Introduction to Complex Biological Systems
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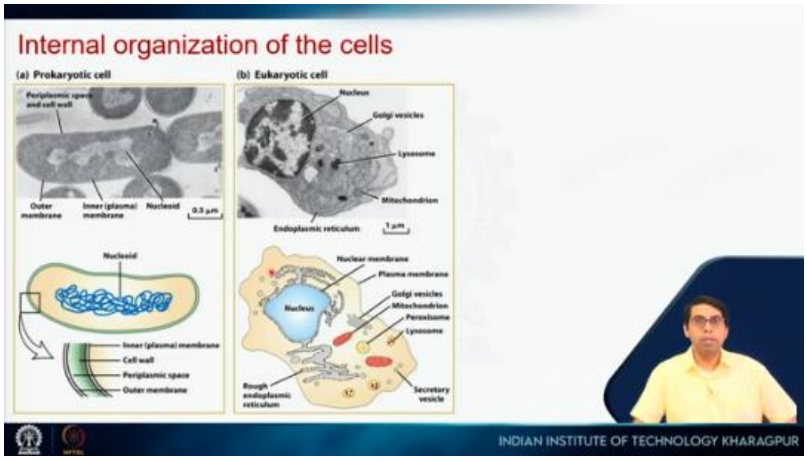
Lecture 22
Visualizing cells

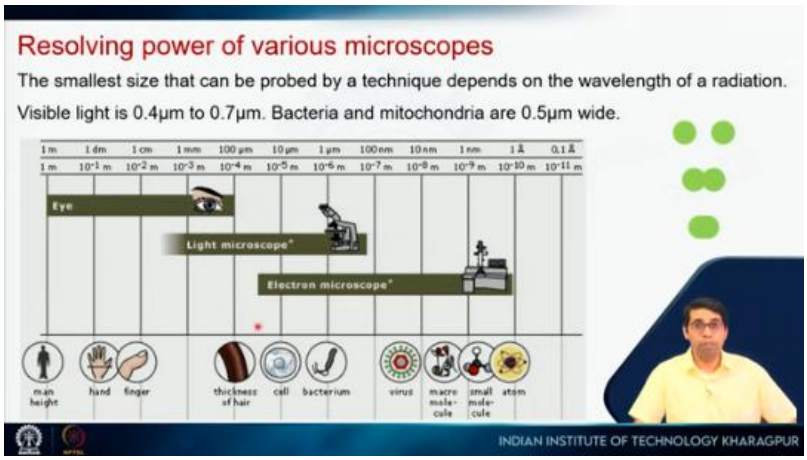
Hello again. So, in this week's lecture, we are looking at cells, which are the unit of life, and today I am going to talk about how we see cells because seeing believes, and looking at things is a major function in science. So, we are going to look at how we see cells and how we visualize these cells using different microscopic techniques. So, I have already shown you this image where this is a bacterial cell and this is a eukaryotic cell and these are all cartoon diagrams where we draw DNA like this, and we draw all the intracellular organelles like this. But I have also shown you these real images, which are taken by different means of microscopic techniques. So, the images that you see up here are taken by transmission electron microscopy. So, today I'm going to discuss all these microscopic techniques, which have enabled us to see inside these structures in great detail and understand a lot about these cell structures. I will discuss all these in detail in the next slides.



So, the important thing that I am going to talk about first is something called the resolving power of various microscopes. So, what do I mean by resolving power? Let us say inside a cell we are looking at something, maybe two proteins, maybe two different organelles. If they are separated like this, we can clearly see them as two distinct dots.

If they are close, then you can still make it. So, this will be at the limit of resolving power, which means that we can still tell that these two are separate entities, but if they are closer, then we cannot tell them apart. So, this will be the resolving power; the distance between these two will be the resolving power of that particular technique, and that resolving power depends on the particular technique. It turns out that the smallest size that can be probed by a technique depends on the wavelength of the radiation. For example, if I talk about a light microscope, this is a light microscope. It uses visible light, and the wavelength of visible light is from 0.4 micrometers to 0.7 micrometers, which means that the smallest structure that we can see using a light microscope will be in this range.

So, it turns out that it is around 0.2 micrometers. That's the smallest size you can see. For example, bacteria and mitochondria, so bacteria and mitochondria inside a eukaryotic cell, are around 0.5 micrometers apart or wide. So, you can see them using a light microscope. If you want to probe something that is even smaller, then we will have to use a more powerful technique, which is an electron microscope, which uses electrons whose wavelengths are even smaller.

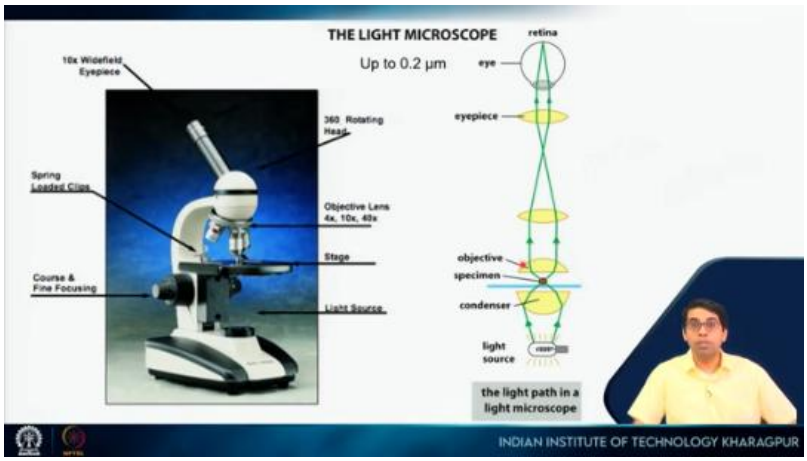


And then, if you want to look at proteins, we use X-rays so that we can get atomic details where the wavelengths are even smaller. So, this is a light microscope and you can see there are all these different parts.

This is called the stage where we place the sample. There is a light source below. So, light passes through this. And then there are all these different lenses here which will magnify it. And then finally, through this eyepiece, we will see the image.

Right. All microscopes have this basic structure. But of course, this light source and the lenses will change depending on the type of wavelength. So, the schematic diagram shows the same thing that is shown here. So, this is the light source; there is a condenser which will condense these light rays and focus them onto the specimen. Then, from the specimen, it passes through an objective lens.

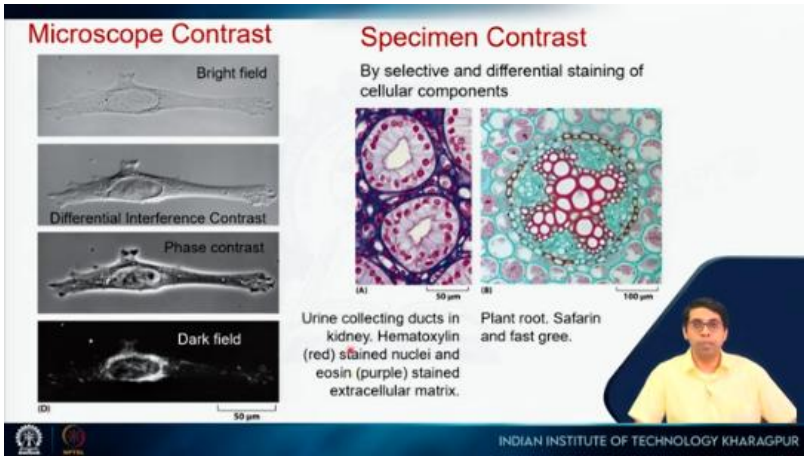
So, you get magnification through all of these, and then finally, you see it. We can see it, or we can also put up a camera so that we can take pictures and show it, like I'm showing you in these slides. Light microscopes can be of various types. So, there are four different types of images that are shown, which all are taken by different types of light microscopy techniques. All of these depend on the fact that when light is passing through a medium, there is refraction because the refractive index is different.



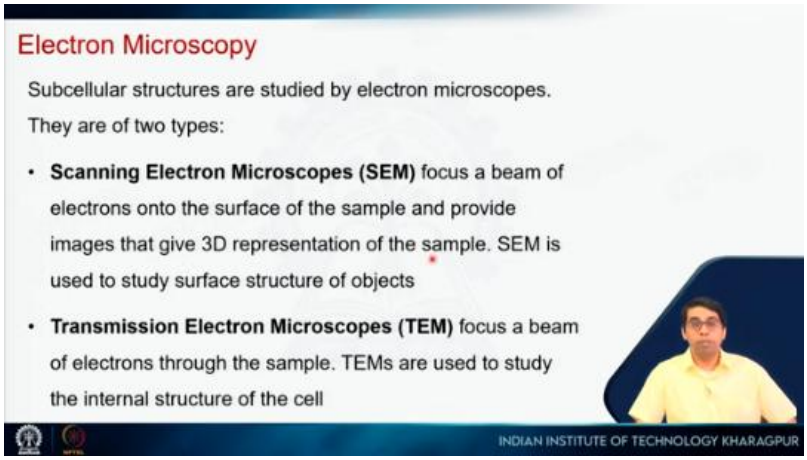
So, you have seen that if you put a spoon inside a cup of water, then the spoon appears to be bent. That is because of refraction. So, the same thing will happen if light passes through this cell. Then, because of the matter that is inside it, the phase of the light will change, and that difference in phase is used to create contrast. So, this is basically microscope contrast, and then there are different ways you can utilize that. That leads to this bright field image, differential interference contrast image, phase contrast image, or

dark field image. So, what you see here is a fibroblast cell, the same cell visualized using these four different techniques.

Another very common technique is to use certain dyes, and that is called staining. So, we can use these different dyes to stain different components of a cell and then see it under a light microscope. For example, you can see what you see here is the urine-collecting ducts in the kidney. So, all these cells you can see from the outline. The cells are there, the nuclei are stained red, and that is stained using hematoxylin. So, hematoxylin gives a red color, and then this extracellular matrix is colored purple by another dye, which is eosin. So, these are dyes that are very commonly used. Similarly, this is a plant root, and that is differentially colored using two different dyes: safranin, which gives you this red color, and fast green, which gives you this green color. So, the light microscope has its limitations because the light wavelength is of a certain length.



So, you cannot see anything less than 0.2 micrometers in size. If you want to see something smaller, then we have to use some other type of microscope. Here, the light source or energy source is used in electron microscopy, and this can be used to see subcellular structures. There are two types of electron microscopy. One of them is called SEM, and the other one is called TEM so the SEM stands for scanning electron microscope and TEM stands for transmission electron microscope. SEM focuses a beam of electrons onto the surface of the sample and provides images that give a 3D representation of the sample. So, you can see the three-dimensional shape of a particular cell or any object that you want to see.



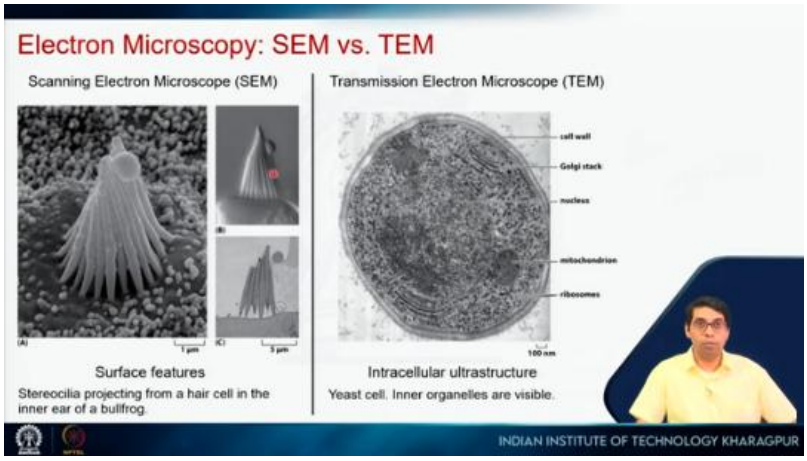
On the other hand, the transmission electron microscope focuses a beam of electrons through the sample, which means that it can be used to see the internal structure of the cell. The construction of these two microscopes is slightly different. On the right-hand side, we have the TEM or transmission electron microscope, and on the left-hand side, we have the SEM or scanning electron microscope. You will see that they again look very similar to what we saw in the light microscope. So, in this case, there is an electron gun. So, that is the source, the light source. There are condenser lenses in both cases. So, in this case, the beam is concentrated, and all that, and here the specimen is present.

So, in this case, what is done is the specimen is coated with a very thin metal layer. and that will scatter the electron beam, and that scattered electron beam is detected. So, you can move this detector around and construct a three-dimensional image of your specimen. In this case, the beams pass through your specimen, and then you can collect the image on a digital camera or some viewing screen. So, this type of technique requires a lot of sample preparation. So, here I say that you have to coat your sample with a thin layer of metal so that you can scatter the electrons from the specimen. In this case, the specimen is prepared because we want to see inside. What is done is all the proteins inside the cell are crosslinked using a chemical called glutaraldehyde, and then you also want to freeze the membrane structure, which is done using osmium tetroxide.

Then using certain polymeric resins, it is frozen into a particular space so that things do not move, and then very thin slices are made because the penetration power of these electrons is not very high. So, you have to cut your specimen into very thin slices using

special instruments called microtomes and they are around 100 nanometers or 200 nanometers in thickness. That is what you put here and then we see images like this.

So, using SEM, this is a stereocilia projecting from a hair cell in the inner ear of a bullfrog. So, you can see the three-dimensional shape, the three-dimensional arrangement of these projections. This is the image of the same specimen using a transmission electron microscope and this is taken using a light microscope. So, you can see that the details are much more here because the resolution is much better.



This is the inner structure of a yeast cell. You can see all the organelles. You can see the mitochondria. You can see the nucleus here. You can see the Golgi stack. You can see the cell wall and you can also see the ribosome as these black dots. So, a transmission electron microscope shows you the inner structure of the cell, and a scanning electron microscope shows you the three-dimensional structure of any object. So, another very important microscopic technique is the fluorescence microscope. So, we have seen the light microscope, we have seen the electron microscope, and the third one is the fluorescence microscope. So, what is a fluorescence microscope? It uses molecules which give fluorescence.

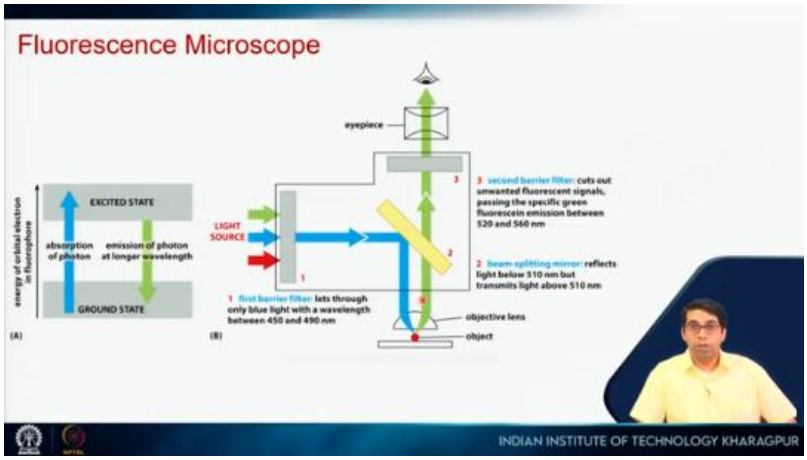
So, these molecules will be excited by some energy source. So, there will be some wavelength at which they will get excited, and then they will give an emission spectrum, which will be of shorter wavelength because some of the energy will be lost. So, this will be of longer wavelength because some of the energy will be lost. So, fluorescence molecules will be excited at a certain wavelength, and we will observe them at a different

wavelength. This is the construct of the microscopes. You can see it's very similar. There is your specimen, you want to see this, and there are all these different lenses which will magnify the image, and then finally, you will see it. The light source in this case is not here; it is here, and typically, these are lasers.

So, you have a light source, and then we have a barrier. So, this, in many cases, is called a monochromator. So, what it does is it will let through only a certain wavelength of light. So, in this example, this one lets through only blue light with a wavelength between 450 to 490 nanometers. So, the light source has all these wavelengths, but only one wavelength is passed, and it hits this beam-splitting mirror.

So, this is a mirror, and it is a very interesting mirror because it will reflect light at a certain wavelength and become transparent for light at a different wavelength. So, it reflects light below 510 nanometers, but it will transmit light above 510 nanometers. So, this is blue light, which is 450 to 490, which means it is less than 510. So, it acts as a mirror; it will reflect it, which will be condensed using this lens, the objective lens, onto or focused onto your specimen. The fluorescence molecules, which get excited at this particular wavelength, will get excited, and then they will emit photons at a different wavelength. So, in this case, they emit in the green wavelength, which gives you a longer wavelength than 510 nanometers.

So, this becomes transparent. So, it will pass through, and then you can see it either by eye or you can again use the camera to capture the image. So, let us see an example. There are many dyes that have been developed over the years that occupy this different range of the spectrum. So, on the left-hand side, the wavelength excitation wavelength is listed, and on the right-hand side, the emission wavelength is listed.

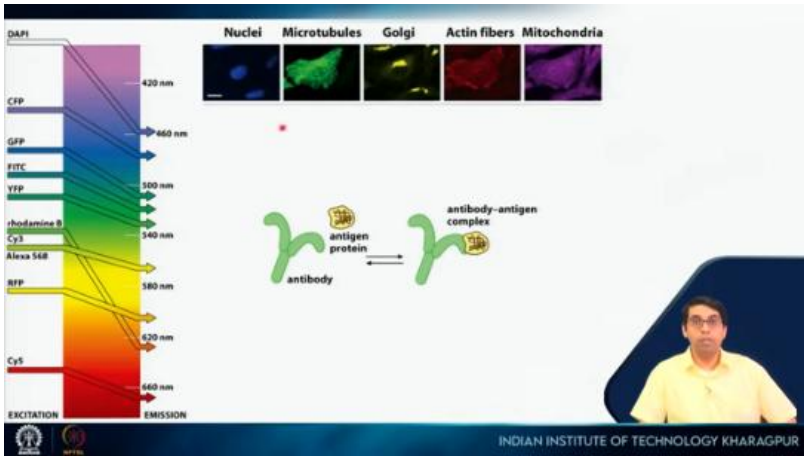


So, you can see that for all these dyes, the excitation wavelength is more than the emission wavelength. So, this is shorter than the emission wavelength. So, this is shorter; this is longer and these different dyes are all listed here.

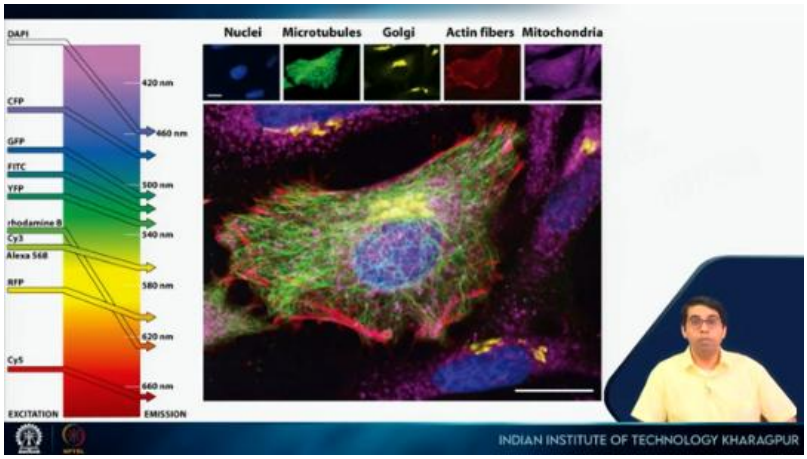
For example, DAPI, which is excited almost in the violet range, emits at 460 nanometers, which gives you a blue color. DAPI is a dye that very specifically stains the nuclei. So, we can use DAPI to look at the nuclei. We can use different dyes to stain different parts of a cell and in many cases that is done either using dyes which bind to these proteins.

So, you can have a dye which will bind to microtubules. Microtubules are made up of tubulins. We will see that in a few minutes in today's lecture. So, they can either go and bind to it specifically, or we can use antibodies. So, antibodies are proteins which have very high specificity to their target proteins.

So, this target protein will be called an antigen protein. So, in this case, let us say the tubulin of microtubules is the antigen. We can develop an antibody which is specific to it, and then we will attach a dye at the other end, so, it will go and bind only to microtubules. Similarly, we can use antibodies again for Golgi, actin fibers, and the mitochondria and we can conjugate dyes which are of different colors. So, using blue color, we can see the nuclei; using green color emission, we can see the mitochondria; Golgi is yellow, actin fibers are red, and mitochondria are purple. Then, what we can do is we can superimpose all these images to see how they are localized relative to each other, and the resulting image will be something like this.

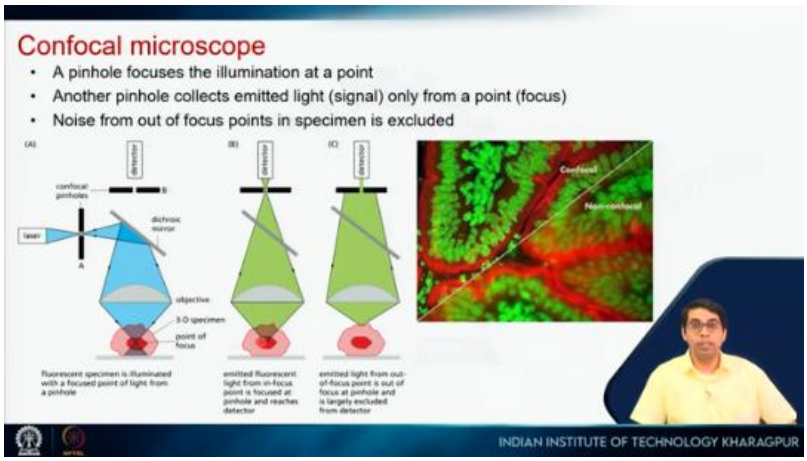


So, you can see that the nucleus is at the center. Microtubules are spread throughout the cell; actins, it turns out, are localized mostly towards the periphery of the cell, around the cell membrane. Golgi is here, which is almost touching the nucleus, and mitochondria are spread throughout the cytoplasm of the cell. So, this type of image is very useful because it gives you the relative orientation of these different entities inside the cell.



We will see that this is very important because we are going to talk about these different filaments: microtubules, actin filaments, and intermediary filaments, which are present inside the nucleus. A very specific type of fluorescence microscopy is a confocal microscope. So, this is very similar to the microscope that we have seen before, but in this case, we can focus at different positions inside the cell. So, you can focus your space. You can collect images. So, if this is my specimen, you can collect images at the bottom, at the center, or at the top. So, we can scan along this vertical dimension with high precision. So it is a confocal microscope. So, in this case, what happens is that there are

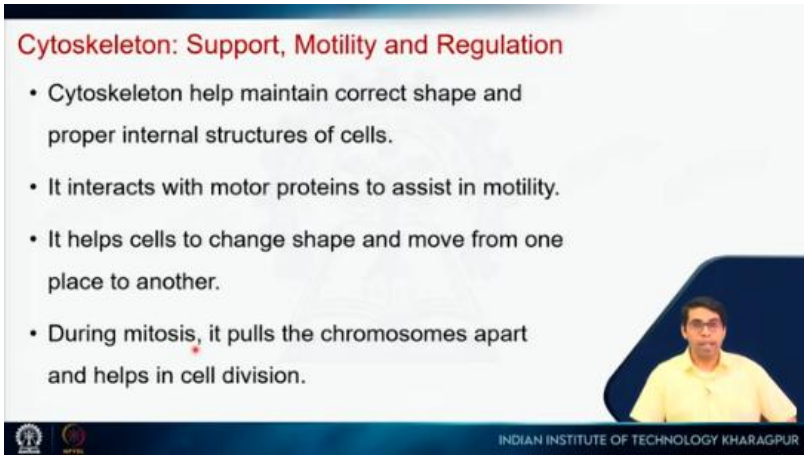
two pinholes, one for the source and other one for the detector. So, in this case, the blue light emitted from the laser is focused at a particular point on your specimen, and then the light, the green light that comes out, is again focused into the detector. Any light that comes from a different part of the cell will not be focused, and it will be eliminated. So, we can get images from a very thin slice of the sample.



So, you can see that if the focusing is not right, it will be blurred, but if you can focus it properly on a particular part, you can see very sharp images. So I have already talked about microtubules, actin, and intermediate filaments. These are called the cytoskeleton, just like our skeleton. We have a skeleton that supports our body structure. Similarly, cells need a particular skeleton to have a particular shape and size. So, the cytoskeleton helps maintain the correct shape and proper internal structures of the cell. Not only that, they also interact with motor proteins to assist in motility, which means that if a mitochondrion has to move from one part of the cell to another, it will use this cytoskeleton to move. There are vesicles which carry all materials inside from one part of the cell to another, and again, that is done by these motor proteins which move along the cytoskeleton. Cytoskeletons also help to change shape and allow the cell to move from one place to another.

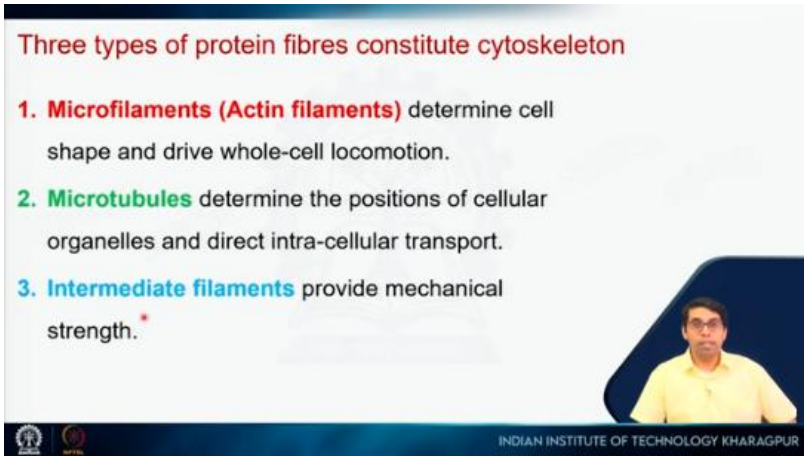
So, the shape of the cell can change because of the cytoskeleton. They can help in that, and they can also allow the cell to move from one place to another. In the next lecture, we will see that during mitosis, cytoskeletons, more specifically microtubules, pull the chromosomes apart and help in cell division. So, we will see how these cytoskeleton

proteins, such as microtubules and actin, actively participate in cell division. So, there are three types of cytoskeleton or protein fibers. One, the first one is called microfilament.

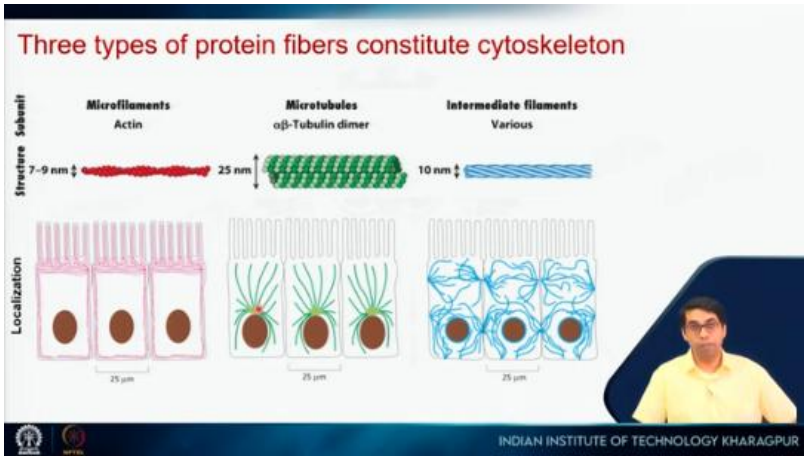


These are also called actin filaments. They determine the cell shape and drive whole-cell locomotion. So, the cell can move from one position to another, maybe to find food. So, that cell locomotion is driven by actin filaments. Microtubules determine the positions of cellular organelles and intracellular transport.

So, we will see that intracellular transport, the movement of vesicles and other things from one end of the cell to another, is driven by microtubules. These two filaments also play important roles during cell division, which we will see in the next lecture. Intermediate filaments provide mechanical strength to the whole cell structure. These are the three filaments, the microfilaments, microtubules, and the intermediate filaments. Interestingly, all these three filaments are polymers made up of proteins. So, we have seen that proteins are themselves polymers, which are made up of amino acids. In this case, these filaments are made up of polymers where the subunit, or the monomer, is a protein itself. So, actin or microfilaments are made up of proteins called actins.



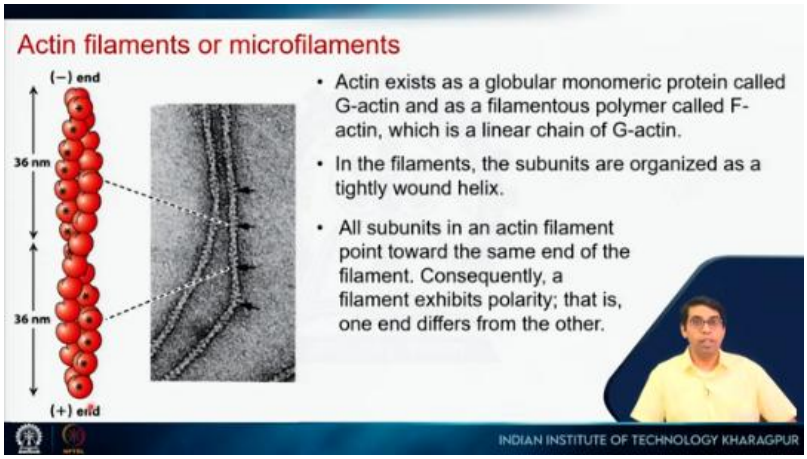
Microtubules are made up of two types of proteins called tubulins, the alpha and the beta tubulins. So, these two tubulins form a dimer, which are shown in two different colors: light green and red. They form a hollow cylinder-like structure. So, in the case of actin filaments, these proteins form two fibers that are intertwined. In this case, we have a much bigger structure. So, it forms a hollow cylinder-like structure and in the case of intermediate filaments, there are multiple proteins present. So, these are called intermediate filament proteins, and these proteins form the intermediate filaments. You can see the dimensions. The actin filaments are the narrowest, at 7 to 9 nanometers in diameter; intermediate filaments are around 10 nanometers in diameter; and microtubules are the thickest, at 25 nanometers in diameter. These filaments are present in different parts of the cell. So, this is a cell, and you will see that actin filaments are mostly present along the cell membrane. On the other hand, microtubules are more inside the cell, and they originate from a particular structure called centrioles.



We will see that in more detail in the next lecture, and they radiate out from that structure and intermediate filaments, these are present around the nucleus and throughout the cytoplasm, and they provide this particular mechanical strength to the cell. So let us look at actin filaments. This is an electron microscope image of actin filaments, and you can see that these are the monomers. So actin filament monomers are classified into two types. One is called a globular monomer, or it is called G-actin.

So actin exists as a globular monomeric protein called G-actin and when it forms this filament, it is called the filamentous polymer or F-actin, which forms this linear chain of G-actin. Now, remember that these monomers are not covalently connected, unlike a protein. So, in protein, the monomers are covalently connected. The monomers or the amino acids are covalently connected with each other, but in this case, these monomers are not covalently connected with each other, which means that they can assemble and disassemble much faster.

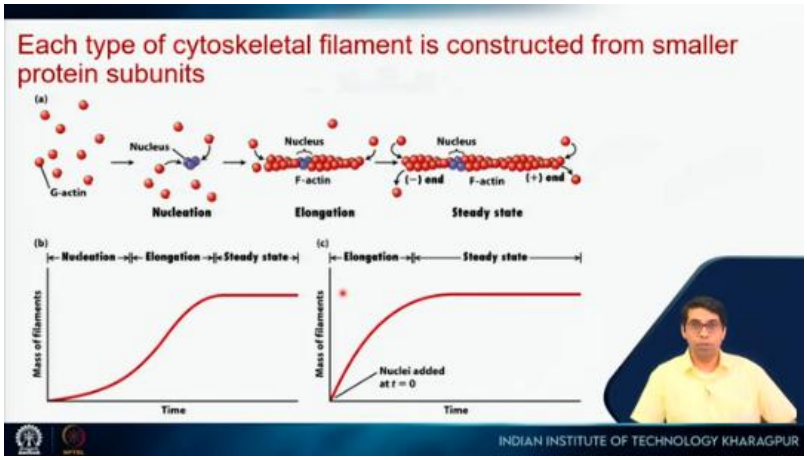
In filaments, the subunits are organized as a tightly wound helix. All subunits in an actin filament point towards the same end of the filament. Consequently, the filament exhibits polarity; that is, one end differs from the other end. So you can see that one end is labeled as a negative end, and the other end is labeled as a positive end. The way we can differentiate these two ends is by seeing how fast the actin polymer grows. So it grows faster at the positive end compared to the negative end. So from the rate of growth, we can tell this is the negative end, and this is the positive end. So the formation of the polymer is shown here.



Again, this is very different from what we have seen in the case of protein, DNA, or RNA, where polymers are constructed in a particular sequence. You have 20 different monomers in protein and 4 different monomers in the case of nucleic acid, but in this case, all the monomers are G-actin, so the sequence is immaterial. However, there is no template, so how do you start polymerization? It turns out that several G-actins will come together to form something called the nucleus. Once this nucleus is formed, more and more G-actins can come, and it will start elongating. At both ends, one end the elongation is slower, and the other end the elongation is faster because of the asymmetry in the structure, because all these actin proteins are oriented in the same direction. So, this end versus this end will be different. So, this end will be called negative, and this end will be called positive.

Now, it turns out that this nucleation is a very slow process, and if we plot the length or the mass of the filament versus time, we will see that this length or the mass increases very slowly. So, this slow part is where this nucleation is happening. Once this nucleation happens, it will start growing rapidly because this is elongation. So, we have the nucleation now; it will grow from both ends. So, the mass is increasing, and then ultimately it will reach a steady state where the rate of addition of actin monomer and the rate of dissociation of actin monomer becomes equal.

So, it will not change in length much. So, it will reach a steady state. If in a solution of G-actin, we add a nucleus, then we will see something like this. So, you see that the nucleation phase is absent. It directly goes into the elongation phase and reaches the steady state.

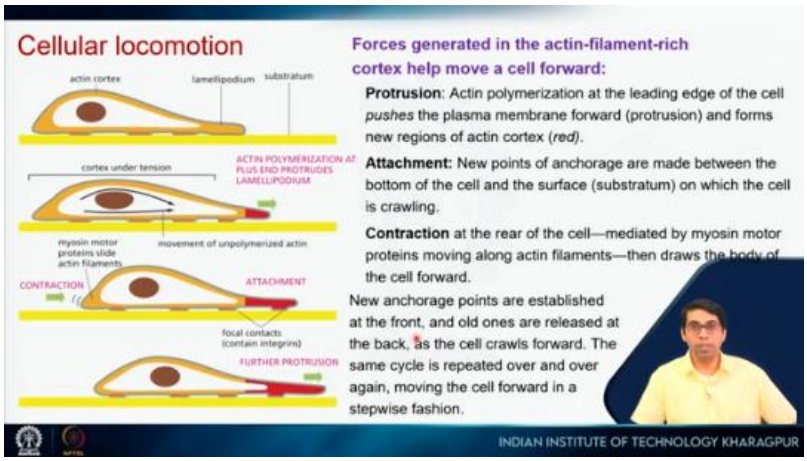


So this is something that is very useful for the cell because this polymerization and depolymerization of actin helps the cell to move, which is called cellular locomotion. So forces generated in the actin filament reach the cortex and help move a cell forward. So what is a cortex? This is a cortex. This is where the polymer of actin is present, and it is just below the cellular membrane.

So the first step is the formation of a protrusion. So the cell, let us say, detects some food in this direction. So that will drive the polymerization of the actin filament in this part of the cell. So actin polymerization at the leading edge of the cell pushes the plasma membrane forward. This is called protrusion and forms new regions of the actin cortex, which is shown as red here.

So actin polymerization will happen here, and it will form this protrusion, which is the actin cortex. New points of anchorage are made between the bottom of the cell and the surface, which is this yellow part, which is the surface on which the cell is moving, which is the substratum on which the cell is crawling. So you see that the cell addition is here now. New cellular addition is formed here as the cell will keep on protruding further. Such focal contacts will be created, so this allows the cell to attach to this surface and move forward. Now, since the cell is moving in this direction, it has to move out from this direction, so contraction of the actin filament has to happen on this side, which is the rear of the cell. So contraction at the rear of the cell is mediated by myosin, the motor proteins moving along the actin filaments, which then draws the body of the cell forward. So there are motor proteins; we will see them in one of the last slides in today's lecture.

So myosin pulls the rear end of the cell. So actin pushes here, myosin pulls here, so the whole cell body will move further. So these new anchorage points are established at the front, old ones are released at the back, and as the cell crawls forward, the same cycle is repeated over and over again, resulting in the movement of the cell in this particular direction. And again, the movement of the cell is driven by external signals.



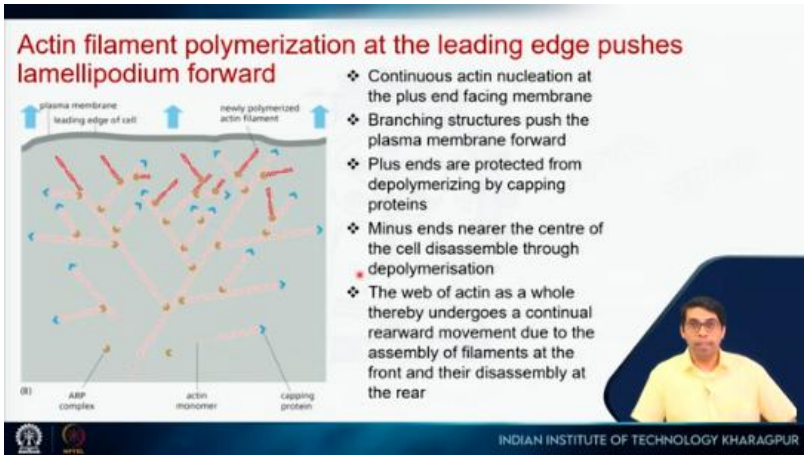
So, the same event is shown with more details. So, active filament polymerization at the leading edge pushes the lamellipodium forward. So, this is the lamellipodium that we saw as the protrusion. So, continuous active nucleation at the plus end faces the membrane. So, this will be the plus end.

These are the minus ends. The branching structure pushes the plasma membrane forward. So, there are actin filaments in this direction, and there are actin filaments in this direction. So, these are all pushing the plasma membrane forward. Plasma ends are protected from depolymerization by capping proteins.

So, now there are other proteins that come into this picture. So, these blue proteins are capping proteins. So, they will prevent depolymerization at this end. So, they will allow growth but not shrinkage. Minus ends nearer the center of the cell disassemble through depolymerization.

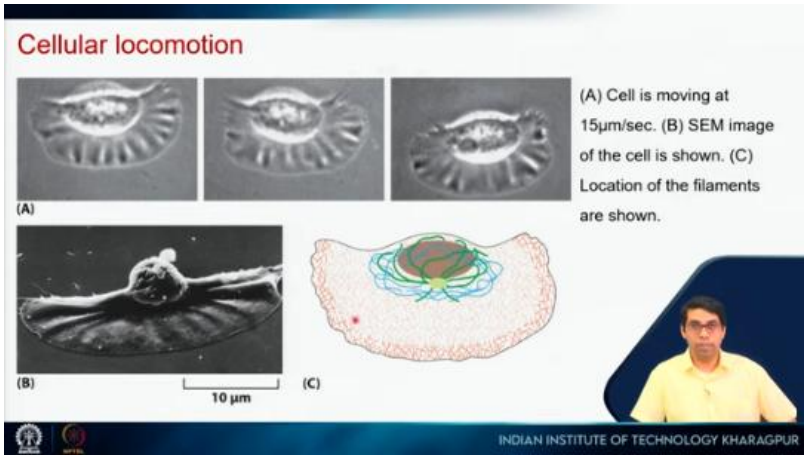
So, these minus ends will start depolymerization, and these monomers will be added at this end. So, the actin is recycled. The actin monomers are recycled. This wave of actin as

a whole thereby undergoes a continuous forward movement. So, it gets depolymerized here, polymerized here, and pushes the cell in this particular direction.



So, this is an actual image of a particular cell. So, you can see that this cell is moving. So, if we take snapshots using a light microscope, you can see the cell is here. So, this is the lamellipodium. The cell has moved.

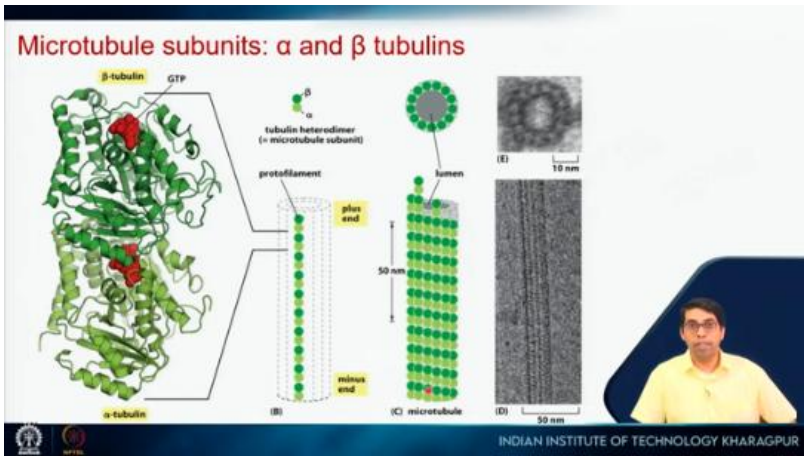
It has moved further and we can time the speed of the movement of the cell, and in this particular case, the cell is moving at 15 micrometers per second. This is the same image of the same cell, and this is the schematic which shows the location of the filament. So, you can see that the actin filaments are here, which are driving this motion. Green is the microtubule, and red are the intermediate filaments.



So, here is the structure of a microtubule. As I mentioned, a microtubule is made up of two types of monomers, the one is called the alpha monomer, and the other one is called

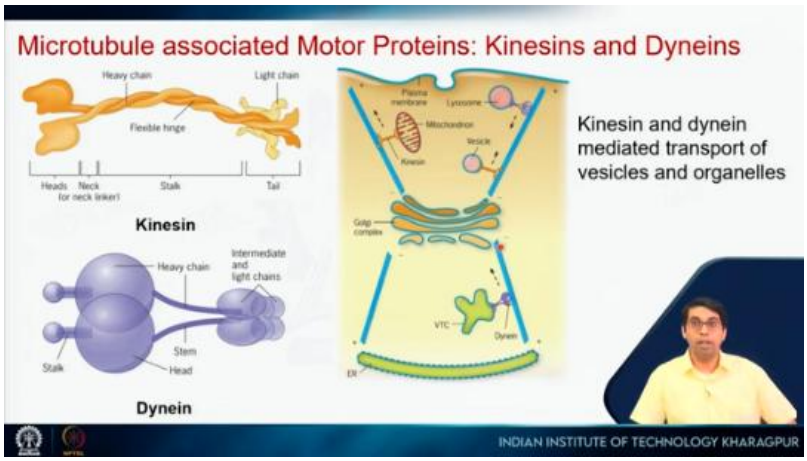
the beta monomer so alpha tubulin and beta tubulin. So, here are the structures of the alpha tubulin and beta tubulin, and you can recognize the alpha helices and the beta strands in these two monomers. What we have here in red is GTP, which is bound to this alpha tubulin and beta tubulin. So, these two monomers form a dimer like this, and then this dimer forms a linear chain, which is called a protofilament.

It turns out that there are 13 such linear chains, which form this hollow cylindrical structure, and this is a microtubule. This is again an electron microscope image, so this tubular structure can be seen here, and you can see the protofilaments. If we see from this end, you can see the hollow structure. It turns out that GTP The presence of GTP is something that stabilizes the microtubule structure at this end, and later on, this GTP is hydrolyzed to GDP inside for this other part. Now, at this end, if GTP is hydrolyzed to GDP quickly, then it will result in destabilization of the structure, and these monomers will start falling off, or the protofilaments will start peeling off from the tubular structure of the microtubule.

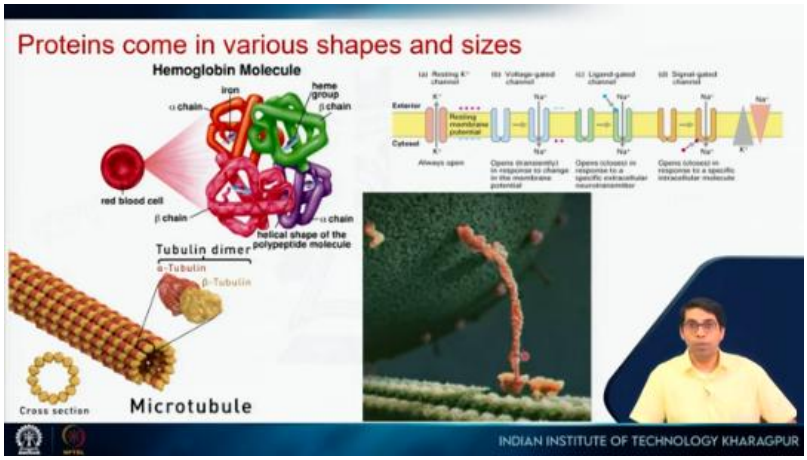


So, I mentioned motor proteins. Here, I am going to discuss two motor proteins, and we will talk about another type of motor proteins later on when we discuss tissues and organs inside a particular organism. So, here I am going to talk about kinesin and dynein. So, these are motor proteins which walk on microtubules. You can see that these are microtubules, and again, they have this plus end and minus end. This is a dynein motor protein which is walking on this microtubule from the plus end to the minus end.

So they can carry vesicles, they can carry lysosomes, they can carry mitochondria, so they can carry all these different organelles inside the cell. So dynein goes from the plus end to the minus end, and kinesin goes from the minus end to the plus end. So, you can think of these microtubules here as a railroad, and these are your trains which are carrying this cargo from one end of the cell to the other end of the cell.

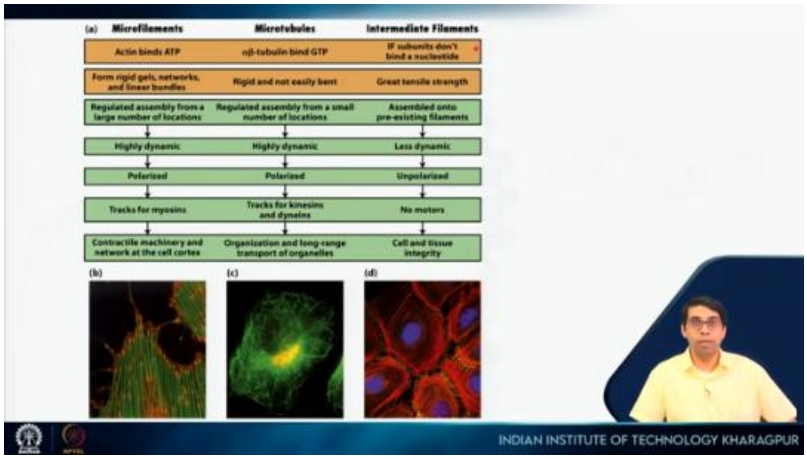


So, you have already seen this image. So, this is the microtubule. Alpha and beta tubulins are there and they form this hollow structure. You can see the microtubule down here, and this is a kinesin. So, it is carrying this huge vesicle along the microtubule from one end of the cell to the other end of the cell.

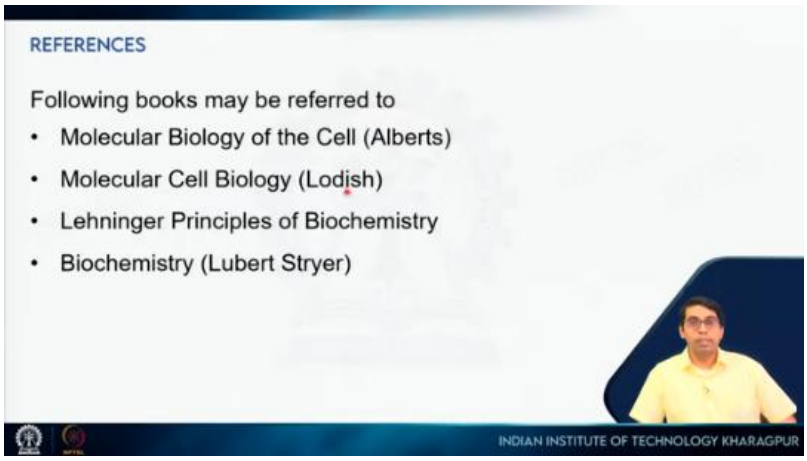


So, this is a summary. You can pause the video here and just go through it. I am not going to go through this in more detail. So, I have already mentioned microfilaments. They bind actin; microtubules bind GTP, and these are intermediate filaments.

They don't bind to any nucleotides. So, you can see that the similarities and differences between these three different filaments are listed here.



The books that you can follow are Molecular Biology of the Cell and Molecular Cell Biology by Alberts and Lodish. These are two very good books you can follow for this particular lecture.



Thank you.