INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

NPTEL

NPTEL ONLINE CERTIFICATION COURSE

Biomedical Nanotechnology

Lec-05
Characterisation of Nanoparticles

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Hello everyone I welcome all to the fifth lecture of this course. So today we are going to see characterization of nanomaterials. So there are several methods available for characterization of nanomaterials.

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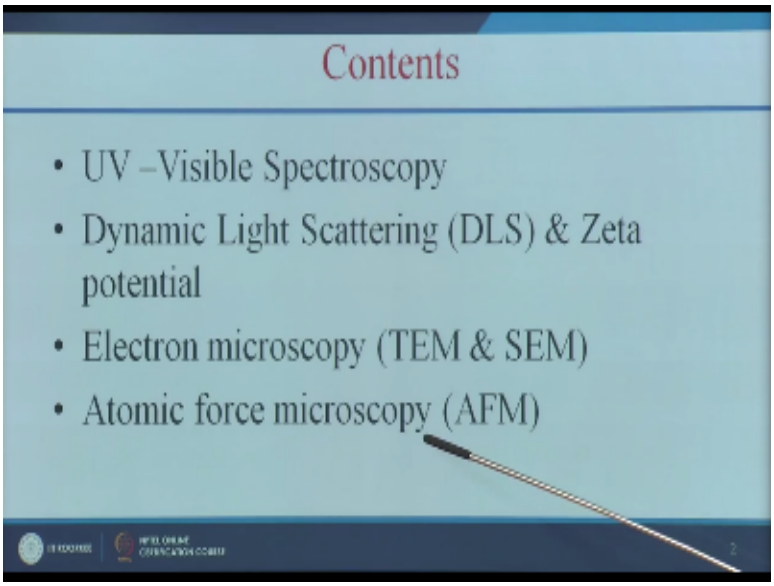

But in this lecture we are going to focus on UV visible spectroscopy and dynamic light scattering DLS and zeta potential, and we are also going to focus on electron microscopy like TEM and SEM, and atomic force microscopy. So when you synthesis any metal nanoparticle the first thing you observe is formation of color. So what is the reason for the beautiful colors, the reason is surface Plasmon resonance.

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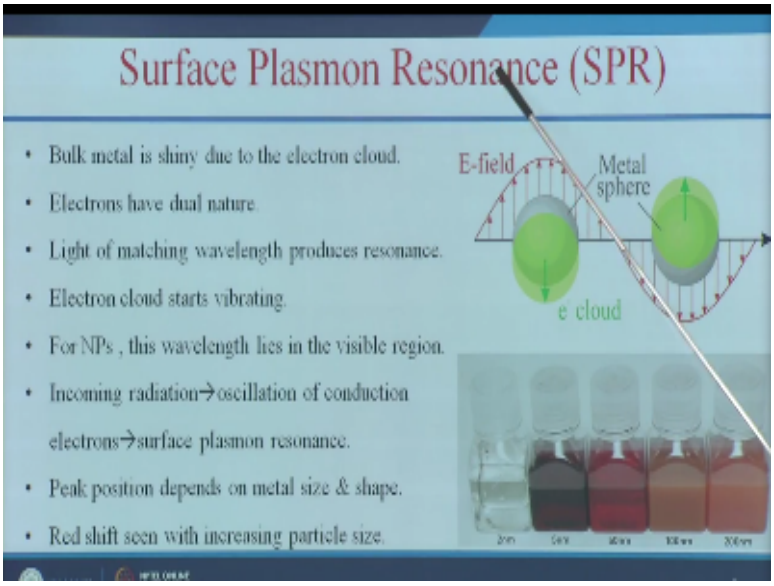

So in the previous lectures I have already explained what is surface Plasmon resonance, let us see once again. So usually the bulk metal is shiny due to the electron cloud. So these electrons have dual nature, so the light of matching wavelength produces the resonance then electron clouds start vibrating. So for nanoparticles this wavelength lies in the visible region, so that is why you are getting these kinds of colors.

So when the incoming radiation induces the oscillation of conduction electrons which leads to surface Plasmon resonance. And when you study these metal nanoparticles under the UV visible spectroscopy you will get different kind of peaks depends on the size as well as shape of the nanoparticle. And there will be rd shift with the increasing in the particle size.

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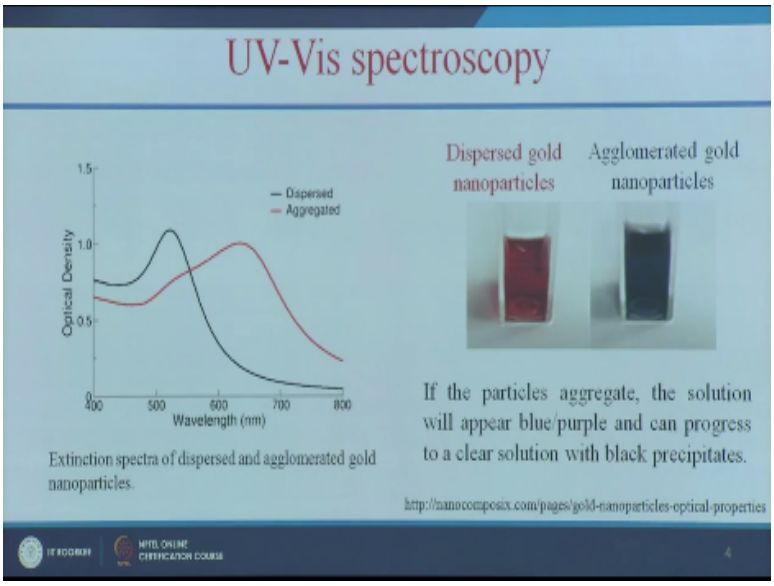

So when you study the metal nanoparticle in UV visible spectroscopy for example you can take the example of gold nanoparticles, so usually the gold nanoparticle is ruby red color, and if that gold nanoparticles agglomerated or aggregated it will be like a purple color and if it is studied under the UV visible spectroscopy usually there are disposed gold nanoparticles you will get peak at around 550 nanometer and the agglomerated or aggregated particle it will give peak at around 650 nanometer. So any metal nanoparticle the first step of characterization is your UV visible spectroscopy.

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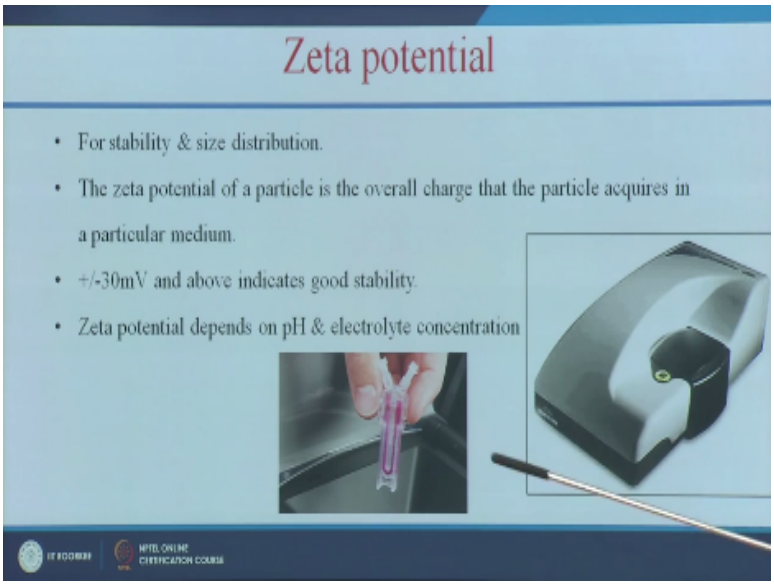

And for any nanoparticle the stability and size distribution is very, very important okay. The stability of the nanoparticle can be studied by using zeta potential measurement. So what is zeta potential, the zeta potential of the particle is the overall charge that the particle acquires in a particular medium that is called as zeta potential. And the zeta potential balance should be +/- 30mV, and or it should be above the +/-30, so that indicates the good stability of the nanoparticle.

And the zeta potential depends on the pH and electrolyte concentration of the particular material. And it is zeta or size equipment, so and for zeta potential we have to use this kind of cubit and when you put this cubit inside this zeta size it will give the zeta potential of your nanoparticles.

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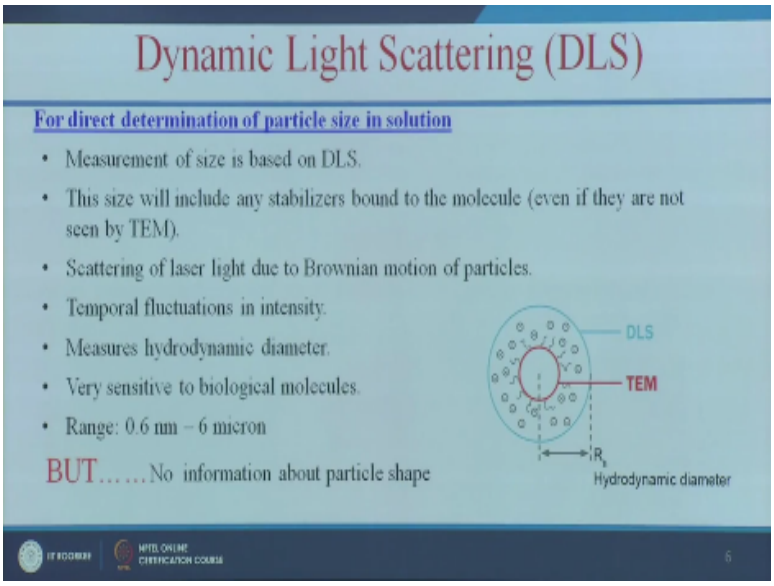

So let us see dynamic light scattering, using this same equipment we can also measure the dynamic light scattering of the particles. So this is for direct determination of particle size in solution. If you want to measure the particle size in solution we have to use the DLS that is called the dynamic light scattering. So here the measurement is based on the principle of DLS. And here the size of the nanoparticle will include even the stabilizers bound to the molecule okay.

So which cannot be seen by the TEM, and here the principle is the scattering of laser light is due to the Brownian motion of the particles, so due to it what happens is there will be a temporal fluctuation\ in intensity, so which measure this hydrodynamic diameter of your particle. And it is very, very sensitive to other biological molecules and it can measure 0.6 nanometer to 6 micron, but the drawback of this DLS is we cannot get any information about the particle shape okay.

So we can see here this picture in transmission electron microscope TEM you will get the particle size exact particle size but in case of DLS that dynamic light scattering you will get the hydrodynamic diameter okay so the particle size will be slightly bigger because you are determining the particle size in the solution.

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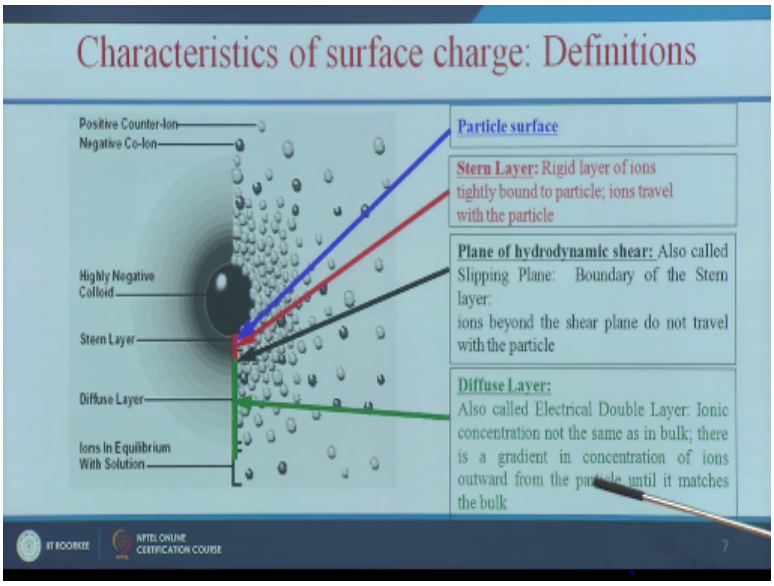

So before we understand what is vita potential let us see some of the definitions so this is your highly negative colloid metal particle okay so this is your particle surface the blue color is the particle surface and the red color is the stern layer this stern layer is a rigid layer of irons which is tightly bound to the particle and these irons travel with the particle and this black color minor line is also called as slipping plane or it is a plane of hydrodynamic shape this is the boundary of the stern layer.

And irons beyond this stern plane do not travel with the particle and this green color is defused layer also called electrical double layer so here the ironic concentration not the same as in the bulk so there is a gradient in concentration of irons out word from the particle until it match the bulk.

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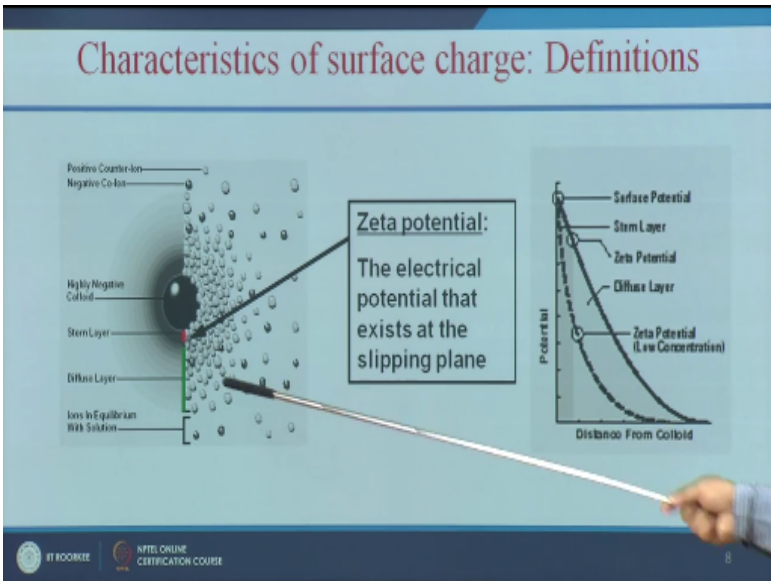

So let us see what is zeta potential the electrical potential, that exits at the slipping plane is called as Zeta potential.

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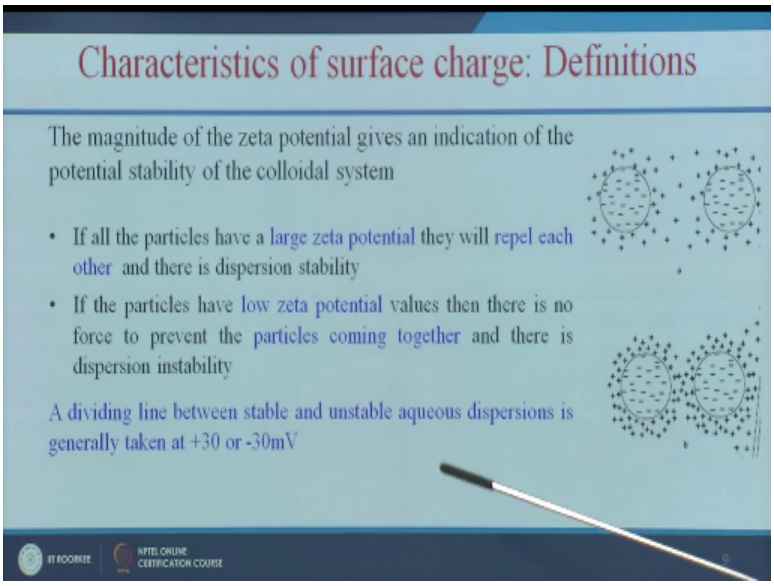

And this zeta potential mainly depends on the stabilizing agent as well as the coating agent or capping agent okay so the magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. Suppose if all the particles have large zeta potential that means if it is more than + 30 or if it is more -30 that is it will repel each other so the particles having uniform charges will repeal each and there is at dispersion stability.

Suppose if the particles have low zeta potential value that is know force to prevent the particles coming together so the particles come together and it will aggregate or agglomerate okay so that is why there is there is as they made dividing lines between stable and unstable aqueous dispersion so which is+30 or -30 mV so we have your zeta potential +30 or -30 or above that means your particle is highly stable nano practical.

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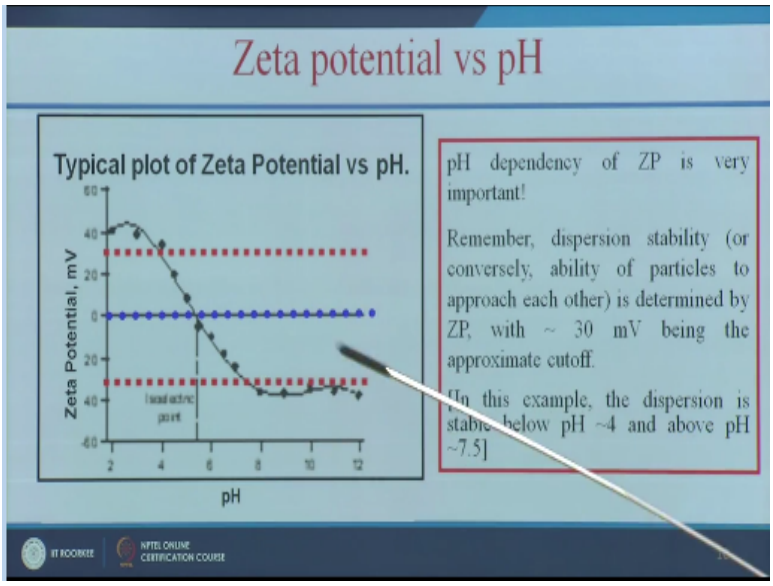

And again this zeta potential is highly depend in the pH so in this example you can see here so as I told you earlier so the +30 or -30 above is a stability of nano particle being very high very good so in this example you can see here so the pH below 4 as well as pH as well as pH above 7.5 then the nano particle is stable in this example. So let us see how we can use microscopy as means of nano characterization.

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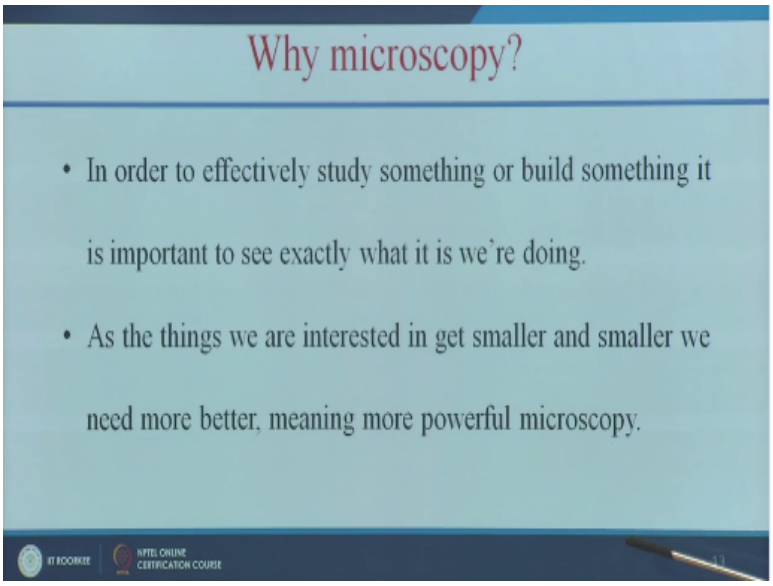

So first what is microscopy so microscopy is any technique for producing visible images of structures or details which is small or otherwise we cannot see weather human eye okay so why we need microscope because in order to effectively study something or build something we need microscope okays o we can understand the structure shape and everything further we can understand a structure we can modify the structure or shapes okay.

So as the things we are interested is getting smaller and smaller we need a more and powerful microscope to understand the nano structures.

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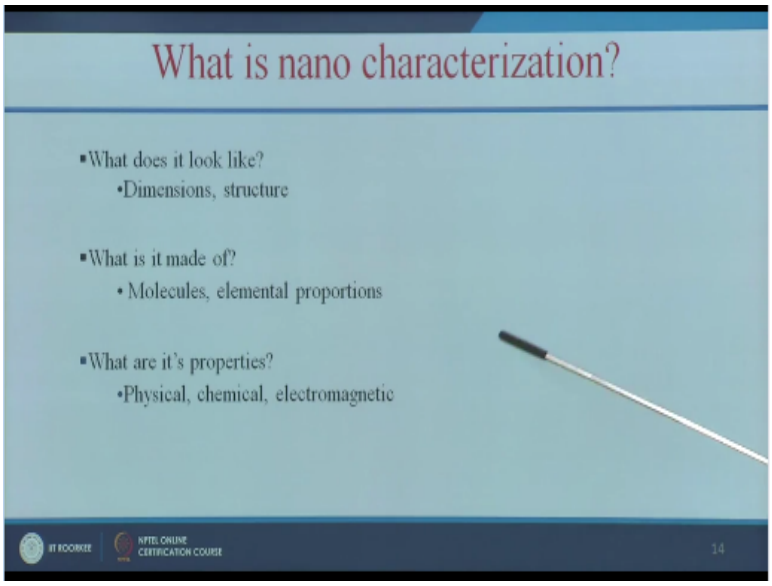

So what are the things you will understand by this nano characterization so you will get a idea about what is the dimension and what is the structure of the nano particles ad also you will get a idea of what kind of materials is made up of what are the elemental proportions and what are the physical chemical properties so these are the things you can understand by nano characterization.

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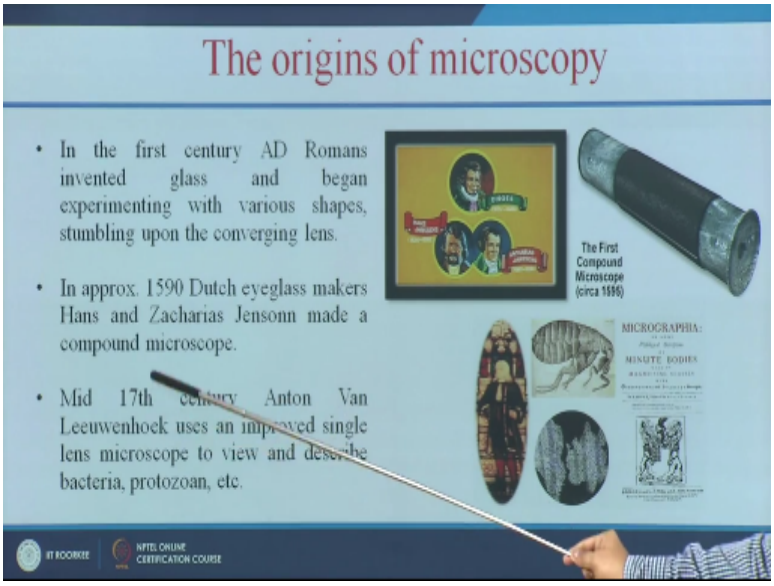

So let us barely see the origin of microscopy so in the first century Romans invited the glass okay and they are experimenting with various shapes and converging lens in 1590 Dutch eyeglass maker so they made a compound microscope and in the mid of the 17th century Anton Van Leeuwenhock he first absorbed the bacteria and protozoan under the small microscope simple microscope.

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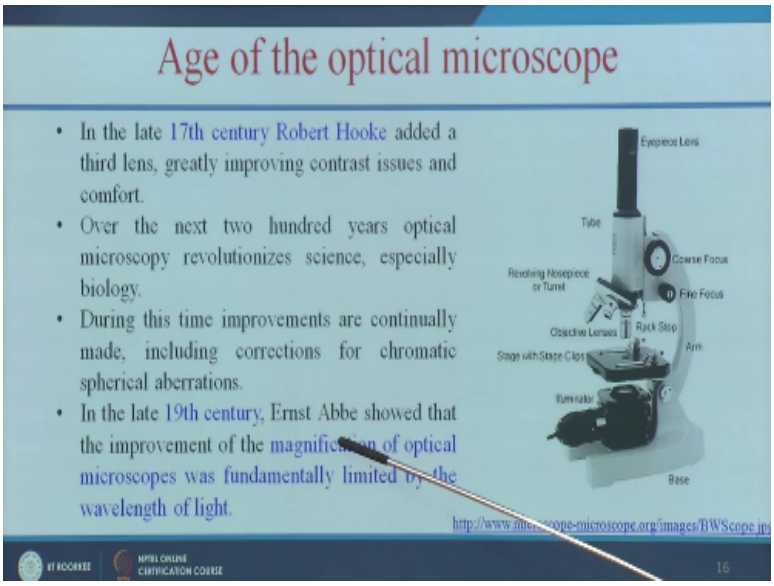

And in the later in the 17th century Robert Hooke so he made lot of modification to the microscope and he made a kind of compound microscope okay so the next 20 years with optical microscope it played in many major role in the biology and other fields okay so in the late 19th century Ernst showed that improvement of the magnification of the optical microscopes as fundamentally limited by the wave length of the light so the wave length of the light is very important and deciding your magnification of your optical microscopes.

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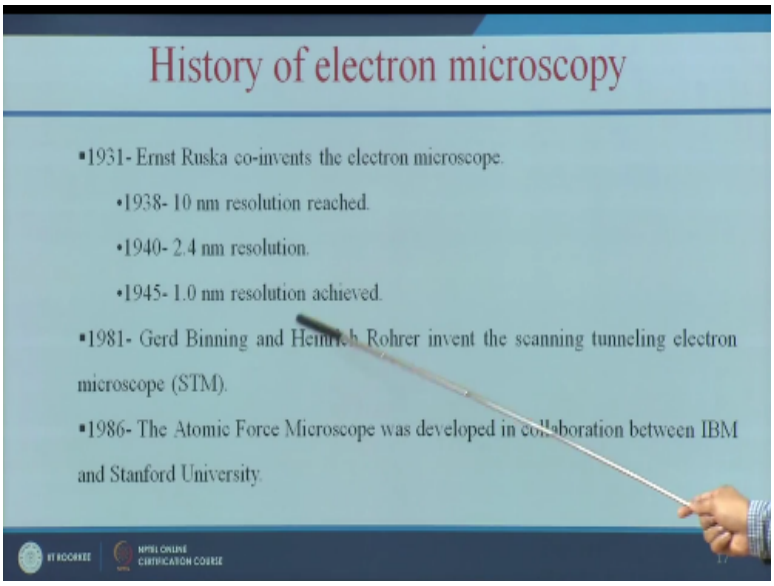

So that is why they move to electron microscope okay so in 1931 Ernst Ruska they invents the electron microscope and in 1938 you think that the pm electron microscope they reach the resolution of 10 nanometer and 1940 2.4nm resolution and 45 the range 1 nm resolution and 1981 so this people how invented scanning tunneling microscope and in 1986 atomic force microscope all develop a by the same group.

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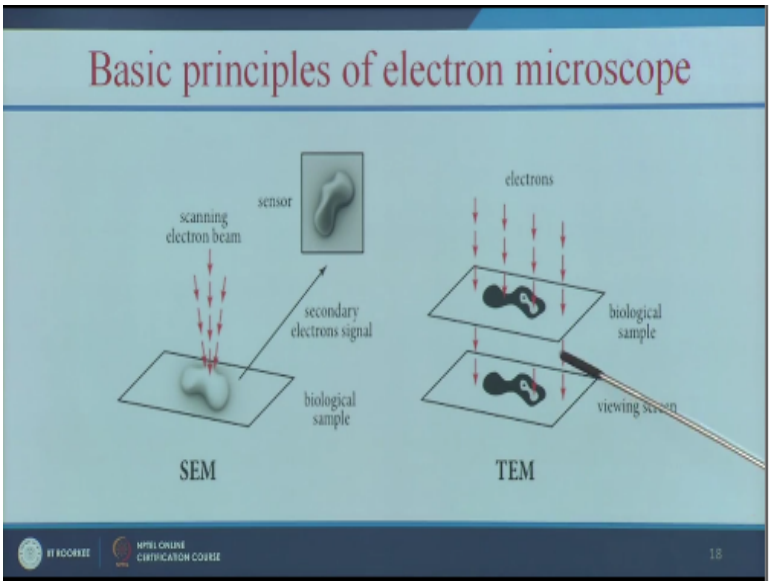

So let us see the basic principle between the SEM and TM so here they are same scanning alter microscope it is similar to your scanner so it will scan the sample are it will give the image and in the TM the transfer electron microscope so the electrons will be transmitted and we will get the image.

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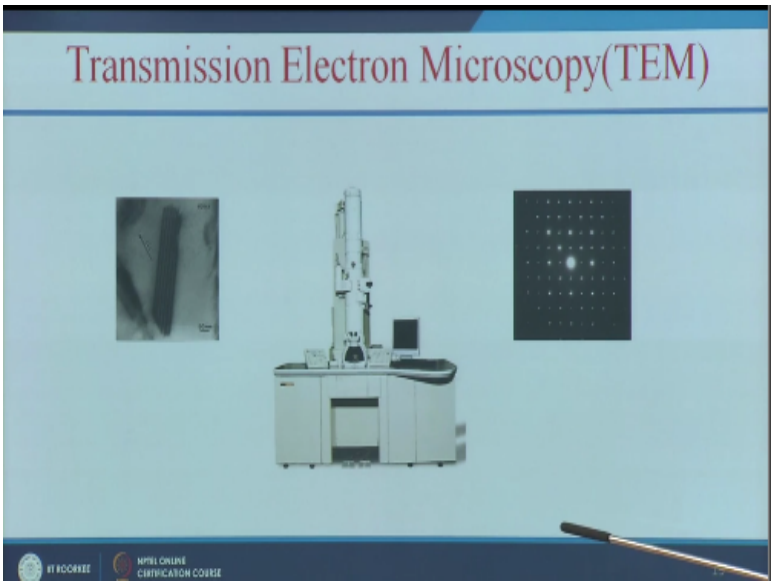

So this is the picture of the transmission ultra microscope so there are two modes bright fin as wells as dark fin so we will get disconnect the majors using this transmission ultra microscope and.

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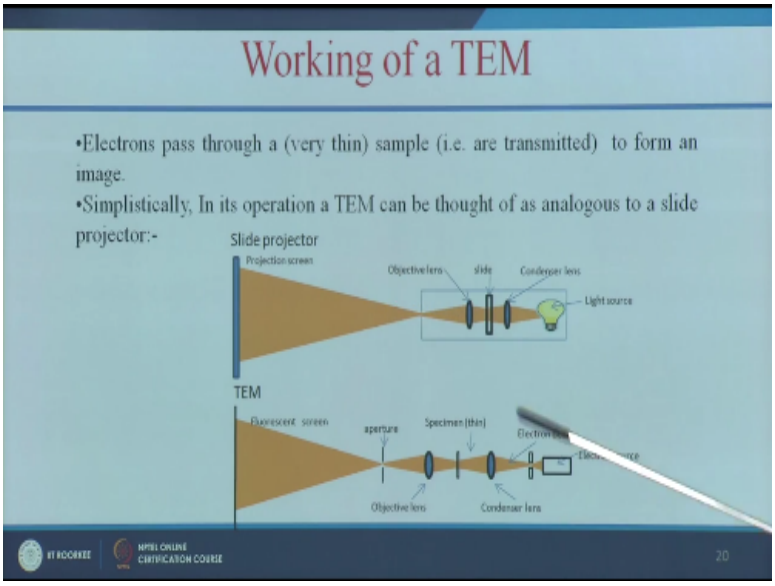

So here there are microscope the electron pass through a very thin sample to form a image in a simple term we can understand it is similar to the your slide projector.

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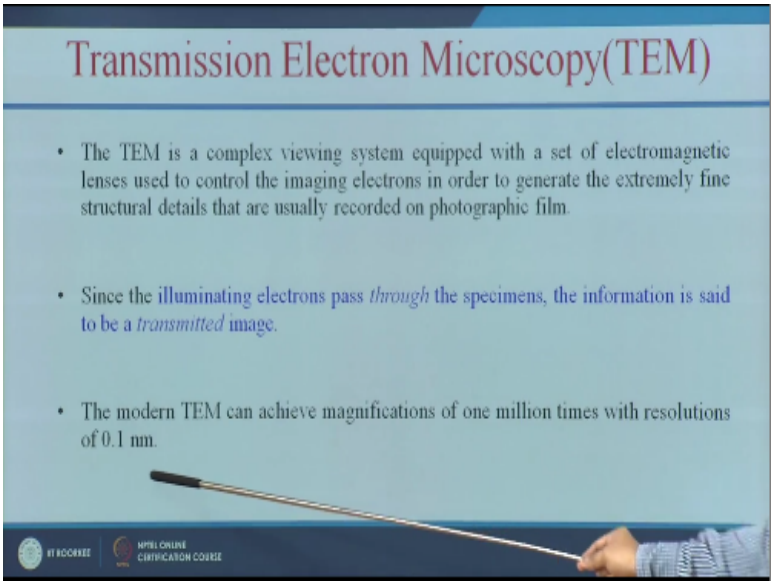

So in TEM that is a transmission electron microscope it is a complex viewing system equipped with a set of electromagnetic lenses okay and which used to control the imaging electrons in order to generate the extremely fine structural details that are usually recorded on a photographic film so here the eliminating electrons pass through the specimens that is why the information said to be transmitted image that is why we are calling like transmission electron microscope image and the modern TEM can achieve magnification of one million times with the resolution of 0.1nm.

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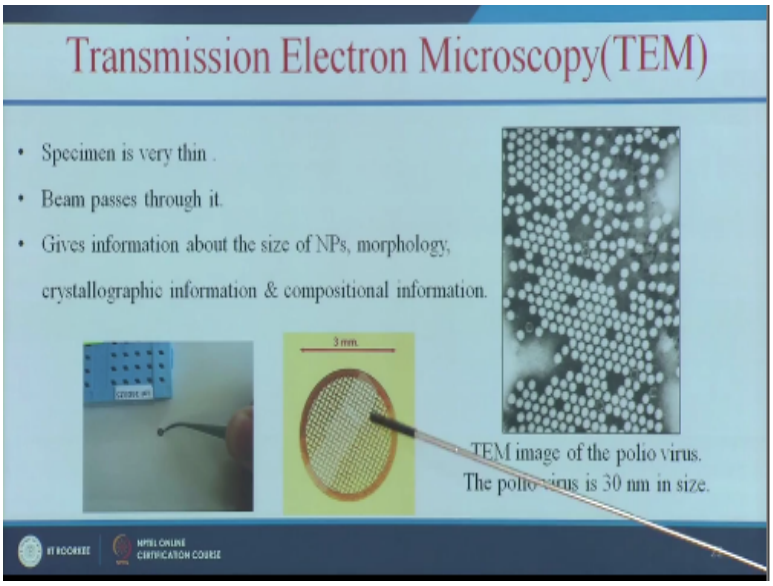

And for transmission electron microscope we need a specimen very, very thin specimen so that the Beam can pass through it and it can gives the information about the size of the nanopartcile morphology and also it can give the information about the crystallographic nature as well as composition information so this is a your TEM grid very, very small size TEM grid and the jule measure of the TEM grids is this is a TEM grids so the Tem grid has two sides one is shinning side an non shinning side.

So you have to deposit your sample on the non shinning side which is coated with the carbon okay.

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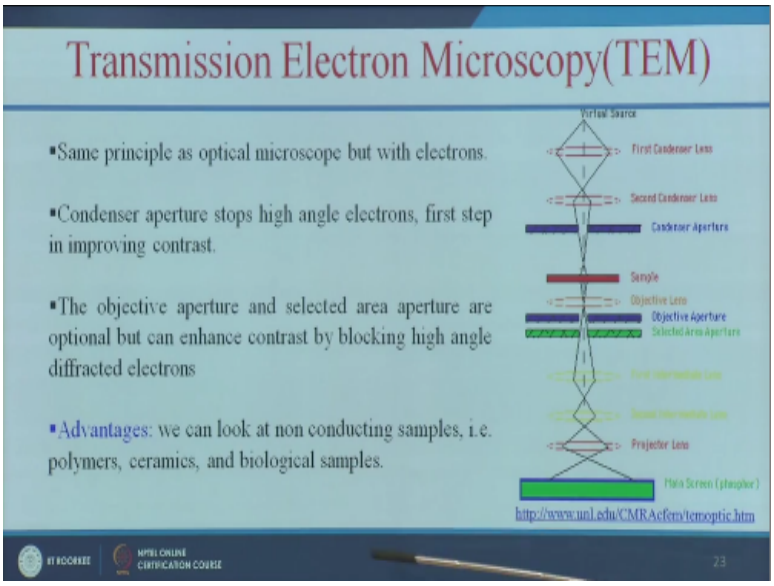

So let us see the principle of this TEM so the principle is similar to your optical microscope but here instead of using the light you will be using this electron has the light shows okay so the condenser apertures stop the high angle electron that is a first step in improving your contrast and this objective aperture and the selected area aperture optional but it can enhance that contrast by blocking the high angle different electrons so what are the advantage of this we can look at the non conducting sample that is your you can measure we can study the size everything you of the polymer samples ceramics of biological samples.

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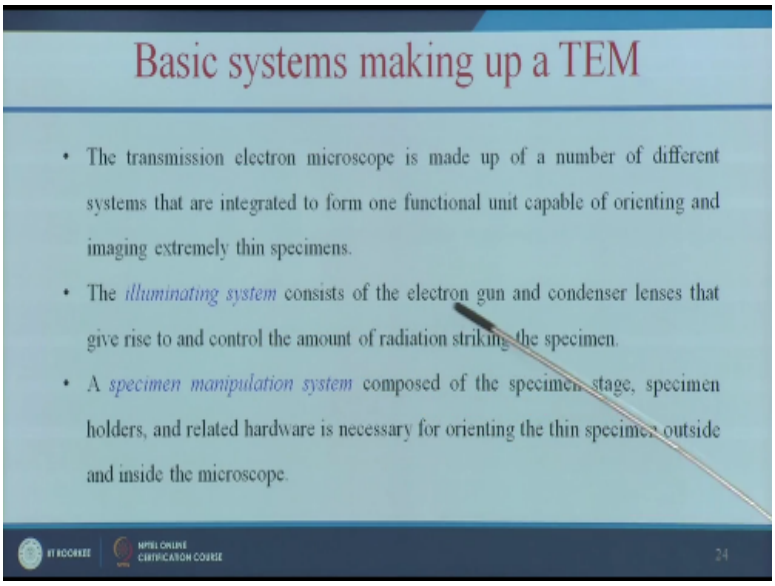

And this these are the basics systems of the TEM so the transmission atomic scope is made up of a number of different systems okay and it forms one function unit of capable of orienting and imaging extremely thin specimens so it has a illuminating system as well as a specimen manipulating system so in the illumination system consist of electron gin and condenser lens so that give rise to and control the amount radiation striking this specimen and in this specimen manipulation system it is composed of specimen stage and specimen holders and also related handwork which is necessary for orienting the specimen inside or outside the microscope.

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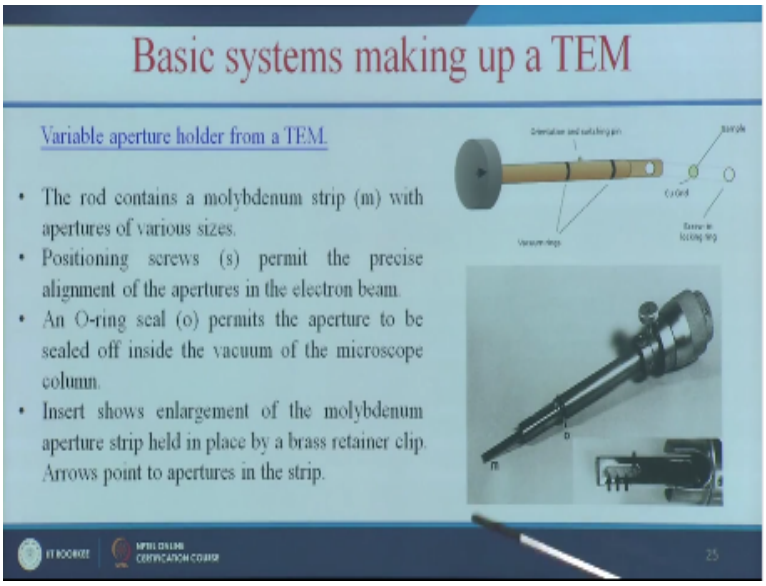

So here you can see here this is a variable aperture holder of TM so your temp grids will be placed here and you can keep this oaring filed so that will permits the aperture to be sealed of inside the vacuum of the microscope column.

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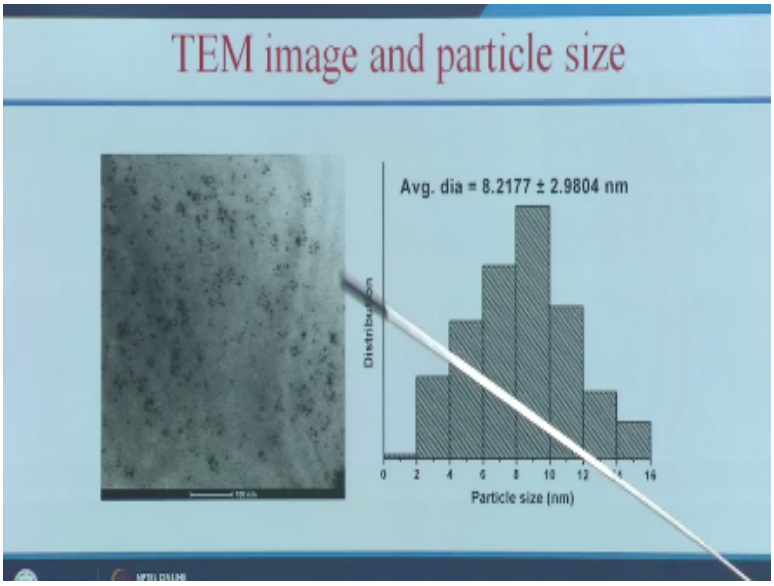

So here the example of your TEM image how it look like so when you study this still on the particular under this transmission electron microscope we can see here this black color dots are your cellular on a particle and as I told you the previous lecture we have to include the scale bar so using this image J software we can measure the particle size so in this case the particle size is approximately 8.2 nano meter in diameter.

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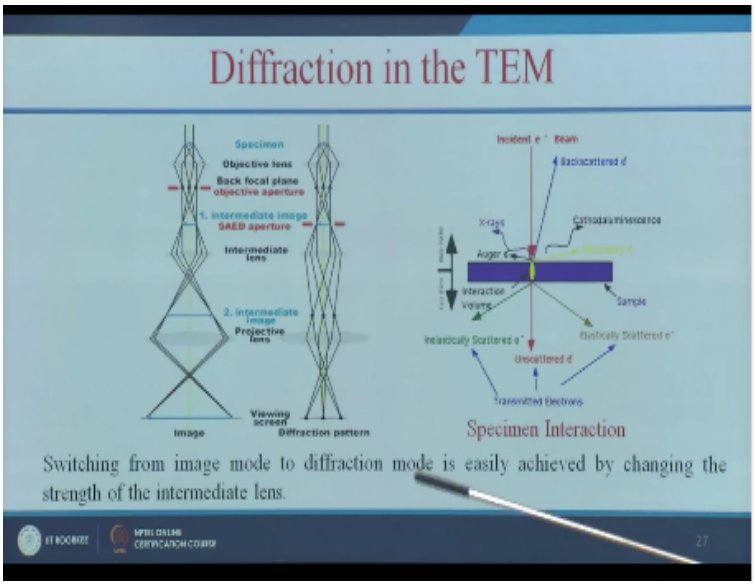

Also we can study the diffraction of your sample by switching your sample from image mood to diffraction mood and which can be easily achieved by changing this strength of the intermediate lens.

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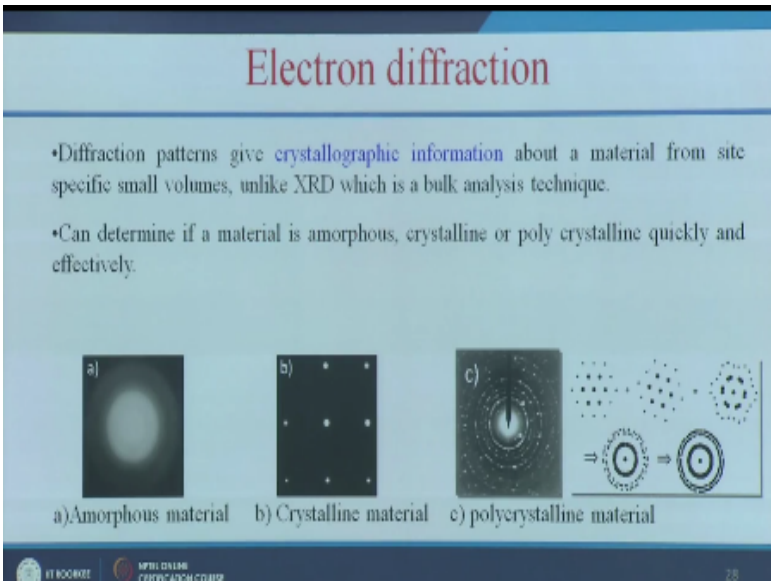

So when you see that electron diffraction pattern of your sample so this diffraction patterns give crystallographic information about your material, okay. And it can determine whether your material is amorphous or crystalline or polycrystalline and it can measure your crystallographic information quickly as well as effectively, in case of amorphous material you will get this kind of diffused reign and in case of crystalline material you will get this kind of spotted.

And in case of polycrystalline material you will get the rings with this kind of bright spots so how you are getting this kind of spots in the polycrystalline material, you can see here in case of mono crystalline or crystalline material you will get this kind of spots and when you combine this kind of things you will get rings with the bright spot that is called as polycrystalline material.

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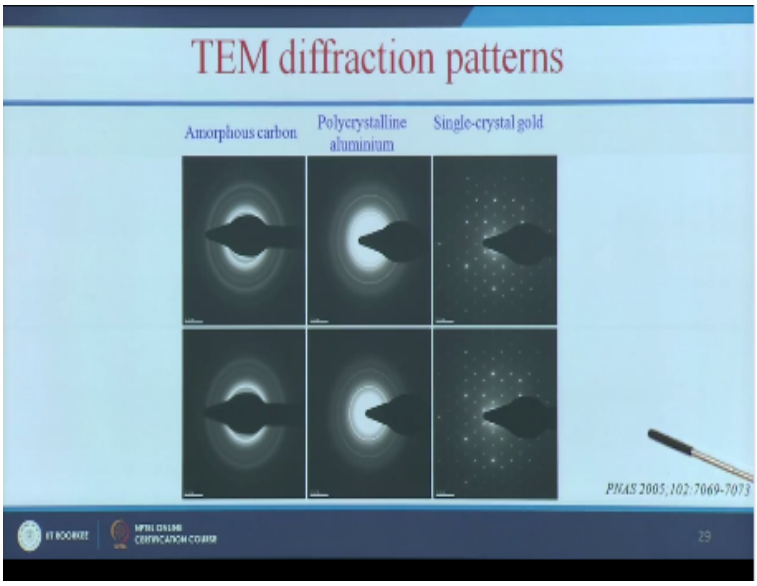

Another example of diffraction pattern you can see here so in amorphous carbon you will get the diffused ring pattern and polycrystalline aluminum you will get this kind of rings with the spot and single crystal gold you will get this kind of bright spots.

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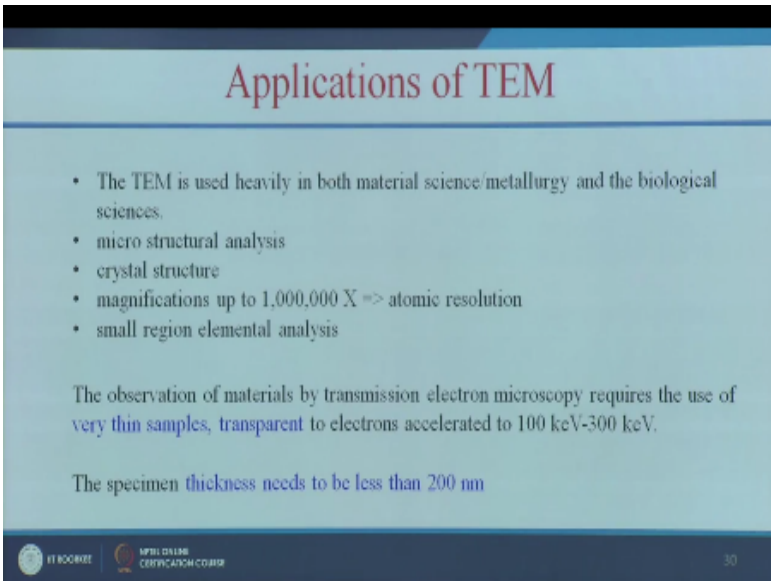

So the applications of Tem so it is heavily used in material science as well as biological science so it can get the micro structure analysis and also we can study the crystal structure and the magnification is very high here so that is the very advantage but the another drawback is we need a very, very thin sample and the sample should be transparent and the thickness of the sample shou7ld be in the range of 200 nm.

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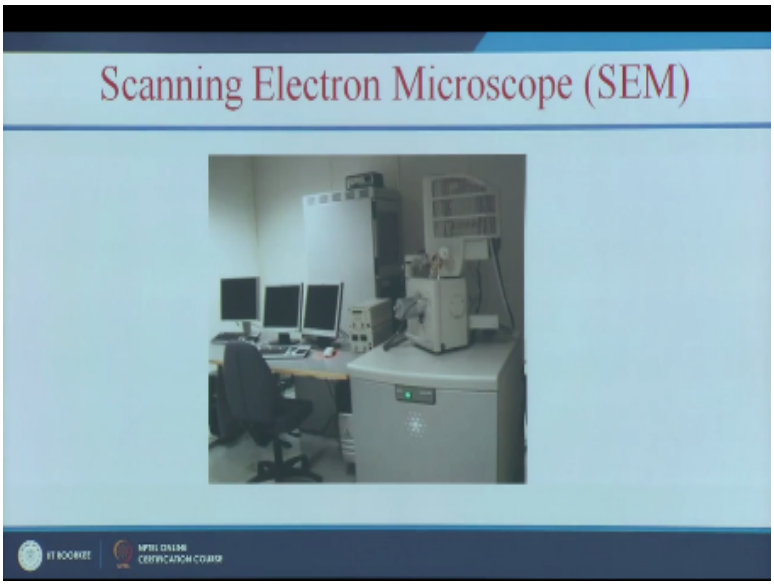

And let us move on to the other microscope that is scanning electron microscope okay so this is the picture of scanning electron microscope.

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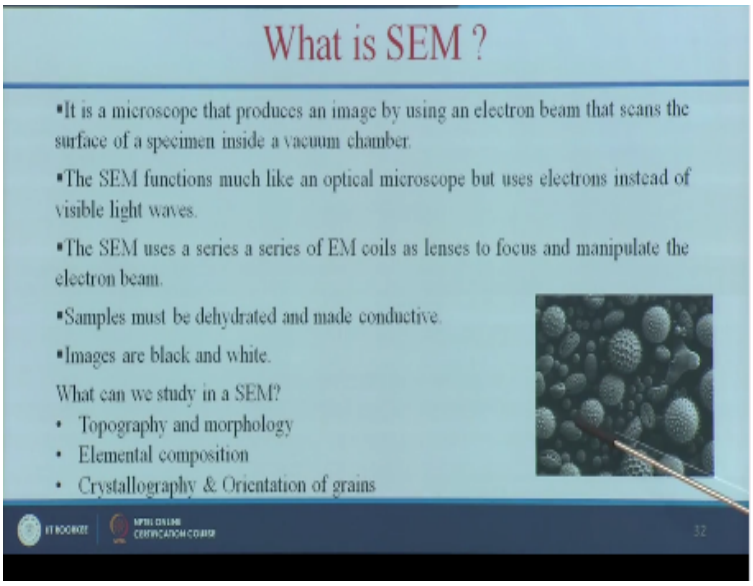

So as I told you earlier so here instead of transmitting the electron here the sample will be scan and it will produce the image, so it is a microscope that produce an image by using electron beam that scans the surface of the specimen inside a vacuum chamber, okay. So here the same function like an optical microscope but as I told you earlier it uses electron instated of light waves and here the same uses serious of electromagnetic coils as lens to focus and manipulate the electron beam.

And here the sample should be in a completely dehydrated form and it should be made conductive okay, so what are things we can study using this SEM, we can understand the topography and morphology of the sample and we can also understand the elemental composition and we can understand the crystallography and orientation of the grains.

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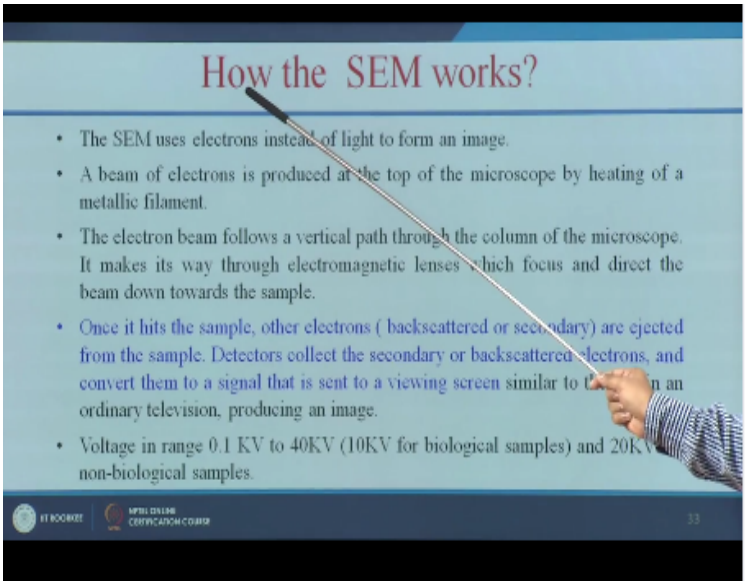

So how the SEM works, so here the electron beams follow a vertical path through the column of microscope okay and it makes its way through the electromagnetic lens and which focus and direct beam towards the sample, once the electrons hit the sample back scattered electrons and secondary electrons are ejected from this sample, so the detector will collect the secondary electron or back scattered electrons and convert them into a signal that is send to a viewing screen and it will produce the image and this is similar to your ordinary television how it produce the image and here the voltage should be like 0.1KV to 40KV we can go in this range and for biological sample it is 10KV is recommended and for our non biological sample we can go even 20KV.

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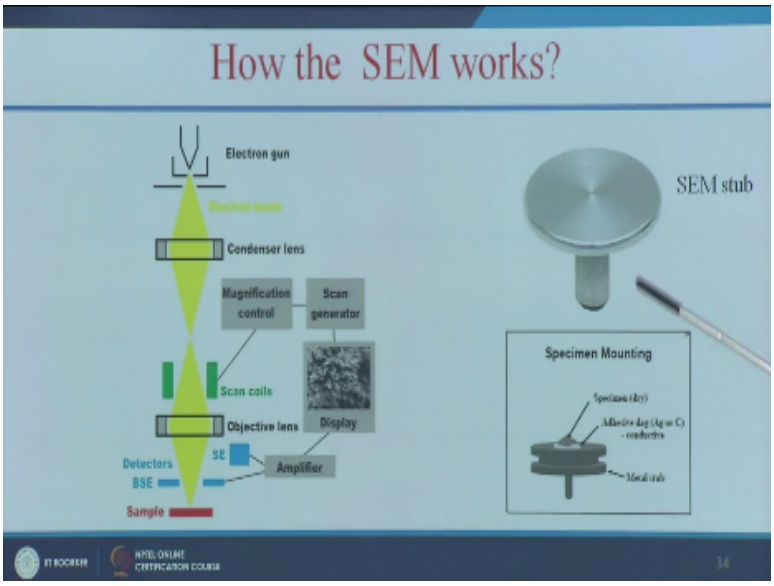

How the SEM works we can see that this electron gun passing the electron beam so it is getting the sample and the back scattered electrons will be amplified and it will display the image, so this is your SEM stub where you have to prepare your sample so your sample should be dried okay, and it should be placed on the top of this SEM stub which is having this carbon tape.

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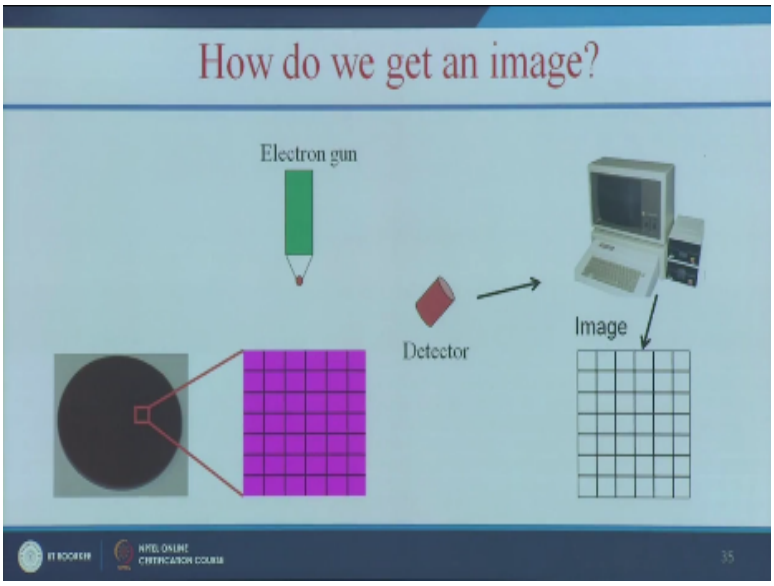

So how do we get an image so when the electron hit was sample for the back scattered electrons will be detected by the detector so it will scan your sample one by one and that will be detected by the detector all the back scattered electrons have been detected by the detector and it will take it to the computer.

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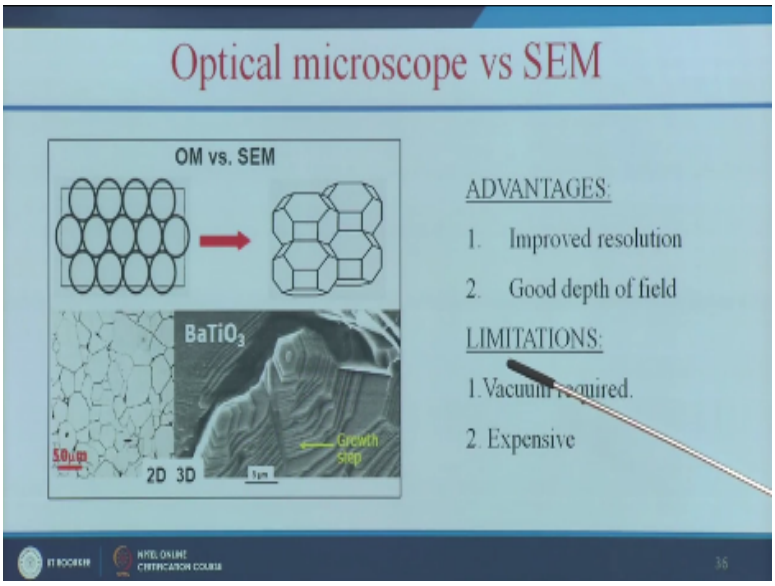

And using the software it will give the 3 dimensional image, so we can compare with the optical microscope verses scanning electron microscope so in optical microspore we will get the two dimensional image for the same compound you are getting this kind of image and when you study under this scanning electron microscope you will get the three dimensional structure, so what are the advantages of using this scanning electron microscope you can have a improved resolution and also you can have a good depth of field and the limitations is required a vacuum and also it is the equipment caused and otherwise expensive.

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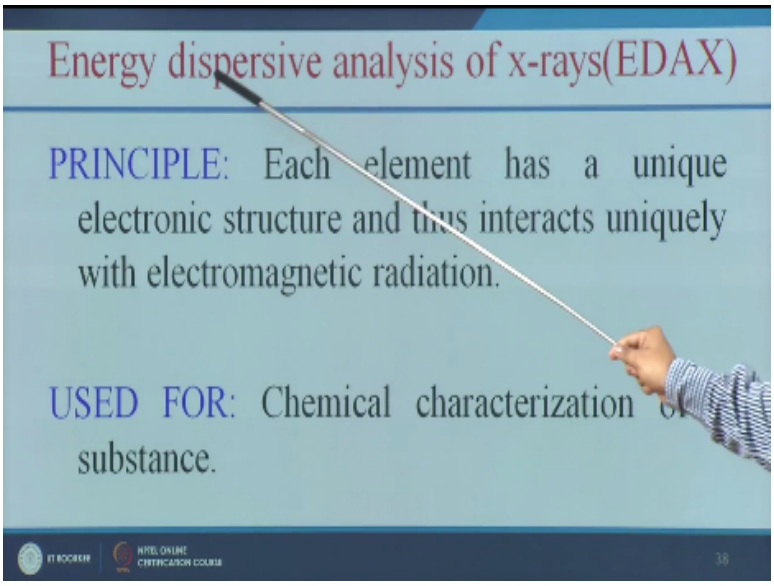

So another advantage is we can do the energy dispersive analysis of x-rays, the principle is each element has unique electronic structure and this interacts uniquely with the electromagnetic radiation, so it can be useful for chemical characterization of a substance.

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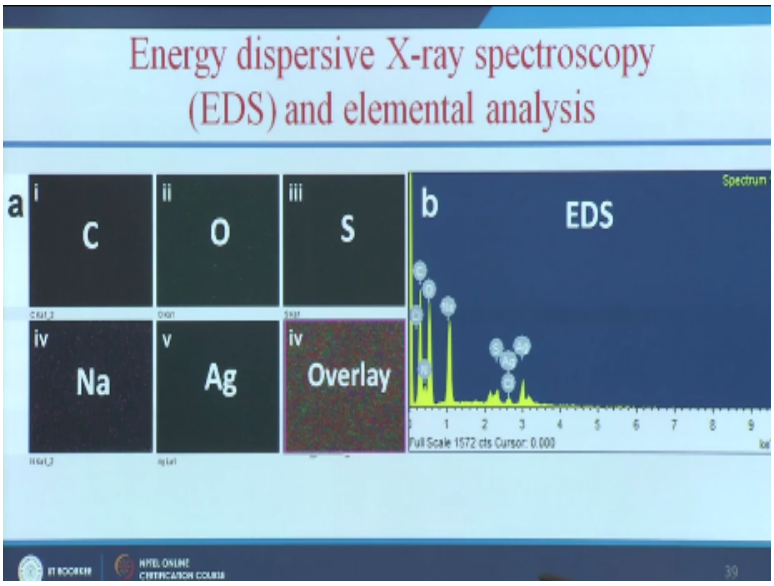

So if you see this is your EDS results that is the elementary analysis result so you will get the what are the elements present in your sample that can be measured using this EDS analysis.

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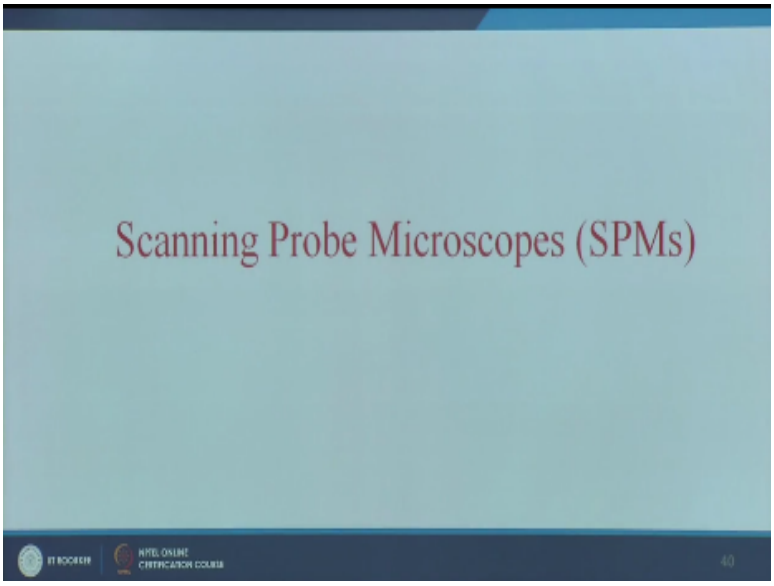

So next we move on to this scanning probe microscopes so under this scanning probe microscope there are two types one is.

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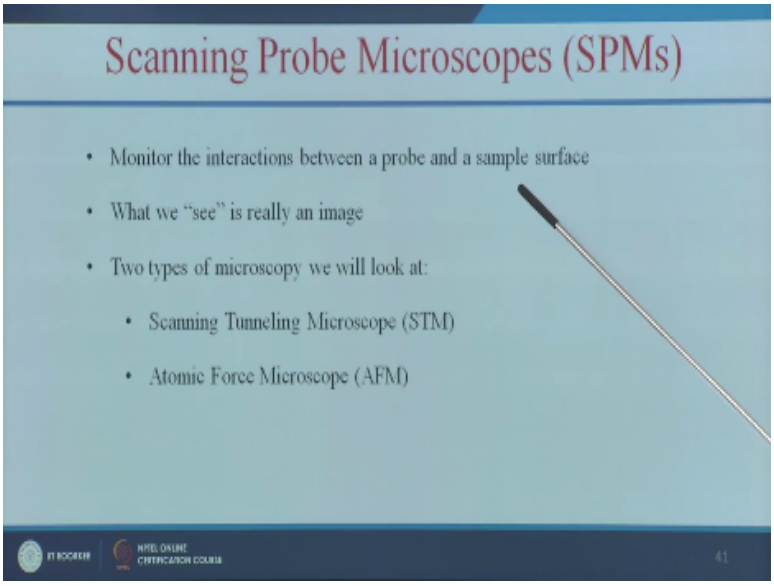

Scanning tunneling microscope and other one is atomic force microscope, so here we can monitor the interaction between the probe and the sample surface.

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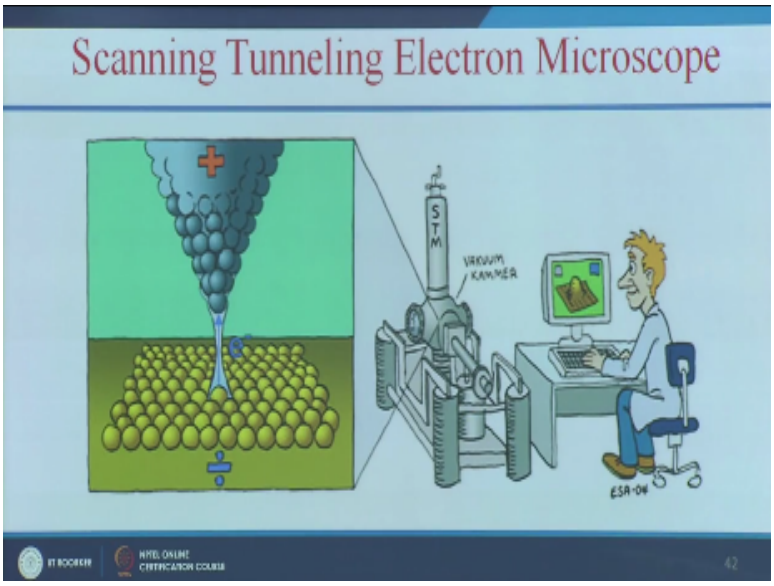

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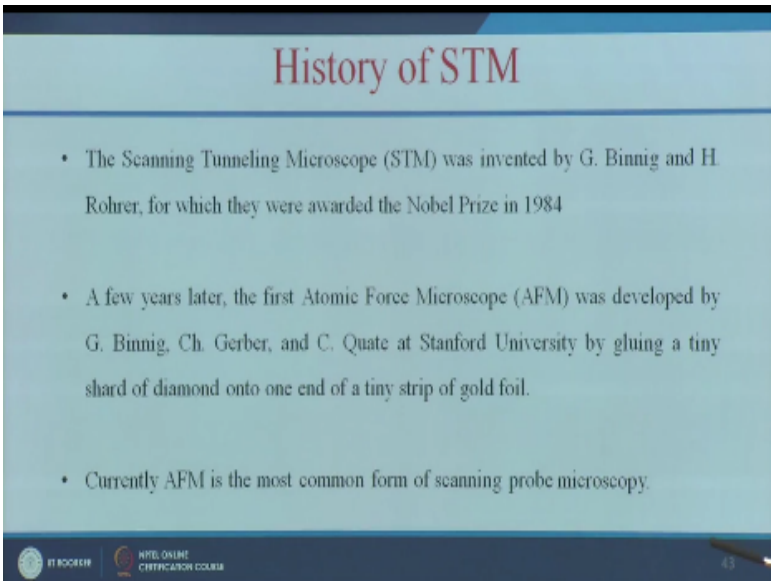

So briefly on this history of STM, the scanning tunneling microscope was invented by G. Binnig okay, and they awarded noble prize in 1984 and after few years they have invented this atomic force microscope okay, and currently the AFM is the most common form of we was scanning probe microscopy.

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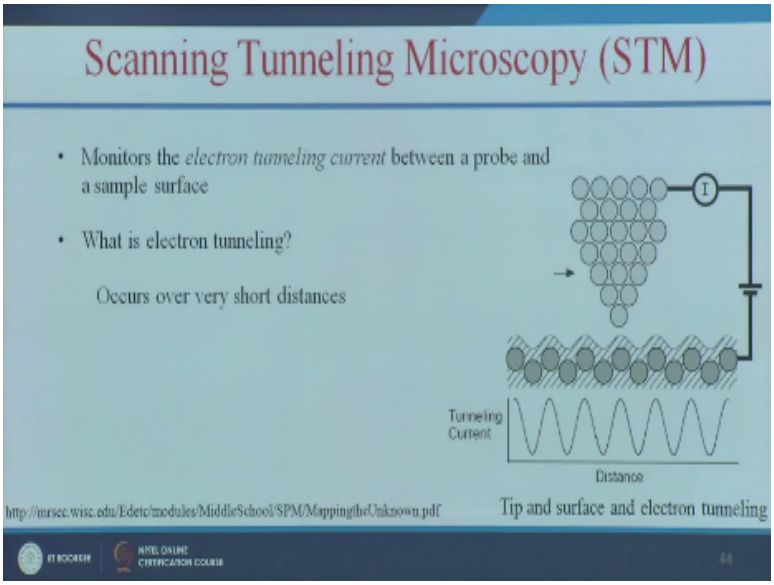

So in scanning tunneling microscope STM what we are going to measure so we are going to monitor the electron tunneling current between a probe and a sample surface, okay. So what is electron tunneling it occurs over a very short distances.

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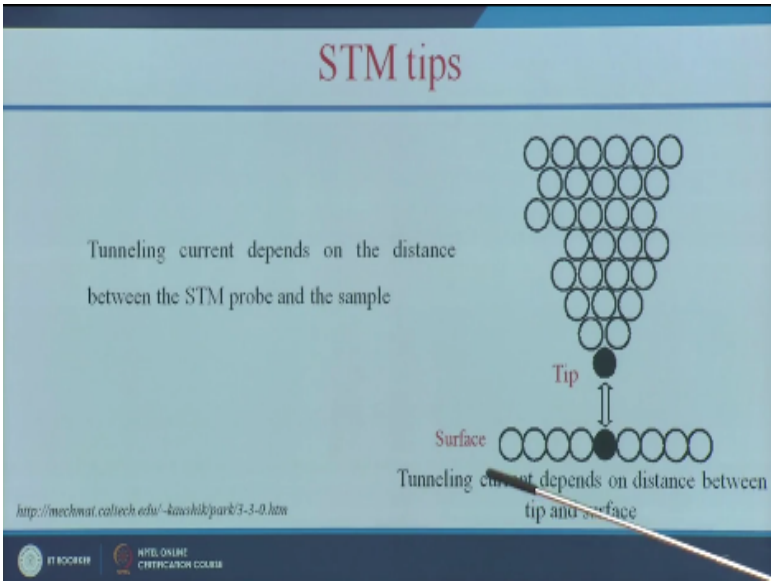

And the tunneling current depends on the distance between your STM probe and the sample surface, so we are going to measure the tunneling current between your tip as well as surface of the sample.

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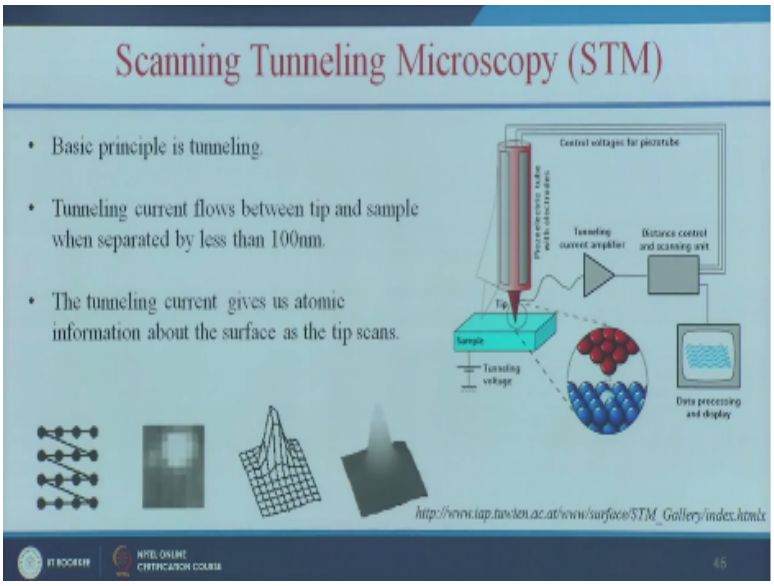

So here the basic principle is tunneling, so the tunneling current flows between the tip and the sample when separated by a less than 100 nano meter, okay and this tunneling current gives us atomic information about the surface when the tip scans the sample.

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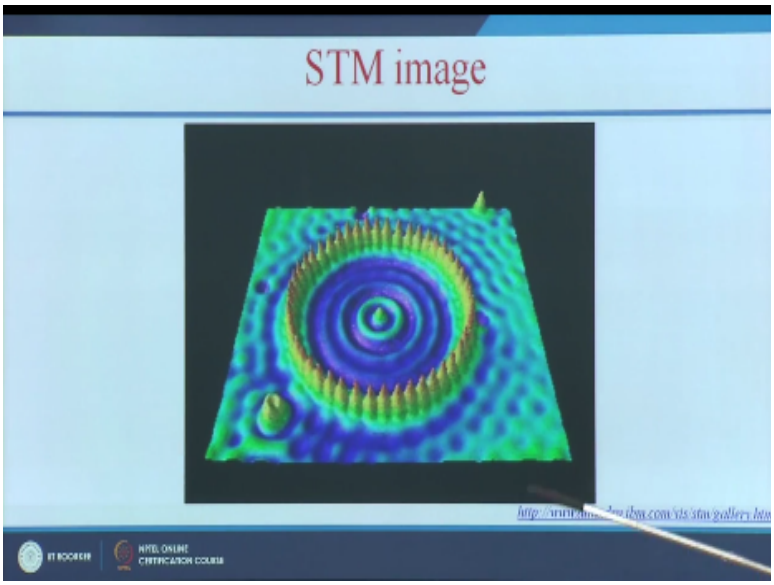

So this is how the STM image looks like.

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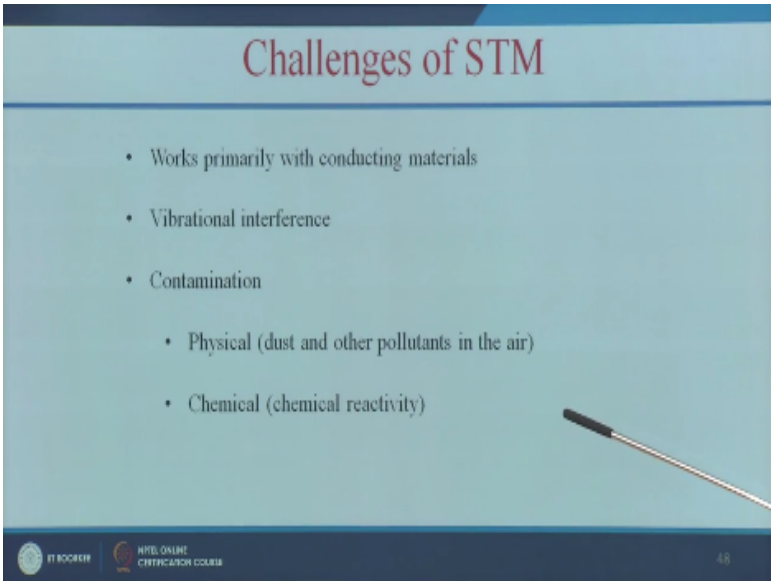

So but the challenges with the STM is like it works primarily with the conducting materials and there is a chances of vibration interference as soon as the contamination by a physical like dust particles and other pollutants in the air and also chemical reactivity of the samples.

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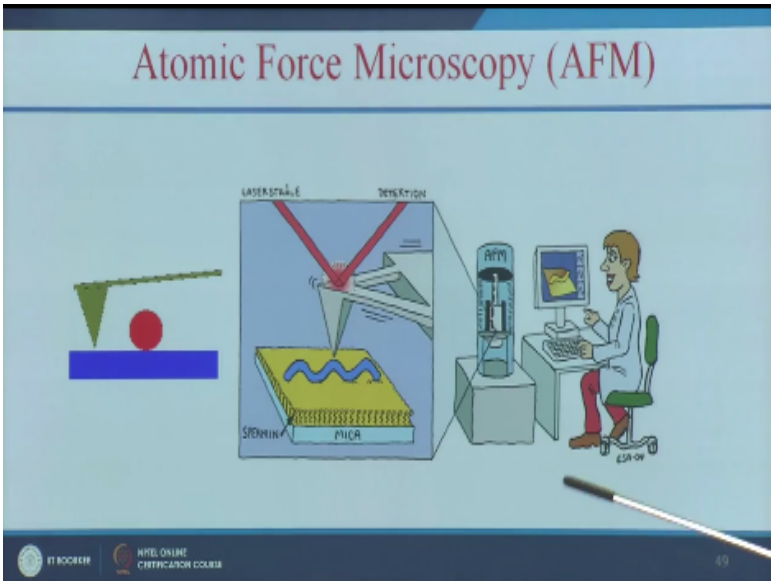

So next one is atomic force microscopy.

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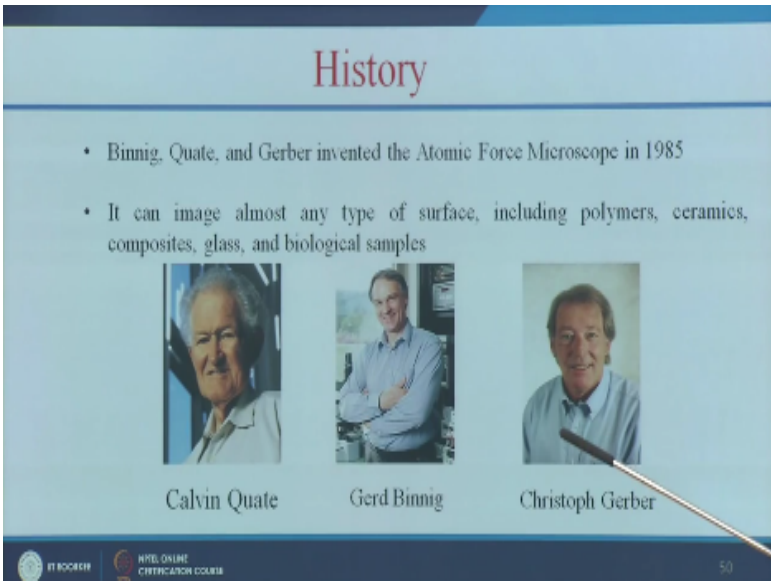

So these three people invented this atomic force microscope in 1985 okay, so using this atomic force microscope we can measure almost it can image any kind of surface including polymer ceramics glass and biological samples anything can be studied using this atomic force micro scope okay.

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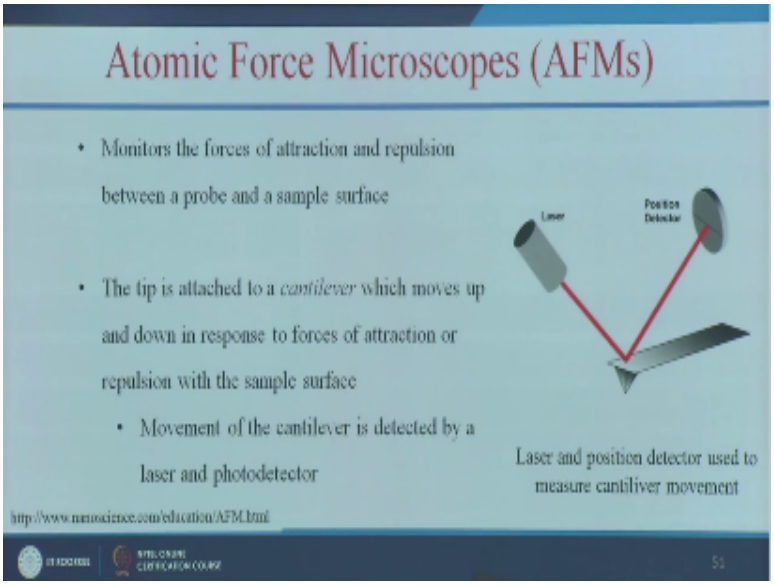

So this atomic force microscope monitors if force of attraction and repulsion between a probe and a sample surface, so here that tip is attach to a cantilever okay which moves up and down in responds to sources of attraction of repulsion with the sample surface. And this movement of the cantilever is related by the laser as well as the photo detector.

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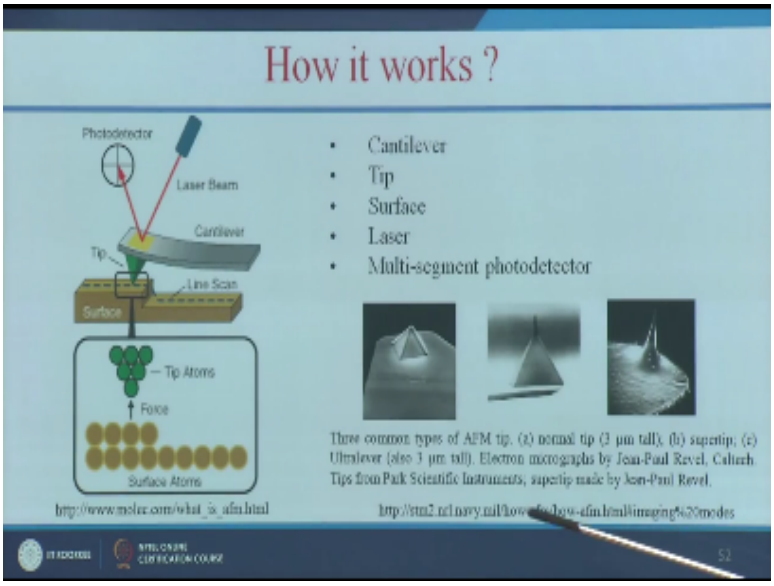

So here you can see here this is your cantilever and this is your tip okay and if its moves on the surface up and down so depends on that the laser being potion will be changed, so those will be measured using the photo detector and it will give the image according to that okay. And we can select he tip according to our sample as well as applications so the first one is normal tip and this is our super tip and this is one is ultra liver tip.

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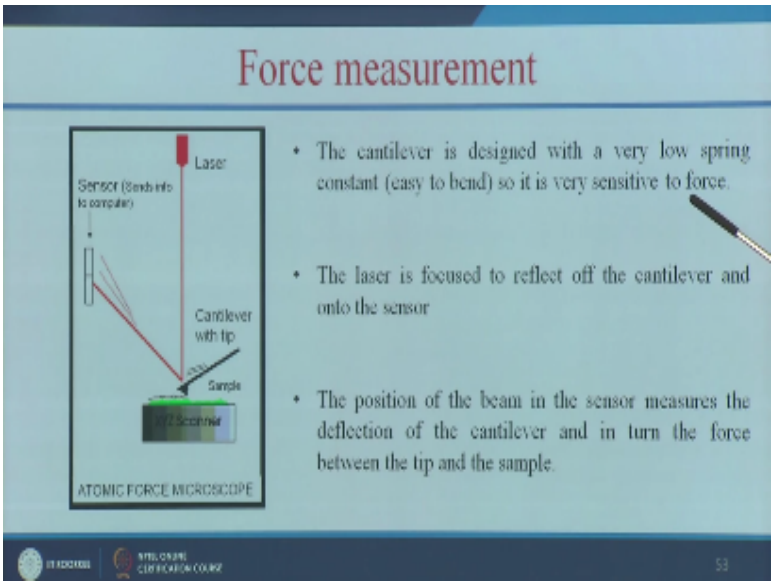

And here we can measure the force this cantilever is design with the very low spring constant that means it is easier to bend okay so it is very, very sensitive to even a small force when the laser is focused to reflect of the cantilever and on those sensor so it will produce the image. So here the position of the beam in the sensor measure the direction of the cantilever and it turn its force between the tip and the sample.

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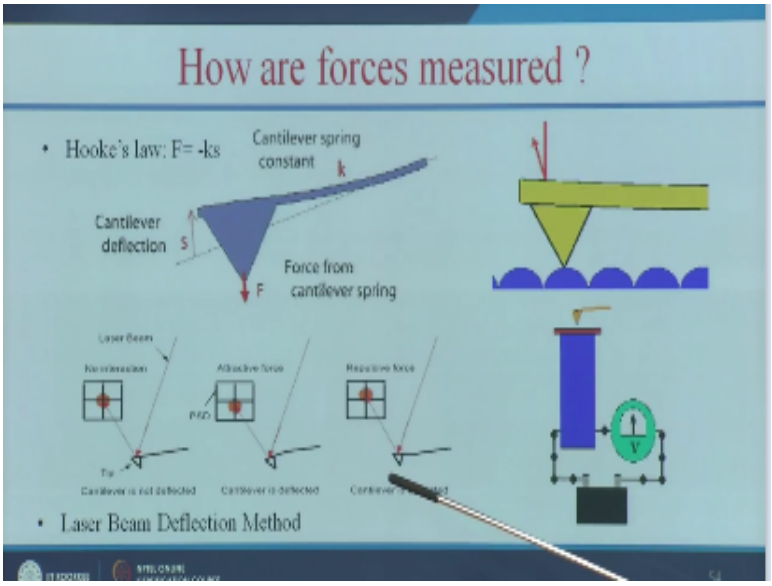

So how the force are measure using the Hook's law and here you can see here this cantilever tip it is going up and down in the sample and you can see here this the laser beam also getting reflected according to the samples nature and if there is no interact mate in the tip and the sample the cantilever is not deflected.

So in this case we will get the laser beam will be directed in this area and in the cantilever is deflected you will get the laser beam potion will be here and if it is having repulsive force and the cant this laser beam optional be here, so based on that it will produce the image.

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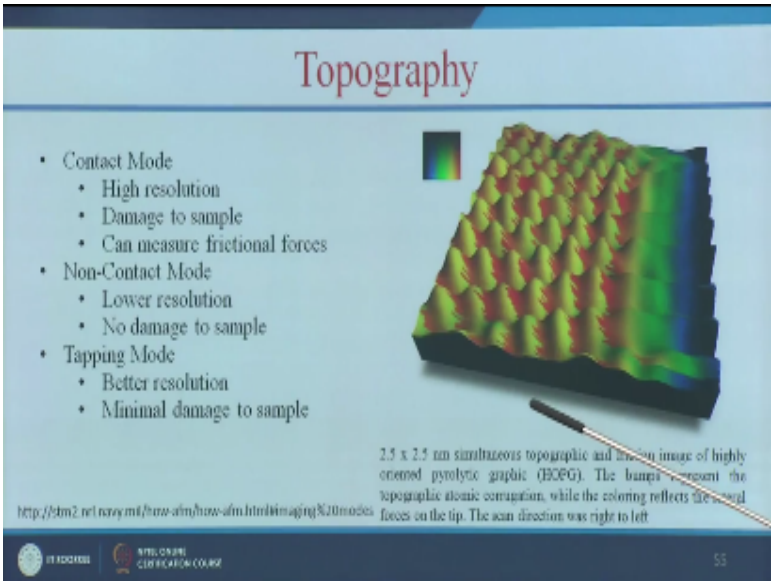

And this air from has three different modes one is contact mode, non contact mode and tapping mode so in this contact mode you will get high resolution image but the problem is it will damage to the sample because the cantilever tip is touching your sample okay. And it can also measure the frictional forces and in the non contact mode it is lower resolution but it would not cause any damage to sample because it is not touching this sample and in the tapping mode it will give a better resolution and it is minimal damage to the sample.

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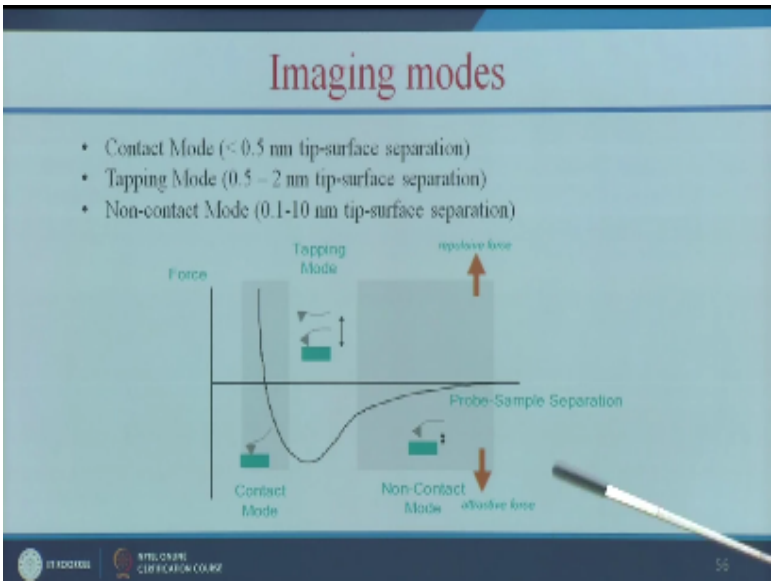

So we can see here in the contact mode the distance between the tip and surface id 0.5 nm and in the tapping mode the result is approximately like 2nm and the non contact mode it can reach even 10nm tip- surface separation okay. So in case of contact mode this cantilever tip is touching your samples in case of tapping mode the repulsive force will be high and in case of non contact mode the attractive force will be low.

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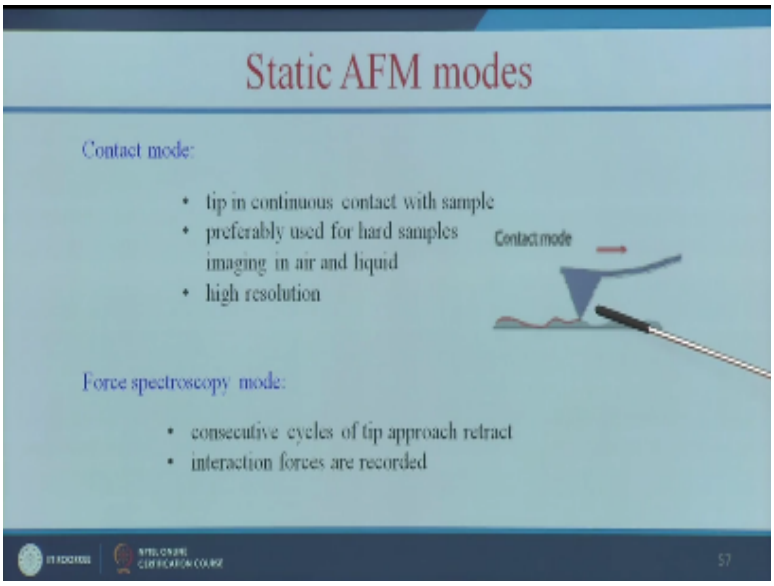

So let us see this modes in detail in contact mode the tip will be continuous contact with your sample this is your tip and this is your sample it is touching your sample and it can give the details of your sample okay. So it is mainly useful for hard samples okay and imaging can be done in air and liquid and it will give the high resolution but the drawback is it can damage your sample or it can damage the cantilever tip.

Next one is force spectroscopy mode here the consecutive cycles of tip approach and attract and here we can measure the interaction forces okay.

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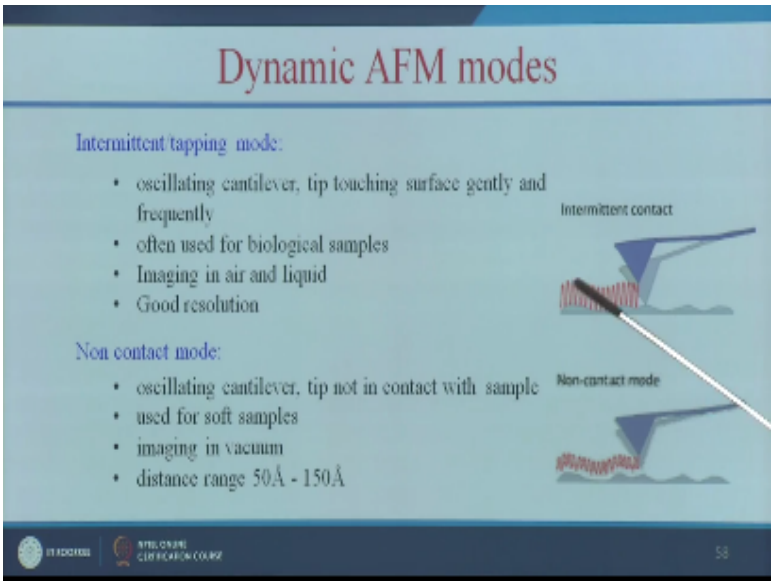

The next mode is intermittent tapping mode here the your cantilever tip will be oscillating it will be oscillating in a uniform frequency okay so that the tip touching the surface and gently and frequently and it is mainly used for the biological samples and here we can do the imaging in the air and liquid and it will give you a good resolution.

And the third one is non contact mode here also we will be having a oscillating cantilever but here the tip is not in the contact with sample okay. So it will be mainly used for the soft samples for non contact mode you have to do imaging in vacuum condition okay and here the distance range will be 50A-150A.

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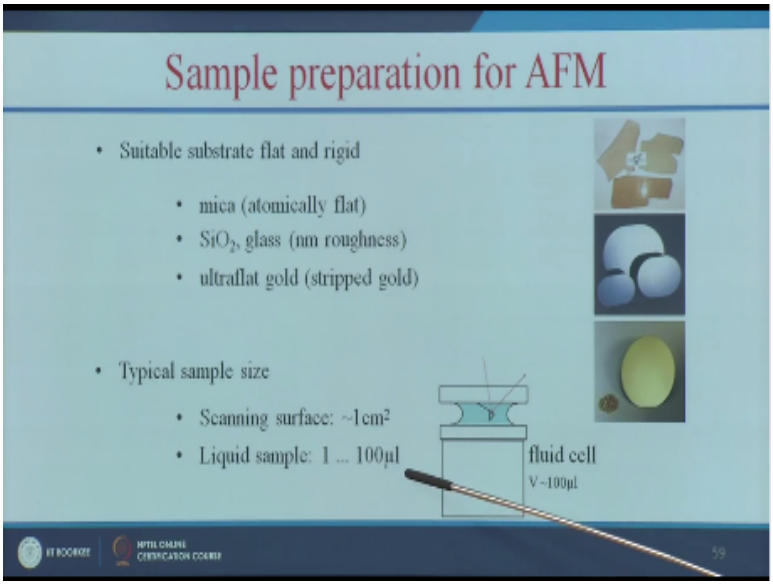

And how do we prepare the sample for the AFM so we need a completely flat and rigid surface , so we can use the mica which is atomically flat surface and also you can use that Sio2 glass and the typical sample size should be like it can be like $1cm^2$ or for liquid sample it should be between 1 to 100µl.

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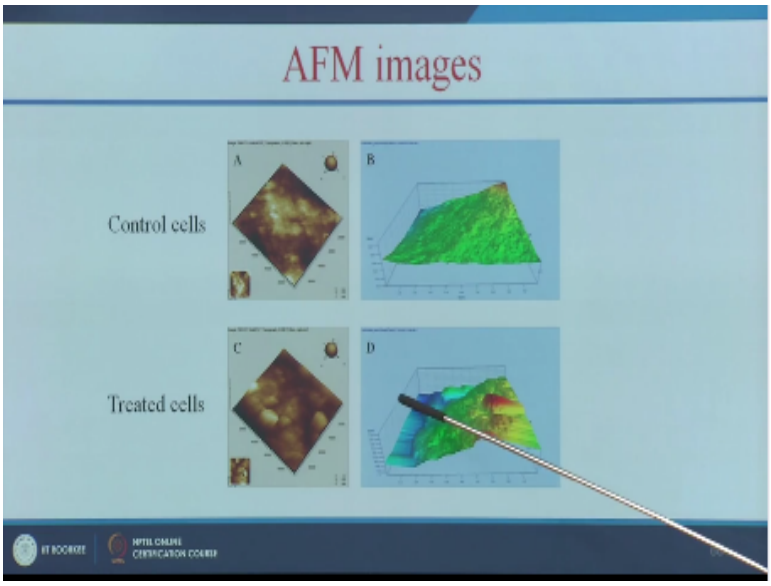

So these are the AFM images of control cells and treated cells okay. In the control cells you can see here the surface is very smooth and these are the treated cells so these are the mammal cells and it has some nano particles, so here due to nano particles treatment the cell will get damage and you can see here the roughness of this cell is getting increased, so we can measure the surface roughness using this atomic force microscope and these are the some of the AFM images you can get the idea how does the image look like.

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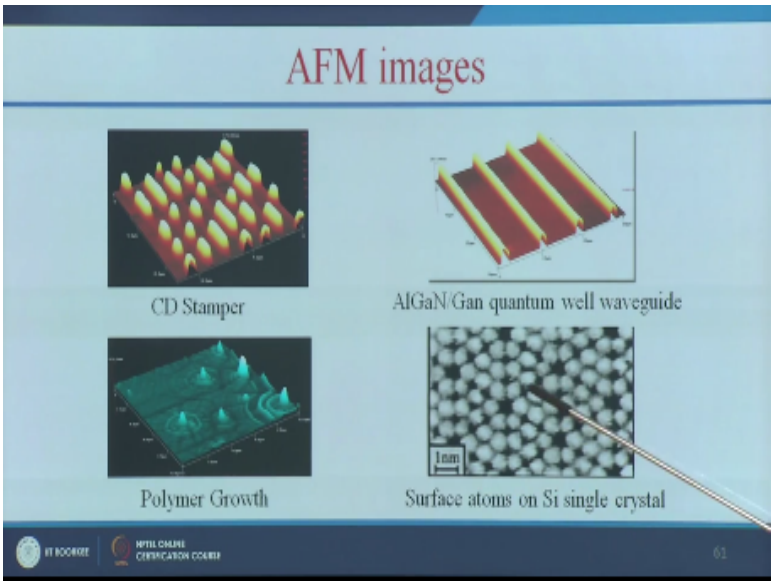

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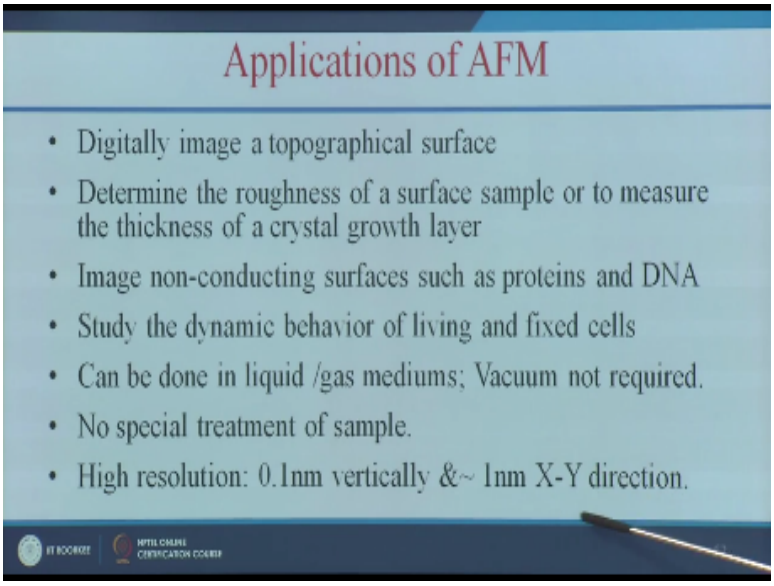

So let us see the applications of AFM so we can study with the topographical surface and we can also determine the roughness of the surface sample and also we can measure the thickness of the crystal growth layer and here another advantage is image non conducting surfaces as DNA proteins. For most of biological samples we will be using the AM

Because if you are using the SVM or any other microscope so you have to do some kind of treatment like you have to sputter with goal. Then only you can make your biological material into a conducting material then only they can use the SVM energy. But in this case of AFM so you can even use the non conducting surface protein and DNA.

And we can also study the dynamic behavior of living and fixed cells in case of electron micro scope or transmission atomic scope we cannot study the living cell okay, so we have to fix the cell and it should be in dried form then only we can study. In case of AFM we can also study the living cells and it can be done in gas medium and here the most of the mode vacuum is not required and here we do not have to do any special treatment of samples. As I told you we have to do gold shuttering and the sample will be made conducting and here the resolution is also high we can reach 0.1nm vertically and 1nm X-Y direction.

As a summary so in this lecture we learnt how to calculate the nano material using usual spectroscopic usually the hydro size of the nano particles will be dl dynamic light scatting and how to study the stability of the nano particles and we have also learn how to prepare sample for ultra micro scope and how to study the sample using the transmission atomic scope and we have

also learn how to prepare sample for atomic microscopic and what are the modes available in microscopic so I will end my lecture here I thank you all for listening this lecture I will see you in another lecture.

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