One and Two Dimensional NMR Spectroscopy for Chemists
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Lecture - 58
NMR Data Acquisition - 2

Welcome back. In the last class, we started discussing about several practical aspects concerning recording of the NMR spectrum. And afterwards we have to discuss about how we have to process this data. Of course in the last class, I gave a lot of information about sample preparation, the type of NMR tube you have to use, you have to choose appropriate solvent, for locking of the field the deuterated solvent is very important, to avoid the drifting of the magnetic field; and I also said how to choose the spectral width. Of course I said we need to acquire the data, so that we should not truncate the FID, at the same time we need to acquire sufficient enough to see the signal. And I also mentioned if you acquire signal for a longer duration more than what is required, that is after the signal dies down, you will be acquiring more noise than the signal. As a consequence, your signal to noise ratio will come down, that is also not advantageous. So, there are many parameters, which you need to optimize before recording the spectrum. So today, we will continue further.

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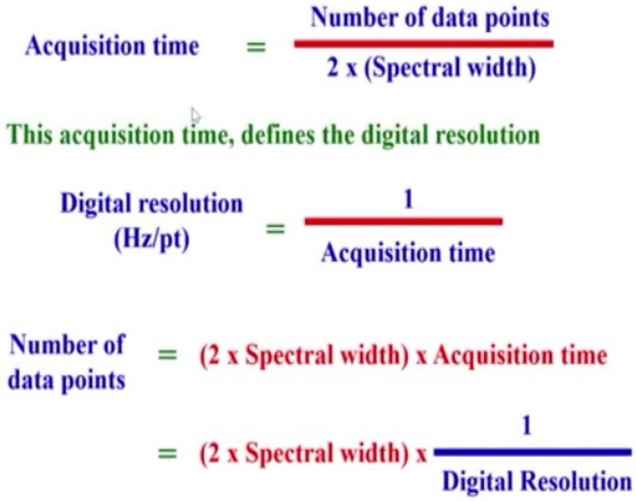

$$\text{Acquisition time} = \frac{\text{Number of data points}}{2 \times (\text{Spectral width})}$$

This acquisition time, defines the digital resolution

$$\text{Digital resolution (Hz/pt)} = \frac{1}{\text{Acquisition time}}$$

$$\text{Number of data points} = (2 \times \text{Spectral width}) \times \text{Acquisition time}$$

$$= (2 \times \text{Spectral width}) \times \frac{1}{\text{Digital Resolution}}$$

And let us see how we have to choose the spectral width. I said we should have a spectral width that also defines your resolution, and how much time the signal is decaying, how long you have to acquire, everything we have to decide. For example, acquisition is a time required to acquire the time domain signal. You send a radiofrequency pulse, start collecting the signal. How long you have to acquire the signal. That is what I said, you have to acquire the signal, and you have to optimize it.

How do you optimize it? That depends upon number of data points. Acquisition time is the number data points is divided by twice the spectral width. It is a very important parameter. The acquisition time is the one that defines the digital resolution. See you cannot have a shorter spectral width. If you have a shorter spectral width, your acquisition time becomes longer. You have to acquire a signal for a long duration, probably your signal would have died within few seconds. But you may still be acquiring signal for few more seconds. That is not good. If you take large spectral width, your acquisition time is very short. Then, you may truncate the FID. You will not be collecting the FID for longer time, for what is required. So you have to decide on this parameter. You have to optimize it. And the acquisition time is the one which decides the digital resolution. That is called Hertz per point.

See NMR data, when it is collected in a time domain, it is digitized. After Fourier transformation, you will see the frequency domain is also digitized. When you are digitizing the time domain data, how many times you have to digitize? and let us say I start digitizing here, there is a free induction decay. Let me just tell you here, I have a peak here. I have to start collecting the signal, at different points like this. I will digitize, and I will collect. The time between this and this or between any two adjacent data points, the Hertz between these two data points, is called digital resolution. The digital resolution is defined by these two data points. Let us say, I collect one data point here, another data point here, then you will not be able to resolve the spectrum at all. There is no digital resolution for it. On the other hand, let us say we have two peaks. Actually, it is a peak like this, it is a doublet. But you collect one data point here, one data point and one data point here, then you will miss the doublet, because you are not getting good resolution. So, the number of data points you acquire and the duration for which you are going to collect the signal defines your digital resolution. It is called Hertz per point and the time between two adjacent data points is called dwell time. That is very important. This defines the dwell time.

The shorter the dwell time, better is the resolution, remember. So how you decide the number of data points, say I manipulate this equation. If it is number of data points, you have to decide how much you have to give; this is acquisition multiplied by twice the spectral width. I said acquisition time and digital resolution are inversely proportional to each other. And this is the equation. This tells how many number of data points you need to collect, to get good resolution. You can decide that.

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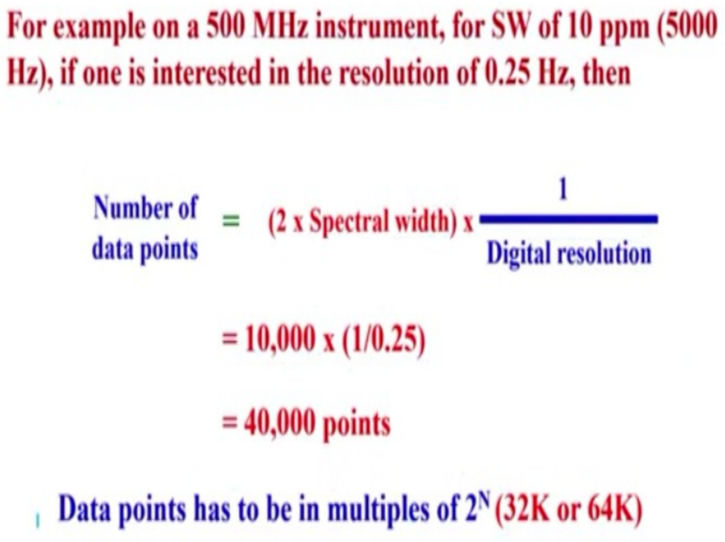

For example on a 500 MHz instrument, for SW of 10 ppm (5000 Hz), if one is interested in the resolution of 0.25 Hz, then

$$\text{Number of data points} = (2 \times \text{Spectral width}) \times \frac{1}{\text{Digital resolution}}$$

$$= 10{,}000 \times (1/0.25)$$

$$= 40{,}000 \text{ points}$$

Data points has to be in multiples of 2^N (32K or 64K)

For example, I have a 500 MHz instrument, for which I have to acquire data for 10 ppm. You all know, 10 ppm in 500 MHz corresponds to 5000 Hz. Let us say, I have two peaks separated by 0.5 Hz. I want to get a resolution of less than 0.5 Hz, so that I can see, I can resolve two peaks like this, which are separated by 0.5 Hz, let us say. So if that is the case, how many number of data points I have to choose.

That you can decide based on this equation, twice the spectral width divided by digital resolution. And this will tell me about my resolution. So my digital resolution should be at least 0.25 Hz. So dwell time between two adjacent data points, it should be 0.25 Hz. Now for 10 ppm, I have to take 5000 Hz, this is twice the spectral width, so it is 10,000 Hz; divided by 0.25 is the digital resolution, I expect or I want.

So the number of data points, I must choose should be minimum of 40,000 points. What you call as data points or SI, the size of the memory, what you are going to collect should be at least 40,000 points. And you cannot take the odd number like this, especially for acquiring data. This is called, in your spectrometer, it is called SI. But the time domain data points are different. This is the data point, it is size of the memory, SI.

So these data points have to be in multiples of 2 to the power of N; and you cannot take 40,000. Of course, time domain points you can choose any number. That is not a problem. We have provision for it; but at the time of acquiring, if I want to have this SI, the memory of the computer for which you are going to collect the data, it has to be the multiples of 2 to the power of N. It should be 32K or 64K, like that or 16K, you have to choose. Based on number of points you have to choose. Supposing instead of 0.25, I need minimum 40K data points. Let us say I take only 10K data points or 10K points. If I collect the signal and I will use the size of the memory, size is only 10K I will take. Then you will not get that much resolution. So you will be missing two points or two peaks which are close in frequency. Please understand. So the digital resolution, if it is 0.25 in a 500 MHz spectrometer, minimum I should have size of the memory should be at least 40K.

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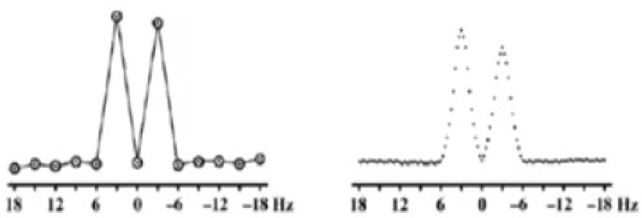
Digital resolution is important in getting better resolution



Low digital resolution
3.0 Hz/pt.

High digital resolution
0.3 Hz/pt.

Now what happens? Based on that, of course after acquiring data, you can play with the time domain data also. There also you can increase the number of data points. It is as good as telling indirectly you are increasing the size of the memory, size of the data points, memory size. See now consider this example of a spectrum, where you have two peaks, which are resolved, no problem. But look at this number of data points here. As a consequence, you are not getting good spectrum. Supposing, if this data point is missing, you would have got a peak like this, then you would not get resolution; and you will not observe this as two peaks. This is a situation, the Hertz per point at dwell time is quite large, and the resolution is poor. On the other hand, now instead of 3 Hz, I will take the digital resolution to be 0.3 Hz. That is data between these 2 points; any 2 points you take adjacent points. This is called the dwell time, and this will be 0.3 Hz, let us say. I take digital resolution, the resolution between any 2 adjacent data points is 0.3 Hz. Look at the type of spectrum you are going to get. It is better resolved. You can see the resolution. You cannot miss this doublet at all. But in this case, if you make a mistake or slightly more than 3 Hz is there, you will definitely not be able to see doublet. That is the important point you must remember. Digital resolution is very important in getting better resolution.

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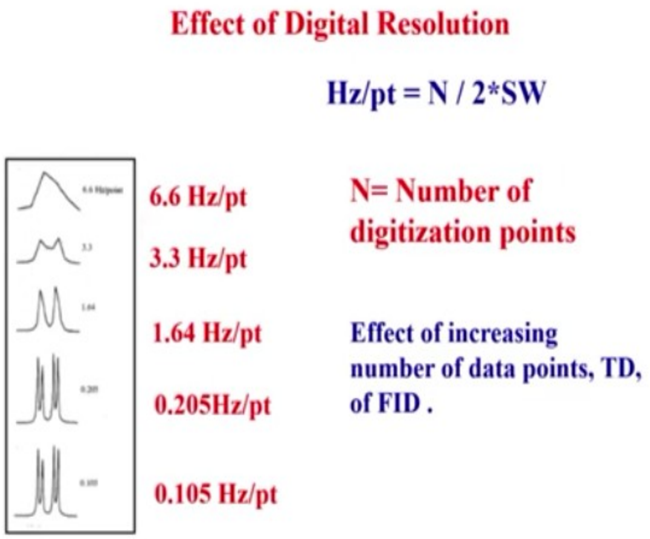

Effect of Digital Resolution

Hz/pt = N / 2*SW

6.6 Hz/pt

3.3 Hz/pt

1.64 Hz/pt

0.205Hz/pt

0.105 Hz/pt

N= Number of digitization points

Effect of increasing number of data points, TD, of FID .

You see the effect of digital resolution on a some standard example like this. This is a situation number of digitization points is N. Let us say number of digitization points is N; and spectral width is some value, it is taken. When the Hertz per point, dwell time is 6.6 Hz. Look at the spectrum, so broad. In the same thing, reduce the Hertz per point to 3.3. See you already started seeing the resolution.

Keep on reducing like this, come to 0.105 Hz per point, by playing with number of digitization points or the spectral width. Reduce the spectral width or increase the number of the digitization points, size of the memory, size of the digitization points, in which case you see what you would have seen with a poor resolution as a broad hump, now you will see as doublets of doublets. The effect of digital resolution is so good and so important. So proper choosing of the spectral width and the number of digitization points is very important to achieve very good digital resolution to see well resolved spectrum.

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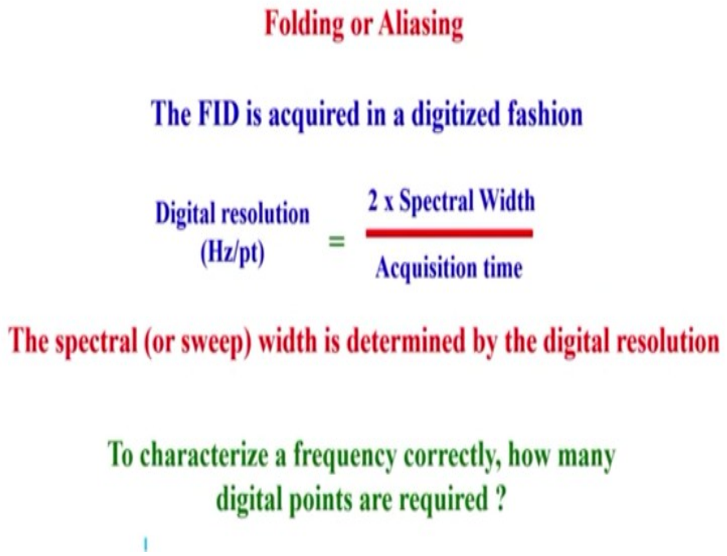

Folding or Aliasing

The FID is acquired in a digitized fashion

$$\text{Digital resolution (Hz/pt)} = \frac{2 \text{ x Spectral Width}}{\text{Acquisition time}}$$

The spectral (or sweep) width is determined by the digital resolution

To characterize a frequency correctly, how many digital points are required ?

So now when we are discussing about digital resolution and also dwell time, I will introduce one more term called folding or in Fourier transformation, it is also called aliasing, folding or aliasing. As you know FID is acquired in a digitized fashion and this is the digital resolution that is what we discussed, just now. The spectral width is determined by the digital resolution, that is correct. That is what we said.

Now the question is, to characterize the frequency correctly. How many digital points are required? For example, in this case, look at this one. Now, not this one, you look at this one, here we have 1, 2, and 3 points to define a peak. Here, there are 3 points to define a peak, but still you are able to identify two peaks here. Here, you have more number of digital points that is better. Supposing, if I had missed this peak, what would happen? You would not have seen the doublet. Now another extension of this is, if you do not have enough number of digital points, you get into a problem called folding or aliasing. This is because of what is called Nyquist theorem in Fourier transformation.

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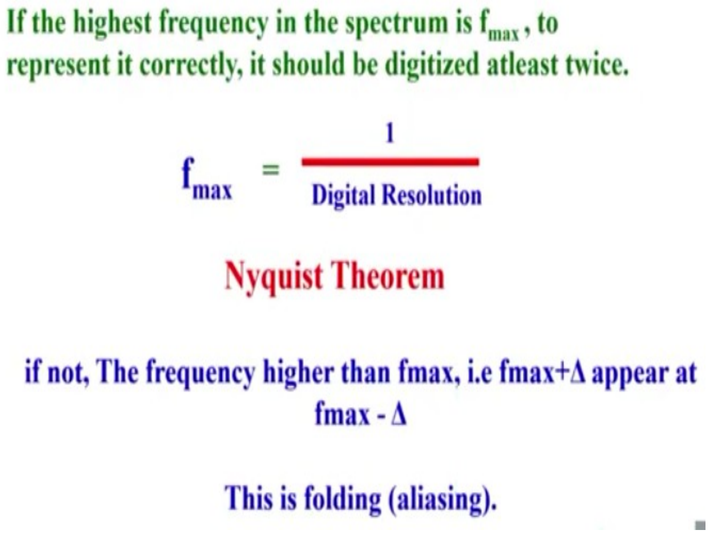

If the highest frequency in the spectrum is f_{max}, to represent it correctly, it should be digitized atleast twice.

$$f_{max} = \frac{1}{\text{Digital Resolution}}$$

Nyquist Theorem

if not, The frequency higher than fmax, i.e fmax+Δ appear at fmax - Δ

This is folding (aliasing).

Let us say, I have a spectrum with different frequencies from 0 to 10 ppm. Let us say, I have taken the spectral width 0 to 5000 Hz. 0 to 5000 I have taken, at 5000 I have a peak. This is my largest frequency and if the highest frequency, the spectrum is f maximum. If I have to represent it correctly and not only that, all the frequency lower than that will be represented correctly, then digitization should be such that f of maximum will be at least equal to 1 over two times the digital resolution. 1 over two times, 2 is missing here; 1 over two times of digital resolution. If not, the frequency higher than the f maximum that is if I have a frequency beyond 5000, that is at 6000. It is 1000 Hz more than maximum; then f + delta appears as f – delta. Remember this is 1 over twice the digital resolution. Remember do not get confused with the Nyquist theorem.

The 2 was missing. At least, you have to digitize twice. The highest frequency in the spectrum should be at least digitized twice, so that frequency can be represented correctly. What happens if the frequency is, let us say like this, you digitize one point here and one point here, you will definitely miss it. So it cannot happen. Higher the number of digitization points, of course, it should be minimum 2, you have to have two data points per peak. More than that does not matter.

So this is what it is, according to the Nyquist theorem, it says, the highest frequency should be at least 1 over 2 times the digital resolution, that is Nyquist theorem. Otherwise folding will occur.

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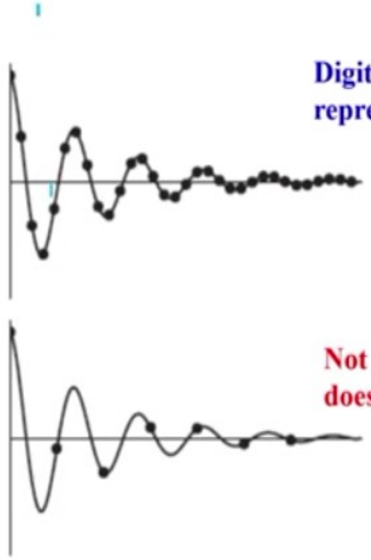

Digitized atleast twice, correctly represented

Not digitized atleast twice, does not obey Nyquist rule

For example, look at this spectrum this is a free induction decay. It is digitized. Of course, I am showing you only single frequency oscillation here. The damped oscillation is only for single frequency. If there are more frequencies, you would have got an interferogram. For representation purpose, I have taken only one frequency. Now is it represented correctly here? look at it. Number of data points 1, 2, 3, 4, 5, 6, it is definitely digitized precisely, because you can measure the frequency from this oscillation. I know the frequency.

So in this, here to here is lambda, you know the frequency, that shows 1, 2, 3, 4, 5, 6 points, minimum 6-7 points are there. It is digitized much, much better, more that what is required. It requires at least twice digitization; this is digitized much more than that. So this is very well characterized, very well represented correctly. On the other hand, look at the free induction decay at the bottom here. There is one point here and one point here and one point.

So this frequency is not well characterized. At least there must be two points for each frequency, but it is not there. So it is not digitized at least twice and does not obey Nyquist rule. The Nyquist theorem says the free induction decay should be sampled at least twice of that of the highest frequency available. So f maximum = 1 / 2 times the digital resolution. That is the condition we have to maintain.

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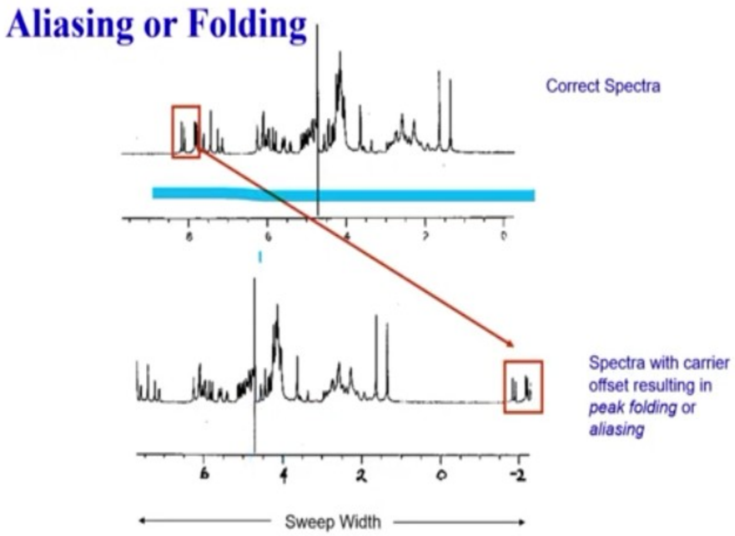

Aliasing or Folding

Correct Spectra

Spectra with carrier offset resulting in *peak folding* or *aliasing*

Otherwise you will get a spectrum like this. Let us say, this is the correct spectrum, but if the digital resolution is, let us say, I am sitting here. This is a carrier offset, I will tell you how to set it and if there is a peak somewhere here, well it appears as if the peak which is here gets folded here. Let us say sitting from here, I cover this much range. I have a frequency here, lots of peaks are here. These peaks are not very well represented, because my digital resolution is taken for only highest frequency within this region. So, the peaks, which are coming with a higher frequency than this chosen range will get folded here. This is called folding or aliasing. So that is what happens. Now well, I said sitting here and covering here, this is folded here; or sitting here covering this much, this gets folded here. Either ways is correct.

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The NMR signal (FID) can be co-added in the time domain

Resulting in the enhancement of signal intensity

What happens to thermal noise? Noise has all the frequencies present and has random phase

This also gets added, but not linearly

So now the question is, I collect one-time domain signal FID. Signal is very poor, let us say. Intensity is not very good. I have to enhance the signal intensity. Can I do that? Can I keep on adding the signal once, twice, thrice like that, keep on adding? Is it possible to do? Yes, you can do that. The NMR signal can be co-added in the time domain. In the time domain, you send a radiofrequency pulse, collect the free induction decay. Send another one, collect the free induction decay over this, keep adding it. Then what happens, at the end, when you do the Fourier transformation, you will see the enhancement in the signal intensity. But you may also ask me a question. What happened, there will be also be a thermal noise.

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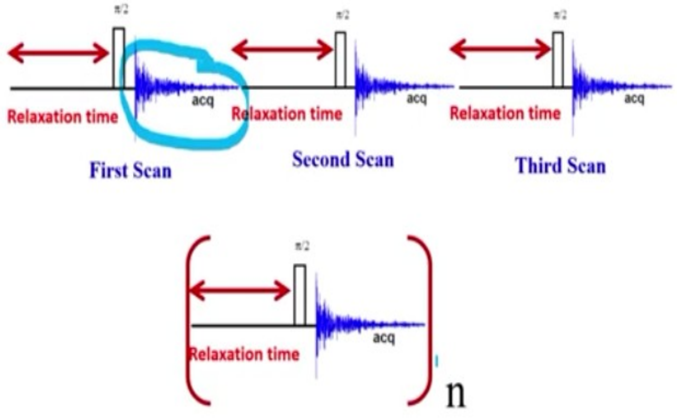
Signal averaging. The time domain signal is accumulated at regular intervals



Noise invariably present, generally we will have white noise. White noise is the one, which has all the frequencies. So what to do? So, noise has all the frequencies present and has random phase, whereas the peaks are always in phase. They get added up. Co-addition of this is easy whereas noise cannot be added up in phase. They are random in phase. As a consequence, this also gets added up, but not linearly. Free induction decay, the peak from the sample, which are in phase, they get added up linearly, but noise will not get added up linearly. So as a consequence after you keep on acquiring for a long duration, because it is a random phase, when you take the average, this noise keeps on reducing. Whereas, what happens to this signal, you are co-adding in the phase. They keep on adding up, then signal to noise ratio becomes better.

This process of co-addition of free induction decay in the time domain is called signal averaging. Remember it is called signal averaging. The time domain signal is accumulated at regular intervals. Like this, you start collecting the signal here. There is a time, which I will tell you what is this relaxation time, how much you have to give. Apply a radiofrequency pulse, there is a time delay here, this is called dead time delay and then start collecting the signal. This is one experiment. This is one time, when you want to acquire a signal, this is what you do.

Please understand. Send a radiofrequency pulse and collect the free induction decay, that is all. If the signal intensity is very good, then you do not have to bother about co-addition. You do not have to bother about signal averaging. Fourier transformation of this one will give you frequency domain spectrum. But generally it is true for highly abundant spins, like proton and fluorine, you may get the signal very fast.

What happens if you take a less abundant spins? For example, you want to see nitrogen, in natural abundance. You cannot get the signal in one scan or one accumulation. So, we acquire data once, we call it as scanning once. So it is one scan. So you keep on adding, you have to increase the number of scans, so what you will do. This is the first scan, and afterwards wait for some time, and then send another second radiofrequency pulse, acquire signal. Then after some time, send third radiofrequency pulse, acquire the signal. So what you will be doing, same one dimensional experiment of sending the pulse and acquiring the data, you will be repeating it N number of times. And N number of times, you will be co-adding the data. This will give you enormous gain in the signal intensity. Enormous means how much? If I acquire once and twice, is it enough? If I acquire twice, then how much gain I will get? If I acquire 100 times, how much gain I get? These questions we have to answer.

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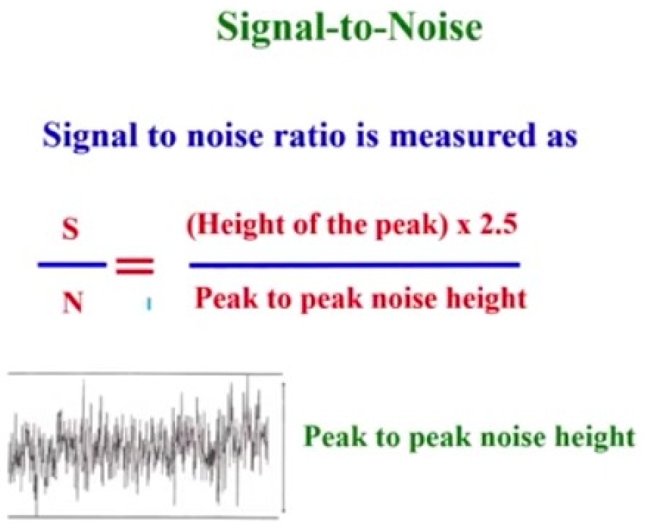

Signal-to-Noise

Signal to noise ratio is measured as

$$\frac{S}{N} = \frac{\text{(Height of the peak)} \times 2.5}{\text{Peak to peak noise height}}$$

Peak to peak noise height

That depends upon a parameter called signal to noise. A signal to noise ratio is measured as S/ N. I will say I am selecting a region for which I have to find signal to noise ratio. In that region, if there is a peak, I will find out height of the peak, and multiply by 2.5 divided by peak to peak noise height. You may ask me the question why I have to multiply by 2.5. It comes by noise, if you want to understand how to get the signal to noise ratio etc, you will get it, do not worry. We will not go into the theory, but I am giving you the final equation for S/N. You have to get the height of the peak, multiply by 2.5 and divide by peak to peak noise height. Peak to peak noise height, you will get like this. If this is the noise, let us blow up the signal several times, increase the vertical intensity.

Now you get the maximum noise, in the representable form. Measure from this point to this point, what is the height of the peak. That is peak to peak noise height. Put that in the denominator. If I have peak here like this, and measure this height, this is the height of the peak. Aand then you will rather get signal to noise ratio.

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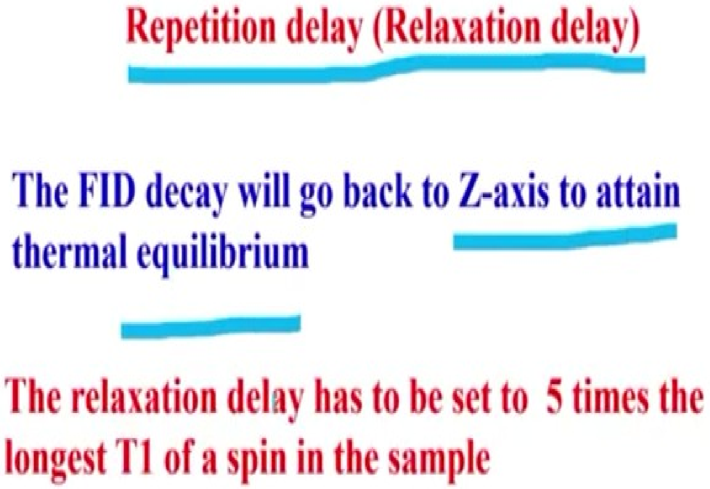

Repetition delay (Relaxation delay)

The FID decay will go back to Z-axis to attain thermal equilibrium

The relaxation delay has to be set to 5 times the longest T1 of a spin in the sample

Now the question is, if you want to do a proper experiment, you need to repeat the experiment with appropriate delay. In the previous example here, I gave you relaxation time here. What is this relaxation time? If I have to repeat it N number of times, like this. Let us say, I send the pulse here, immediately I send another pulse here, immediately I send another pulse here, without waiting for the nuclear spins to relax, then what will happen?

You must understand what is happening. We also discussed at stretch, after the free induction decay starts decaying, this is called time domain decay, relaxation in the transverse plane. It is called T2. It depends upon the relaxation time T2. It has to decay. If it decays completely at the same time, the signal will be growing along z axis. The decay in the xy plane is called transverse magnetization. I remember we discussed this at one of the classes before. The decayed magnetization will grow along z axis. It is an exponential growth. Decay is also exponential, but the time periods for decay and growth are different. Now I must know, how much time it takes for the spins, for the magnetization to go back to z axis and attain thermal equilibrium. The time it requires to attain thermal equilibrium is called spin lattice relaxation time. That is the one, which defines what should be the relaxation time.

Remember the relaxation time should be such that FID has to go back to thermal equilibrium. In principal you must wait for 5 times the longest T1. Why, because remember when I explained to you about the decay of the magnetization and growth of the magnetization, I gave you an equation. The magnetization MZ coming back to z axis = $M_0(1 - e^{-t/T1})$. That is what I said. So it is an exponential function.

It means, remember you put t = T1 what happens, it turns out to be 0.67. If T1 is equal to, let us say, 2t like that you calculate. t/ T1 is there, make it equal, then make it double, then make it triple, find out. When the time delay t =5T1. $1 - e^{-t/T1}$, that is equation 1 minus. If it has to be equal to 1. Then MZ = M_0. That is the thermal magnetization, equilibrium magnetization.

If MZ has to become completely M_0, which was there before application of the pulse, then T should be equal to 5T1. You put the value into that equation MZ= $M_0(1 - e^{-t/T1})$, then you will find out this exponential factor turns out to be 99.999. Whatever that number, second or third decimal you can take. That means MZ is almost equal to M_0. The magnetization would have recovered 99.999% along Z axis.

Then, you have to apply another pulse to get the signal. You understand. Supposing you do not wait for that 5T1, I will say I have sent the radiofrequency pulse. Signal will be there. I start collecting signal and I do not wait for 5 times the T1. Then, I send another pulse, immediately without waiting for even 1T1, what will happen? Magnetization would not have grown along z axis. As a consequence, what will happen, you will not get the signal. You will be only acquiring the noise. So waiting to send the second pulse for signal averaging is essential, that relaxation delay is essential, and it should be at least 5 times the longest T1 of your sample. If there are many protons, each would have a different T1. You have to find out the longest T1, give 5 times that value and you will get all peaks of nearly equal intensity, and get maximum signal. Otherwise, you will be acquiring only noise.

Now you may ask me a question, why should I wait for 5 times the T1. Suppose my T1 is several hours, not seconds or minutes, hours, I am wasting my time. There are many methods, especially in the solution, to bring down the relaxation by using certain methods, like some shift agents everything we can use or chromium acetyl acetate, we can add a microgram quantity to bring down the relaxation. There are several methods.

Also you do not have to wait for 5T1. If you wait for 1T1, find out, when T = T1, 67% of the magnetization would have come back. find out how much it is for 2 times; find out for 3 times T1, already 95-96 would have come back, that is enough. Only you are losing 5% of intensity, 5% signal. If you wait for only one time T1, you will be losing 33% signal. So we can play with that. So you do not have to bring the magnetization to z axis by 90 degree pulse always.

You can use different angle and calculate how much is the magnetization you will get back and how much time you have to wait and you can optimize the experiment. This is only the tip of the iceberg, I am giving you. There are number of things you can do. There are number of improvements, modifications are there, where we know how much is the pulse width, what should be the time you have to wait to get a good signal to noise ratio, what we have to do. Many, many pioneers have contributed significantly and you can follow that, but right now, I am giving you only the basic idea.

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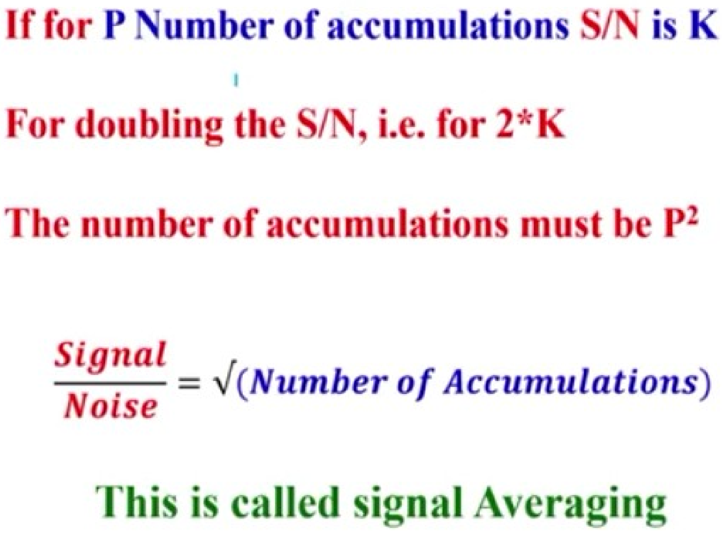

If for P Number of accumulations S/N is K

For doubling the S/N, i.e. for 2*K

The number of accumulations must be P²

$$\frac{Signal}{Noise} = \sqrt{(Number\ of\ Accumulations)}$$

This is called signal Averaging

If you want to do the signal averaging, you must wait for atleast 5 times T1. Now if the number of accumulations is let us say for P, then signal to noise ratio we calculate is K. for doubling of that, what we have to do? Let us say, I take 2 times the signal to noise ratio I want to get. The number of accumulation must be P^2. Remember signal to noise ratio will go by the square root of the number of accumulations.

To give you an idea, if signal to noise ratio, let us say, is 2. I want to make it 4, what you will do? If the signal to noise ratio is 2, I could get in 4 scans. If I have to make this 4, I have to acquire 4^2 number of scans. I have to acquire 16 scans, so that the signal to noise ratio which was 2 can become 4. Remember, the signal to noise ratio always goes by square root of this one, number of accumulations. And this type of acquire the signal one after the other for improving the signal to noise ratio is called signal averaging. Please remember, it is signal averaging.

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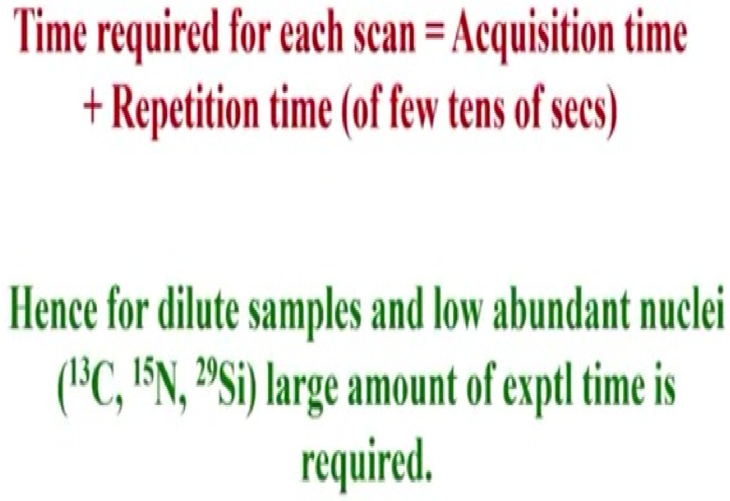

Time required for each scan = Acquisition time + Repetition time (of few tens of secs)

Hence for dilute samples and low abundant nuclei (^{13}C, ^{15}N, ^{29}Si) large amount of exptl time is required.

So now what I am going to tell you is the time required for each scan is, the acquisition time plus the repetition time. Acquisition time is the time for which you acquire a signal. So, if you have dilute sample, or less abundant spin like carbon 13, nitrogen 15, etc. we have to wait for longer time because such nuclei also have longer T1.

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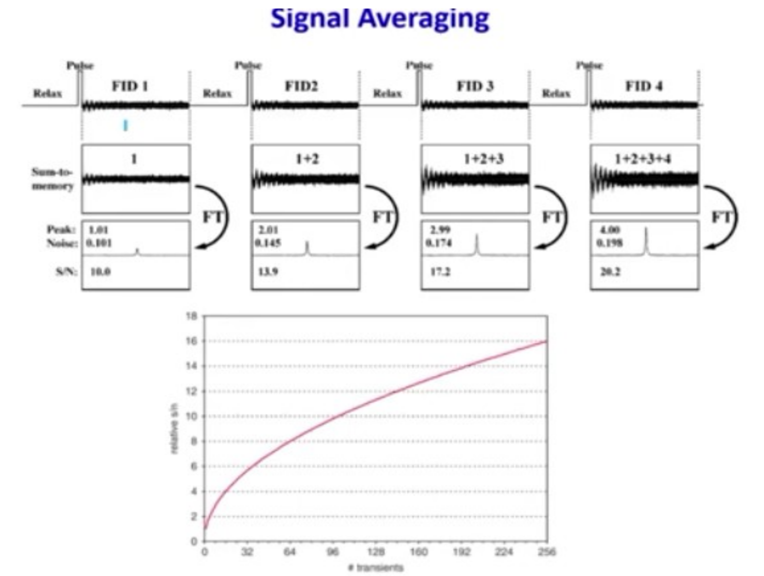

And this is a picture, which shows, if I have one free induction decay, you acquire one signal and do the Fourier transformation, this is signal to noise ratio. Co-adding 1 + 1, now it is 2, like this. Add 3 FIDs, this is signal to noise. Add 4 times it should be exactly double, you see that will be 10, signal to noise ratio was 10 here, it became 20, you see. Signal to noise ratio, if you have to double it, the number of scans, number of accumulations should be square of that.

Signal to noise ratio goes by square root of the number of accumulations. So, I am going to stop it today here. We will come back and continue further tomorrow with some more information about data acquisition and processing.