Experimental Biotechnology
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Module-VIII
Cell Biology Techniques (Part-1)
Lecture-33
Cellular Fractionation

Hello, everybody this is doctor Vishal Trivedi from department of biosciences and bio engineering IIT, Guwahati. And what we were discussing in the previous lecture is about the growth as well as the propagation of the prokaryotic cell as well as the eukaryotic cell. So, in that lecture we have also discussed about the different types of media and as well as the media component, what you require to successfully grow as well as manipulate the cells. So, in today's lecture we are going to discuss about the fractionation of the cells. So, what we have discuss in the previous lecture is that.

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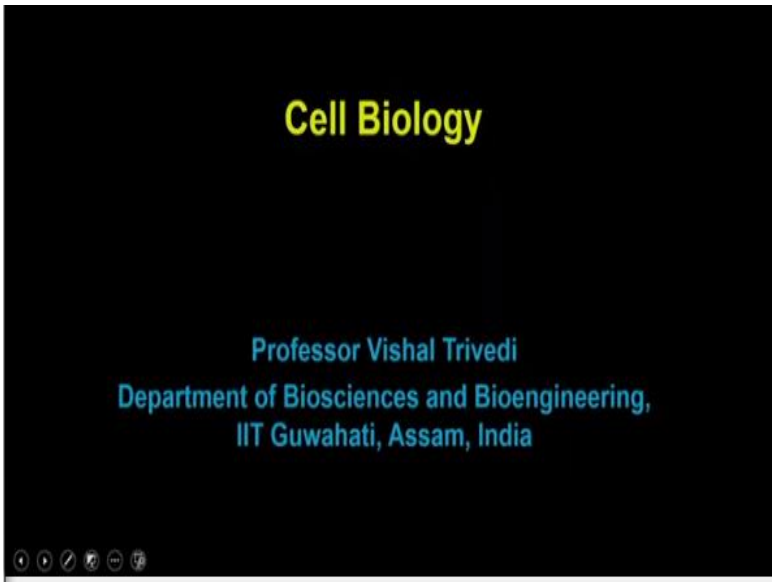

We have discussed about the different types of cells.

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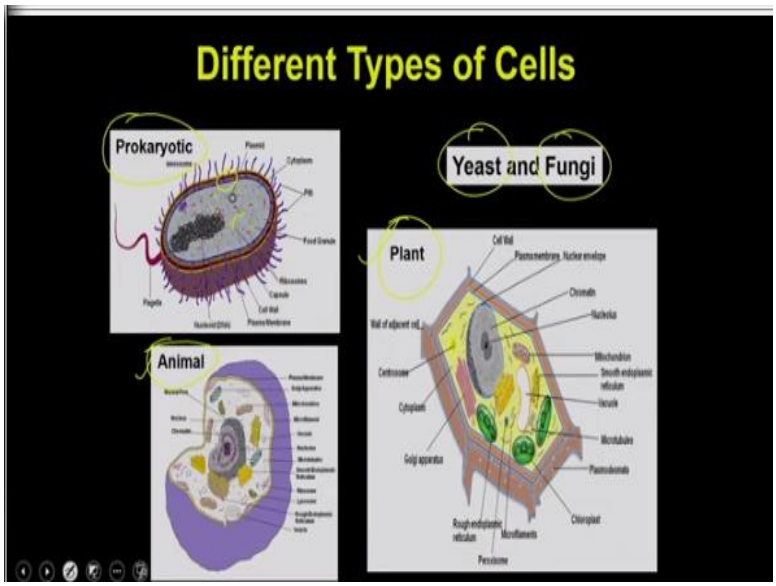

So, we have discussed about the prokaryotic cell, we have discuss about the yeast and fungi which are actually within the eukaryotic cell as well as we discuss about the plant cell as well as the animal cells. So, when you have the different types of cells, these cells are actually are very, very complex in structures. And you might be sometime interested to explore a particular molecule or particular substance from a particular location of that particular cell.

For example in some cases you might be interested to see the molecule which are present onto the plasma membrane of a prokaryotic cell or you are looking for a molecule which is present within the cytosol of a prokaryotic cell. The situation is in more and more complicated when we talk about the animal cell as well as the plant cell. because compared to the prokaryotic cell which actually has a single cell body where you do not have the multiple organelles except the plasma membrane and the cytosol.

Apart from that you have also have the periplasmic fractions but besides very simple structure the eukaryotic cell actually offers many more complications. For example you have the nucleus, you have the different types of organelles like endoplasmic reticulum, mitochondria, lysosomes, golgi bodies and all other kind of organelles. And in addition to that when we talk about the plant cells, the plants are also contains the cell wall as well as the plasma membrane and all other kinds of organelles chloroplast, nucleus answer.

So, when you are looking for the isolation of a particular factor from the particular region of a cell whether it is belonging to a mitochondria or chloroplast or endoplasmic reticulum or lysosomes, the things becomes more and more complicated. So, in today's lecture we are going to discuss about how to fractionate the prokaryotic cell as well as a eukaryotic cell to isolate the protein of your interest from a particular cell organelles or a part of the cell. So, we will start with the prokaryotic cell.

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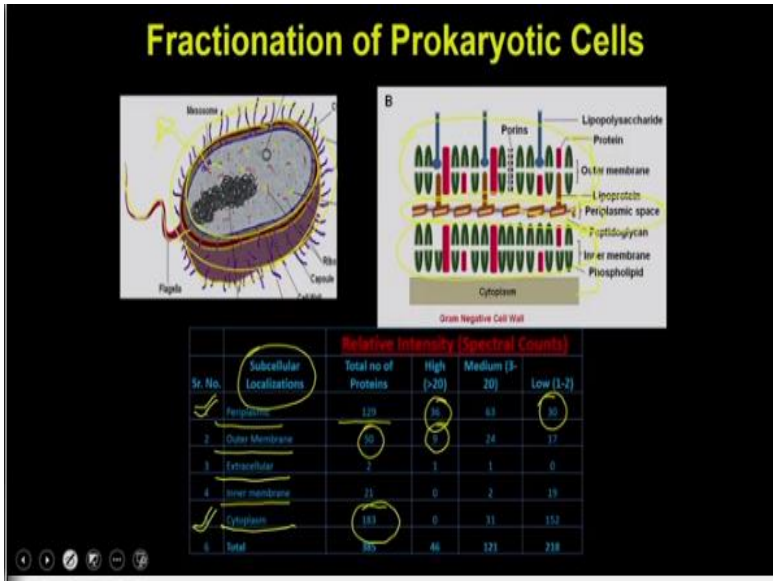

So, in prokaryotic cell, you have so this is a typical bacterial cell where you have the plasma membrane and outside the plasma membrane you have a cell wall. And this is a flagella which actually allows the movement of a typical prokaryotic cell. And within the cell wall what you have is you have the two layer one is the outer layer and then you have the inner layer and in between the outer layer as well as the inner layer you have a space which is called as the periplasmic space.

So, what you see here is the distribution of the different types of proteins what is present within the bacterial cell. So, what you see is that in the periplasmic fractions you have the 129 different types of proteins where some proteins are of high molecular weight and some proteins are of low molecular weight. Similarly you have the outer membrane where you have the 50 different types of proteins, 9 proteins are only belonging to a high molecular weight protein.

Then some of the proteins are extracellular in nature, which means they will be going to be secreted from the bacteria and they will be present into the media of that particular culture. Then you have the inner membrane and as well as the cytoplasm. So, the major chunk of the protein are always been present within the cytoplasm. And you have the two region which are of your interest to isolate the protein, one is the periplasmic fractions and the second is the cytoplasmic fractions.

So, the first we are going to discuss about the periplasmic fractions. And remember, the periplasmic fraction means, the fraction which is present within the outer layer as well as the inner layer. So, the region which is present between the outer layer and inner layer is called as the periplasmic region and that region is always been used by the bacteria to store the proteins for or sometimes they also use that protein into that periplasmic fractions. Sometimes they also use the proteins to even sequester the drugs and related complexes.

And periplasmic fraction is very important even in suppose you would like to use the bacteria for over expression purposes because the periplasmic fraction is giving a suitable environment for the proper folding of the protein. So, in some cases what happen is that you are actually putting a signaling sequence or you are putting a localization sequence into your protein of your interest. And as a result the protein is getting into the periplasmic fraction. So, let us see how you can be able to isolate the periplasmic fraction from the bacteria.

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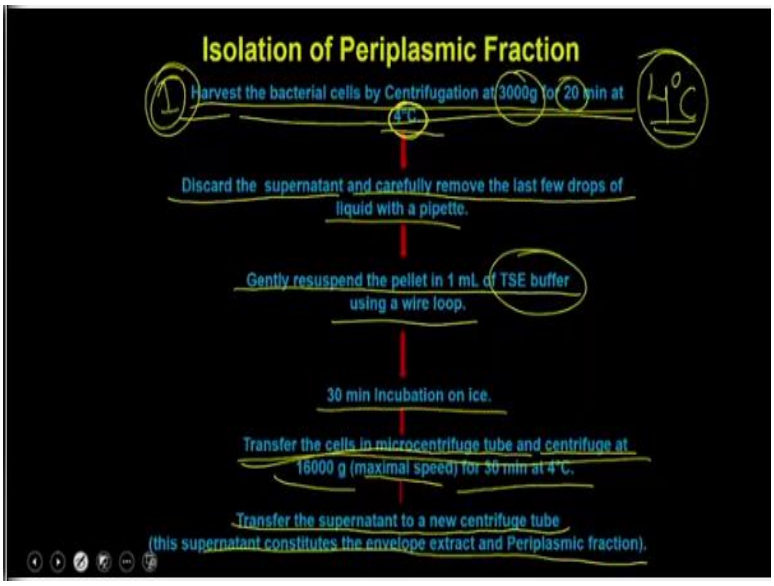

So, the periplasmic fraction first you have to harvest the bacterial cell by the centrifugation at the 3000g for 20 minutes at four degrees Celsius. So, remember the periplasmic fraction isolation is a very, very sensitive for the proteins that is why the most of these procedure has to be performed at 4 degrees. Because the once you isolate the bacteria and when you are going to break open and you would like to isolate the periplasmic fractions, the all the proteases and all other kinds of protein degrading machinery is going to be activated in that process.

That is why the whole procedure has to be performed on 4 degree. So, in the first step, you are going to harvest the bacterial cell by centrifugation at 3000g for 20 minutes at 4 degree Celsius. Once you have got the bacterial pellet, then you are going to discard the media and then you are going to carefully remove the last drop of liquid, so that you will not going to suck up the bacterial cells.

Then the bacterial pellets you are gently suspend in one ml of TSE buffer using a wire loop and then you incubate this mixture at on ice for 30 minutes. So, in this particular incubations what will happen is that the bacteria is going to swell and it is actually going to give you the periplasmic fractions. Then what you have to do is you have to transfer the cells in a micro centrifuge and centrifuge at the 16,000g which is the maximum speed in the microfuge for 30 minutes at 4 degrees Celsius.

Then you transfer the supernatant to a new centrifuge tube, this supernatant constitute the envelope extract as well as the periplasmic fractions. So, in this particular protocol, what we have done actually is we have actually given a heat shock we have given the osmotic shock to the bacterial cell after removal of the cell wall and in that process, the periplasmic fraction is been removed from the bacteria or the protein what is present in the periplasmic fractions are been extracted.

So, what you see is that first you have harvested the cells, then you have incubated the cell in a TSE buffer and that actually is good enough to give them a shock. And then you are actually centrifuging that, so that the supernatant will going to contain the periplasmic fractions and the pellet is going to contain the other part of the cell.

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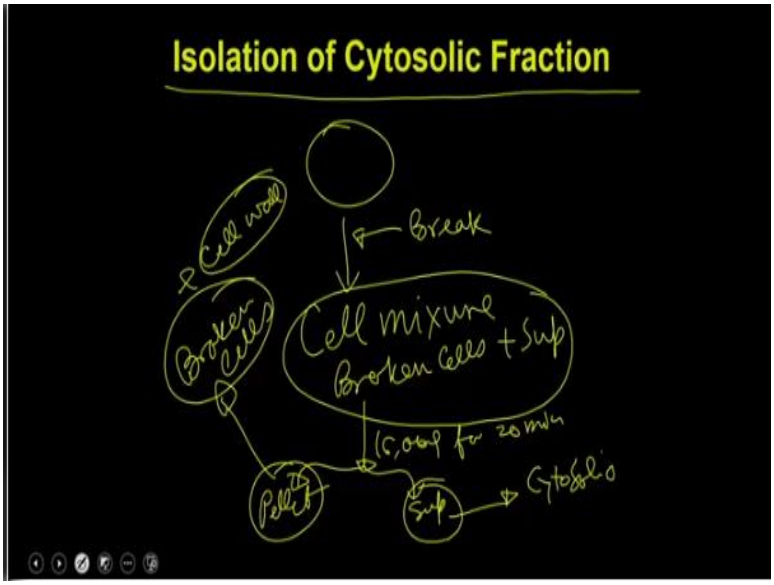

Apart from that you can also do the isolation of the cytosolic fractions. So, there is no special way of doing of isolating heteroplasmic fractions. What you have to do is, you have to take the bacterial cells, and then since the bacterial cell does not contain the organelles, the isolation of the cytosolic fraction is very easy. Because what you have to do is you have to take the bacterial cells, then you have the multiple options like through which you can actually break the cells.

So, you have to first break the cells with the help of the different types of the cell disruption methods. So, there are mechanical method, there are the enzymatic methods and there are the

physical methods. For example, you can use the thermo lysine you can take the osmotic fragility you can take the help of the detergents. In case you are looking for only to prepare the cell lysate you are not interested to isolate the active protein.

But if you are interested to isolate the active protein, you can even use the mechanical methods like you can use the homogenization, you can use the other kinds of mechanical methods. And as a result what will happen is it is actually going to give you the cell mixture like where it is actually going to contain the cells self and plus the supernatant. And then what you have to do is you have to centrifuge this mixture at the 16,000g for 20 minutes.

And as a result you are going to get 2 fractions, you are going to get the pellet fractions, you are going to get the supernatant fractions. This pallet fraction is only going to contain the broken cells and that you can actually discard. Because this broken cells as well as these are actually going to have the cell wall and all those other kinds of material whereas in the supernatant you are actually going to have the cytosolic fractions.

So, this is the way you can actually be able to fractionate the bacterial cells either you can isolate the periplasmic fraction or you can isolate the cytosolic fractions.

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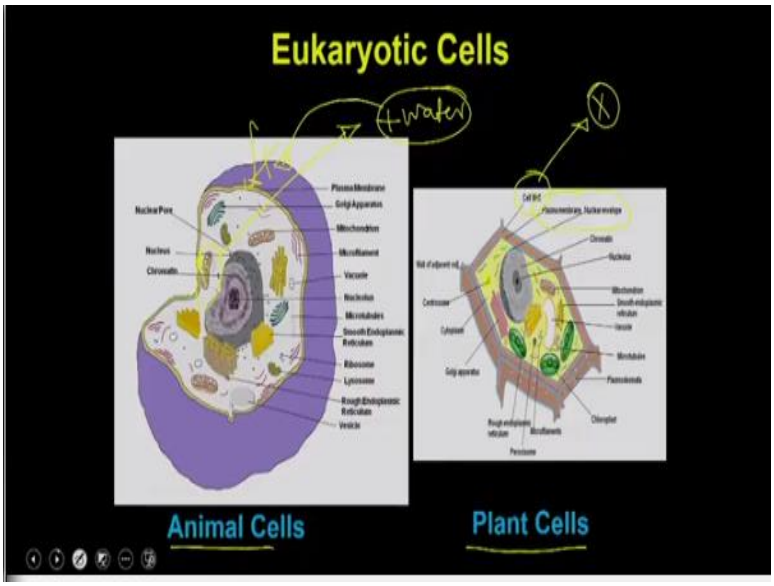

Now compared to the prokaryotic cell which are actually very simple and where you do not have the multiple organelles, the isolation of the proteins from the different organelles or even within the presence of cytosol is very, very complicated when you talk about the eukaryotic cell, for example you are talking about the animal cell or to the plant cells.

So, in the case of plant cell you are going to have the problem of cell wall which is actually going to be broken down. Then only you can be able to access the plasma membrane, you can be able to access the organelles. Whereas in the case of animal cell which is actually going to be very, very sensitive for any kind of treatment. You have to be very, very careful about the osmatic fragility and you always have to be very sensitive about the.

There should be no change in the osmolarity of the solutions because as soon as you change the osmolarity of a solution and the mammalian cells are present. That itself is going to break the cells they might also disturb the overall distribution of the protein within the organelle as well. And as a result you are not going to get the good recovery of your protein from a certain organelles.

For example, if you are interested to isolate the protein from the mitochondrial fractions but by mistake if you add the some amount of water ok. So, what will happen is the water is going to break the plasma membrane which is going to be the first barrier and then it is actually going to destroy the mitochondrial membrane as well. And ultimately what will happen is that the recovery of your particular protein is going to be very, very less.

So, that is why we have to be very careful when we are handling the eukaryotic proteins because they are very sensitive for different types of treatment what you are going to perform in during the course of different types of experiments or when you are actually going to isolate the different proteins from the different locations of the cell.

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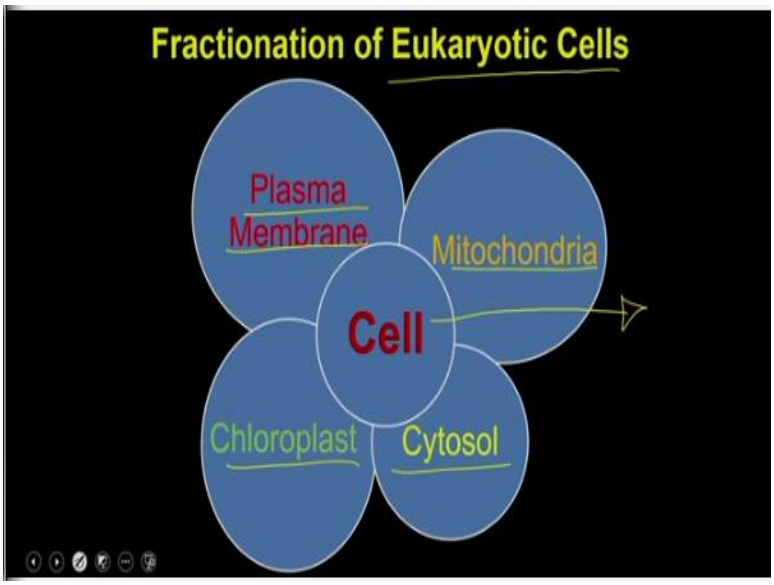

So, within the eukaryotic cell majorly you have the multiple options like you have the locations like plasma membrane, you have the mitochondria, you have the chloroplast, you have the cytosol. And apart from that, you also in the case of plants, you might have the chloroplast and you might have the cell wall as well. So, how to fractionate this because so to understand the fractionation first you have to understand the underlining the instruments and as well as the principle.

And then we were going to discuss about the fractionation of the eukaryotic cell and how you can be able to isolate the different organelles and even how you can be able to isolate the protein from those organelles.

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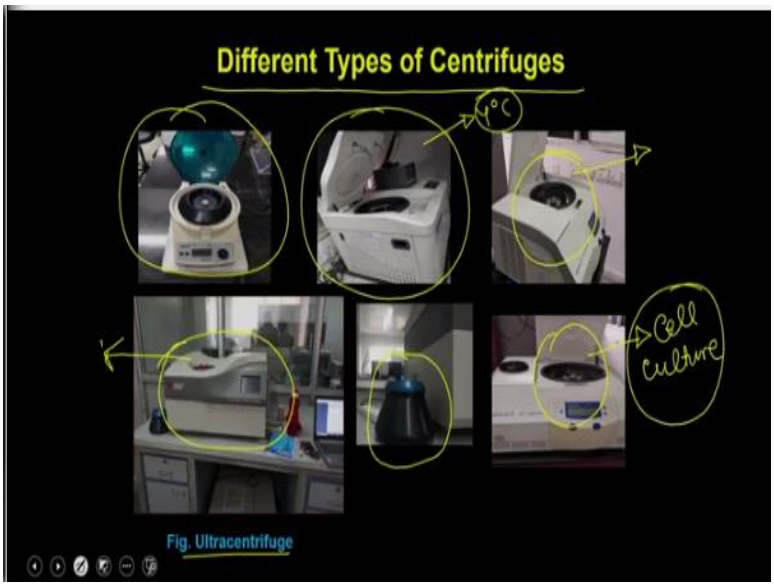

So, for the fractionations, you might have to require the different types of centrifuges. So, this is the microfuge which is actually very low speed centrifuge then you have the high volume high speed centrifuge. It actually can also go to the 4 degree as well as can be 37 degrees Celsius. And then this is the high speed centrifuge, this is the centrifuge what you use for cell culture purposes.

Because it also can have the flexibility of putting the different types of rotors like you can have the fixed angle rotors or you can have the plate rotors. And this is the centrifuge what you have is called as the ultra centrifuge because it actually can go up to the ultra high speeds and it actually can go even up to the one lakh g actually. So, that actually is required to isolate the different components of cell.

This is a typical rotor what you use in a ultracentrifuge when you are using and these rotors are actually been made with a very, very strong metal. So, that when they are rotating at a very, very high speed, it actually withstand that kind of high pressure.

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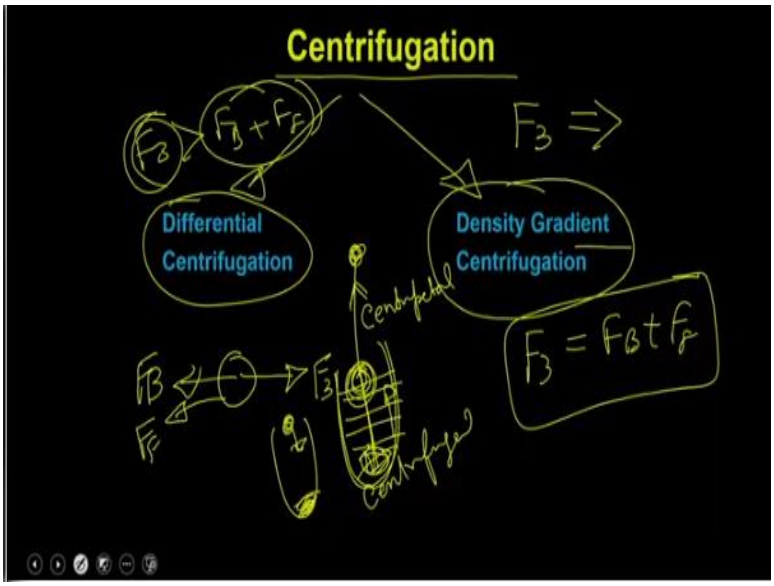

As far as the centrifugation is concerned, the centrifugation can be done in two ways either you can do a differential centrifugations or to the density gradient centrifugations. The basic principle or the underlining principle of the certification remains the same. And I think you remember when we were talking about the maintenance as well as the operation of the centrifuge we have discussed about the basic principle.

So, the basic principle is that when you are rotating an object around the axis, it actually experiences the two forces, the centripetal forces which is actually towards the axis and the centrifugal forces which is actually away from the axis. So, if you are actually rotating a object and around an axis, so, what will happen is you are going to have the centripetal force towards the center of the axis, where as you are going to experience the centrifugal force which is away from the axis.

So, you can imagine that if you keep this object into a eppendorf or into a tube this object will try to move towards this side but this object is present in a liquid. So, this liquid is actually going to oppose the movement of this object. So, what will happen is when the object is trying to move away from the center because of the centrifugal force which is actually going to be F 3, it is actually going to be opposed by 2 forces one is called as the buoyancy forces.

So, that you can imagine that FB and then you also can have the frictional forces because when the molecule is moving to through the viscous material it actually going to experience 2 things, one is the buoyancy. Because of the density of the molecule and other one is the friction because the molecule has some size. So, F 3 is actually going to equal or bigger than in both the cases.

So, F3 can be equal to in the case of F B + F F when you are talking about the density gradient centrifugations, F 3 is going to be bigger to the F B plus the F F. Because in that case what will happen is the F 3 is going to push this object towards the end of this tube. And ultimately what will happen is that because the tube is closed from the lower end the object will go and form the pellet.

So, if it is travelling and it is travelling because the opposing forces are very, very less. Then what will happen is this object will reach to the end of this tube and it is actually going to be pelleted down. So, this is the basic principle of the centrifugation as far as differential centrifugation is concerned as well as the density gradient centrifugation is concerned. Both of these centrifugations are actually utilizing the centrifugal forces as well as they are exploiting the size as well as the density of the particular material.

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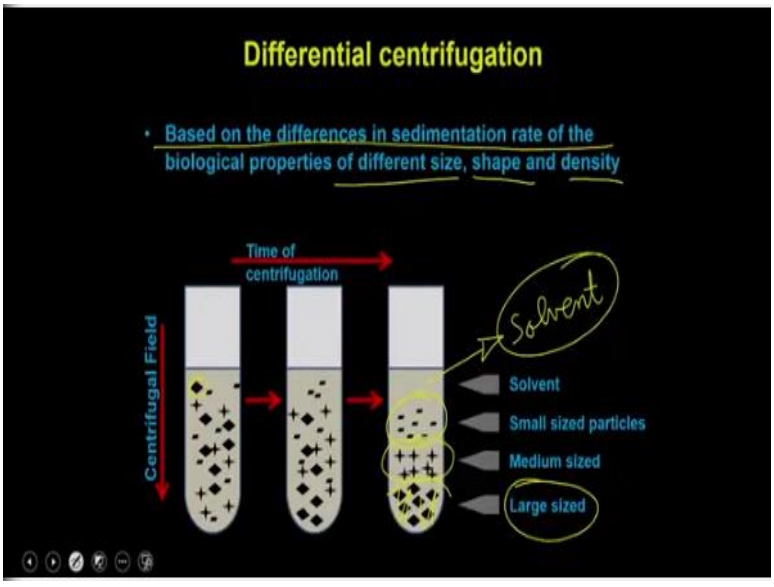

So, that is why the sedimentation of a particular object is depends on to the different size, shape and the density. So, you can imagine that if you have a molecules or different sizes as well as the

densities. And if you start doing the centrifugations what will happen is that the molecules which are of large size which means of a high molecular weight are going to be pellet down first, then you are going to have the medium size object.

And then you are going to have the small size object and on the top you are going to have the solvent which means the differential centrifugation is actually exploiting the 2 phenomena. One is the size of the object and the second is the density of that particular object because these are the 2 parameter which are actually going to decide at what centrifugation speed they are actually going to overcome the buoyancy forces as well as the frictional forces, so that they will be get pelleted down.

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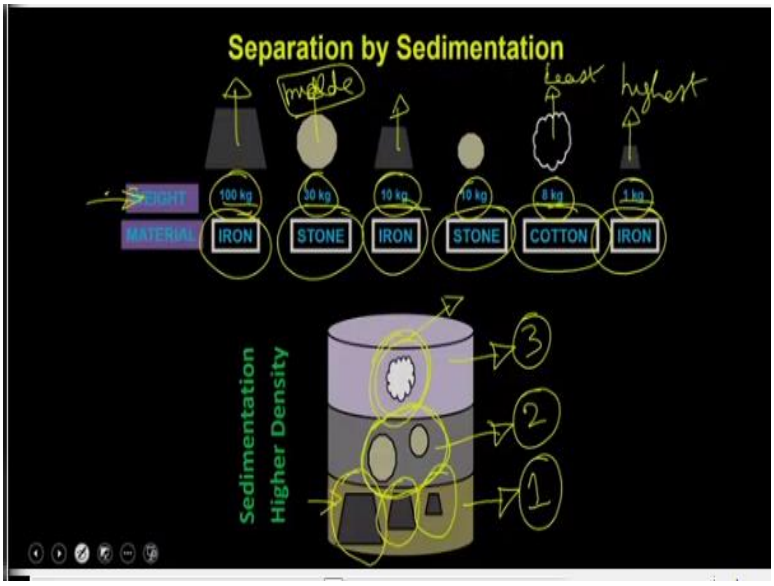

So, you can understand that with the simple thing that suppose we have the different types of objects. For example you have an iron which is of 100 kg, you can have this stone which is of 30 kg, you can have another iron block which is of 10 kg and you can have the stone which is of 10 kg. And then at the end you also have the cotton which is of 8 kg and you have another block of iron, which is 1 kg.

So, remember you know that the iron is the highest density and the so this is iron, right. So, iron is actually having the highest density whereas the stone is going to have the middle density and the cotton is going to have the least density. So, when you are actually going to take this mixture

and you are going to spin what will happen is that irrespective of their weight means irrespective of whether they are 100 Kg, 10 kg or 1 kg the iron is going to be pelleted down at the bottom.

So, it is actually going to be the heaviest particle and then the stone is going to be pelleted down later on and the cotton is going to be remained at the top. So, which means if you do a differential centrifugations, what will happen is that the iron is going to be pelleted down first or the iron is going to be pelleted down at a very, very low speed. Because it is actually going to help the centrifugal forces and actually it is going to.

So, even if you spin at a very slow speed the centrifugal force of the iron because of the density is going to be so heavy that it is actually be good enough to nullify the effect of the buoyancy forces as well as the frictional forces. Whereas at that particular time the stone as well as the cotton will not be getting pelted down. But when you increase the speed little high then the stone is going to be pelleted down but the cotton will still remain within the liquid ok.

And then if you increase the speed further up because the cotton is going to have the least density, it requires more force or more speed. Because then only the centrifugal force is going to be good enough, so that it will be get pelleted down. Because at the end the centrifugal force has to nullify the effect of the frictional forces as well as the beyond forces. And that is how actually you can be able to palette down the particles of the different densities.

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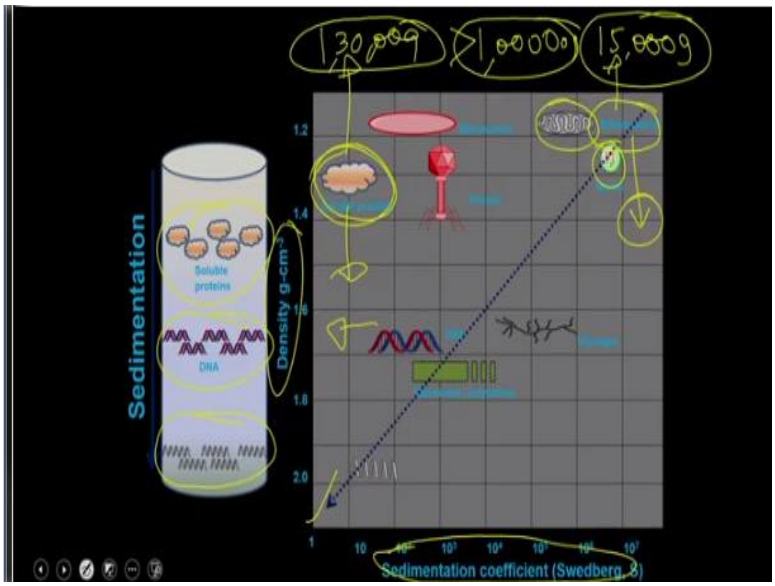

Let us see in the biological world what is the situation. So, this is the graph what is being shown and this is the densities. So, what you see is the sedimentation rate or the sedimentation coefficients of the different molecules. And what you see is that the proteins which are actually having the very high molecular weight is actually having the least sedimentation rate. Whereas the molecules which are of as you go from this side to this side the sedimentation rate is getting down.

And when the sedimentation rate will go down which means you have to run these molecules at a very high speed. Similarly what you can see here is the it is showing as the sedimentation rate of the protein, DNA As well as the other biomolecules. And, so in this graph, what we are trying to show is that as the density of a molecule will go up, you are actually going to have the higher sedimentation rate.

And that is how you might you do not have to run the centrifuge at a very high speed which means because the molecule itself has a tendency to sediment it is own. That is why you do not have to spin at a very high speed. For example the mitochondria is actually going to be pelleted down at a speed of 15,000g. Whereas the protein which is present in the soluble fraction is actually going to be pelleted down at a speed of 1 lakh 30,000g which means or any speed which is more than 1 lakh g.

So, which means and as you can see the sedimentation rate or sedimentation coefficient of the soluble protein is on a lower side. Whereas the sedimentation coefficient of the mitochondria as well as the nucleus is on the higher side which means these molecules are does not require a high speed to be get sedimented.

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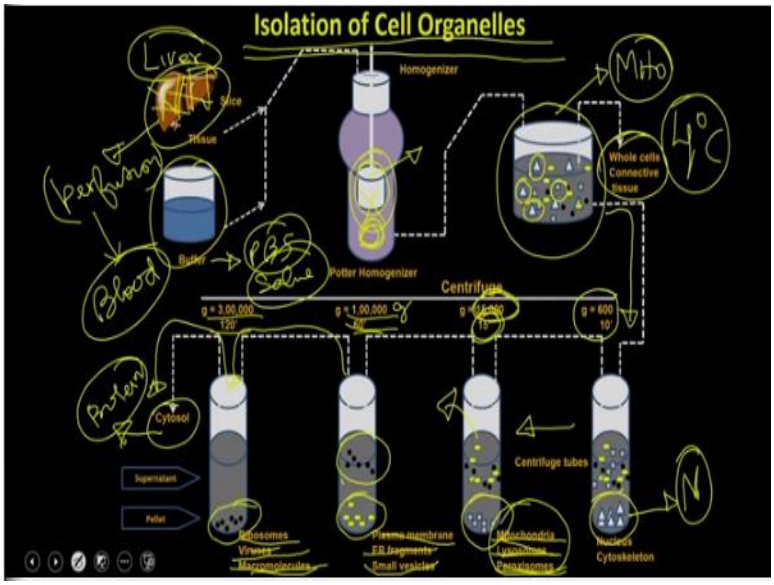

So, let us discuss about the isolation of the cell organelles. So, I have taken a 2 examples, in the first example we are trying to process the liver cells. So, in the liver cell what will happen is that the if you want like to isolate the cells and then if you would like be interested to isolate the cell organelles, what you have to do is that first you have to do a process called the perfusion. So, perfusion is a process which actually removes the blood from the liver because you know that the liver is a vascularized organ.

So, liver is having the full supply of blood and because of that when you are trying to isolate the liver cells you are also going to have the contamination of the blood cells. Because the blood is present in the liver, so the liver can be perfused with a isotonic solutions like you can use the saline or the phosphate buffer saline. And that actually is going to remove the blood what is present inside the liver and that actually is going to remove the contaminating cells.

Otherwise when you are trying to isolate a organelle, for example, if you are interested to isolate the mitochondria from the hepatocytes which are basically making the liver cells you will not be

able to isolate the mitochondria only from the liver cell. Because the mitochondria will also going to be contribute from the (()) (27:54) cells and the other kinds of immune cells but is present in the blood.

So, in the first step you remove the blood from the liver in a process called perfusion. And then what you have to do is you dissect this liver into small pieces and you homogenize in a buffer which is isotonic either you can use the PBS means like phosphate buffer saline. Or you can use a saline, simple saline like so both are going to be isotonic and then you do the homogenization. So, homogenization you can do in a homogenizer, homogenizer is a kind of a mechanical cell disruptors.

So, where what will happen is that when you do homogenization this particular type of Teflon blade is actually going to rotate into this particular chamber. So, homogenization is like simple if you might not have seen the homogenizers, it is just like a as you are using the mixer grinders in your home actually. So, mixer grinders are nothing but it is having a blade which rotates and because it is rotating the blade, it actually cuts the cells it cuts the tissue into a small particles and that is how it actually going to break the cells.

So, once you do the homogenization you are going to get the whole cell or the cellular particle which means you are going to have the different types of organelles what is present. So, homogenization is never going to break the cell into such a way that the individual organelles are also going to be broken down. Because the homogenization is going to be done in a isotonic condition.

If you do homogenization under the hypertonic conditions then only it is actually going to disrupt the cell organelles as well. So then what you have is, you have a mixture of the broken cells, you are going to have a mixture of different types of organelles. Like you are going to have the mitochondria, you are going to have the chloroplasts, you are going to have the mitochondria, you are going to have the nucleus, you are going to have the lysosomes, you are going to have the endoplasmic reticulum, the golgi bodies and all that.

Then what you have to do is, the first step what you have to do is since we are using the differential centrifugations, you first spin this mixture at 600g for 10 minutes. And what will happen is when you do that it is actually going to pellet the most heavy particles. So, it is actually going to remove the nucleus from the mixture. And now what you have is you are actually going to have the remaining stuff like you are going to have the mitochondria, you are going to have the lysosomes, you are going to have the peroxisomes.

And now what you do is you spin this again at 15000g for 15 minutes. And remember whole this procedure has to be done at 4 degree as we discussed before also that whole cell fractionation process is very, very sensitive for the proteases as well as other cell lytic enzymes. So that is why it is important that you perform all this procedure at a low temperatures. So, now what you have, you have a mixture of these and then if you spin at 15000g for 15 minutes.

It is actually going to pellet all the other heavy particles like mitochondria, lysosomes, peroxisomes and so on. Now what do you have, so you take out the supernatant into the next tube and then again you are going to spin. So, now what you are doing is you are spinning at the 1 lakh g for 60 minutes. And in that process what will happen is that the plasma membrane as well as the ER as well as the small vesicles are going to be pelleted down.

Whereas the ribosomes and the viruses or the macromolecules are going to be remained in the supernatant they are not going to be get pelleted down. Now what you have to do is, you take this supernatant again and you spin it at 3 lakh g for 2 hours. And that is actually going to pellet the ribosomal fractions, you are going to get the virus particles as well as you are going to get the macromolecules.

Now after this whatever the supernatant you are going to get is actually going to be the cytosol which actually going to contain the monomeric proteins. And it is actually going to contain all the so it is actually going to contain the cytosol which is actually nothing but a protein solutions. So, this is what the differential centrifugation can be used to fractionate the different types of organelles starting from the liver.

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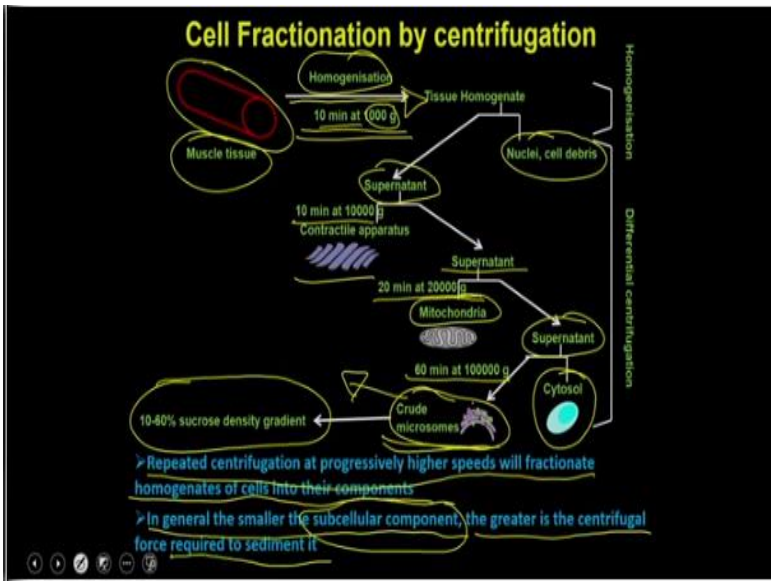

Now you can do the same thing from the muscle cell as well. So, in the case of muscle cell because the muscle cells are not very vascularized you do not need to do a perfusion step to remove the blood cells. But you can do is you can first the step itself you can do the homogenization for 10 minutes at 1000g so after the homogenization you are going to get a cell mixture where you are going to have the broken cells, nuclei, mitochondria and chloroplasts and everything, all the cell organelles.

Then what you do is you spin at 1000 g for 10 minutes and that is actually going to remove the nucleus as well as the cell debris. And then you got the supernatant, you spin at 10000g for 10 minutes and that is actually going to remove the mitochondria and all other kind of thing. And then you take the supernatant and spin it at 20000g that is actually going to remove the mitochondria.

Then you take the supernatant and spin it again for 1 lakh g for 60 minutes and that is actually going to give you the microsomes or the endoplasmic reticulum fractions and the plasma membranes. And then you are going to get the cytosol and if you are more interested to even isolate the plasma membrane further then what you can do is, you can do a density gradient centrifugation of this fractions.

And that actually is going to give you the pure fractions of different microsomal fractions. So, repeated centrifugations at progressively high speed to fractionate the homogenate of cell into the individual components. In general, in smaller the sub cellular component the greater is the centrifugal force required to sediment it. So, this is the basic principle where the smaller the object, the lesser the density, it is actually require the higher centrifugal force. And higher centrifugal force means you have to run the centrifuge at a high speed.

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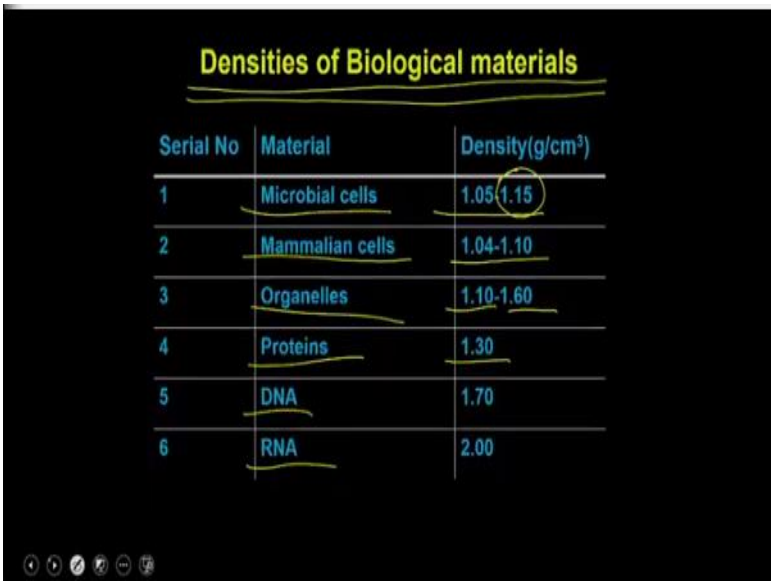

Then we are talking about the density gradient centrifugation. So, you can see the densities of the different biological molecules like the microbial cells which goes into 1.05 to 115. Then you have the mammalian cells which is actually in this range, then you have the different types of organelles which goes from 1.1 to 1.6. Then you have the proteins which goes into 1.3 DNA and RNA.

So, the density gradient centrifugation actually exploits the physical property of a molecule that where the centrifugal force is equal to the beyond forces and the frictional forces. So, what you have seen in the differential centrifugation that we are actually increasing the centrifugal force and that is how you are actually you know you are nullifying the beyond forces as well as the frictional forces.

Whereas in this case you are simply doing the reverse which means you are keeping the centrifugal force constant**.** Now what you are doing is you are actually increasing the beyond forces as well as the frictional forces, especially the beyond forces. And that is how what will happen is the molecule which can be pelleted down in the absence of the beyond forces. It is now actually not going to be pelleted down because you are increasing the beyond forces. And as a result the molecule is going to be remain in the supernatant and it is going to be localized in a particular region.

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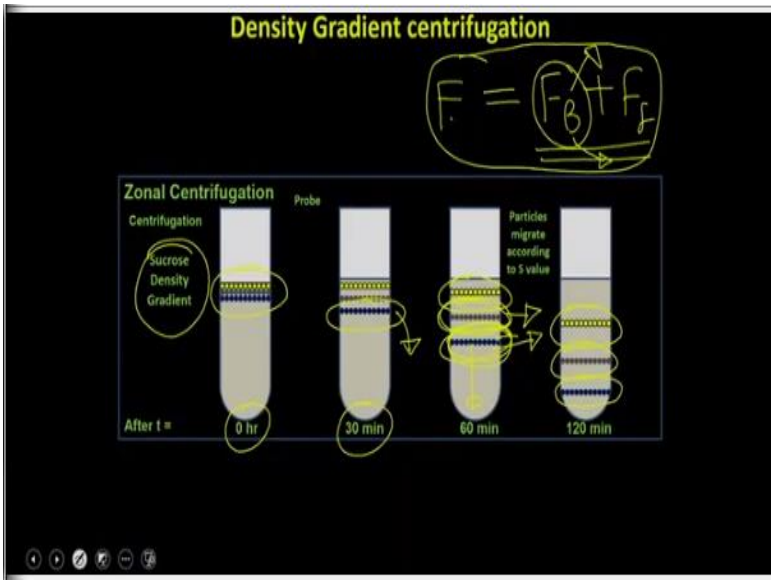

So, what you are going to do in the density gradient centrifugation is that suppose I have use a sucrose density gradient centrifugations. And then I have loaded the molecules of the different densities onto this. So, at the beginning all the molecules are mixed and you have a mixture. Then what will happen is when you run this for 30 minutes what will happen is that since all these molecules are having the differential sedimentation rate because they are actually associated with the differential centrifugal forces.

So, what will happen is that centrifugal force is actually going to oppose by the beyond forces as well as the frictional forces. So, if I increase the beyond forces or if you manipulate the beyond forces, what will happen is that the molecules are going to be localized in different zone within that particular supernatant instead of getting pelted down. Because what you are doing is you are

actually manipulating with the beyond forces and at a place where they are actually going to stop.

Which means at this point for these blue color pellets what will happen is that the centrifugal force for these molecules are actually equivalent or the equal to the beyond forces to the frictional forces, so as a result it is actually going to be localized to this. Similarly if this is the location for this, so at this particular location the forces are now been equalized. And what will happen is if you keep doing this the molecule will localize to a particular region.

And that is how they are actually going to be separated which means the molecules are actually going to utilize their densities to generate a particular type of centrifugal force. And that centrifugal force is actually going to be opposed by the beyond forces plus the frictional forces. But at a particular point the frictional forces plus beyond forces are going to be equalized by the centrifugal forces and that is the place where the molecule is going to remain there, it will not going to be pelleted down.

Because if you go further up and if you increase the centrifugal force further then the molecule is going to be pelleted down and it will remain at the bottom of that particular tube. So, compared to the differential centrifugations the density gradient centrifugation actually exploits the densities of the molecule as well as the densities of that particular the medium as well.

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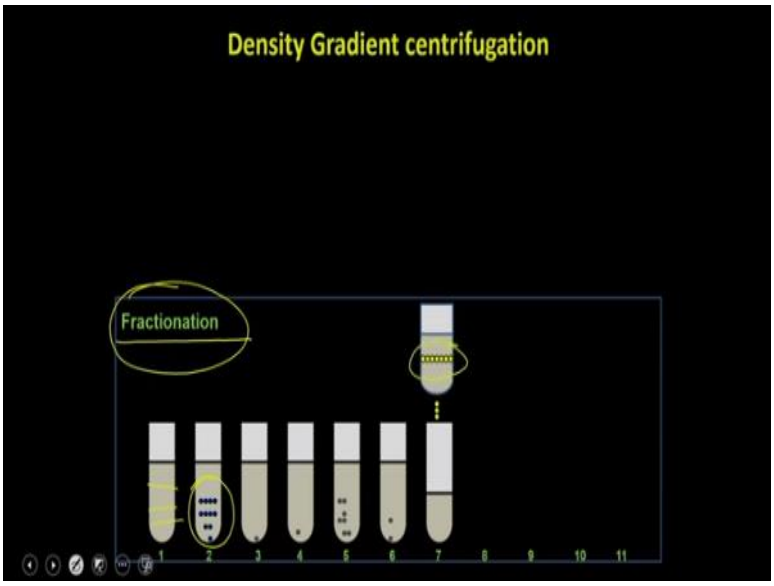

So, these are the fractionation that is the way you are going to do the fractionation which means when you are going to start. So, the molecules are going to be localized and then if you want you can actually break open these tubes and you can be able to collect all the individual fractions.

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How to collect the fractions when you are going to do the density gradient centrifugation. So, you can imagine that I have crude mixture when I run it for the 3 hours at 1, 50,000g, what will happen is it has form the different types of bands. Like the band which are actually been corresponding to the heavy fractions, light fractions, triads and as well as the surface membrane.

Then what will happen is if I have to remove these what I can do is, I have the 2 options either I can just use the pipettes and I can just suck it. So, I can put pipette to next to this particular fraction and I can just suck this whole liquid and that is actually going to remove. Automatic fraction collector for unstable gradients and the second, third case is you can do the freezing and slicing.

So, what happen is in the freezing and slicing what you do is suppose this is the fractions you have the different types of fractions. So, what you do is you freeze this and then you actually going to cut all these into small slices and then individual slice you can remove and thaw and that actually is going to give you that individual fractions. So, this is all about the cell fractionations and how you can be able to exploit the different types of centrifugations.

Either you use the differential centrifugations or the density gradient centrifugations to isolate the different types of organelles from the eukaryotic cells. We have also discussed about the prokaryotic cell and how you can be able to fractionate at prokaryotic cells to isolate the periplasmic fraction as well as the cytoplasmic fractions. So, with this I would like to conclude my lecture here, thank you.