Basics of classical and emerging tools

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Lecture-3

Objects at different length scales, numerical aperture, Abbe diffraction limit, types of light microscopy, principles of electron microscopy, types of electron microscopy, types of probe microscopy, STM, and AFM

welcome back, in this video lecture we will look at basics of classical and emerging tools, ok, we will look at we will try to give you some insight into some of the, you know, classical and emerging tools so why we should ,you know, understand ,you know, these tools now in the module one itself ,right, so that is the question that one would one may ask ,so the idea is we want to introduce this ,you know, give overview of these tools now itself so that as we move on So, you will be coming across, you will be dealing with many material systems. Let us say, you will come across, let us say, you may work with, let us say, colloidal particles, right? Or you may work with surfactant systems. or polymer molecules or polymer systems right. So, you may be wondering, you know, what kind of techniques what is the, right, technique that one should use, you know, to characterize them. Let us say somebody may be interested in, you know, understanding or performing the size distribution analysis of given particle system. Or somebody may be interested in knowing what is the morphology of the given nanomaterials.

Some may want to just understand the structure. Okay, so depending on the application and end use one would want to use the, right, technique and to characterize the materials and different nanomaterials are the materials that you will be, you know, dealing with you may want to know what what suitable techniques one has to use, right, So, this is the, right, time for us to introduce. some of these techniques are very relevant to interfacial engineering now itself so that as you move on you will have some clarity about, you know, the various tools that are available, okay, yeah let's begin,

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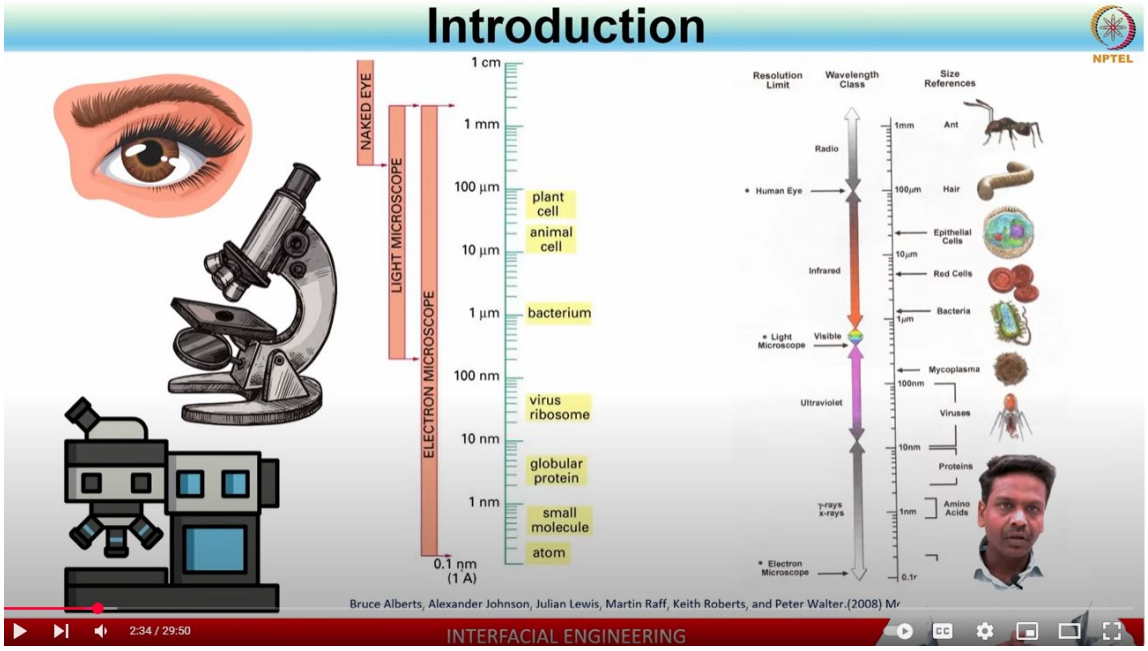

yeah so first let us start with ,you know, brief overview of length scale, ok ,as you can see ,you know,our human height we can look at we can ,you know, visualize objects ,right, any objects ,you know, up to certain ,you know, length scale only ,you know, through our naked eye say for example Anything beyond, let's say, 200 µ or maybe 300 µ, it is very difficult to resolve through Naked eye.

So, one should resort to the modern techniques that are available. let's say you may use light microscopy okay so that will be helpful to resolve any objects of size let's say up to 200 nanometer okay so so that so so that with that you will be able to ,you know, look at the samples let's say for example you want to see bacteria how bacteria look like okay you can see using the light microscopy but if you want to understand let's say virus how virus will look like then you may not see them through the light microscopy So, in such case you may have to resort to the electron microscopy ok. So, with electron microscopy you may be able to resolve up to let us say 0.1 nanometer using the transmission electron microscopy one can resolve any object a specimen of size up to 0.1 nanometer. With FESEM or SEM one can resolve up to one nanometer ,right, so these are various length scale that you can connect ,you know, well with the different living matter also ,right, and on the right hand you can easily map ,you know, what kind of technology one should use and what are the for example, example system say, for example you can use radio frequency up to about 100 µ meter So, when we talk about 100 µ meter you can relate with the our ,you know, hair ,right, our ,you know, human hair ,you know, airy particle is in the size range up to 100 micrometer ,right,. Which is more or less same as 100 micrometer or slightly above 100 micrometers. So, it is easy for us to, you know, resolve, right, I mean, you can see up to 100 micrometers through your naked eye. We

are able to see your hairs, right, Hairy particle, right, But beyond 100 micrometer, then one has to ,you know, result through different technology say for example less than 100 µ meter let's say up to 10 µ meter so you can give an example of epithelial cells ,right, they are slightly above 10 µ meter so you cannot directly view them through naked eye so then you need some technology like light microscopy that will be of use ,right, so you can resolve them under light microscopy okay and so anything so the bacteria and red cells you can easily map say bacteria is about one µ in size ,right, whereas the red cells is slightly ,you know, above 1 µ, okay, so you can easily connect ,right, now between 1 µ to 100 nanometer you can give some example systems that is like mycoplasma mycoplasma is a good example and ,you know, then comes viruses so between 100 nanometer to 10 nanometer ,you know, there are ,you know, various viruses okay range between 10 nanometer to 100 nanometer you cannot see them under light microscopy so one has to use a special or modern I mean sophisticated technique like ,you know, electron microscopy ,you know, to look at them to visualize them okay and if you want to relate anything between less than 10 nanometer then you can talk about proteins ,you know, protein molecules are of this of the size range up to 10 nanometer between 1 nanometer to 10 nanometer if you are talking about 1 nanometer you can connect easily with amino acids, amino acids are of that range okay What about less than 1 nanometer? You can talk about atoms, right, So, if you want to look at the atoms, right, of size let us say 0.1 nanometer, one can use electron microscopy, right, So, these are various length scale and various example systems, okay, to relate with the length scale, right,

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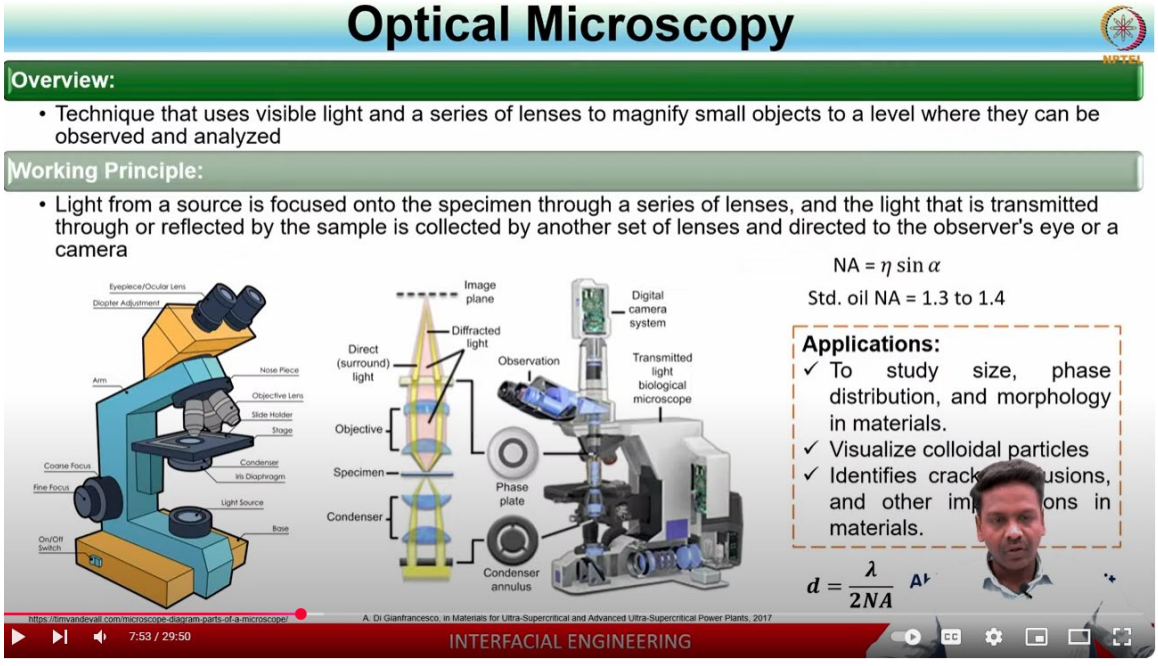

Okay, let us now look at the optical microscopy. So, thanks to, you know, the discovery of this, I mean, the people who invented optical microscopy in 16th century itself, but the much development came, you know, after, you know, 18th or 19th century ,right,? So, still lot of, you know, development ,you know, taking place in this domain that is optical microscopy, so optical microscopy is very useful technique ,you know, to visualize any object okay ,you know, which are not possible to look through naked eye ,right, so say for example if you want to understand how the bacteria look like you can easily look at them under microscopy okay some of the particle system that you want to let's say which are let's say 1μ in size or 2 μ in size you can look at them easily under microscopy optical microscopy okay all you need is just one light source and magnifying power just like how we use the magnifying lens you have objectives, objective lens in the microscopy that has got the magnifying power and ,you know, whenever you shine a light on the object okay whatever is transmitted from the space i mean object or specimen is collected by the objective lens and that is what is reflected in the ,you know, the viewing lens or eyepiece okay you can also ,you know, fit a camera digital camera and collect those image and project it directly on the screen ,you know, computer screen you can also record or you can also ,you know, record video video of ,you know, the live sales ,right, that is also possible you can also click capture a snapshot of the samples ,right, using this digital camera okay So these are ,you know, very much possible nowadays and there are some advanced stuff also but the basic of microscopy is you have got some important components like ,you know, condenser. So what condenser does is it collects the light and it focus the light on to the specimen and there is something called specimen stage and on top of which you will keep the sample and there is objective so objective lenses are available at different magnifying power say for example you have 5x objective lens 10x, 50x up to 100x objective lenses are possible so when I say 100x ,50x meaning you will be able to see specimen ,you know, several times say for example when I am weaving the specimen through 10x objective and my eyepiece is also 10x ,right, so then I would be able to see the object uh ,you know, ten hundred times more than the object ,right, say for example if i look at them through 100x objective i am magnifying the object specimen by thousand times ,right, so that is how you are able to see the the specimen ,you know, very clearly okay, which is not possible through naked eye ,right, so these are various components what is more important is that there are two there are a few i mean many types of microscopes that are available okay let's say for example the commonly used are ,you know, inverted microscopy or upright microscopy, upright microscopy is available in two category one is transmitted mode and another one is reflected mode whereas the inverted microscopy is available mostly in transmitted mode ,right, so whenever you deal with upright microscopy you will see that the objectives are fitted at the top okay whereas the light source is coming from the bottom in the case of inverted microscopy you will see that the objectives are fitted at the bottom whereas the light source is coming from the top okay so that's the difference that you will see whenever

you deal with upright or inverted microscopy Mostly biologists use inverted microscopy, biological scientists use inverted microscopy whereas material science scientists prefer ,you know, the upright microscopy.

Sometime the upright microscopy comes with ,you know, both reflected as well as transmitted ah ,you know, features so that you can see a sample of any kind transparent sample as well as opaque samples, ok ,so more importantly you must understand what is known as the numerical aperture, so numerical aperture tells the tells us about the magnifying or resolving capacity of the lenses Let us say, the more the numerical aperture, ,you know,, greater are the magnifying or resolving power of the lenses. Okay. So, the maximum angle that can go up to, in this case, the alpha can go up to 90 degree, which means the maximum, at maximum numerical aperture case is sin alpha will be 1.

Let us say if we deal with oil immersion objective, this eta is refractive index, right, So, this may vary between 1.3 to 1.4 for, you know, the ah oil immersion ah objective, right, So, this is about numerical aperture ah there is something called Abbe's diffraction limit this sets the ah the limiting point. Let us say ah if your ah wants to resolve some object ok which are kept at a distance of 200 nanometer you will be able to, you know, resolve them under the microscopy. Say for example, if I substitute lambda as 500 nanometers because this light microscopy works in the visible region visible light. So, lambda can be 500 nanometers then NA if I put 1.4 here. I will be getting about 178.6. So, one will be able to resolve a specimen which are kept at a distance, you know, of 178.6 nanometer, right, that is the resolving power of this light microscopy. The limitation is coming from the wavelength of the light itself ok, right.

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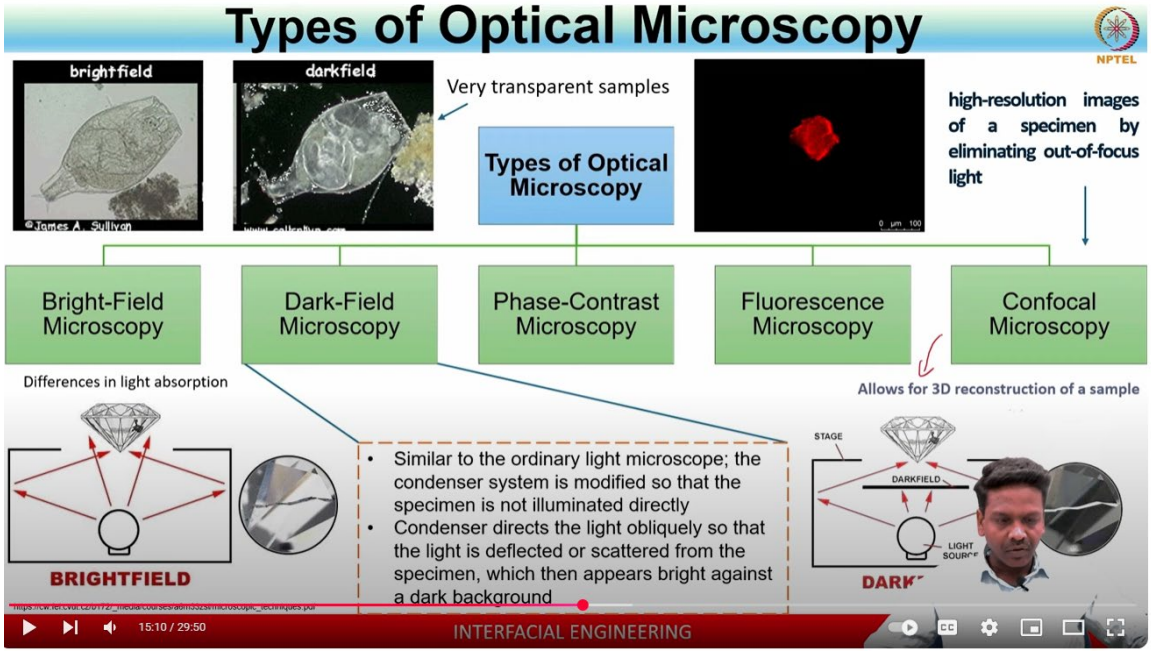

So, let us look at types of optical microscopy ok. So, more importantly you will see what is bright field, dark field and fluorescent microscopy ok. So, the difference between bright field and dark field is in the case of the dark field we the objective collects only the scattered light from the specimen. which means that ,you know, the light does not directly fall on the sample instead the sample is eliminated from the side so that only the scattered light comes out of the sample is collected by the objective lens ,right, so in doing so the because ,you know, all light that are transmitted from the sample are not collected so the background here will look very dark whereas the specimen will appear bright okay this is because of this feature in the case of bright field microscopy why we are able to see the specimen ,you know, distinctly is because of the differences in the light absorption okay although both will appear bright in this case Because specimen absorbs light in a different way, whereas the background absorbs in a different way, because of these differences in light absorption, we are able to see the specimen distinctly, although background and specimen are appearing bright in this case, ,right,? so there is something called phase contrast microscopy this is used let us say whenever you whenever the light passes through the sample because there are two kinds of changes that happen one is the there will be a magnitude change and the other one will be contrast change so the phase contrast microscopy what is what it does is it adjusts the contrast so that ,you know, the images are seen very clearly so it can avoid any blurry images ,right,

So, for that purpose this phase contrast microscopy is very much useful ,there is something called fluorescent microscopy this is very much in use whenever you work with ,you know, the stained samples a sample that contains dye or fluorescently labeled samples In those scenarios, fluorescent microscopy is very useful. Confocal is useful

whenever you want to obtain high resolution images by eliminating out of focus light. You can also construct 3D image of a sample using the confocal microscopy.

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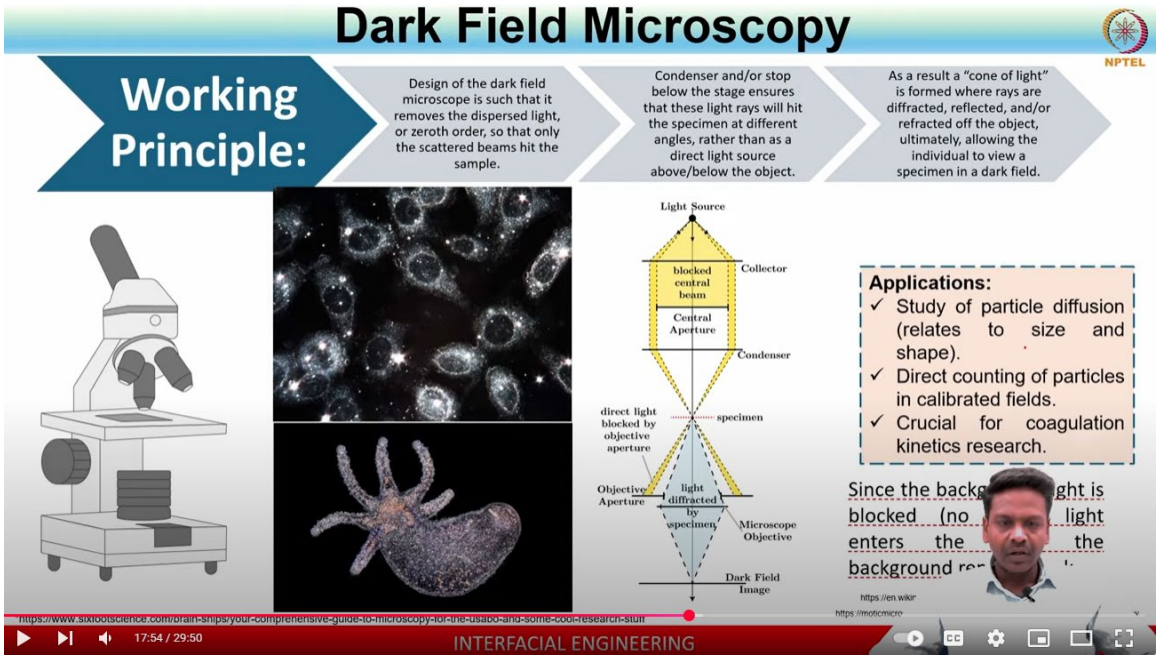

So, this dark field microscopy we have already seen, the difference here is because it collects only the scattered light from the sample, you are able to block the background light coming from the sample. you are able to see that only the specimens, that is the samples or whatever light that is scattered from the sample is only collected. The rest of the information is, you know, blocked in this case. That's why the specimen objects bright, you know, whereas the background appears dark in this case, right? Right.

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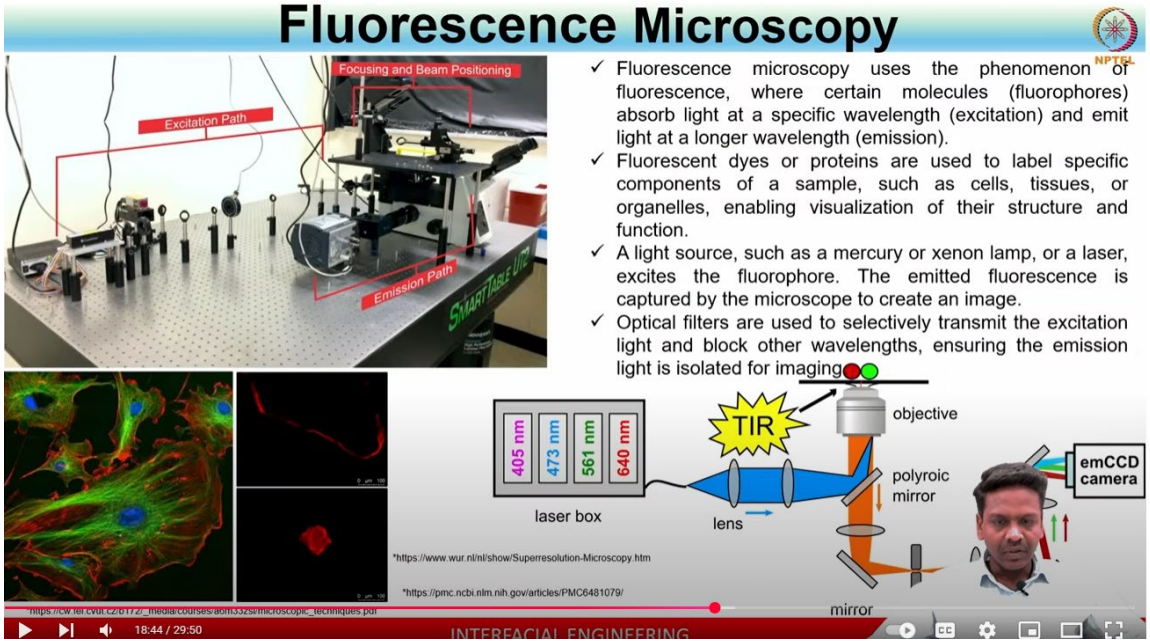

So, the fluorescent microscopy that we see that we talk about is basically it has what it has magnifying power lens, right, and light source that is basically mercury or xenon lab unlike the bright film microscopy you will have to use mercury or xenon light source This will be useful to excite the fluorescent part, right. So, whenever this fluorescently labeled molecule or the object absorbs the light, it will emit the light at a different wavelength, right, at a longer wavelength, okay. ,right, and this will be collected by the specific filter okay that are available in the microscopy okay let us say if you want to ,you know, understand ,you know, a sample okay stained sample you can use specific filter okay so that except those light, I mean light of that particular wavelength only will be allowed to pass through, the rest of the wavelength will be blocked. So, in such case, you will only be collecting the information about that stained specimen, okay, or fluorescently labeled specimen. This is very useful, you know, to visualize a particular part of the specimen sometime, okay, or only those which is fluorescently labeled.

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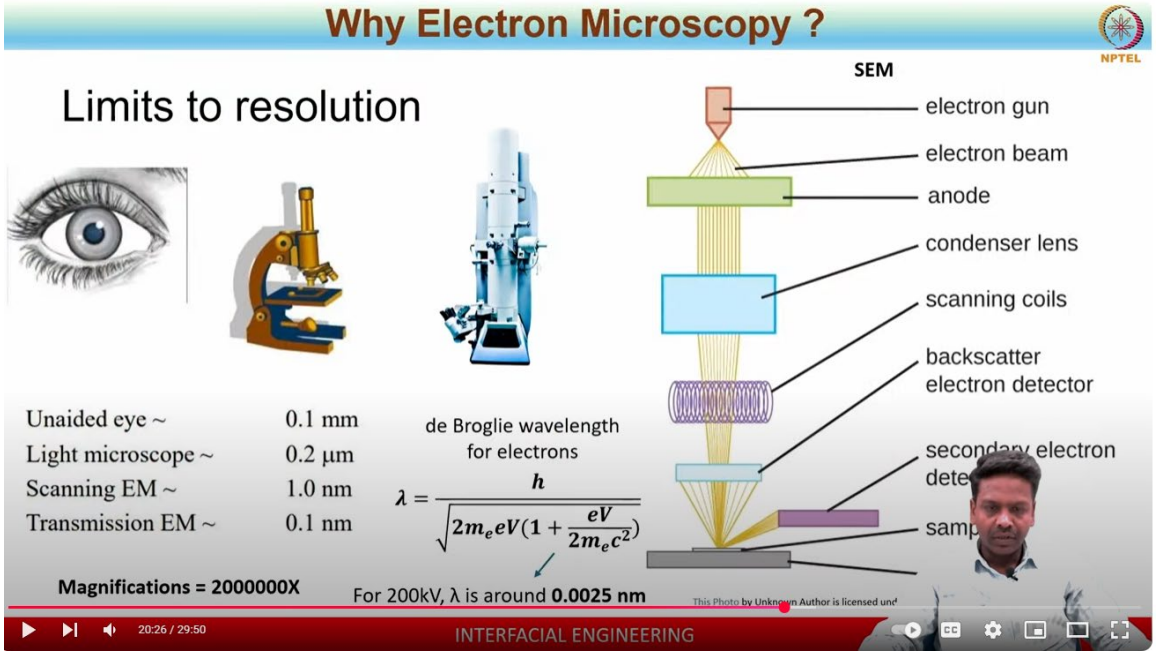

So, here comes the electron microscopy. Why electron microscopy one can use? So, the answer is when you want to resolve a specimen or object up to 0.1 nanometer. electron microscopy is quite often used ,right, so we have just now seen that ,you know, light microscopy has some limitation that comes from the wavelength of light itself okay so we have seen that based on the Abbe's diffraction limit even if you set the wavelength of light as 500 nanometer what you will be getting is the any objects which are kept at a distance of let's say 178 nanometer is what can be ,you know, resolved using the light microscopy okay whereas in the case of electron microscopy one can resolve ,you know, samples any objects ,you know, which are kept at a distance of 0.1 nanometer also okay this power this resolution comes from the magnifying power of the electron microscopy that is nothing but ,you know, 20 lakh times ,right, you can use the electron microscopy to magnify up to 20 lakh time Whereas the light microscopy ,you know, offers only up to 1000 times or 1500 times that is the difference. So, where this why this difference occur is because when electron beams are passed on to the sample, electron can access anywhere between the atoms ok.

So, the characteristic size of electron or the de Broglie wavelength for electron you can just calculate, you know, you know, based on this high voltage that is 200 kilo volt the lambda for electron in this case will be 0.0025 nanometer. So, that is the characteristic size of electron. So, electron can access any space between the atoms whereas light cannot pass through because of the wavelength itself, right, because of its limitation, right, So that is the difference. So, when it comes to electron microscopy, let's talk about, I mean, let's start with the SEM scanning electron microscopy. It has got electron gun.

Okay. And through electron gun, the beam of electrons are generated. And that pass on to the sample. As electrons interact with the sample, it produces two kinds of signal. One is backscattered electrons. The other one is secondary scattered electrons.

There are two detectors. These two detectors collect the backscattered electron and secondary electron. Both these signals are collected and processed to form an image that look like a 3D image, even though it is a 2D image, right?

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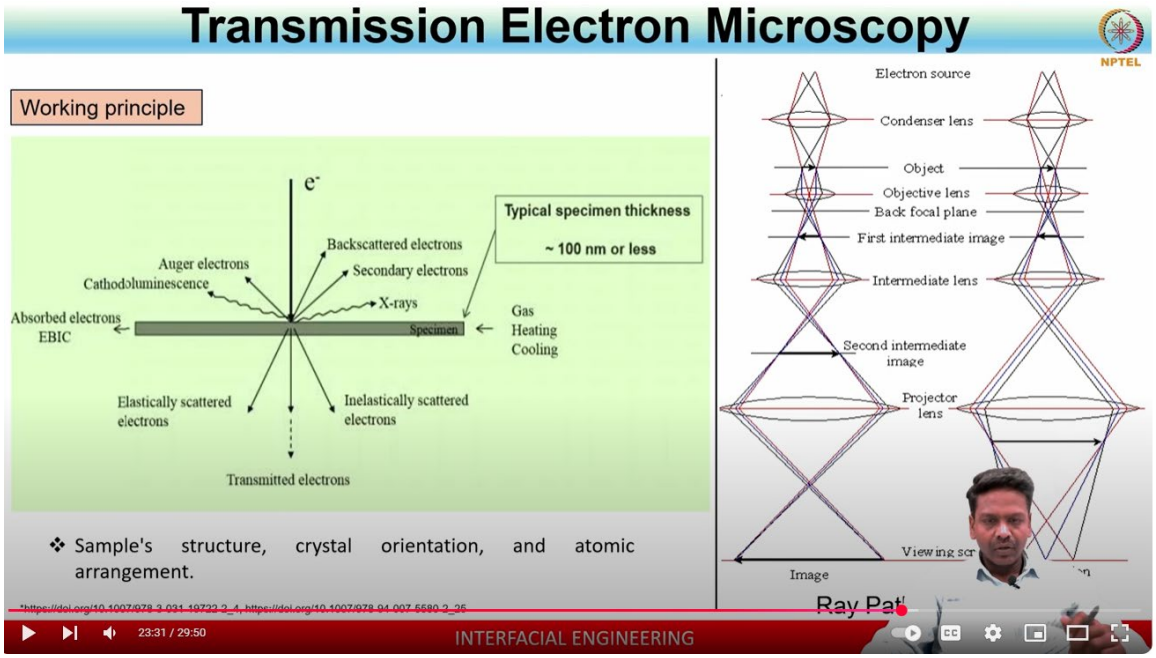

so what is transmission electron microscopy how it is different from the scanning electron microscopy the only difference is the beam of electron transmits through the thin sample okay as it travels from the sample through the sample, it not only collects the backscattered electrons and the secondary electrons in addition to that there are something like elastically scattered as well as inelastically scattered electrons, the elastically scattered electron meaning after interacting they interact with the atom okay and nuclei and other aspects of the sample without losing energy whereas inelastically scattered electrons interact with the sample but it loses the energy ,right, so these two electrons in addition to the backscattered and scattered electrons help us decode the information from the sample that you can collect you can ,you know, get the information about sample structure crystal orientation and atomic arrangement ,right, in addition to the features that we have in the case of scanning electron microscopy with the help of

transmission electron microscopy you can decode these many information as well from the sample that is the advantage of transmission electron microscopy

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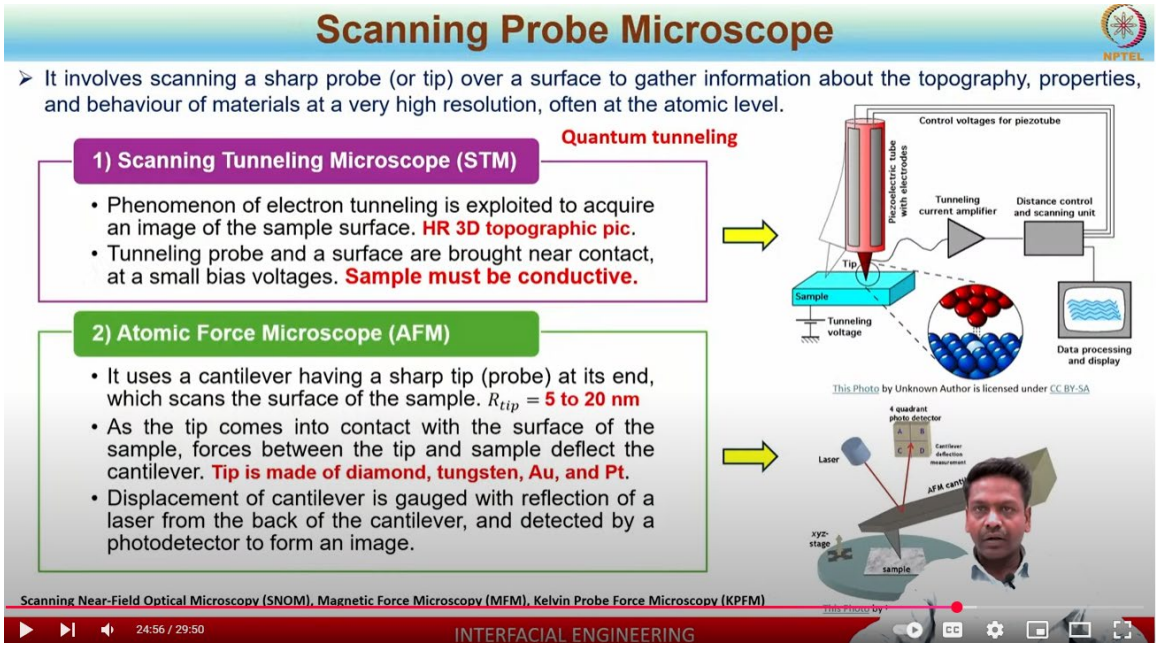

here we will look at the scanning probe microscope Usually it involves scanning a sharp probe over a surface to gather information about the topography, properties and behavior of materials at very high resolution and at the atomic level. So there are several types of probe microscope. Widely used are scanning tunneling microscope and atomic force microscope. Other probe microscopes used are listed over here.

How scanning tunnelling microscope works? Often it is called as STM and we also call atomic force microscope as AFM. Now how scanning tunnelling microscope works? Usually there is a very sharp tip attached to the cantilever and when you bring this tip very close to the surface, maybe at a distance of let us say 1 nanometer, there is something called quantum tunnelling happens.

So, which means that there will be a flow of electron from the tip to surface or vice versa. Now as you move along the surface while scanning, whenever there is a change in the distance occurred and that will be reflected in the flow of electron. So, this change in the electrons signal collected by the detector is again a process to form an image. That's how the STM works. The advantage of STM is you can actually collect high resolution three-dimensional topographic image. But the disadvantage part is you need the sample need to be conductive. I mean conductive, right? Sample must be conductive. That is the one

disadvantage. Apart from that, the atomic force microscope can also be used to collect the surface topography and surface morphology. So, it works in three different modes. One is contact mode, non-contact mode and tapping mode. So, how it works? In contact mode, there is a very sharp tip attached to the cantilever. So here the tip actually touches the surface. So, when it touches the surface, in three ways, force will be experienced by the tip.

One is due to contact force or Van der Waals or electrostatic force due to interaction with the atom and molecule on the surface. Now when this force is experienced by the tip, this force will cause the cantilever to deflect. Now, there is something called laser light that will be sent, you know, at the back of the cantilever. Now, this deflection is traced by the laser light, right? As and when you scan the surface, whenever there is a change in the force, that will cause changes in the deflection as well. Now, this deflection information is collected by the photo detector, okay? and finally this information whatever gathered by the photo detector is processed to form an image this is one way here the radius of the tip that is usually employed is between 5 to 20 nanometer whereas the tip the material construction i mean the material of construction of the tip is usually a diamond tungsten gold and platinum okay In the case of non-contact mode, it works because the tip covers above the surface with a distance of few nanometer.

Whenever there is a change in the distance occurred, the change in the amplitude and frequency is collected by the photo detector and the disinformation is again processed to form an image. In the case of tapping mode, the the tip doesn't touch the surface continuously but in intermittent but intermittently ,right, so it will tap at a regular interval at the same time the tip will over above the surface with a distance of two nanometer, in this way you collect the information again and process to form an image ,okay, we will stop here we will continue from the next lecture, thank you you