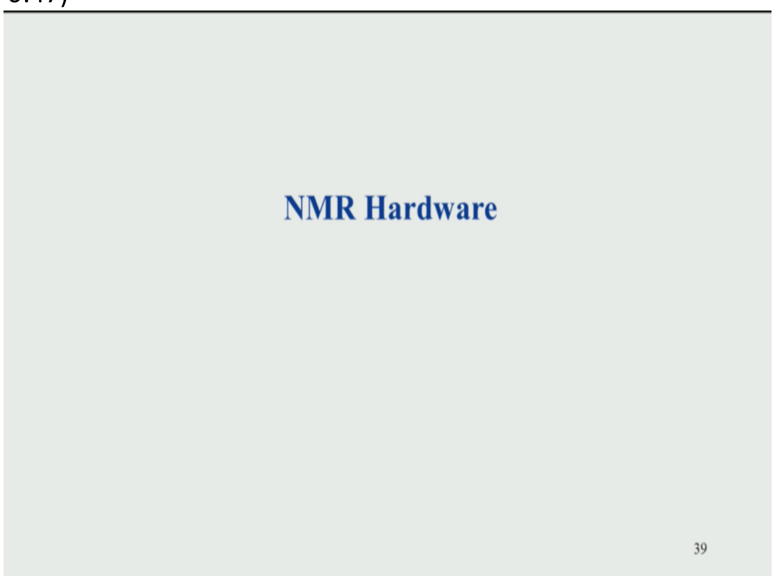
**Principles and Applications of NMR spectroscopy**
**Professor Hanudatta S. Atreya**
**NMR Research Centre**
**Indian Institute of Science Bangalore**
**Module 1**
**Lecture No 05**

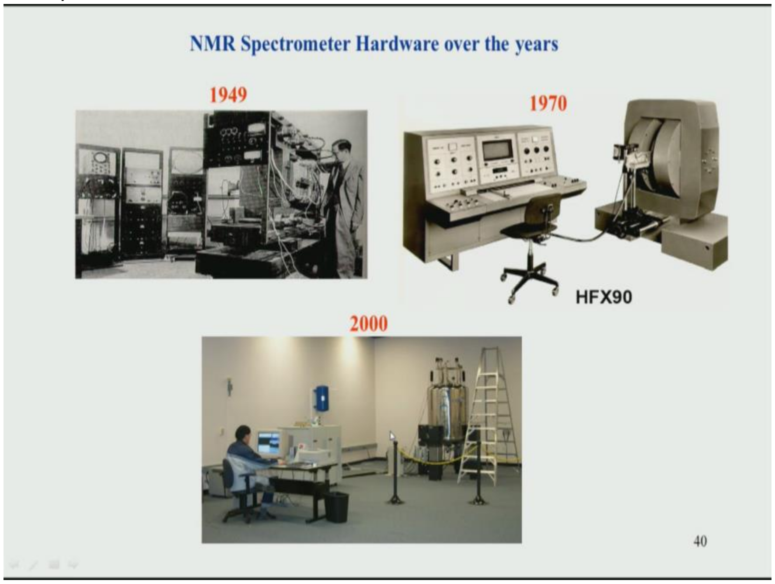
welcome back in the last class we saw how NMR experiments can be performed by applying an RF radiation to the samples which are kept in a magnetic field and the molecules go from the ground level alpha state to beta state and when they come back they emit this signal known as the free induction decay which is captured and digitized and finally it is Fourier transformed to give the spectrum.

(Refer Slide Time: 0:47)




So we will look at today at the hardware NMR hardware aspects, we will look at how an NMR spectrometer is built, what are the different components of this system so that we get a feel of how experiments are performed.

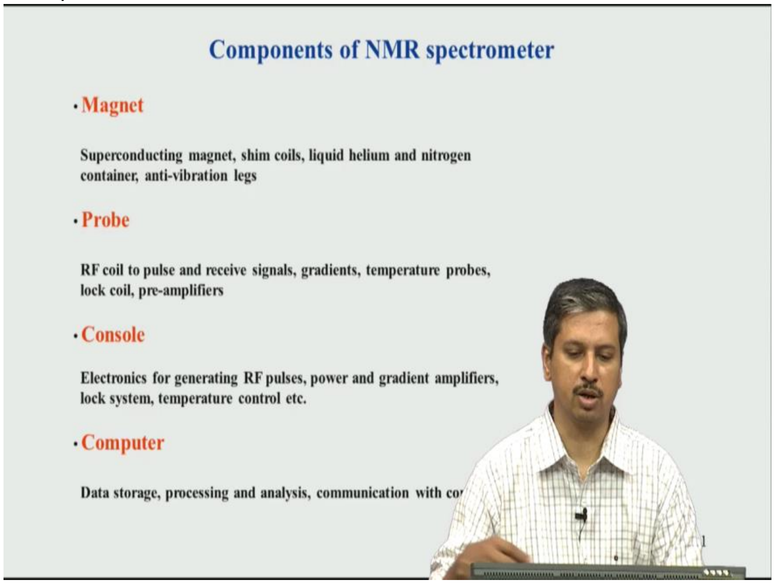
(Refer Slide Time: 0:57)




So NMR spectroscopy has actually moved a come a long way since this invention. So it was discovered, the phenomenon of NMR was discovered as we showed in the last class in 1945 and since then the NMR instruments have really become more and more compact and more and more modern. So you can see here some pictures of very old systems this is now historical and you can see how they developed. So the current day systems what we have nowadays is something which looks like this.

So you have what is called a magnet. So this is what is shown here, we will see more pictures of this as we move on. So this is this is a superconducting magnet and from here is where we keep the sample and there is this this portion, this box here, is electronic components of which controls the this the sample and so on thus experiments; and all this data is finally analyzed in a computer which is gives you the spectrum and all the computer Fourier transform etcetera is performed in the computer here. So you can see this is basically the basic setup of an NMR spectrometer. So let us look at each of these components in more detail.

(Refer Slide Time: 2:06)



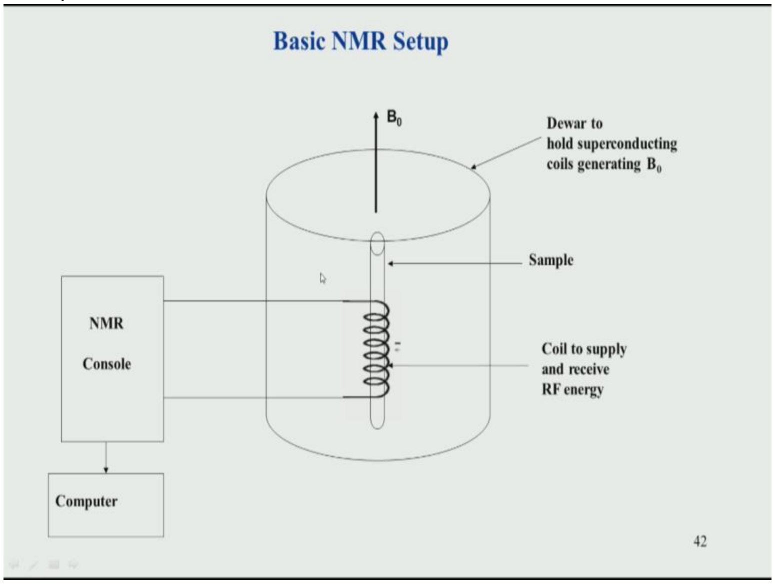

So typically an NMR spectrometer consists of the following components. You have what is called the vein the most heart of the NMR system is a magnet. So it is a superconducting magnet. We will look at this shortly. then there are something called shim coils, again we will talk about this in later slides. so the superconducting magnet, when we say superconducting, basically there is a coil which is at a superconducting state and that is kept in the state because of liquid helium.

So liquid helium is has a very low temperature at a very low temperature of 4 kelvin and at that temperature this material which current carries the coil current becomes super conducting and we will see that shortly.

The next major component of NMR spectrometer is probe. A probe is basically the part of the system which actually where you apply the RF pulse, you record the receive the signals and you control the temperature and so on. So we will also have a look at this components and all this probe and magnet whatever control we want to do is done by this electronics which is in the what is called as console. So console is basically a box which contains all the different boards different circuits and so on.

For generating the RF pulses, you also we have what is called gradient amplifiers and lock system and so on. And finally all this the data which comes out which is basically collected by this hardware is after digitization is stored in the computer which where we do the processing and we analyze the spectrum and that is how we come to look at the data.

(Refer Slide Time: 3:42)




So let us go one by one with each of the components. So this is basic schematic of a magnet. So as we can see magnet is basically a big deewar which looks like this and this contains, so this is of course not in the dimension of the real size. This is a schematic figure. So this particular thing we will see more in detail in next slide contains basically the magnet and in the middle of the magnet, there is a bore, a hole which through which we put the sample in and then the sample around the sample, the sample goes and sits inside the spectrometer, inside the magnet.

There it is surrounded by what is called a RF coil. So you can see this is a coil and this RF coil is basically the coil which where we apply the RF signal to the sample. This is used for transmitting the signal, transmitting the radiation to the sample as well as collecting the signal from the samples. So the same coil is used for both purposes and that now whatever the signal which is induced current FID which is induced in these coils are collected and digitized in the in the hardware in the in the console which has all the electronic components.

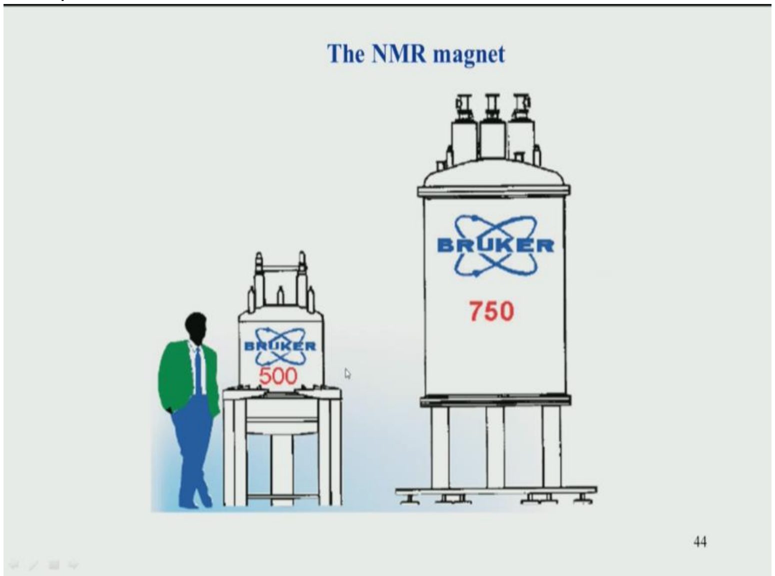
And from here the digitized data is sent to the computer where it is essentially the Fourier transformation and data processing is done. So let us look at more closely to how a magnet in the interior of a magnet looks like.

(Refer Slide Time: 5:04)


So these are some pictures of modern day NMR systems so this is different types you can see these are very huge ones which are tall magnets typically about fifteen, twelve to fifteen feet in case of high field. So you can see the numbers here, basically denote the megahertz. So if you recollect in the last few lectures we saw how does how is a magnet represented I mean the magnetic field strength. We typically talk in terms of proton frequency and the proton frequency is given as in the megahertz regime.

So this is nine hundred megahertz. This could be some seven hundred and so on. So this is some typical pictures of magnets in different labs around the world.

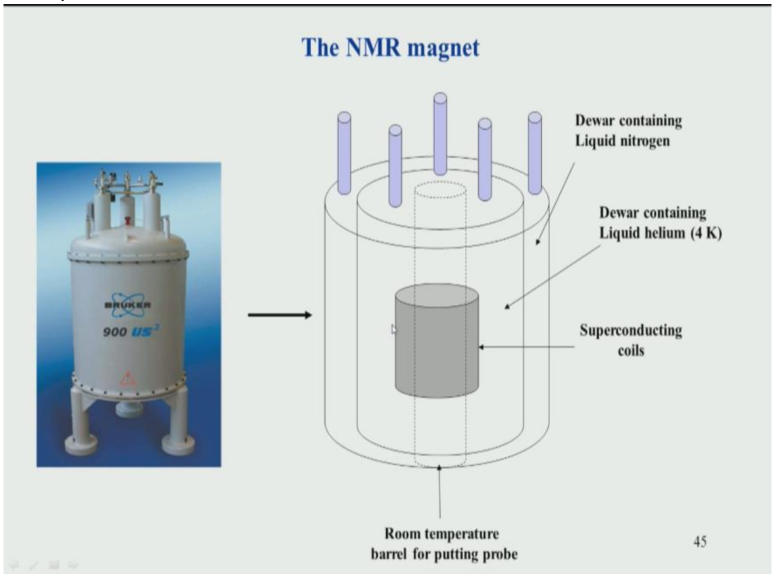
(Refer Slide Time: 5:42)




So if you look at if you want to get a feel of the size of this magnets of these systems, so you can see here; this is a five hundred megahertz machine and this is a person typically about let us say six feet tall. So this is the size of a magnet and when you go to higher fields, the magnet size goes up. So this is basically because as it goes up it comes to that. So this is this is the size of a bigger magnet.

The size goes up because the super conducting coil inside this magnet occupies a larger width compared to what is a smaller magnet. So we will see how these magnetic fields are generated in this particular systems.

(Refer Slide Time: 6:18)



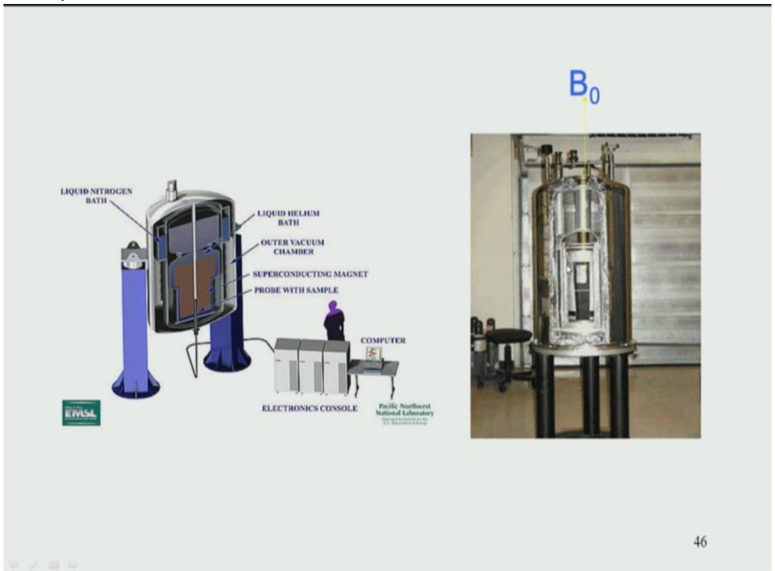

So what happens is that if you look at this again in this picture of the dewar, it has many concentric circles. So this has many concentric dewar inside its the main outside container. So the innermost container, the innermost dewar contains what is called as superconducting coils. So superconducting coils are basically coils which are wound around in a circle and these coils are dipped in liquid helium. So this particular is surrounded by liquid helium here.

So when the liquid helium in the presence of liquid helium which is typically 4 kelvin, this coil becomes superconducting means it has no resistance to the current flowing in the coil. And this basically causes a magnetic field. So whenever there is a current flowing in a coil, we have a magnetic field which is generated perpendicular to the plane of the coil and therefore if this plane of the coil is in this direction with the plane as its XY, we can consider this vertical as a z-axis and the z-axis that magnetic field is generated because of this current. Now the reason it is superconducting is because we need to generate very high currents to generate very high magnetic fields.

So typical magnetic field is about here is about nine tesla, ten tesla or even twenty tesla in the case of high field magnets. So for such high fields, you cannot have an electromagnet, which will generate a lot of heat and it cannot carry such high current density; and therefore we need superconductivity because in a superconducting state, there is no resistance and as a current depending on current density of the coil you can generate very high currents and very high magnetic field in the center. So in the all the modern day NMR machine systems are basically superconducting systems.

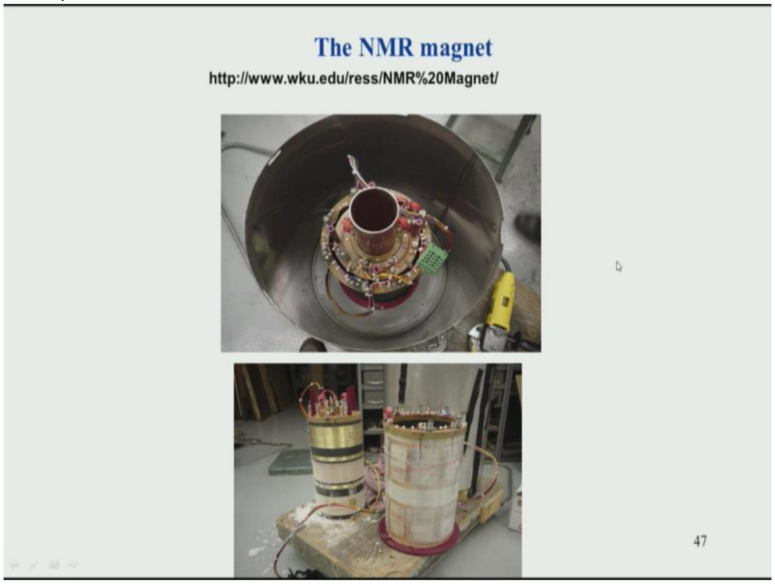
So this is how it is generate so there is a liquid helium surrounding this coil. We will see those pictures in the next slide.

(Refer Slide Time: 8:06)




So you can see this is a cutout of a magnet. So this is a old system which cutout from not functioning machine, so you can see the interior of this, it is like an anatomy of of a magnet. So if you see interiors, so this is you can see in different layers of the magnet one inside the other and you can see in the middle this this the bore here, this is called a bore and this hole runs from top of the magnet to the bottom and you can see the coil surrounding this bore.So this is basically the coil and we will look at more closely this particular coil in the next slide.

(Refer Slide Time: 8:39)



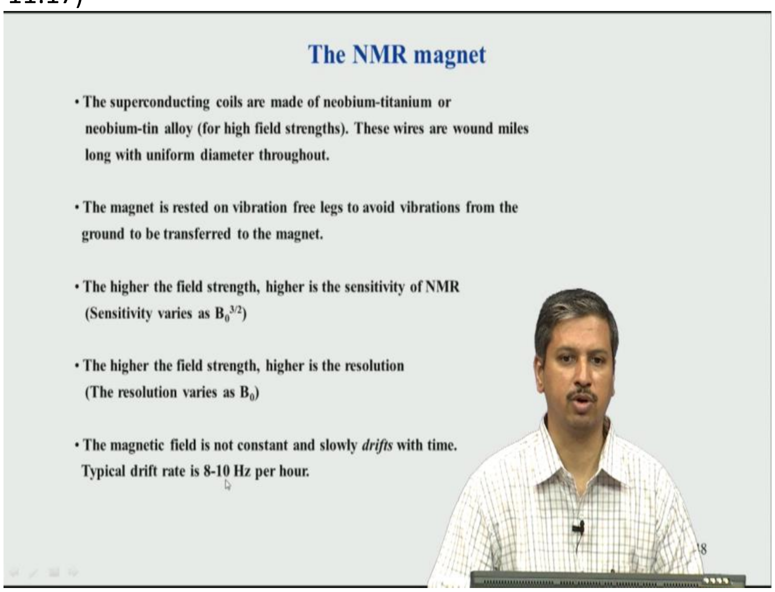

So here is what is shown in the picture so you can see that this is the how the coils are wound around the machine. So this is taken from this website this particular picture more details are also available if you log into this place (())(8:55) this is how the coil looks like and this is a very long coil which is wound very finely. So it is actually making NMR magnets is really a rocket science in that sense. It is very difficult to we have to make sure that there are no joints because joints you no generate resistance and that will create problems when the current starts flowing and these are the problems which very fine technology and this current this coils are typically about a few feet long and that is inserted into the inner most container.

So you see this container here which is surrounding this coil is a inner most and you fill liquid helium around this. And liquid helium basically keeps this whole current under coil in the super conducting state. And then around this this particular container you fill one more container which has a vacuum so that the heat so that this liquid helium which is inside this does not evaporate because we want the liquid helium to stay there and not evaporate very rapidly and therefore we surround it by a vacuum and then also we surround by liquid nitrogen which is another layer of dewar around this the liquid nitrogen helps in reducing the evaporation rate of liquid helium and then again you have a vacuum and so on.

So you have different concentric layers to prevent the liquid helium inside the inner most container to start evaporating, but of course one cannot avoid evaporation, so liquid helium very slowly evaporates and one has to refill helium every few months or few once in few months because their slow evaporation is always unavoidable. In the modern day there are systems where you can actually recirculate the liquid helium, so whatever is evaporated you can condense it and bring it back so you can have what is called a closed closed loop system a the closed system .

The only thing is it causes some kind of vibrations so it is not very popular these days in solution high resolution NMR. But when you go to MRI, MRI essentially relies on that and MRI system that is magnetic resonance imaging which is as I said it is similar to what is NMR, there those systems are the magnets are big and there the systems is closed system and the helium whatever is evaporated from here slowly is brought back condensing brought back. So this is how the NMR in the magnetic system works.

(Refer Slide Time: 11:17)




So let us move on to look at some of the features of this how does the NMR magnet affect the sensitivity of NMR spectroscopy. So these coils which I just now mentioned are surrounded which are super conducting they are basically made of neobium, titanium or neobium tin alloy. And making this alloys and this coil as I said is a very hot area of research people are working on this because we have reached kind of a limit to generate very high magnetic field. So there is a lot of work being done on how to increase a magnetic field.

So there is a (lo) there is a work going on in the area of finding new types of alloys or high TC superconductors which can carry instead of liquid helium we can do it liquid nitrogen temperature. So that is one of the areas of research which is happening. And these magnets have to be on the vibration free because what happens is our ground even though we do not feel the vibrations there is always some kind of motion of the of the ground and that is transferred to the magnet and if that is transferred to the magnet the coil then there is lot of loss in signal to noise or the increase in the drift of the magnet and so.

So therefore the system has to be kept on a vibration free legs, so this is a very important part of the whole machine and then one has to keep in mind that how does this help how does it help to have bigger and bigger magnets. So when we say magnet, we talk about magnetic field and that is given by this letter B0 this is what we have been using in the last few lectures. We have been seeing that, so when you increase B0 the magnetic field increases by 2 the power 3 by 2. So this is a important point here, it is not just linear increase it is increase by factor of 3 by 2 and this is tremendous, so if you go from 500 megahertz to 800 megahertz although you have gone only by 60 percent you actually doubled the sensitivity.

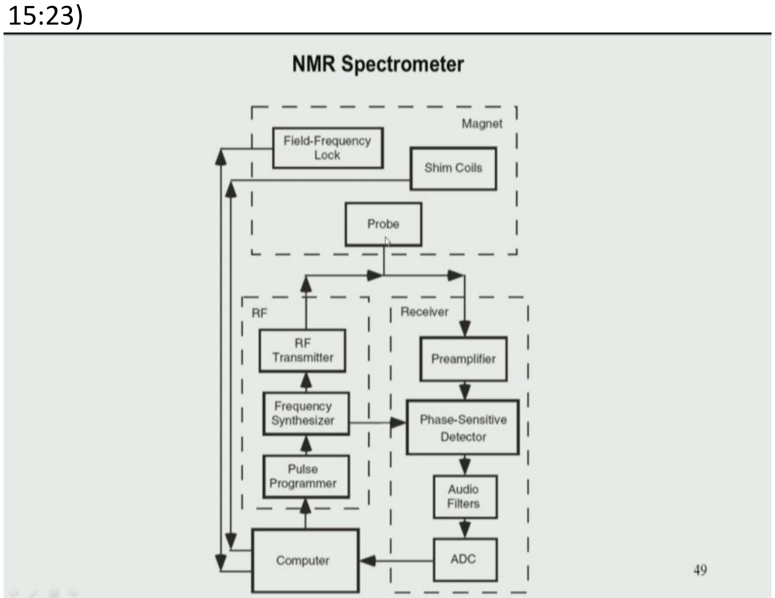
So sensitivity is basically the holy grail in NMR, there is always a there is a push for going to higher and higher sensitivity and therefore lot of money has and research goes on in how to increase the magnetic field and that is the major focus so more the frequency, the other thing which we will see later along when you go along the course is that as we increase B0, one more important gain what you get is resolution. So not only increasing the magnetic field improves this the sensitivity by this factor but it also increases resolution linearly.

So here it is a straight linear relation, so as you double the magnetic field you double the resolution. And what is a resolution? Resolution is basically how easily can you separate two lines two peaks in NMR spectrum. So we will see that more in the coming slides and one important part of magnetic field is even though we say that it is particular tesla let us say we make a magnet with nine tesla, it never remains at nine tesla throughout it slowly starts losing because remember it is a current magnetic generated by current it is not permanent magnet, it is a super conducting magnet where the current is flowing in the coil and this results in slow loss of magnetic field with respect to with function of time.

Typically the drift we use the word drift for this case and we will use this words later on how we improve how we correct for this drift and this drift is typically 8 to 10 hertz per hour. And this is a very important point is because in one hour what it this means is in one hour the magnet has become less by 10 hertz, so it is 900 megahertz it loses 10 hertz let us say in one hour. So this is typically the drift rate for a good magnet for a new magnet and the reason is the lines which we observed the peaks we observed in NMR spectrum is also typically of this magnitude.

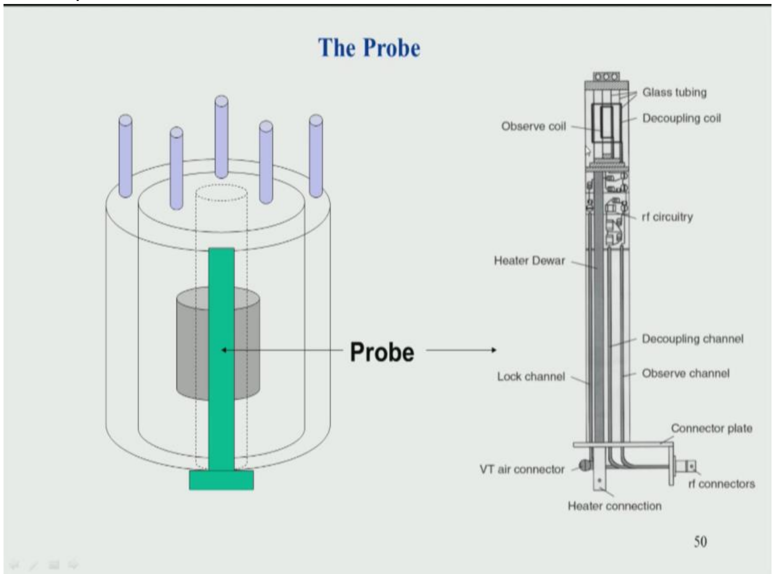
So therefore, if you do not correct for this drift our lines will have an additional 10 hertz broadening increase and that causes resolution problem and therefore there is a mechanism to correct for this drift in real time. So the question the thing is we are correcting for the drift in the spectrum, but we do not correct it in reality, in the sense the magnetic field continues to change to drift but internally we try to correct it by applying a mechanism which we will see that.

(Refer Slide Time: 15:23)




So this is the schematic of the console which I mentioned which contains the hardware the electronics part, so you can see there are different components we will not be able to go in detail for every each and every component, but the major three things which we will look at today is basically this called a lock system and this is called shim coils and a probe. So let us look at a probe first, what is a probe?

(Refer Slide Time: 15:48)

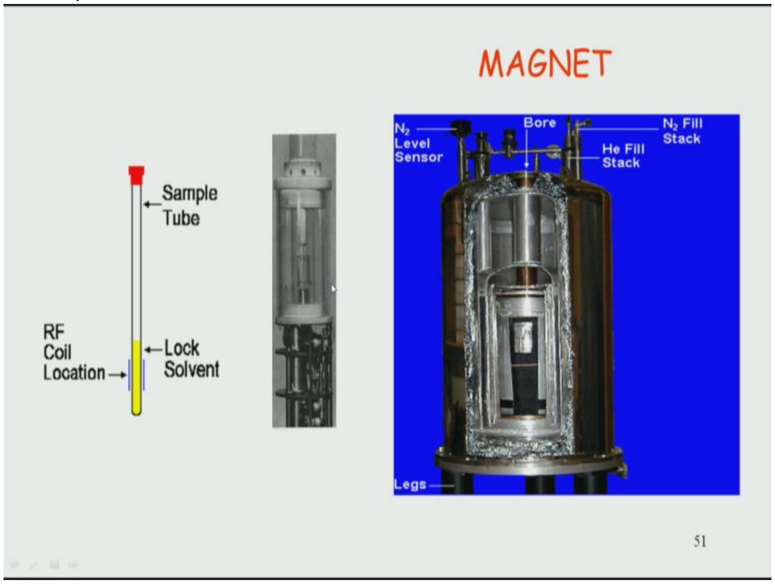



A probe if you again refer to this diagram which we looked at for the magnet in the center of the magnet there is what is called a bore a hole. So that bore runs all the way from top to the bottom and that bore is what contains a thing called probe. So this is green color thing which is showed in a t shape this is what is shown in real picture of a probe look like this. so probe is essentially a the hardware part where we put the samples. So you see this arrow where it is pointing now this is where the sample is stored.

So when you drop the sample we start drop it from here from top of the magnet it comes all the way down and it sits somewhere here and that this part is this part here this black color here the coil. So you can see this is called a RF coil the RF coil is the one which now in the sample is sitting in the middle the RF coil generates energy I mean transmits the RF radiation to the sample and also receives the generates receives the energy from the sample the signal from the sample.

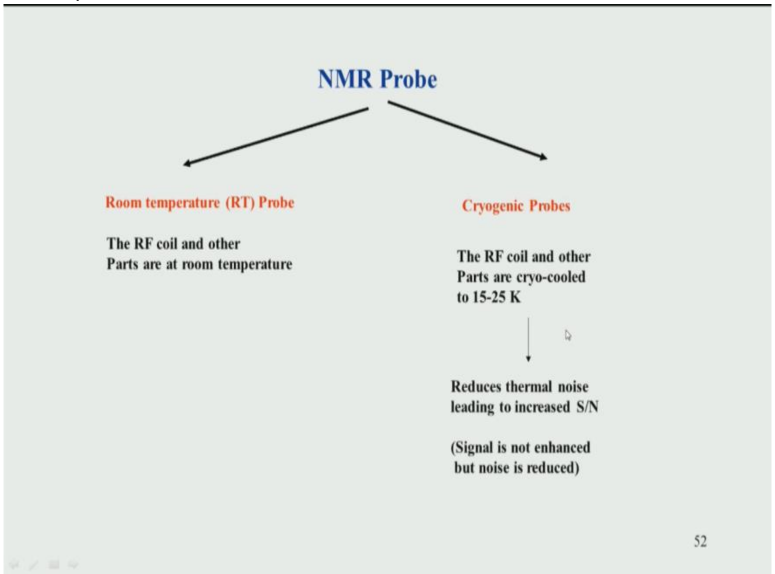
And this signal is further converted and this is how these are different capacitors and resistance and this is this signal which comes out is then digitized and further taken into the next portion part of the spectrometer.So this is probe is a very important part because this is where we actually apply energy to the sample and that is where the resonance concept comes into picture.

(Refer Slide Time: 17:09)




So we look at more of this probe. So this is how a sample tube sample looks like. So this is how we insert a sample. So this typically the height of this tube what you see here is about 6 to 7 inches and the sample is at the bottom part of this tube. So this is how a sample will look like and this is how a tube look like looks like and then you insert this in the top of the magnet and it comes all the way down and it sits here and in the probe. So this is again a figure drawing of a probe.

(Refer Slide Time: 17:38)

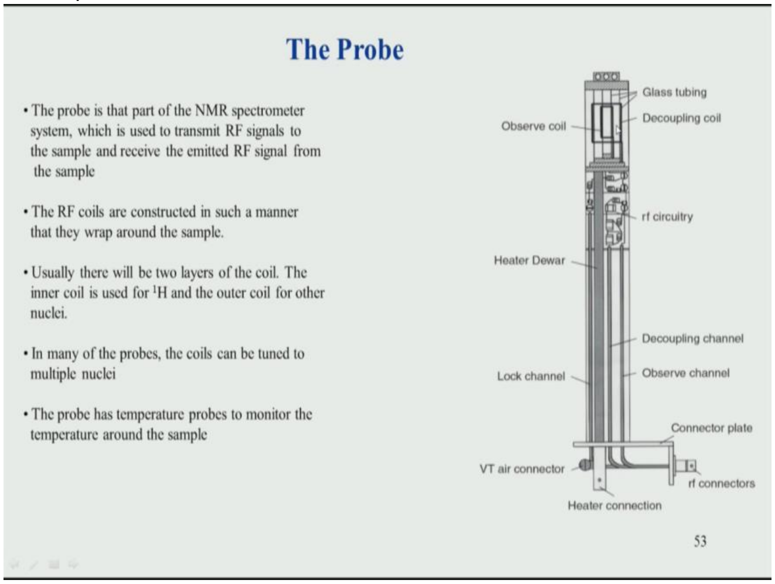


So in the modern day spectrometers NMR spectrometers there are two types of probes which when should know one is called a room temperature probe room temperature as the name suggest it basically the probe is at the room temperature means it is operating at a regular temperature it can increase little bit higher it can go to higher temperature or it can be lower to probably zero degrees or little lower also. So this is called room temperature probe because the probe as such is **is** at room temperature and the sample temperature can be varied.

But this is a standard (op) operation but now a days we have what is called very sensitive cryogenic probes. So in this kinds of probes what happens is this the RF coil which we saw in the previous slide is actually cooled it is not in room temperature it is cooled to a very low temperature by helium gas. So that is the gas this is not liquid and the helium gas is typically kept at this temperature of 15 to 25 kelvin and why is that done? This is done because, when you cool an electronic system, you decrease the noise, the noise which is comes from thermal noise is reduced and therefore when you look at signal to noise, if the denominator is the noise is goes down, the signal to noise goes up because noise in denominator.

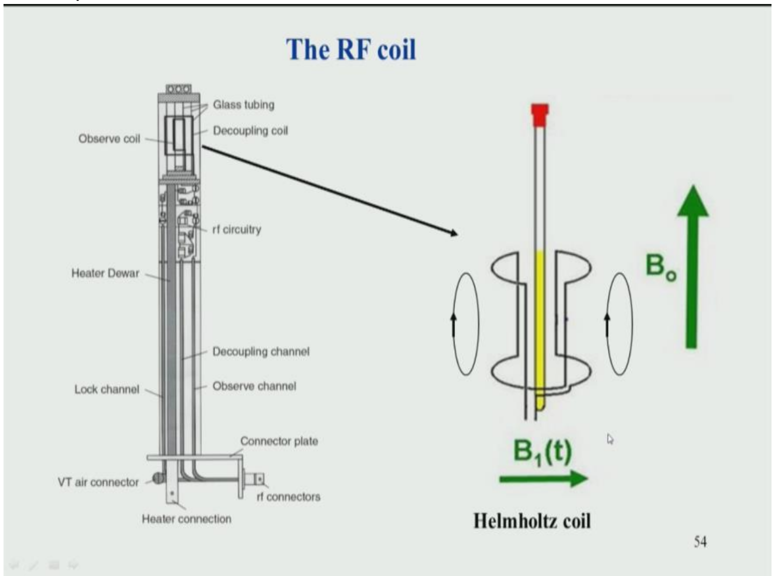
So this is what basically we are trying to achieve the signal is not changed the sample signal remember signal comes from the sample we will look at this also later, the signal comes from the sample and the magnet the signal the signal height but the noise is essentially because of the different contribution from thermal noise and electronics and that is reduced if you go down to low temperatures, so we increase signal to noise so this is very important concept because this is what the modern day probes spectrometers at high field. So when you go beyond 500 megahertz like 600, 700, 800 and a gigahertz those kind of system invariably now a days are come with cryogenic probes.

(Refer Slide Time: 19:30)



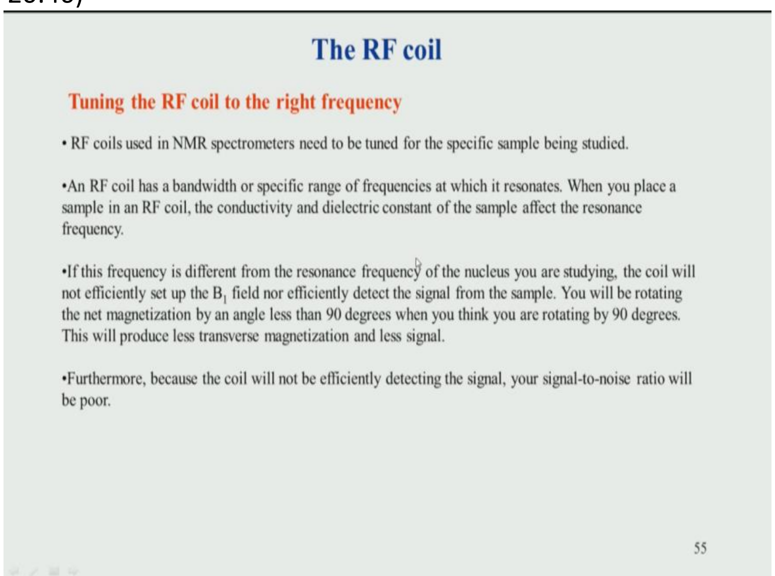

So again this is more detail about a probe how it looks like so the basically the main point here is that this coils they actually wrap around the sample. So you cannot have the sample is very tightly wrapped around by this coil and that is called a filling factor and these are different more technical details. So essentially the point here is that the sample is very close to the coil and whatever signal is given emitted from the sample is immediately taken up by the coil which is in the vicinity.

(Refer Slide Time: 20:01)



So this is this is the picture of a coil so this is Helmholtz coil, so you see this is how the coil is build and the sample is sitting in the centre that is how we insert the sample and the effective volume of the sample is shown here. So and this transmits the current in the perpendicular directions. So if you can remember this idea which we discussed in the last class the RF radiation which is applied is always applied perpendicular to B0 so you see the B0 is facing in the z axis in the vertical z direction and the current we the radiation RF pulse we apply is always perpendicular to B0. So it can be either in x direction or y but this is will be always in the z, so this is how these currents are applied.

(Refer Slide Time: 20:46)

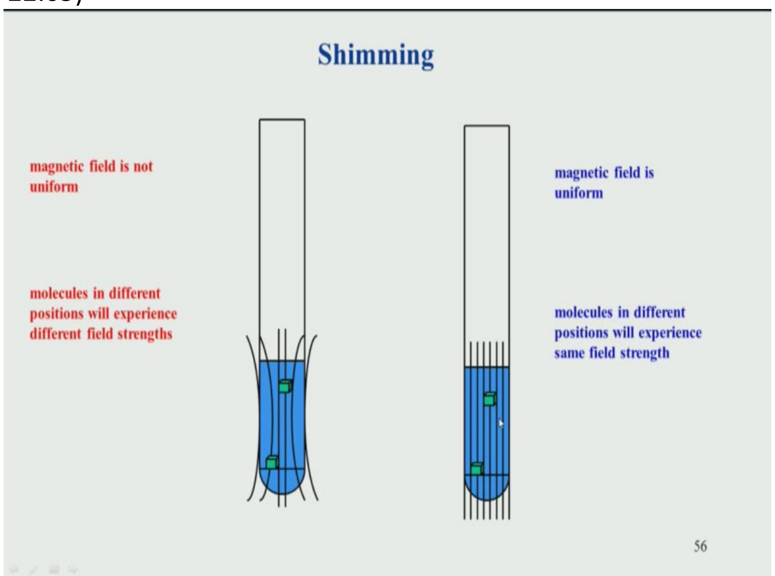



yeah The RF coils actually again depends on what kind of nucleus you are studying, so sometimes we study hydrogen we are looking at proton, we look at carbon 13, we may look at florin 19 florin or we may be studying phosphorous and silver and so on. So there are verities as we saw in very first class that this is called NMR periodic table, here the entire periodic table many elements are available to NMR spectroscopy and therefore one has to now generate frequencies which are tuned to the frequency of a particular nucleus.

For example, when I say 500 megahertz 500 megahertz is basically a frequency of a hydrogen,. But for carbon it will not be 500 it will be 125 megahertz, similarly for nitrogen it will be 50 megahertz. So therefore for different type of nucleus we need to have different types of frequencies and all this is done with the single probe, the same probe has to be used for different nuclei we do not change the probe. So therefore each time when you study carbon 13 or you study nitrogen florin or nitrogen you will have to tune it is like a radio like we tune the radio to different frequencies.

Similarly the coils have to be tuned to different frequencies so that we get maximum benefit or sensitivity for studying that particular kind of coil. So this is a basic idea behind RF coil.

(Refer Slide Time: 22:05)



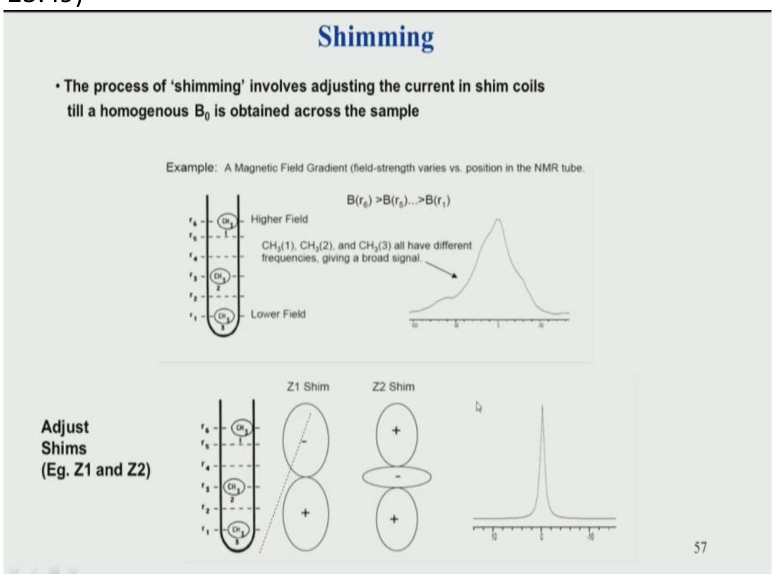

And the next important thing in NMR is what is called shimming. in shimming what is what happens is the following, so you have this is again a schematic of a sample which goes and sits in the centre of the magnet and here what happens is you there are magnet which is whole sample is now surrounded by magnetic field, remember the magnetic field is throughout and it is in the z direction.

But you can see in this picture the molecule let us say this sample contains some molecules and one molecule sitting in the bottom of the tube experiences some kind of a magnetic field which is not the same as the magnetic field generate the experienced by molecule here. Therefore these two molecules will not have the same magnetic field and because of this equation omega a equal to gamma B0 if B0 is different here and here, then omega also becomes different here and here and that basically causes line broadening the lines will look broad we will see that in the next slide also.

So to avoid that kind of a difference we have to make the system uniform means the homogenous the magnetic field has to be same every part of the sample similar. So remember this blue portion which is shown here is only about 2 centimetres long which is 20 millimetre. So in that particular portion of the sample we want to keep the magnetic field as homogenous or as same throughout the sample. And that process of going from this inhomogeneity to this homogenous say state is known as shimming.

So shimming is a word used for so this is basically done by electronics we have lot of coils which are sets around sitting in the sample around the sample and those RF this coils this are not the RF coils, but this are called shim coils. So this shim coils are are operated in electronic in automated manner to generate this particular homogeneity.

(Refer Slide Time: 23:49)

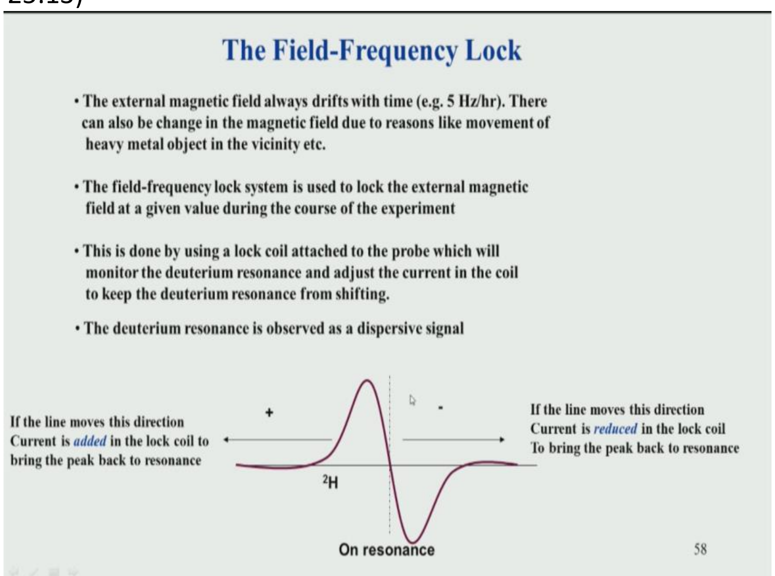



So this is a very important point which is shown here you can see this is our NMR sample tube and here the different let us say we are looking at a particular molecules so we are looking at CH3 and different CH3 is at same molecules CH3 here sitting here one molecule another molecule is here another molecule is here. And because they are in located in different parts of the sample if the homogeneity is not good, then each CH3 will have a different frequency because the each CH3 has a different B value.

So because of that you get something like this peak which looks very broad and difficult to analyse, but if I do a shimming in a shimming process we actually make the magnetic field equal everywhere. So that is what we saw in the last slide and if you do that with different shim coils so here for example there is a shim coil name given Z1 shim, Z2 shim etcetera by doing those kind of shimming you get a very nice line shape and this is very sharp and this is what is very important for analysing.

So we cannot analyse or interpret this kind of spectrum in solution remember we are all talking about this in solution state. In solid state the matter is different where the lines are inherently broad, but we are only talking about solution NMR and there you can see that this particular line is very sharp line and can be analysed. So shimming is very important process and nowadays is done automatically by the computer with very less of manual intervention.

(Refer Slide Time: 25:13)




So the second the thing the other thing which we looked at I showed you what is called lock. So this is this is required because remember I mentioned in the last few slides that the magnetic fields drifts with time, no magnetic is field can be constant throughout. So it always drifts to a some extent sometime it is as low as 5, sometimes it can as high as 20 hertz per hour. So because our the lines which we are studying the peaks which we are studying are very narrow we cannot afford to have this drift happening. So we have to correct for this drift.

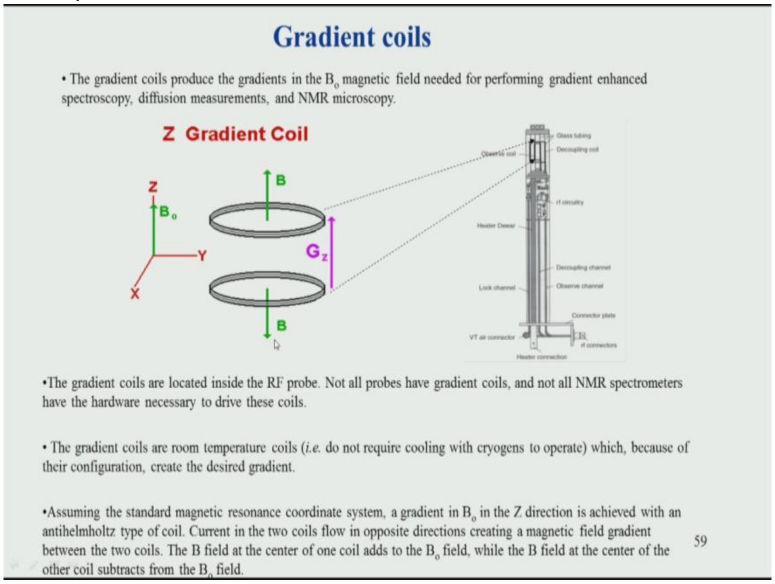
So how do we correct for this drift? This drift is corrected as follows. So what you do is in your sample you have to add a little amount of deuterium, so for example many of the solvents which we use like if we use water, you can add deuterium oxide D2O which is a heavy water that is just a little bit it has to be about 5 percent not not more than 5 percent or you can use completely a deuterated solvent like for example 100 percent of D2O. typically in the organic chemistry people use this solvents called methanol or chloroform CHCL3 in a chloroform you can have a CDCL3 means H is replaced by deuterium.

So the whole solvent is now deuterated. So what you do next is the system looks at a deuterium signal, so a deuterium signal looks something like this and this signal is monitored with respect to time. So if the magnetic field drifts that means a magnetic field is reduces, then this line will also shift because the magnetic field remember omega is equal to gamma B0. So if omega B0 changes omega will also change and omega is nothing but frequency. So this peak starts moving around if there is a drift in the magnet.

So what is done is there is a correction done (())(26:53) current is supplied to a coil the lock coil which is the word used look coil here, such that this **this** drift is subtracted. For example if it moves in the left direction a current is supplied so that this peak goes back to the this direction because when you supply a current, you are applying a magnetic field. So if the main magnetic field shifts by some amount, you can nullify it by applying a small current in the negative direction and move the peak back.

Similarly if the peak moves in this direction you can apply a little extra current so that you add to the magnetic field and it comes back here. So this is done in real time, that means when you are recording your spectrum this continuously keeps happening in the background and that is called a lock drift correction. So lock is basically the word lock means you are locking the magnetic field to a particular value and this a very important point because this has to done for every sample every time we record NMR data and again this is done typically in solution state because the drift which I said matters most in solution not in solid state. So this lock correction is mainly applicable to solution NMR.

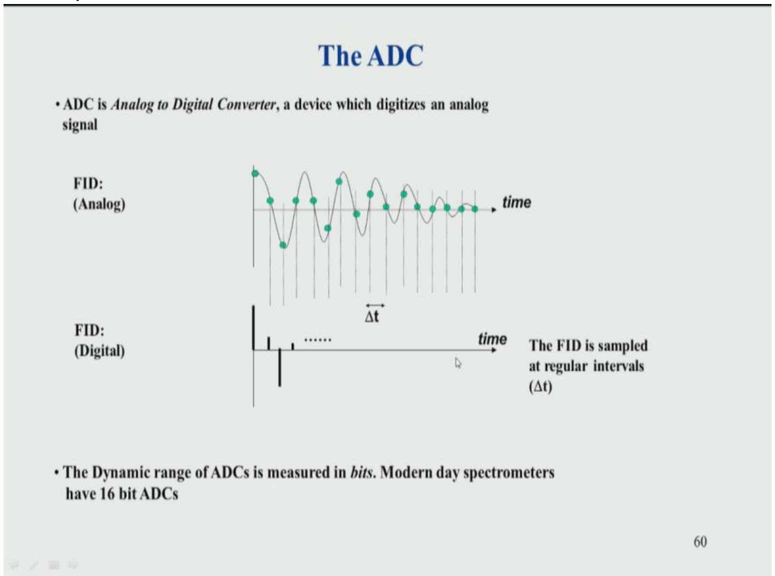
(Refer Slide Time: 27:57)

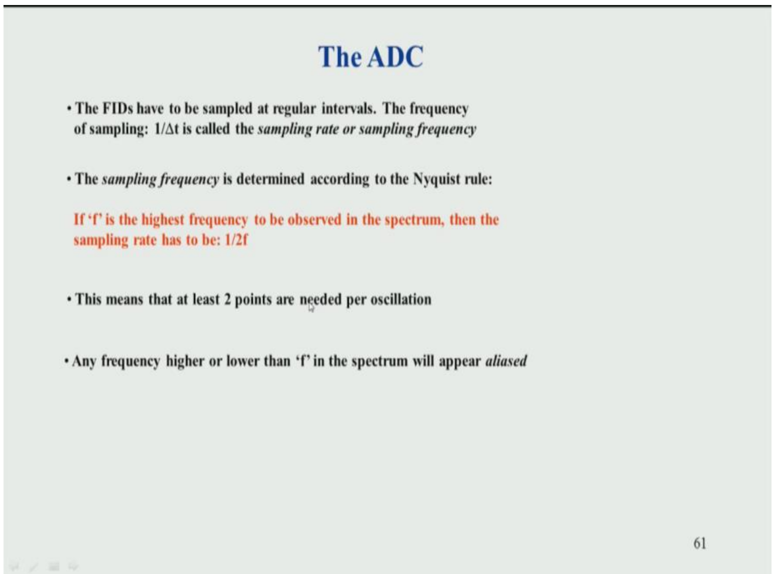



So there is another important thing is called gradience if you again in MRI we rely on images. So this is mainly helpful in MRI as well as in new NMR experiments, the NMR experiments we want to do sometimes what is called solvent separation, water separation as we go on we will see this more in detail, there we have a small gradient this is gradient is nothing but a coil is a RF coil. So in the RF coil you see here is small gradient which is a current is flowing in this coil a current is flowing in this coil which generates the magnetic field.

So you see this is very similar to the superconducting, but it is not a superconducting coil it is a room temperature in a small magnetic field is generated in the z axis. So we will see the applications of this in the later.

(Refer Slide Time: 28:41)




And finally when we look at the FID so this is how the FID comes out of the of the spectrometer and you see the spectrometer gives you signal like this this is called an FID, and I mentioned in the last class we actually digitized the FID. So the FID is actually taken at every point here and this is called digitization and this is how the signal will look like. So you see this is like a sine wave but this is now discretized digitized and the distance between two points between two subsequent successive points is known as dual time.

And this dual time is again very important parameter when you setup an NMR experiments, so this is very useful to keep in mind and this FID is sampled, so we use a word sampling we sample at regular intervals. So this is the finally this little data is what is given to the computer and that is how that is finally what is Fourier transform and a spectrum is given to you.

So this digitization is essentially carried out by what is called an ADC, analog to digital converter. So this is how we digitize a signal FID and it is stored in the computer. So this basically brings us to the end of the hardware this was a very brief overview of what at hardware NMR hardware looks like there are many more details which one can go into but far as far as particle aspects are concerned, we have looked at the main components what now we will move on to the interpretation in the next class we will move on to the interpretation of 1d how a simple 1d experiment spectrum looks like and we will start looking at the different signatures in a 1d.