INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
NPTL ONLINE CERTIFICATION COURSE
Structural Analysis of Nonmaterial's
Lecture-18
Microscopic Structural Analysis
Of Nanomaterials-1
With
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Hello, today we are going to discuss about the, Microscopic Structural Analysis of Nonmaterial's part-1, basically we have divided all the microscopic structural analysis, of different types of nonmaterials, there are several types of experiment so we have divided into different parts, so first let us know that what is nanomaterials.

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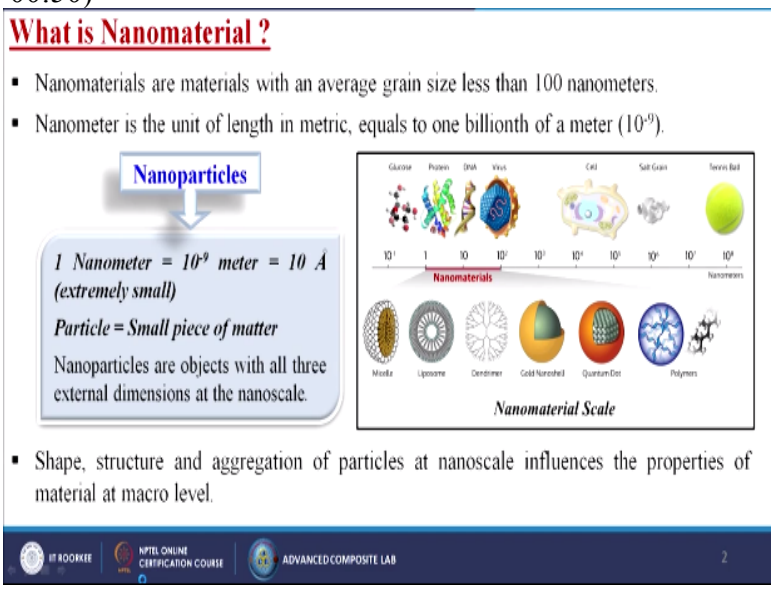

So nanomaterials are materials with an average grain size is less than 100 nanometers, nanometers is the unit of length in metric, equal to one billionth of a meter, that is 10 to the power -9, as we all know that one, so what nano particle, so one nanometer, whatever I have told already that is equal to 10 to the power of -9 meter is equal to 10 angstrom, it is extremely small because 10 to the power – 9 meter, that means we cannot see by our naked eyes, particle small piece of the matter, nano particle are object with all three external dimension.

At the nanoscale, so we are having different derivations in the nano particle like 0D, 1D, 2D,3D like so I will tell you everything in brief, in the next slides, shape structure and aggregations of particle at nanoscale influences the properties of materials at macro level itself, so if you see this images in this particular image you can see that is the nano meters is 10 to the -9 1 to 100 nano meters then 10 to their 3, 10 to their 4,10 to their 5, 10 to their 6, like that, so when we are talking about the nano materials that is 1 to 10 to the power 2 that means 100 nanometers, so generally the miscal, lipozom, dendryma,, gold nanoshells, quantom dots, polymers chains these are the coming, so this is the generally the nano materials scale, but when we are talking about

the 10 to the power 8 nanometers generally, it is the size of the tennis ball, so within in this range, our whole materials more or less 1 to 100 nanometers within this range our whole nano materials lies, so why nanomaterials because now a day's every researcher, every scientists, every word their talking about the nanomaterials, so the first questions is comes to our mind is that, why your using the nanomaterials, what is the beauty behind it.

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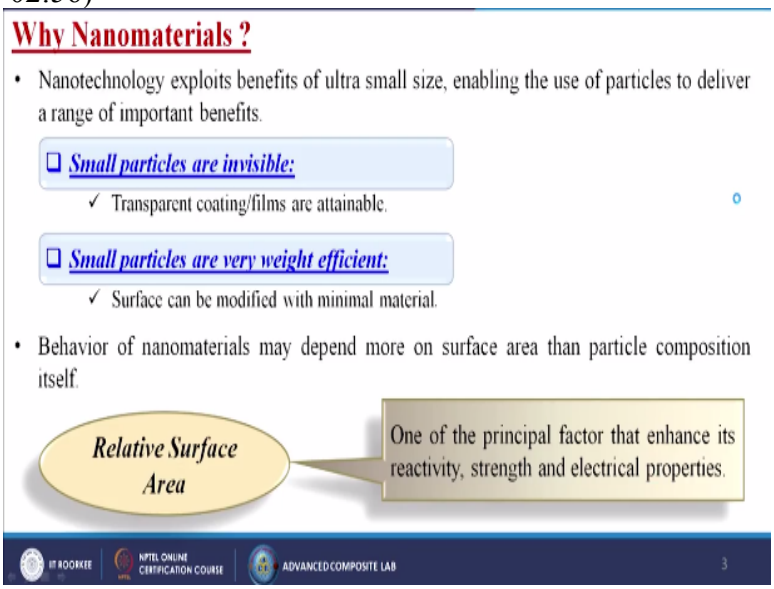

So nano technology exports benefits of ultra small size, enabling the use of particle to deliver a range of important benefits, what are those, first is that small particles are invisible, as I told already, we cannot see it by our naked eyes, transporting are coating films are attainable, then small particles are very weight efficient.

Surface can be modified with minimal material, behavior of nanomaterials may depend more on surface area then particle compositions itself, yes of course, we have prepare some materials, so just changing the outer surfaces, or may be by coating, or may be by Deeping or may be any kind of surface treatment, we can change the whole properties of the particular material, say initial to assume hydrophobic in nature, so just give a coating of hydro filling materials we can make it hydro filling, or may be the our strength was less.

So after doing some high strength materials coating, we can make it harder materials, so like this way, we can change the surface prosperities of that particular material, not the chemical properties, we are going to be changed, so relative surface area one of the principle factor that enhance it, already I told reactive strength and the electrical properties previously it was the conducturing materials, now we are making it totally the insulating materials.

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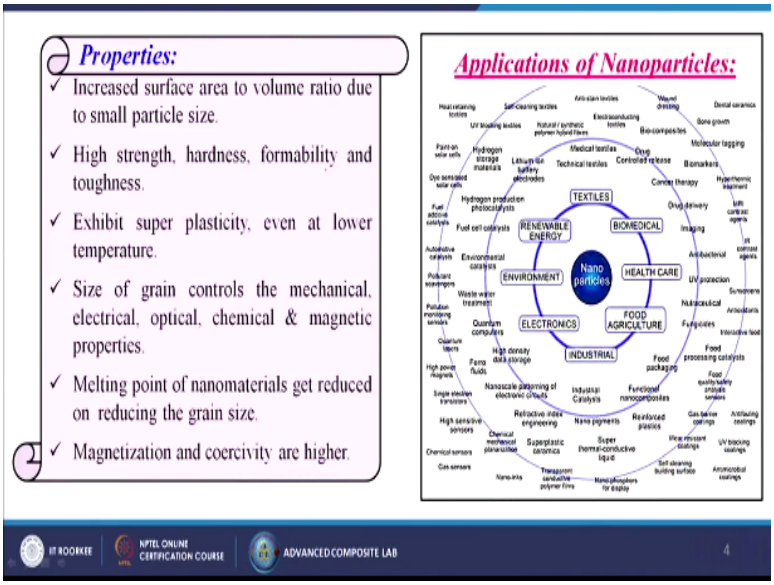

So what are the properties of the nano materials increase surface area to volume ratio due to the small particle size, high strength hardness formability and toughness, exhibit super plastics, even at lower temperature, size of grains control the mechanical, electrical, optical, chemical and magnetic properties, melting point of nanomaterials get reduced on reducing the grains size, magnetizations and cocrcivity are higher, in this particular case right hand side you can see that there are we have shorted the applications of the nanomaterials.

So there are huge, huge number of applications generally, where we are using this kind of nano particles, so suppose in the broad if you divided it, we are using it the text style applications bio medical, health care, food agriculture, industrial, electronics environment, renewable energy and then even after that, if we divided it, or may be the sub divided this broad, so we are using it for the drug delivery, cancer therapy, drug control release, EV protections, food packaging, then high density, data storage, environmental catalyzes, fuel sell catalyzes, so many then after that also again we became it more broader, so we can make it for the sensor applications we are using it for the EB blocking coatings, we are using it for the bone growth, molecular tagging, dental ceramics, so there are N number of applications where we are using nano particles today.

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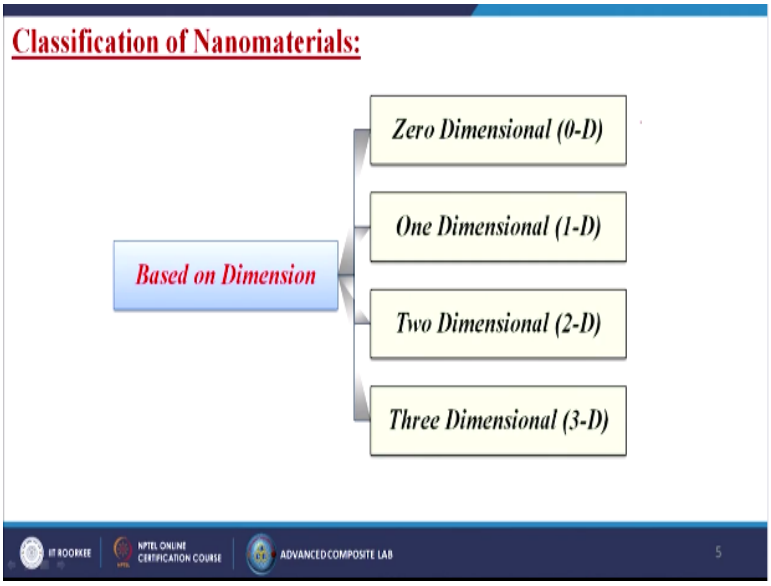

So first we have to classify the nanomaterials, so based on the dimension, it is divided into four parts and one is called the Zero Dimensional, or may be the Zero D, One Dimensional that is 1-D, Two Dimensional that is 2-D and Three Dimensional that is called the 3-D.

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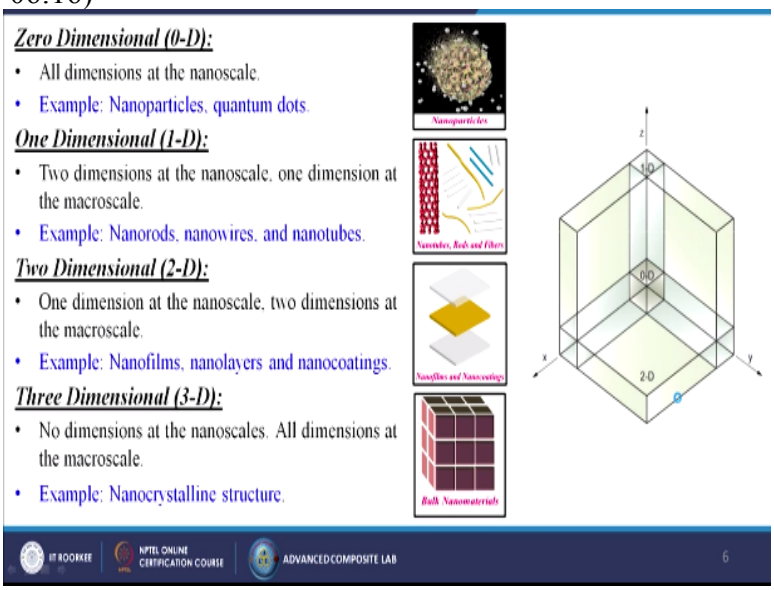

So what is zero Dimensional, all dimensional at the nano scale itself, so that is means XYZ all directions, so Zero D so this small confinement all the dimensions are into the nano scale, so XYZ are into the nano scale examples, like nano particles, or may be the quantum dots, when you are talking about the one dimensional one D, that means we are talking about the one D in this case two dimensions, at the nano scale either may be X or Y or may be Y or Z or may X or Z so in this case X and Y are into the nano scales and Z dimensions is into the micro scale.

So nanorods, nanowires and nanotubes, then two dimensional, one dimension at the nano scale when we are talking about the two dimensionals, so one dimension only the Z dimension over here into the nano scale X and Y are into the macro scale, so like nano films, nano layers and nano coatings, when we are talking about the three dimensional, so no dimension at the nano

scale all the dimensions at the micro scale itself, so it the whole one, so examples, like nano crystalline structures, or may be the Bulk structure, or may be the Bulk nano materials generally we are telling it.

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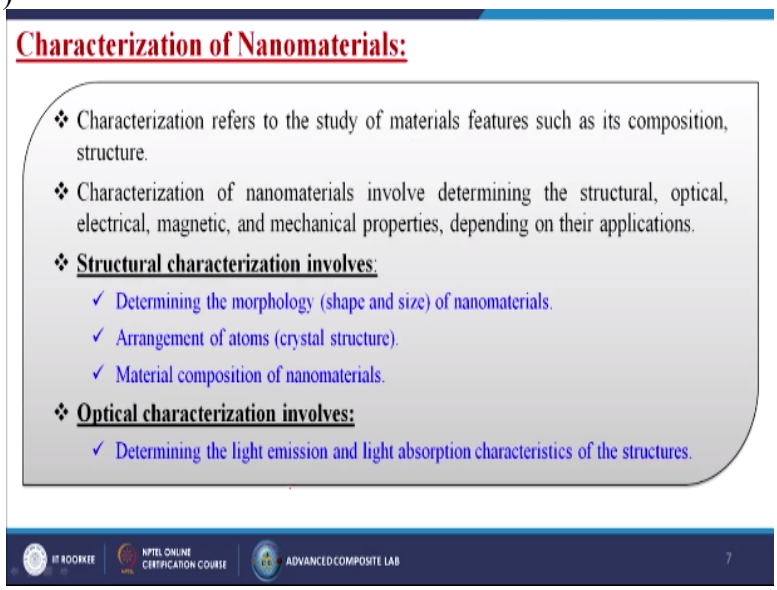

Now we are going into the more wider into this particular topic, so how to characterized this kind of nano materials, so characterizations refer to the study of materials features such as it is compositions and the structure, characterization of nano materials involve determining the structural, optical, electrical, magnetic, and mechanical properties, depending on their applications, structural characterizations involves.

Determining the morphology that means the shape and the size of nano materials arrangement of the atoms, that means crystal structure, materials compositions of the nano materials and when we are talking about the optical characterizations that involves determining the light emission and the light absorption characteristics of the structures itself, now general characterizations and techniques.

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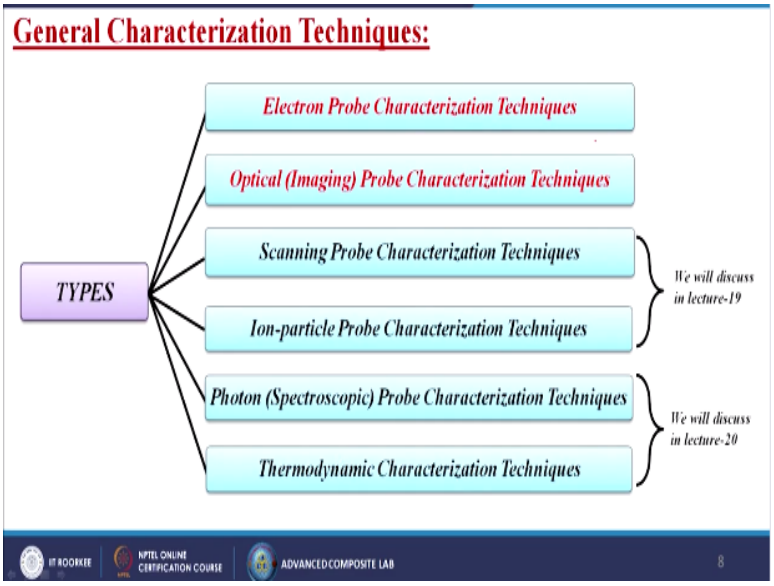

So if we divided into by types, so there are total six techniques, one is called the Electron Probe characterizations Techniques, next Optical imaging Probe characterizations Techniques which we are going to discussed in this particular lecture, then we are having the Scanning Probe characterizations Techniques and Ion-particle Probe characterizations Techniques which we are going to discussed into our next lecture and Photon Spectroscopic Probe characterizations Techniques, thermodynamic characterizations Techniques which we are going to discussed in our last lectures.

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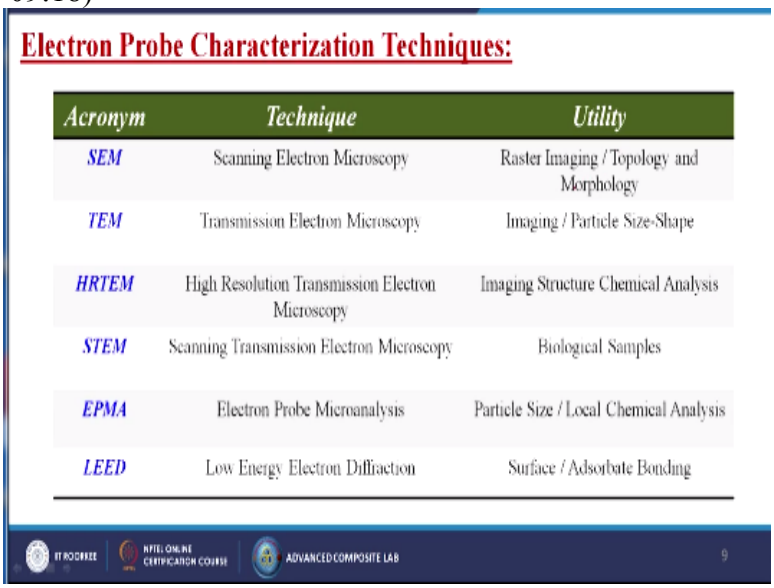

Now first Electron Probe characterizations Techniques that is also divided into several parts first is called the SEM, SEM means Scanning Electron Microscope what is the utility, Raster Imaging and Topology and Morphology of that particular materials, that kind of information generally we are getting, next is called the TEM Transmission Electron Microscope Imaging, or may be the particles Size or shape generally we are getting, HRTEM High Resolutions Transmission Electron Microscope, Imaging structure Chemical Analysis, STEM Scanning Transmission

Electron Microscope, generally for the Biological Samples we are doing, EPMA Electron Probe Microanalysis particle size, or may be the local Chemical Analysis, we are doing LEED that is low Energy electron Diffractions generally for the Surfaces and the Absorbate Bonding, of that particular nano materials, so by doing this kind of characterizations, we are getting this kind of information about that materials.

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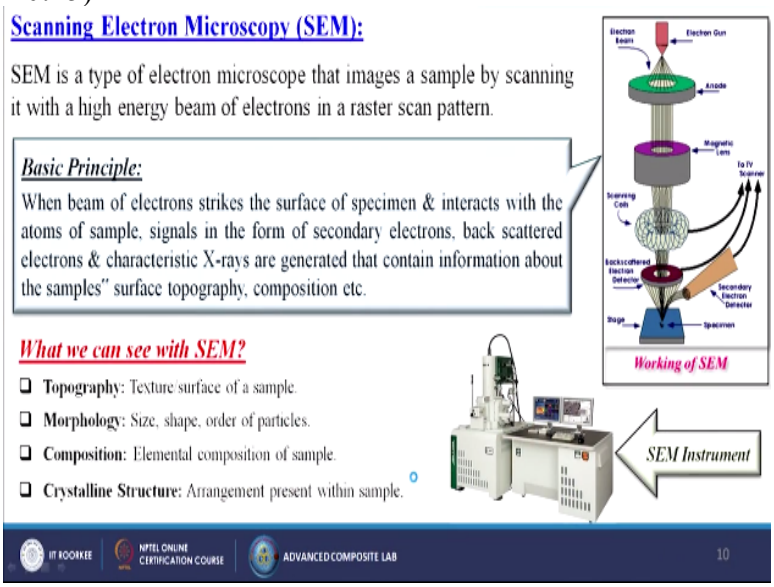

So first we are going to discussed about the Scanning Electron Microscope in small in generally we are calling it as a SEM, SEM is a type of electron microscope that images the sample by scanning it with a high energy beam of electrons in a raster scan pattern, what is the basics principles, when a beam of electrons generally that, we are having that electron gun so through that we are generating the electron and then that electron through some dedicators and some lens and some magnetic lenses it is falling on to our substrate or may be our materials.

Strike the surface of the specimen and interacts with the atoms of sample, signals in the form of secondary electrons back scattered electrons and characterized X-rays are generated that contain information about the samples surface topographic, or may the compositions etc..,

So what we can with SEM, so generally topographic texture surface of the samples we can see the surfaces how it is looks like, morphology size, shape and order of the particles, say suppose I have used some kind of nano particles inside the matrixes that I can see compositions elemental compositions of samples, so I am having a composites, so what are the element I have used and more or less can give the percentage also, then the crystal line structure arrangement present within samples, so this is the same instrument generally, we are using for taking the picture of any kind of nano materials.

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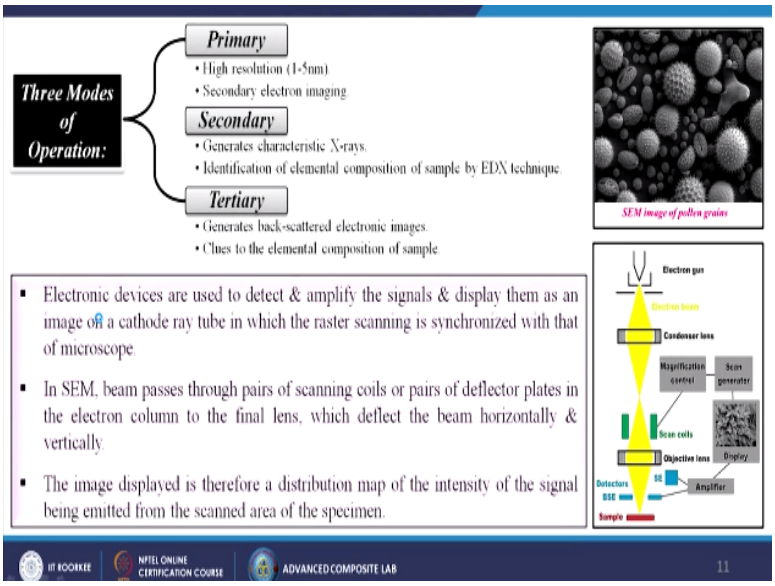

So generally there are three modes operations first one is called the primary, which is the high resolutions generally 1 to 5 nano meters, secondary electron imaging, then second one is called the secondary generates characterized X-rays, identifications of elemental competitions of sample by EDX techniques and last one is called the Tertiary, that is generate the back scattered electronic images, clues to the elemental compositions of sample, so here you can see that is the general feature, we have given that of the pollen grains, so in this particular case you can see that we are having an electron gun.

Then electron beam through condense lens it is coming, then it is falling on to the sample itself, so we are having the three dedicators over there, the dedicators BSC then secondary and another one is the magnifications through magnifications control, that scan generate and we are getting the display over there, so electronic devices are used to dedicate and amplify the signals and display as a image, on a cathode ray tube in which the raster scanning is synchronized with that of microscope, in SEM beam pass through pairs of scanning coils or pairs of deflector plates.

In the electron column to the final lens, which deflect the beam horizontally and vertically, the images displayed is therefore a distribution map of the intensity of the signal being emitted from the scanned area of the specimen itself.

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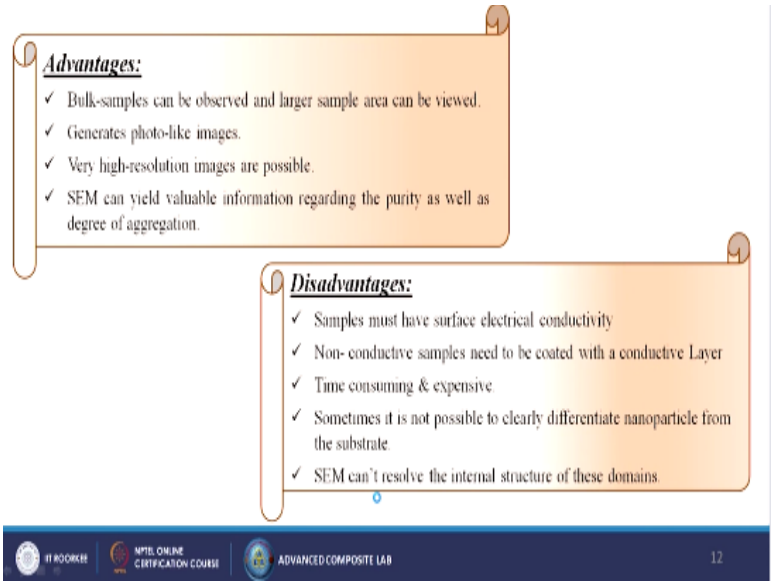

So what is the advantages, so Bulk samples can be observed and larger sample area, can be viewed, generates photo-like images, very high-resolution image are possible SEM can yield valuable information regarding the purity as well as degree of aggregations, then what are the disadvantages, samples must have surface electrical conductive, Non- conductive samples need to coated with a conductive layer, time consuming and expensive sometimes, it is not possible to clearly differentiate nano particles from the substrate, SEM cannot resolve the internal structure of these domains.

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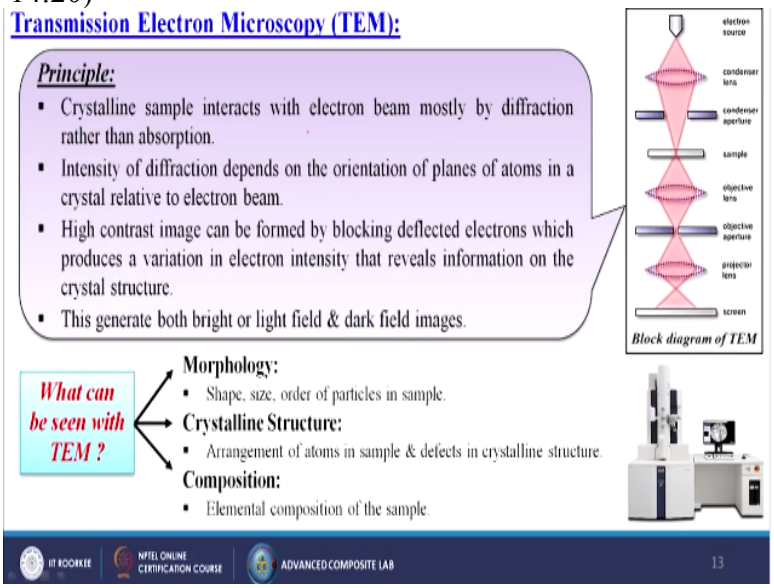

Now next one is called the Transmission electron Microscope, in short generally we are calling it as a TEM, so what is the principle crystal sample interacts with electron beam mostly by diffractions rather than absorptions, intensity of diffractions depends on the orientations of planes of atoms in a crystal relative to electron beam, high contrast image can be formed by blocking deflected electron which produced a variations in electron intensity that reveals information on the crystal structure, this generate both bright or light field and dark field images, so like this

way we are the electro source then we are having that condenses lens then condenses aperture then sample we are keeping through that, we are having that objective lens then objective aperture then, we are having the projector lens and then we are having the screen on which we are getting the image, so what can be see with TEM, on the basic of Morphology generally shape, size order of the particles in sample, if we are going to see the Crystalline Structure we can see arrangement of atoms, in sample and defects in crystalline structure and if we are going to see about the, or may be the, or we are going to know about the compositions, so elemental compositions of the sample, so this is the standard TEM instrument generally.

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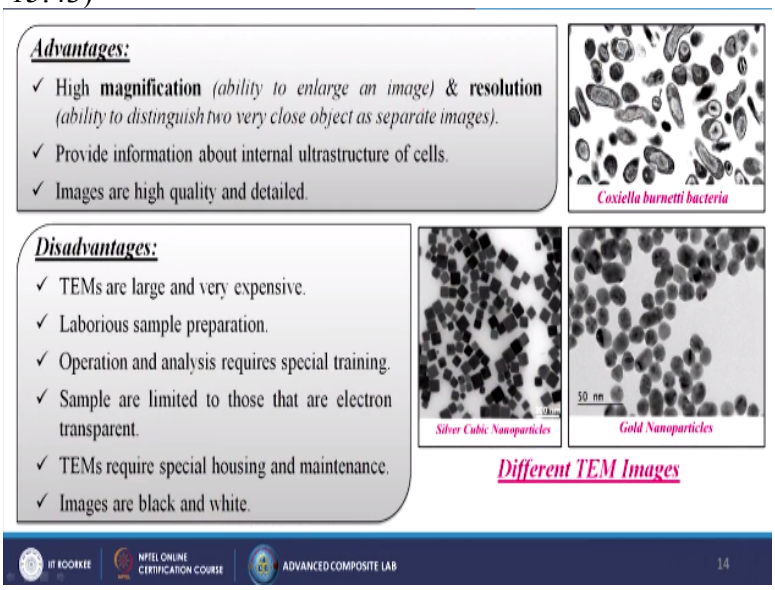

What we are using, what are the advantages high magnifications ability to enlarge an image and resolutions ability to distinguish two very close objects, as separate images, provide information about internal ultra structure of cells, images are high quality and detailed, so here in this cases you can see that we are getting the image into the 50 nanometer scale, or may be the 200 nanometer scale like sliver cubic nano particles, gold nano particles or may be some kind of bacteria image, of course there are certain disadvantages also, so what are those Tem are large and very expensive, laborious sample preparations, operations and analysis requires special training sample are limited to those that are electron transparent, Tem requires special housing and maintenances, images are black and white.

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Comparison of TEM vs. SEM:

	TEM	SEM
Beam voltage	100-400 kV	1- 30 kV
Focus of analysis	Internal or beyond surface	Surface of sample
Modes	Broad beams and scanning probe	Scanning probe
Smallest probe	0.5 nm (5 Å) using STEM	~ 1 nm (10 Å)
Best resolution	0.14 nm (1.4 Å) lattice imaging	~ 1 nm (10 Å)
Contrast	Forward scattered electrons	Secondary emission and backscattered electrons
Insulators	No charging	Charging effects
Sample thickness	10-200 nm (100-2000 Å)	1-10 mm
Sample diameter	< 3 mm across	Full wafers
Minimum preparation time	~ 4 hours	~ 1 min
Image presentation	2-D	3-D
Display of image	On TV monitor	On fluorescent screen

Now we are going to do the comparison study of Tem verses SEM so if we talk about the TEM so beam voltage is generally 100 to400 kilo volt, in the case of SEM it is 1 to 30 kilo volt, focus of analysis it is internal, or may be the beyond surfaces for same it is the surfaces of the sample, modes broad beams and scanning probe, in this case it is only the scanning probe, smallest probe 0.5 nanometer that is 5 angstrom using stem, it is more or less 1 nanometers that means 10 angstrom, best resolutions 0.14 nanometer that means 14 angstrom, lattice imaging, in this cases it is more or less 1 nano meter 10 angstrom, contrast far-ward scatted electrons SEM it is secondary emission and the back scatted electrons, Insulators here no charging here is the charging effects sample thickness it is 10 to 200 nano meter, that means 100 to 2000 angstrom, here in this case it is 1 to 10 millimeter sample diameters less than 3 millimeter across it is full way wafers, so that means you can cover the whole area but of course it is with the limit, minimum preparations time it is more or less 4 hours it is less than one minute, image presentations that is the vital one, it is giving you the 2D image, it is giving you the 3D image display of images on TV monitor it is on florescent screen.

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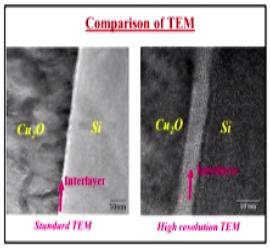
High Resolution Transmission Electron Microscopy (HRTEM):

- It is also known as phase contrast, an imaging mode of TEM.
- It allows the imaging of crystallographic structure of a sample at an atomic scale.
- Independent interaction with sample results the electron wave to pass through the imaging system of microscope where it undergoes further phase change & interferes as image wave in the imaging plane.
- Recorded image is not a direct representation of the samples crystallographic structure.

Uses:

- To study local microstructures like lattice fringe, glide plane or screw axes.
- For surface atomic arrangement of crystalline nanoparticles.



Now we are going to discussed about the High Resolutions Transmission Electron Microscopy which is known as the HRTEM, it is also known as the phase contrast, an imaging mode of TEM,

it allows the imaging of crystallographic structure of a sample at an atomic scale, independent interactions with sample result the electrons wave to pass through the imaging systems of microscope, where it undergoes further phase change and interferes as image wave in the imaging plane, recorded image Is not a direct representations of the sample crystallographic structure, what is the uses to study local micro-structural like lattice fringe glide plane.

Or may be the screw axes, for surface atomic arrangement of crystalline nano particle, so when we are going to do the comparison of the TEM image, so you can see in the standard TEM we are having two samples CU_2O and silicon and in between that we are having one inter layer of course, but in this case it is not clear, but when we are see the same sample under the high resolution TEM, so scalar SEM but totally the resolutions is different, so in this case we can see a clear layer that means the inter layer in between the two materials.

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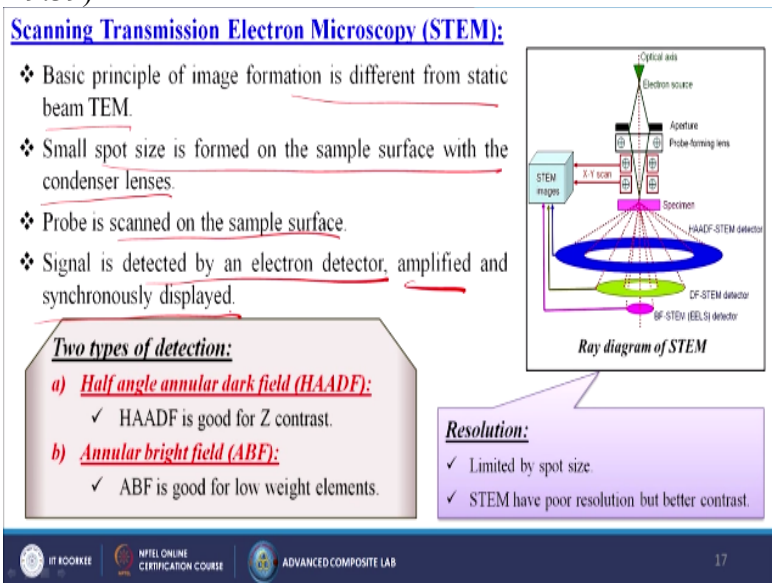

Next Scanning Transmission Electrons Microscopy or may it is known as the STEM basic principles of image formations is different from static beam TEM, small spot size is formed on the sample surfaces with the condenser lenses, Probe is scanned on the sample surfaces, signal is detected by a electron detector, amplified and synchronously displayed this is also two types or may be by two types generally we do this deductions, so one is called the Half angle annual dark field in short it is called HAADF it is good for Z contrast, another one it is called the Annual bright field or may be the ABF which is good for low weight elements, so generally the resolutions limited by the spot size because you can see that thus on the sample it is falling as a small spot STEM has poor resolutions but better contrast.

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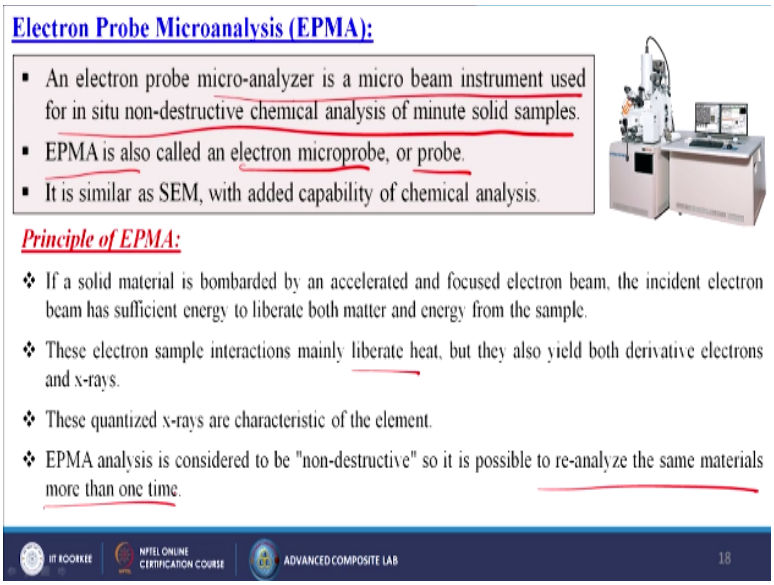

Now next one is that Electron Probe Microanalysis, in short it is called the EPMA and electron probe micro-analyzer is a micro beam instrument used for in situ non-destructive chemical analysis of minute solid samples, EPMA is also called an electron microprobe, or may be the probe, it is similar as SEM, with added capability of chemical analysis, so this is the overall or may be the normal picture of the EPMA machines, principle how it is working.

If a solid material is bombarded by an accelerated and focused elections beam, the incident electron beam has sufficient energy to liberate both matter and energy from the sample itself, these electron sample intersections mainly liberate heat, but they also yield both derivative electrons and X-rays, the quantized X-ray are characteristic of the element itself, EPMA analysis is considered to be non-destructive testing, we are not going to harm the samples on may be the hitting the samples, are may be the breaking samples, so it is possible to re-analyze the same materials more than one time that means several we can do this testing on to the sample itself.

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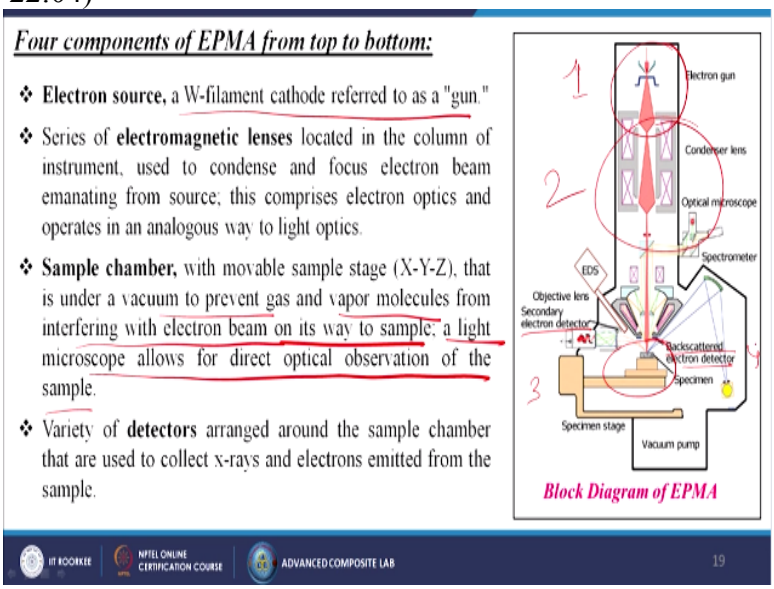

Four components of EPM from top to bottom, so generally there are four components, first one it is called the electrons source, a tungsten filament cathode referred to as a gun, so this is the number one series of electromagnetic lenses located, in the column of instrument used to condense and focus electron beam emanating from source this comprises electrons optics and operates in an analogous way to light optics, so this zone is known as the number two.

Then sample chamber with moveable sample stage X,Y,Z that means any directions you can move you are samples that is under a back vacuum to prevent gas and vapor molecules from interfering with electron beam on it is way to sample a lighter microscope allows for direct optical observation of the sample itself, so here this is known as the number three zone over there, then variety of detectors arranged around the sample chamber that are used to collect X-rays electrons emitted from the sample itself, so we are having the secondary electron detector we are having the back scattered electron detector and this is the, so these zone is number three and this all are the detectors are known as number four.

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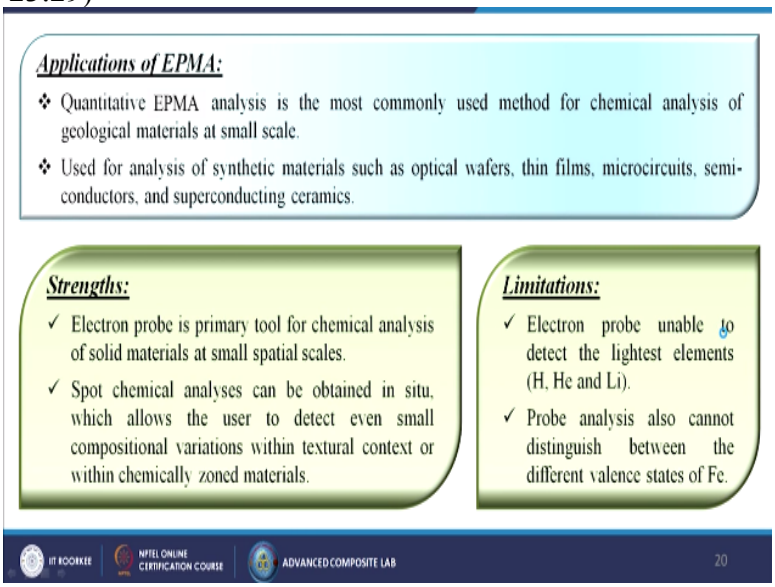

Applications quantitative EPMA analysis is the most commonly used method for chemical analysis geological materials at small scale, used for analysis for synthetic materials such as optical wafers, thin film microcircuits semi-conductor and the super conducting ceramics, strength electron probe is primarily tool for chemical analysis of solid materials at small spatial scales, spot chemical analysis can be obtained in situ, which allows the user to detective even small compositions variations within textual context or within chemical zoned materials, what are the limitations electron probe unable to detect the lightest elements like hydrogen, helium or may be the lithium, probe analysis also cannot distinguish between the different valence states of iron.

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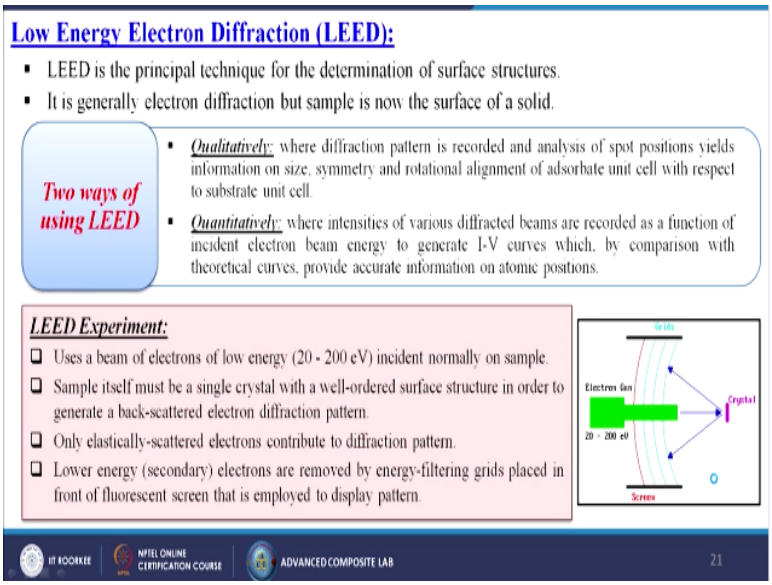

Next is called the Low Energy Electron Diffractions, in short it is calling at, as a LEED, LEED is the principal techniques for the determinations of surfaces structures, it is generally electron diffractions but sample is now the surface of a solid, two ways of using the LEED, first one is called the Qualitatively, where diffractions pattern is recorded and analysis of spot positions yields informational size symmetry and rotations alignment of Absorbate unit cell with respective to substrate unit cell and then next one is called the Quantitatively.

Where intensities of various diffracted beams are recorded as a functions of incident electron beam energy to generate the I-V curves which by comparison with theoretical curves accurate information on atomic positions, how we are going to perform this LEED experiment use the beams of electrons of low energy generally 20 to 200 electron bolt incident normally on the sample itself.

So do you have the electron gun over here so directly it is falling on to you are materials or may be the crystals sample itself must be a single crystal with a well ordered surfaces structure in order to generate a back scattered diffractions pattern, so after falling it is back scattering, only electrically scattered electrons contribute to diffractions pattern lower energy secondary electrons are removed to by energy filtering grid placed in front of florescent screen that is employed to display the pattern itself, so initially we are putting the grids and then after that we are putting the screen.

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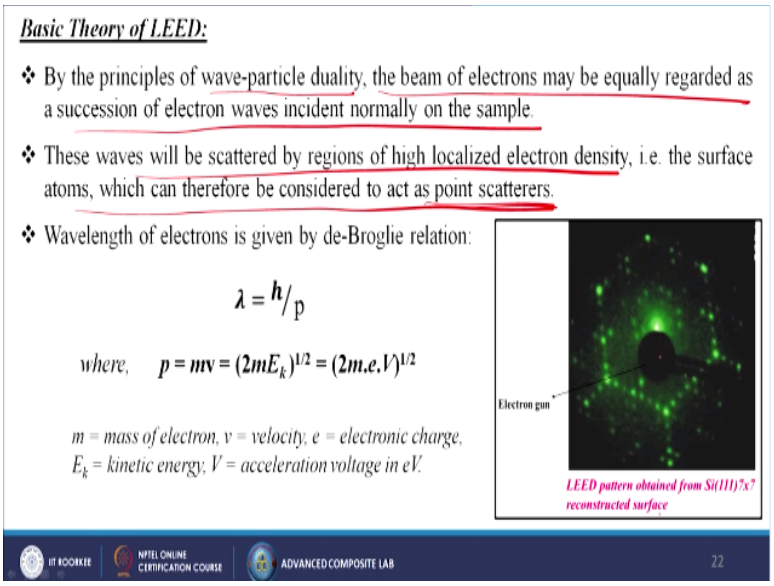

Basic theory how it is working by the principles of wave particle duality, the beam of electrons may be equally regarded as a succession of electron waves incident normally on the sample, these wave will be scattered by regions of high localized electrons density, that is the surface atoms which can therefore be considered to act as point scatterers, wave length of electrons are given by de-Broglie relations that is lambda is equal to H by P, so where P is equal to MV which is equal to $2ME_K$ root over is equal to root over 2MEV, M is equal to mass of electrons, V is equal to velocity, E is equal to electronic charge EK is the kinetic energy, capital V is the accelerations voltage in electron volt, so this is the generally the electron gun, so this is the lead pattern obtain silicon 111 7 into 7 reconstructed surface itself.

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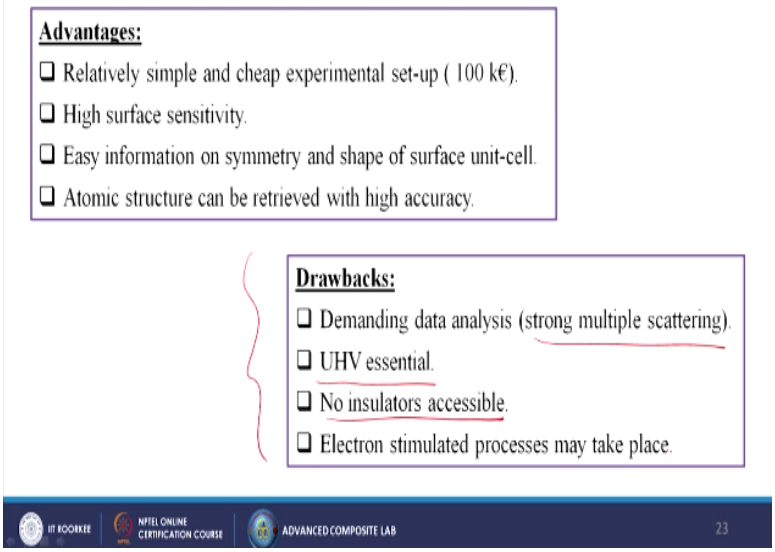

What are advantages relatively simple and cheap experiment set up, that is less than 100 k€ high surface sensitivity, easy information on symmetry and shape of surface unit cell, atomic structure can be retrieved with high accuracy, of course there is certain draw backs what are those

demanding data analysis strong multiplying scattering, UHV ultra high vacuum is essential, no insulators accessible, electron stimulated processes may take place.

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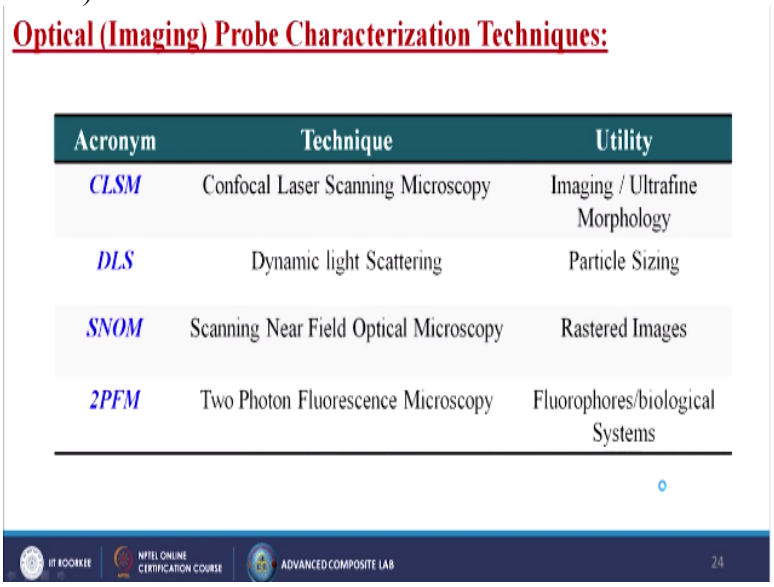

So now we are going to discussed about the Optical Imaging Probe Characterizations Techniques, so generally we are talking about the CLSM which is nothing but known as Confocal Laser Scanning Microscope, what is the utility imaging, or may be Ultrafine Morphology generally we are getting DLS Dynamic Light Scattering that is for the particle sizing, SNOM Scanning Near Field Optical Microscope this is for the Rastered Images and 2PFM Two Photon Fluorescence Microscope generally it is for the Fluorophores or may be the biological systems.

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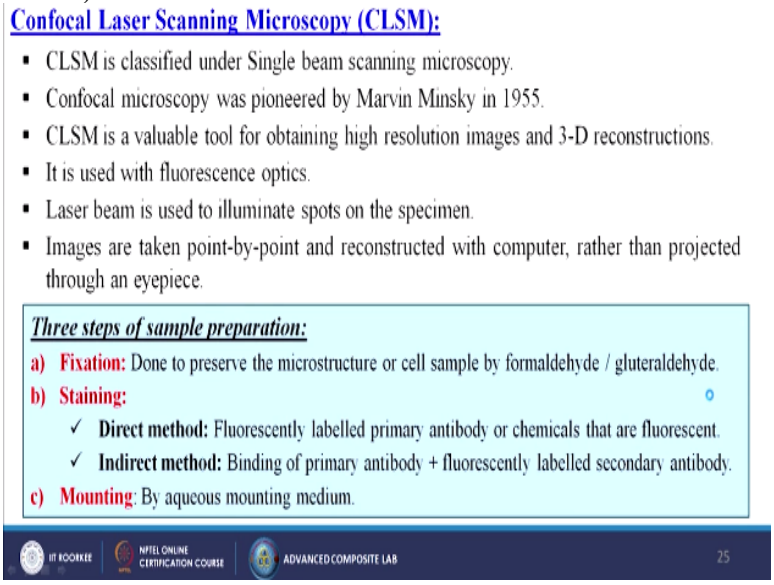

So first we are going to discussed about the CLSM, which is nothing but the Confocal Laser Scanning Microscopy CLSM is classified under single beam scanning microscopy, it was by pioneered by Marvin Minsky in year of 1955, it is a valuable tool for obtaining high resolutions

images and 3-D reconstructions, it is used with Fluorescence optics, Laser beam is used to illuminate spots on the specimen itself. Images are taken point-by-point and reconstructed with computer, rather than projected through an eyepiece. There are three steps of sample preparations. First one is called the fixation, done to preserve the microstructure or cell sample by formaldehyde or maybe the gluteraldehyde. Staining, direct method: fluorescently labeled primarily antibody or chemicals that are fluorescent.
Indirect method: binding of primary antibody + fluorescently labeled secondary antibody. And, mounting: by aqueous mounting medium. Then, how actually we are performing this test?
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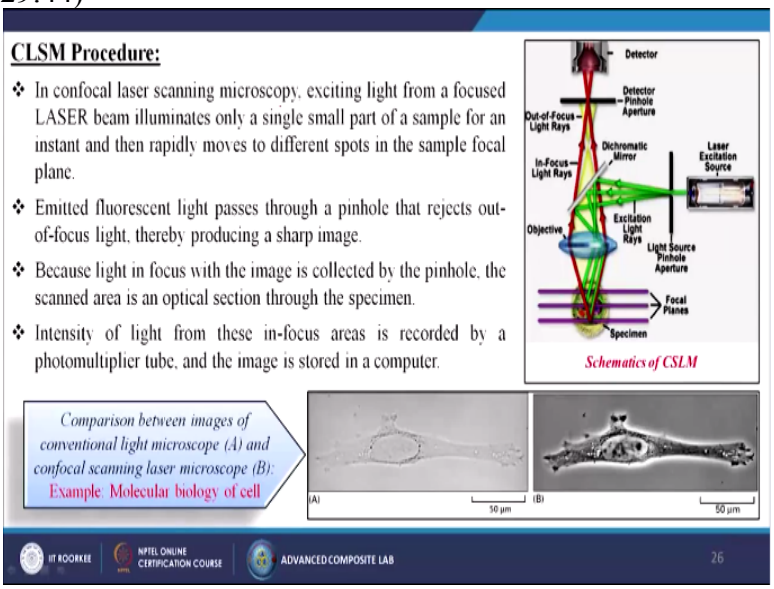

In confocal laser scanning microscopy, exciting light from a focused laser beam illuminates only a single small part of a sample for an instant and then rapidly moves to different spots in the sample focal plane.
Emitted fluorescent light passes through a pinhole that rejects out of-focus light, thereby producing a sharp image over there. Because light in focus with the image is collected by the pinhole, the scanned area is an optical section through the specimen. Intensity of light from these in-focus areas is recorded by a photomultiplier tube and the image is stored in a computer itself.
So here, you can see the two images both the scales are same, but first one is by the comparison of image of conventional light microscope and second one is the confocal scanning laser microscope.
So, you can see that how the contrast of that image is totally changing and how we are getting the clear picture from the CLSM.
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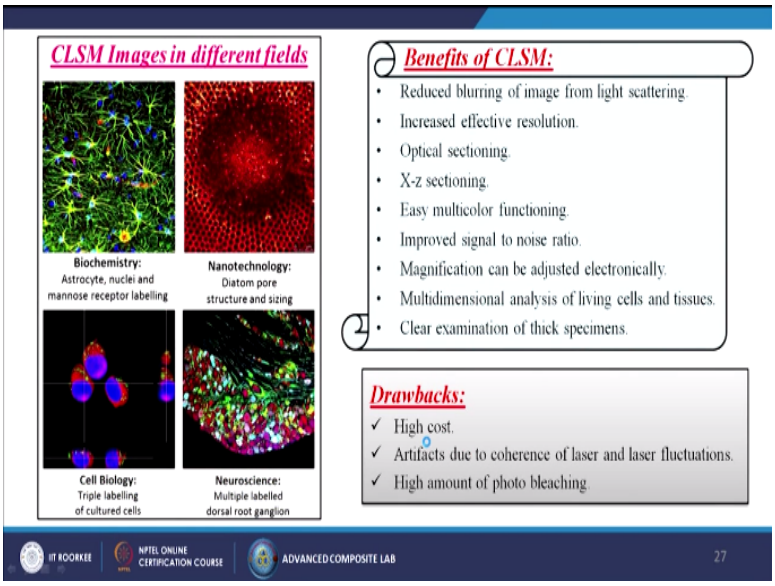

So, we are going to show you, there are several types of CLSM image in different fields like biochemistry like astracyte, nuclei and mannose receptor labeling, for nanotechnology it is diatom pore structure and sizing, for cell biology triple labeling of cultured cells, neuroscience multiple labeled dorsal root ganglion. So, what are the benefits of CSLM reduced blurring of image from high scattering, increased effective resolutions, optical sectioning, x-z sectioning. Easy multicolor functioning, improved signal to noise ratio, magnification can be adjusted electronically, multidimensional analysis of living cells and tissues, clear examination of thick specimens. Of course, there are certain drawbacks also. It is the ecomet cost is too high, artifacts due to coherence of laser and laser fluctuations, high amount of photo bleaching. Next is called the dynamic light scattering or maybe the DLS. DLS is also known as photon correlation spectroscopy or maybe the quasi-elastic light scattering.

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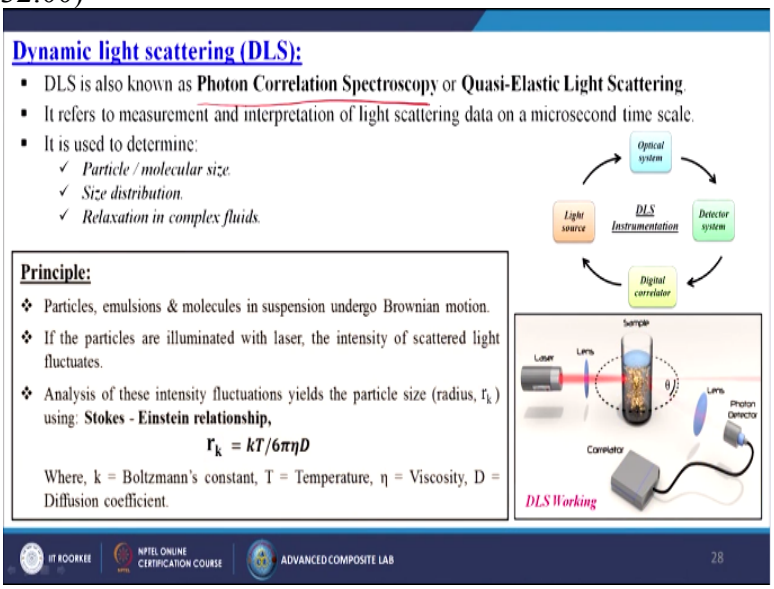

It refers to measurement and interpretation of light scattering data on a microsecond time scale. It is used to determine particle or maybe the molecular size, size distribution relaxation in complex

fluids. So, generally we are having that light source then optical systems, the detector scheme and then the digital correlator. How it works? Particles, emulsions and molecules in suspension undergo Brownian motion.

If the particles are illuminated with laser, the intensity of scattered light fluctuates in this particular case. Analysis of these intensity fluctuations yields the particle size (radius r_k) using: Strokes- Einstein relationship which is $r_k=kT/6\pi\eta D$ where, k is the Boltzmann's constant, T is the temperature, η is the viscosity and D is the diffusion coefficient.

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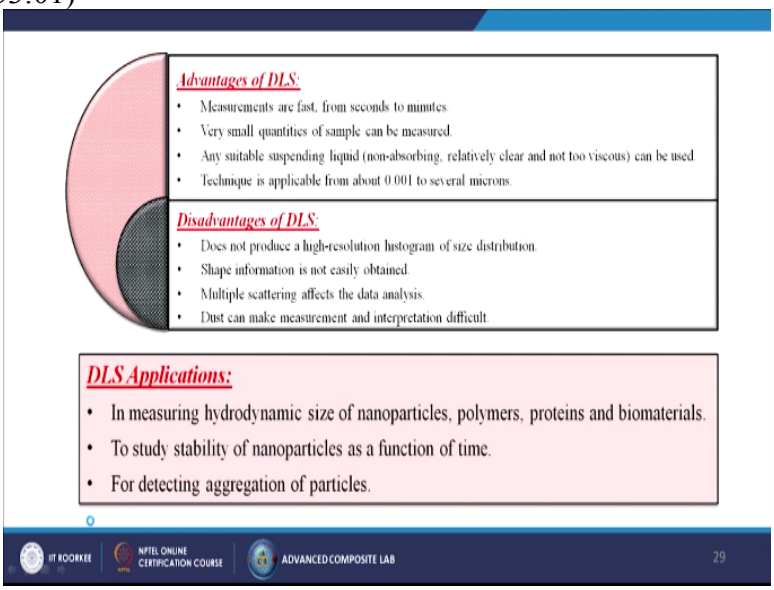

What are the advantages of DLS? Measurements are fast from seconds to minutes. Very small quantities of sample can be measured by this method. Any suitable suspending liquid non-absorbing relatively clear and not too viscous can be used. Technique is applicable from about 0.001 to several microns. There are certain disadvantages also. It does not produce a high resolution histogram of size distribution.

Shape information is not easily obtained. Multiple scattering affects the data analysis. Dust can make measurement and interpretation difficult. What are the applications in measuring hydrodynamic size of nano-particles, polymers or proteins or maybe the biomaterials, to study the stability of nano-particles as a function of time, for detecting the aggregation of nano-particles?

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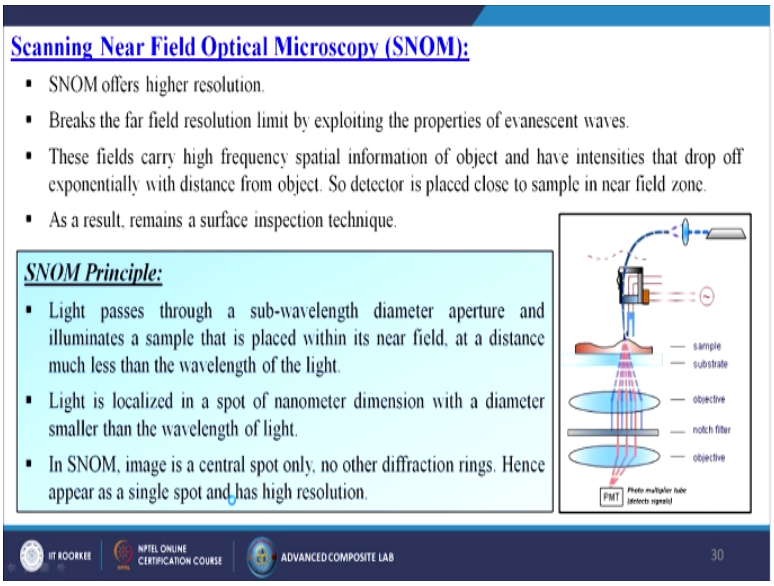

Next, scanning near field optical microscopy in short, generally we are calling it as a SNOM. SNOM offers higher resolution, breaks the far field resolution limit by exploiting the properties of evanescent waves, these fields carry high frequency spatial information of object and have intensities that drop off exponentially with distance from object. So, detector is placed close to sample in near field zone, as a result, remains a surface inspection techniques.

So, we are having the samples on the substrate itself then, we are having the objective not filtered. Then, we are having the objective and then, after that we are having the photo multiplayer tube which actually detects the signals. SNOM principle, light passes through a sub-wavelength diameter aperture and illuminates a sample that is placed within its near field, at a distance much less than the wavelength of the light is localized in a spot of nanometer dimension with diameter smaller than the wave length of light. In SNOM, image is central spot only, no other diffraction rings. Hence appear as a single spot and has high resolution.

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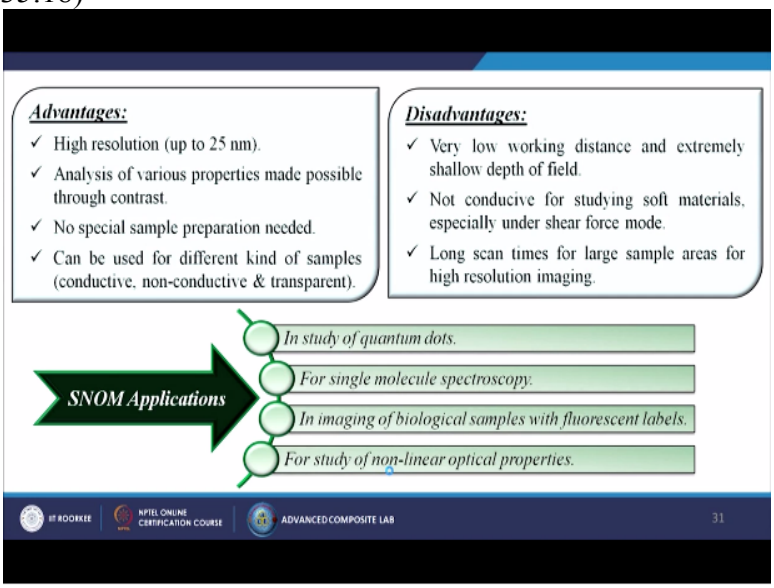

What are the advantages High resolutions (up to 25 nanometer) Analysis of various properties made possible through contrast. No special sample preparation needed. Can be used for different kind of samples like (conductive, non-conductive or maybe the transparent). Disadvantages: very low working distance and extremely shallow depth of field. Not conductive for studying soft materials.

Especially under shear force mode long scan times for large sample areas for high resolution imaging. SNOM applications: In study of quantum dots, for single molecule spectroscopy in imaging of biological samples with florescent labels, for study of non-linear optical properties.

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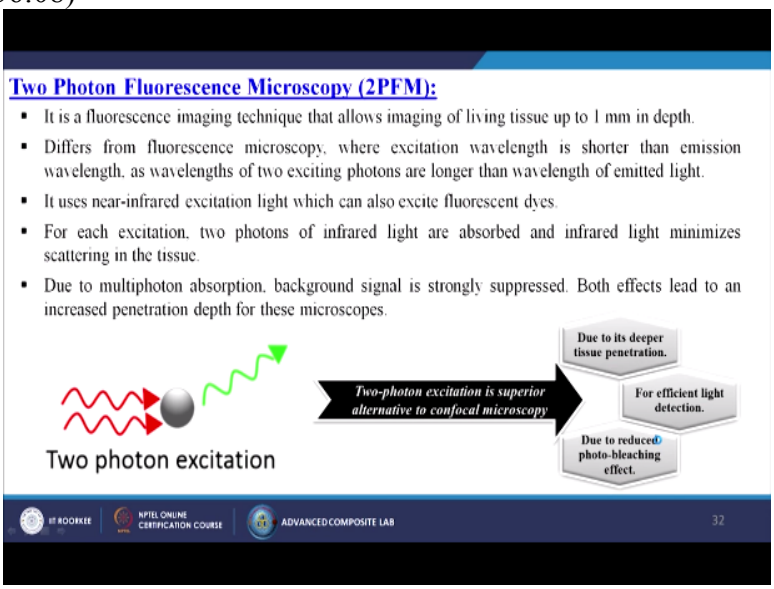

Then, the last one is called the Two Photon Fluorescence Microscopy or maybe, the 2PFM. It is a fluorescence imaging technique that allows imaging of living tissues up to 1mm I depth. Differs from fluorescence microscopy, where excitation wavelength is shorter than the emission wavelengths, as wavelengths of two exciting photons are longer than wavelength of emitted light. It uses near-infrared excitation light which can also excite fluorescent dyes. For each, excitations two photons of infrared lights are absorbed and infrared light minimizes scattering in the tissue.

Due to multi-photon absorption, background signal is strongly suppressed both effects lead to an increased penetration depths for these microscopes. So, generally we are doing it by the two photon excitations. So, due to it is a deeper tissue penetration for efficient light detection, due to reduced photo-bleaching effect. So, of course it is better to photon excitations. It is alternative to the confocal microscopy.

So basic, phenomenon two-photon excitation occurs through the absorption of two lower energy photons via short -lived intermediate states.

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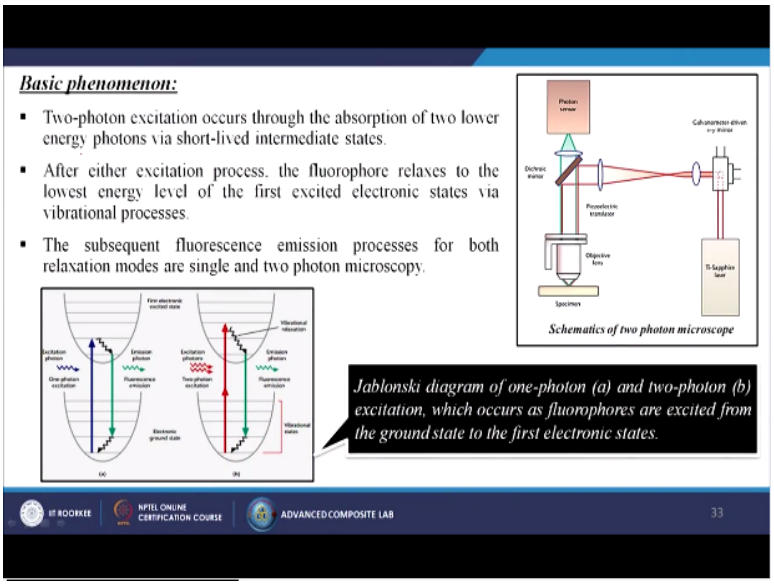

After either excitation process, the fluorophore relaxes to the lowest energy level of the first excited electronic state via vibrational processes. The subsequent fluorescence emission processes for both relaxation modes are single and photon microscopy. Here, it is the Jablonski diagram of one-photon. This is here the one-photon excitation is taking place and two-photon here the two-photon excitation is taking place, which occurs as fluorophores are excited from the ground state to the fast electronic state.

So, you can see that this one is our electronic ground state so it is going to the first electronic excited state.

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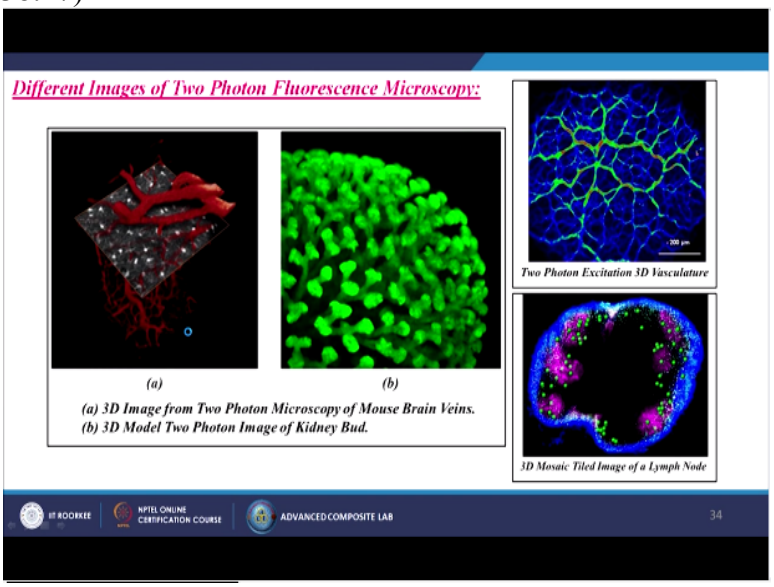

So, these all are the examples of Two Photon Fluorescence Microscopy: So, first is the 3D image from Two Photon Microscopy of Mouse Brain Veins so, this one. Next (B) is the 3D Model Two Photon image of Kidney Bud. This is the Two Photon Excitations 3D Vasculature and this is the 3D Mosaic Tiled image of a Lymph Node.

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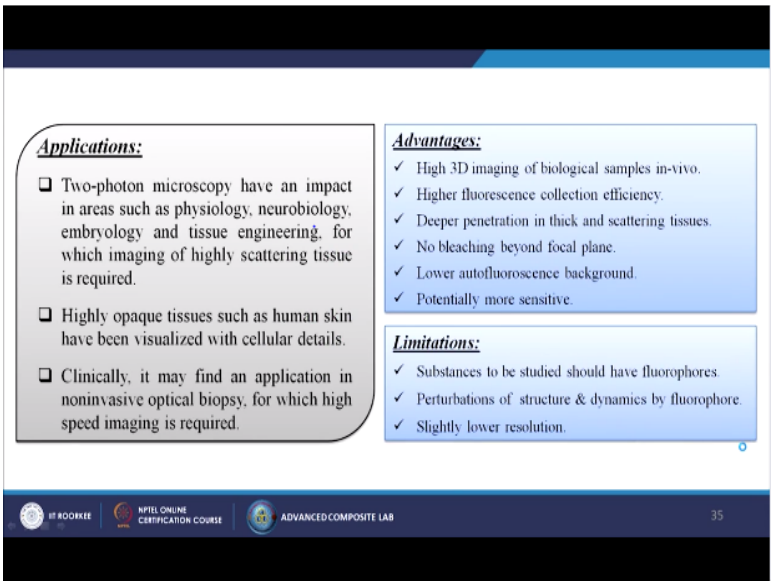

So, what are the applications: Two-photon microscopy have, an impact areas such as physiology, neurobiology, embryology and tissue engineering, for which imaging of highly scattering tissue is required. Highly opaque tissue such as human skin, have been visualized with cellular details. Clinically, it may find an application in noninvasive optical biopsy, for which high speed imaging is required.

Advantages: High 3D imaging of biological sample in-vivo, higher fluorescence collection efficiency, deeper penetration in thick and scattering tissues, no bleaching beyond focal plane, lower auto fluorescence background, potentially more sensitive. What are the limitations: Substance to be studied should have fluorophores, perturbations of structure and dynamics by fluorophore slightly lower resolution. So, now we have come to the last part of this particular lecture. So, we are going to we are going to summarize the whole lecture. So, in this particular lecture we have discussed about the nonmaterials. So, nonmaterial's characterization is necessary to establish understanding and control of nanomaterials synthesis and applications.

For characterization of nanomaterials a large number of aspects might be of interest like Size, shape, structure, chemistry, crystallography, etc.

Due to wavelength, electron microscopy in its different variations is most employed method for characterization of nanomaterials. Spectroscopic methods and 3D-related methods like, tomography complete the results. Nanotechnology has a lot of potential as a futuristic approach but would we be largely governed by simultaneous progress in the newer, faster, simpler and more efficient characterization techniques for nanomaterials.

See, of course after generating the nanomaterials, we can as much as we can able to generate the data or maybe the about the information about those materials. So, it will be beneficial or maybe it will be helpful for the studies. Thank you.

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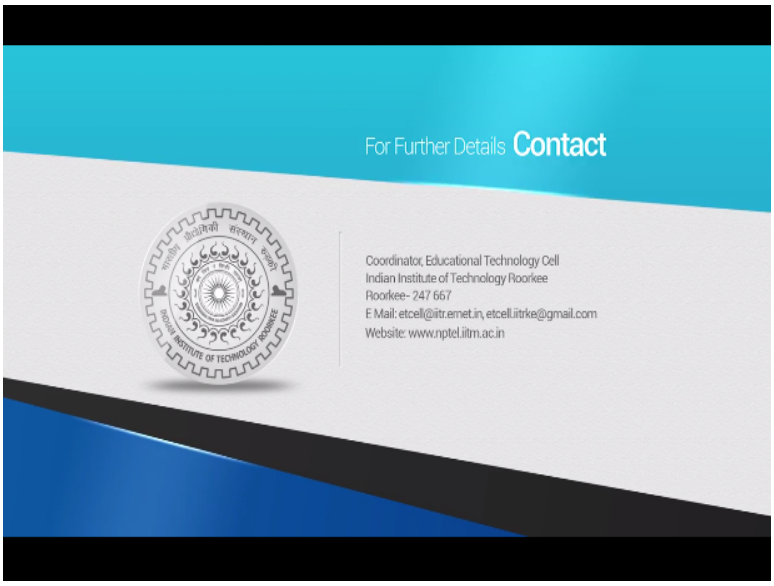

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Acknowledgement
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