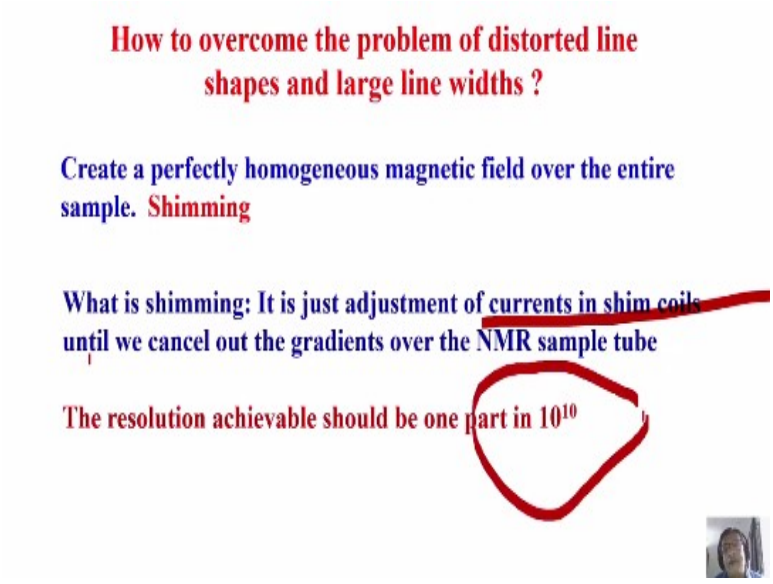

Means there will be lot of distortion inherently in the homogeneity the magnetic field. And at the site of the nuclear spins, we need to get magnetic field homogeneity of the order of one part in 10 to the power of 9 or 1 part in 10 to the power of 10. Very good homogeneity is required. So we have to create a perfectly homogenous magnetic field over the entire sample volume by what is called shimming.

So what is shimming? It is nothing but adjustment of currents in shim coils, until all the inhomogeneity because of field gradients are cancelled out. Then you will get a better resolution and the resolution should be one part in 10 to the power of 10. Remember that is very important. Resolution should be one part in 10 to the power of 10.

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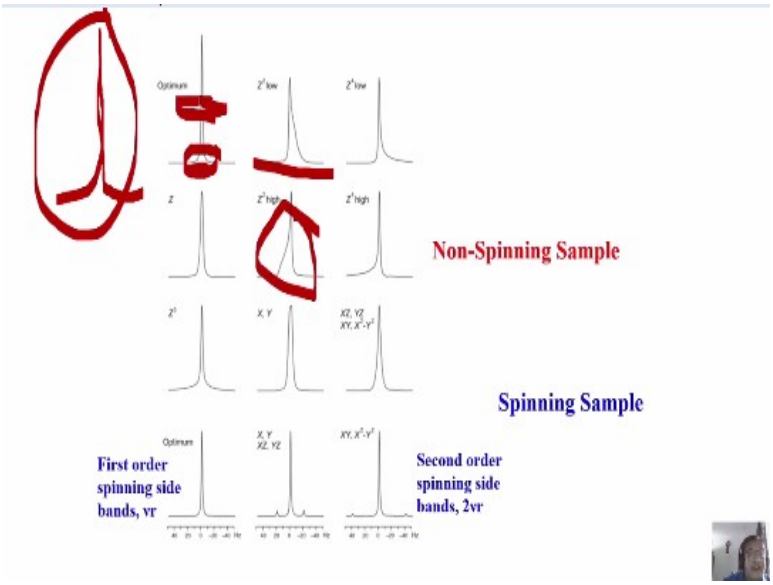

And there are, in all the spectrometers you have about 24, or sometimes 32 or 36 shim coils. There is a shim unit in the magnet. These are called room temperature shim coils, and there are about 36 of them. In some of them we will vary the magnetic field gradient along Z axis. By varying the current you can create counteracting field along Z axis, sometimes along Z square, the X and Y combination; varieties of things you can see.

And in each of them here you can see what is going to happen. This is the optimum one where the line shape is very good. If let us say, Z square is bad, you can see the shape here. Z to the power 4 is bad, you can get pedestals like this. Sometimes the X and Y can be bad; it is uniformly broad, you see a whole this thing is broad. If you are spinning the sample sometimes you get side bands, first order side bands like this, second order side bands like this.

This comes because of the $X^2 - Y^2$ the second order gradients along X and Y will be bad. So, varieties of these shim coils will be there, and mind you getting the homogeneity is not a easy job. You have to iterate upon all of these things. Every time you change these things, there is change in the currents in shim coils. As a consequence, we are creating the counteracting

gradient fields to cancel out the imperfections; or in homogeneities which are inherently present.

These are things which you had to practice, and it is not an easy job and finally when you are expert, when you do all those manipulations you will get a extremely good homogeneous field and lines will be very, very sharp; not only sharp the line shape has to be very good. See remember, here this line shape is very sharp, but this is not a very good line shape. So the line has to be perfectly a Lorentzian. Start like this, go like this, and then come like this. This is not a good shape what I have written. But in principle you will have to get a very good line shape and there is a way to test this.

You have to test the full width at half height and then also width at 0.55% of the height of this peak and 0.11% of height of this one. So, there is a reason for it, I will tell you later. So, there is a way to find the line shape, whether it is good or bad.

For doing all these things you have to use a sample called chloroform, CHCl3, get the proton peak, measure its line width, measure the line width at 0.55% of the height of the signal of this full width at half height, and 0.11% of the full width at half height. Exactly at the center of the peak you have to measure the line width, that is called full width at half height and then get the line shape. I will tell you when we go ahead further.

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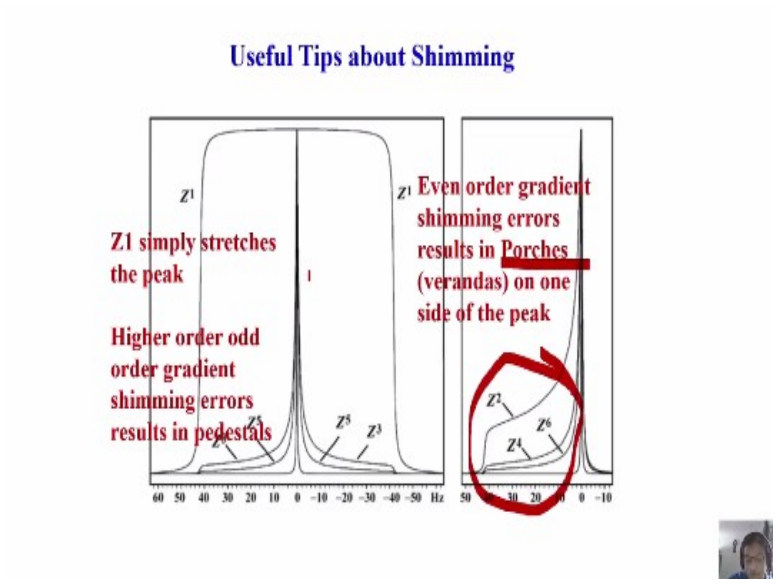

These are some of the useful tips about shimming. You see if Z^3, Z^5, odd order gradients are bad we get like this. These are all called pedestals. See you feel that sample is sitting in a

pedestal, our signal is sitting on a pedestal like this. And these are all called sometimes porches are called verandas. When even order gradients are bad like Z^2, Z^4, and Z^6 on either side, or may be on the left side or on the right side we will get verandas, or also called porches.

So these are the things which tells you that your shimming is bad. You have to make sure that the field homogeneity is very good, then you must really get a peak like this. Look at the peak here, central peak, exactly at zero. On either side you have no pedestals, no verandas, the peak should start beautifully like this go up and come back to enhance the intensity of this peak, vertically.

And measure the full width at half height, and find out 0.5% or 0.55% of this intensity and 0.11% of this intensity what is the line width. There is a formula how much it should be. It has to have certain specifications; and that specification have to be matched. I hope you are all with me. The shimming is a very, very important point.

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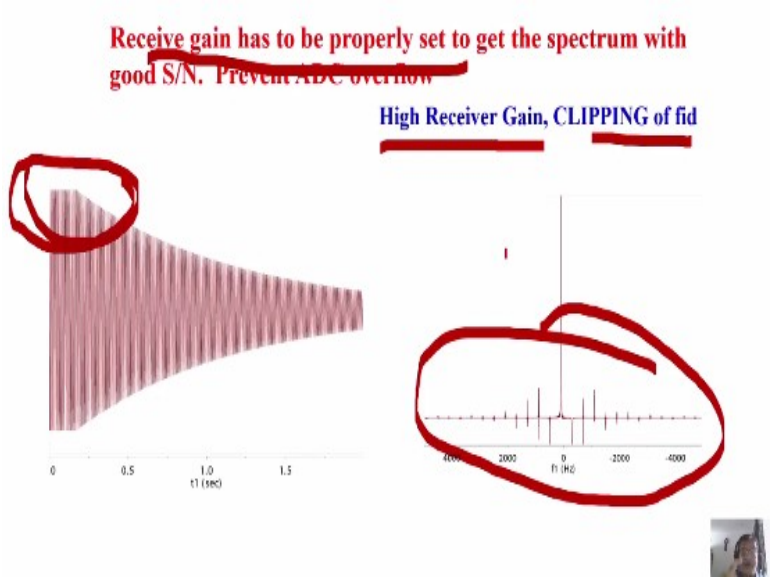

Next, when you are collecting the signal you have to adjust the receiver gain. Higher the gain, signal intensity will be maximum. And there is a limit for it, you cannot keep on raising the receiver gain. When you start doing like this, you know this is the clipping of the free induction decay. The FID gets clipped like this, that is because receiver gain is very high. If you happen to come across such a situation, and without your knowledge you will enhance the receiver gain and collect the signal. Look at it, after doing the Fourier transformation if you get the spectrum, you will get lots of spikes like this. This is an indication your receiver

gain is very high. When you get peaks like this on either side of the peak, lots of peaks like this, this comes because of various reasons. These are called sinc function artifacts, in which case you have to reduce the gain that is very important. please remember these points.

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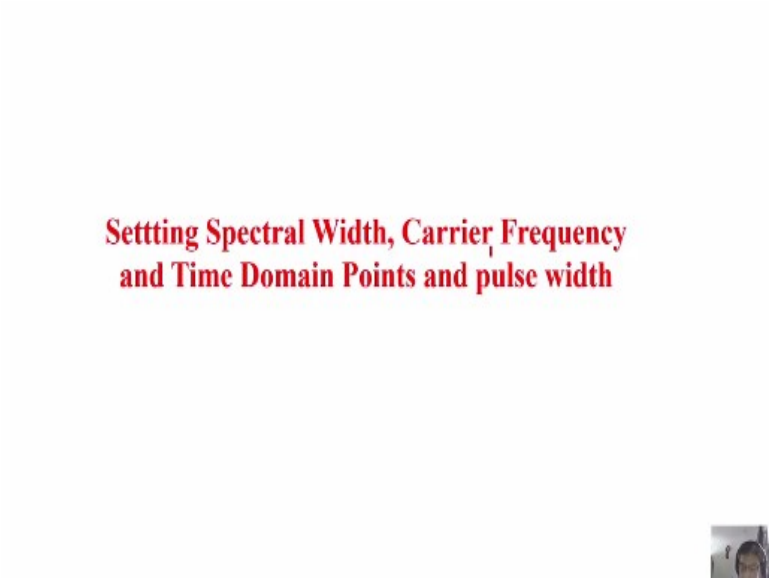

Next, you have to set the spectral width, you have to choose the proper spectral width, you have to put a carrier offset and you have to choose the required number of time domain points, you have to calibrate the right pulse width, 90 degree pulse everything. These are all other things which you must know as how to set these parameters.

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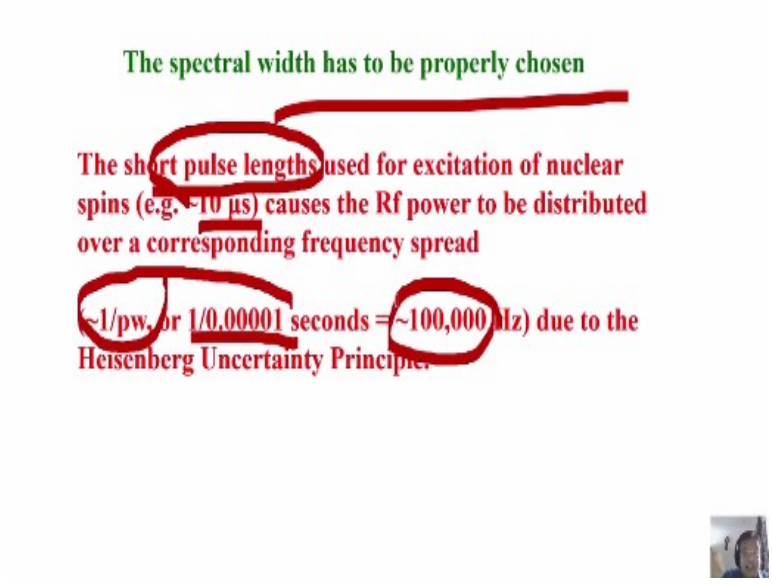

Spectral width has to be properly chosen. That depends upon your pulse width, very interesting, right. RF pulse which are going to apply for excitation; that decides the pulse width that you have to choose. Remember, the excitation profile is inverse of the pulse width

Let us say we are going to use 10 microsecond pulse width, that is the 90 degree pulse. I will tell you how to calculate that later.

If a pulse width, that is 90 degree pulse is 10 microseconds, inverse of that you calculate in seconds and convert into Hertz this is 100,000 hertz. That means your uniform excitation is decided by profile of the pulse. That means when you do the Fourier transformation of this pulse you are going to get a sinc function. You have to find out where the profile.

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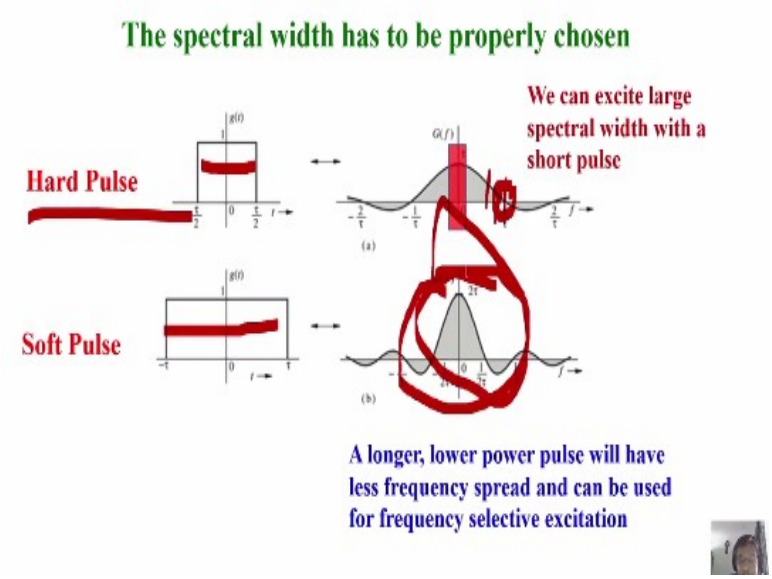

After Fourier transformation we are going to get this type of this thing. This is what you are going to see and this is the region where excitation profile is uniform. This is called power spectrum. This has to be exactly uniform. In the power spectrum you see, suppose you are going to excite in this region, if your spectral width is like this, in which case we have to have a carrier offset, carrier frequency. Your carrier is here; and look for 1000 Hertz or 5000 Hertz whatever your spectral width here, then it is not uniform excitation. You will not be even seeing, sometimes at this point, you may not even excite the spins. So you have to have a uniform excitation. That is why you have choose this region. See and for 10 microsecond pulse width I said, here to here is 100,000 Hertz. So you have to choose this region.

This is the spectral width. This is the region of the spectrum you have to choose, and then in this region all the spins will be excited with a uniform power. The uniform excitation is very important. Suppose, you excite in this region, you will not get anything, you will get some signal of course, I am not saying you will not get completely nothing, but all these spins are not uniform and excited. And the peaks which are coming in this region, may not even get

excited at all. So all this things are important points you have to remember; how to choose your spectral width. So that depends upon the pulse width. A, 90 degree pulse which is sharper, let us say, the width of the pulse is small, the excitation profile is broader. If the width of the pulse is larger, excitation profile because smaller and smaller.

That means if you want to excite large range of the spectral width, your pulse width has to be narrow. Narrower the pulse width, broader is the excitation profile. So let us say, my spectral width is 50,000 or more than 100,000, then you cannot take 10 microsecond pulse width. Your pulse width has to be much smaller, reduce to 5 microseconds. But that profile is also not possible to uniformly excite your spectral width, reduce it further. And beyond some value you cannot reduce; in which case you have to move the carrier offset. I will tell you that as we go ahead. So, remember where do we use soft pulse, for what is called selective excitation experiments. In such experiments we use soft pulse, this is called soft pulse, broader pulse. So it excites only narrow region.

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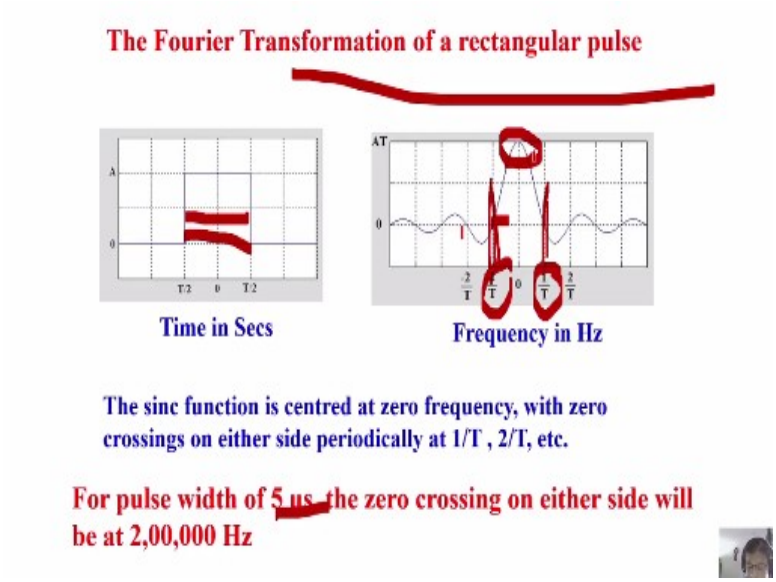

These are the things you should remember. The Fourier transformation of a rectangular pulse is a sinc function and the zero crossings is exactly at –1 / T and + 1 / T. The T is the width of the pulse. So, you have to choose your pulse width such that your excitation profile, that is for this, it should become a larger; for this it has to become smaller. For a pulse width of 5 microsecond the zero crossings will come at 200 kilohertz. That is 100 kilohertz here at + 100 and – 100 kilohertz, there is a zero crossing. You should avoid that. So you have to choose only this region. That is the intelligent way of choosing your spectral width.

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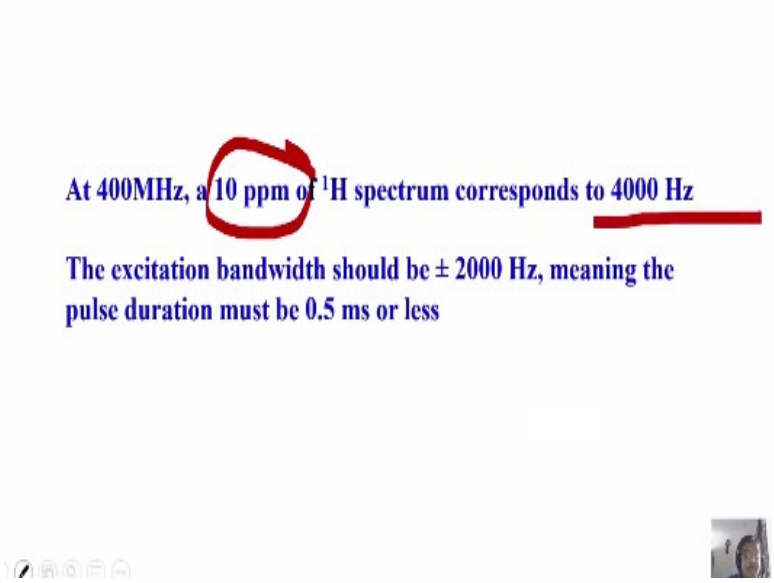

For example, at 400 megahertz if you want to take 10 ppm as your spectral width, which corresponds to 4000 hertz. So your excitation band width should be + 2000 to – 2000 from the center of this power spectrum. From the center of the power spectrum you should have 2000 here, 2000 here; that is much better, the uniform excitation will be there in that region.

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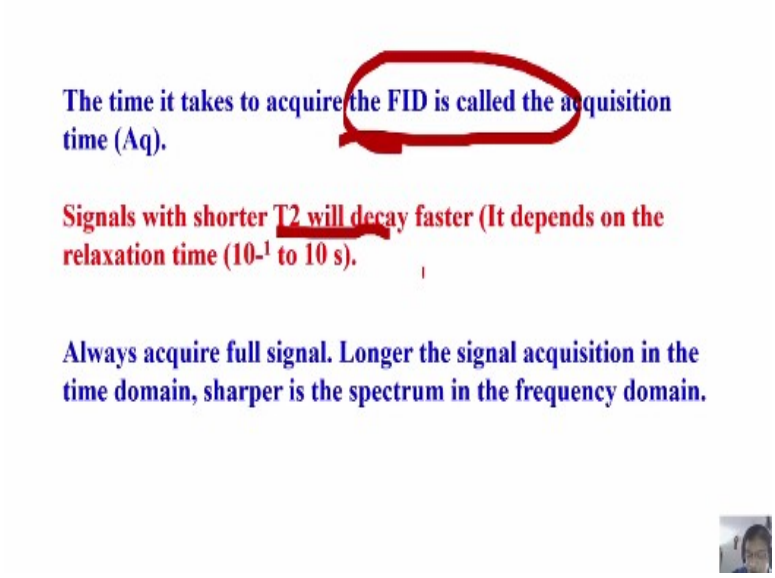

The time it takes to acquire the free induction decay is another point, called acquisition time. You have to acquire only for the period you have signal. And signals, of course, as you know I told you it will decay with time. The FID decays with time, because of T2. When does it decay faster? If the signals have shorter T2, the signal decays faster. So you have to acquire the signal, acquire the FID, before the signal dies down.

Of course, you can keep on acquiring even after signal dies down. But then you will have only noise afterwards. So, the optimum choosing of the period for which you acquire signal that is the acquisition time is important. That also you have to optimize, you have to properly choose.

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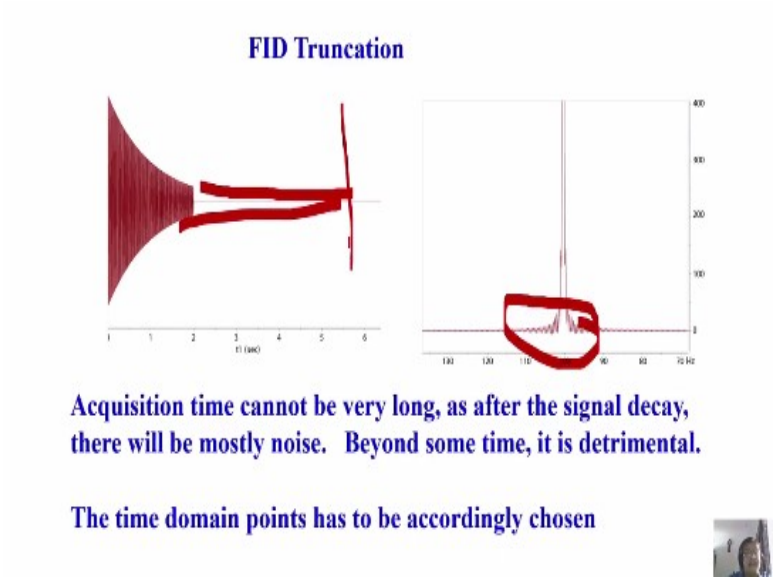

Supposing, you are collecting the signal, there is free induction decay, FID, it is decaying like this. But you are collecting only up to this point, this is called truncation. You have truncated the free induction decay. It is not completely died down. It is coming more towards rectangular pulse or the square pulse. So, what will happen? If you do Fourier transform this, you get a sinc function like artifacts.

So if the FID is truncated; it is not fully allowed to decay, and after Fourier transformation we get spikes like this on either side of the peak. They are called sinc function artifacts. These are called sinc function peaks. So your FID should not be truncated, you have to ensure it decays completely. Till then you have to collect the signal. Like this, it has to decay.

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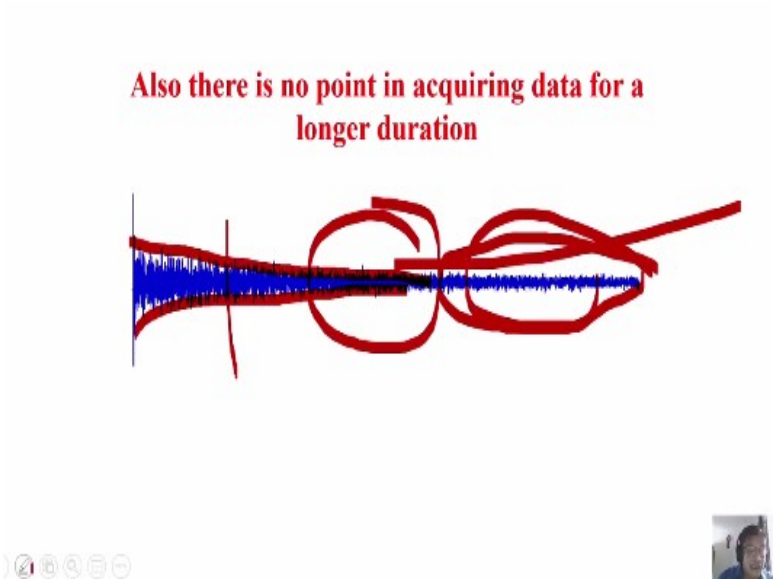

See the FID has died down somewhere here you can see very clearly. I am not a good artist so I am not drawing this properly; see it has died down here. And here if you acquire signal for a longer time what is that you are going to get? it is only noise. So that also has to be optimum; you cannot collect the signal only for a short duration, so that FID gets truncated. At the same time you cannot keep on acquiring the signal for a longer time even after the signal died down, because you will be simply picking up the noise. Then your signal to noise ratio will not be very good, it will go down. If you have more noise your signal to noise ratio will come down.