Electronic Systems for Cancer Diagnosis
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Lecture – 03
Tissue and Cell Culture Techniques: Devices

Hi, welcome to this module. This module is in continuation with our last module and the focus is on Tissue and Cell Culture Techniques right.

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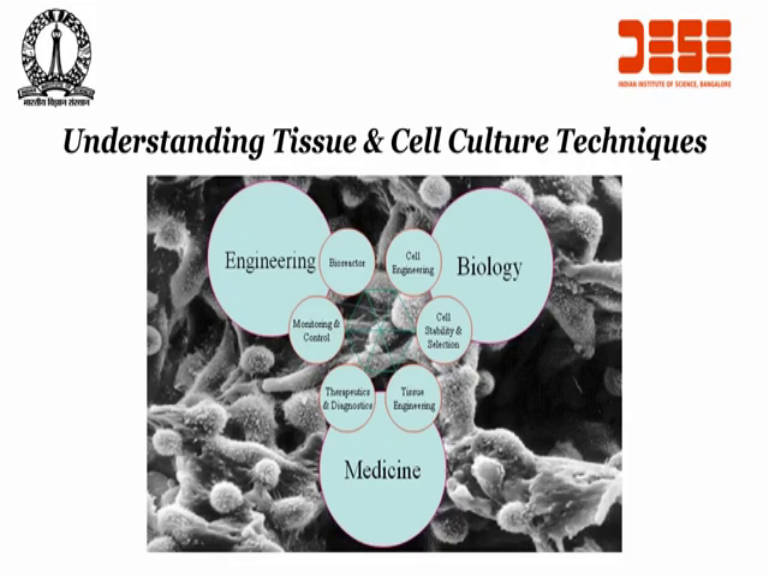

So, if we see what we have seen in the last lecture right, we have seen how the what is the importance of understanding the tissue and cell culture techniques right and how they are interrelated when you talk about developing the bioengineering platform or a biomedical device or in terms of medical device, then how this tissue and cell culture techniques can be used.

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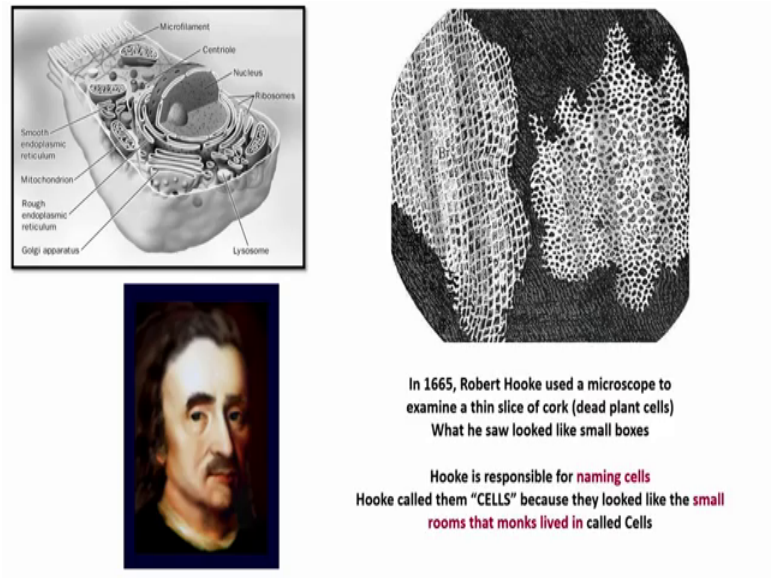

If I go for the next slide and if you see here then, you what you see is that how the cell name came into existence.

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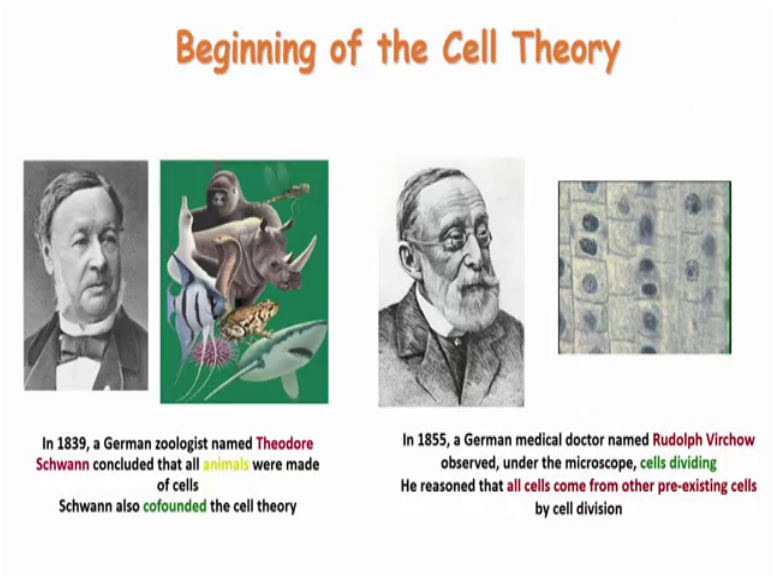

Then we discussed about beginning of the cell theory.

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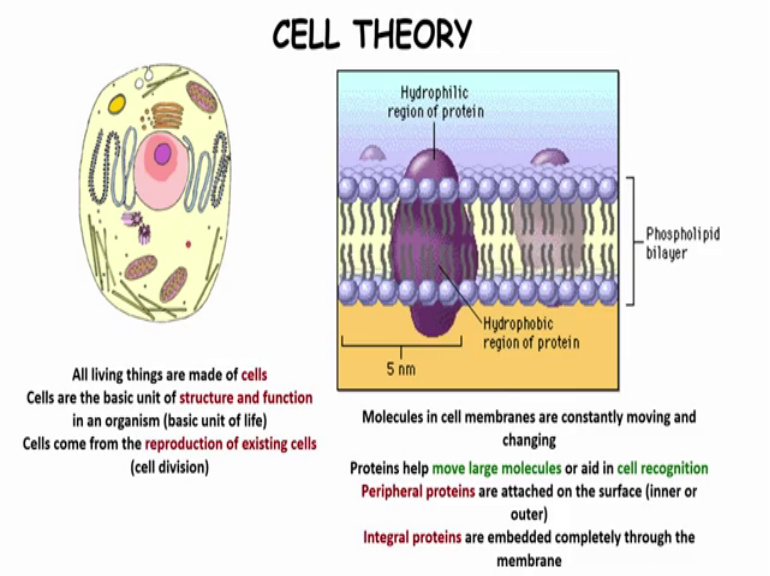

And then we saw what exactly cell theory means.

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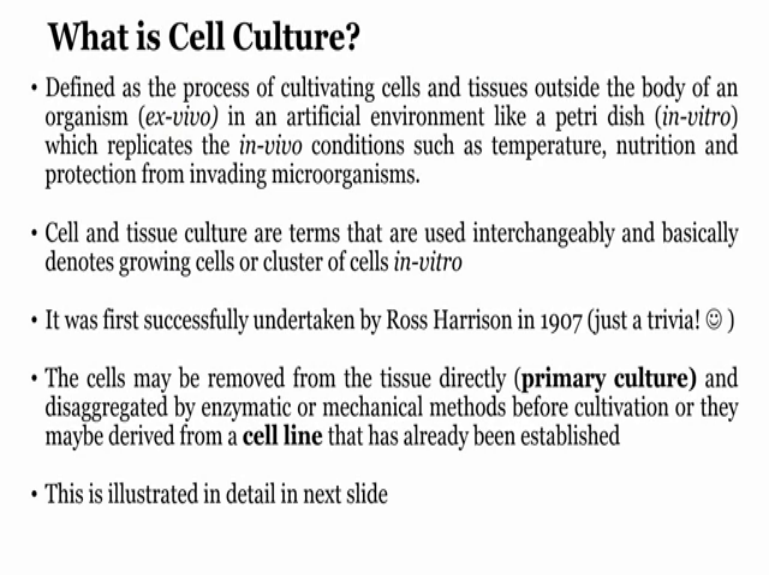

What is cell culture we discussed right and we discussed 3 different points you know or you can say terms one is in-vivo, second is ex-vivo and third one was in-vitro right. In - vivo within the body, ex- vivo and take out the tissue from the body and you do the experiments the in-vitro is when you develop the cells in the laboratory and use the (Refer Time: 01:42) or anesthetic platform like transfer to study the tissue properties or cell properties.

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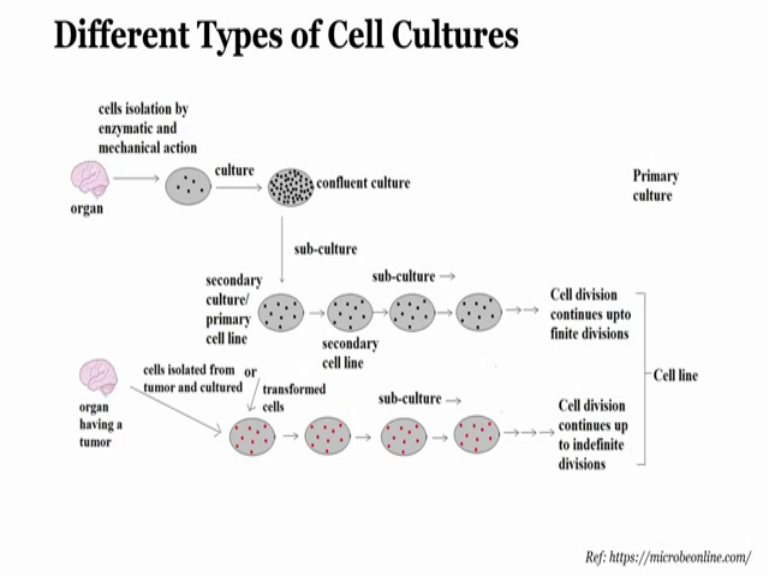

Now, further what we developed is what are the types of cell cultures and how we can isolate the cells or how can we culture the cells from a given organ.

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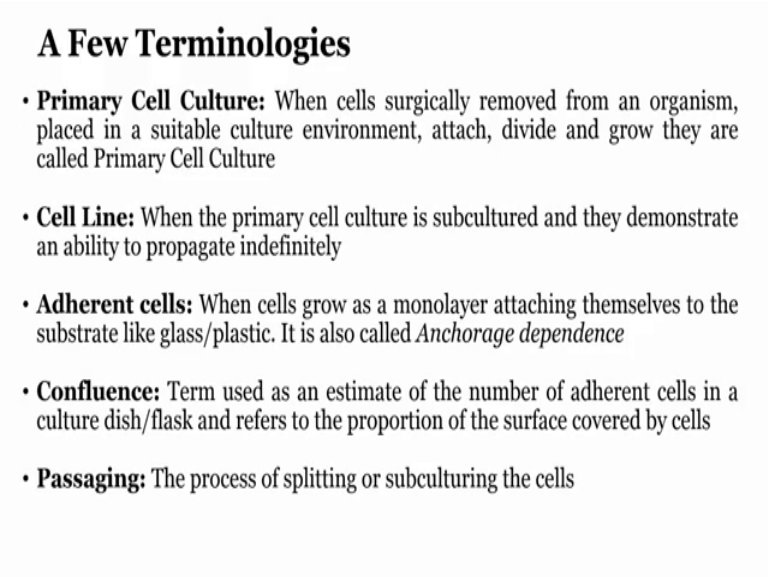

We also saw some terminologies such as primary cell cultures, cell line, adherent cells, confluence, passaging, right.

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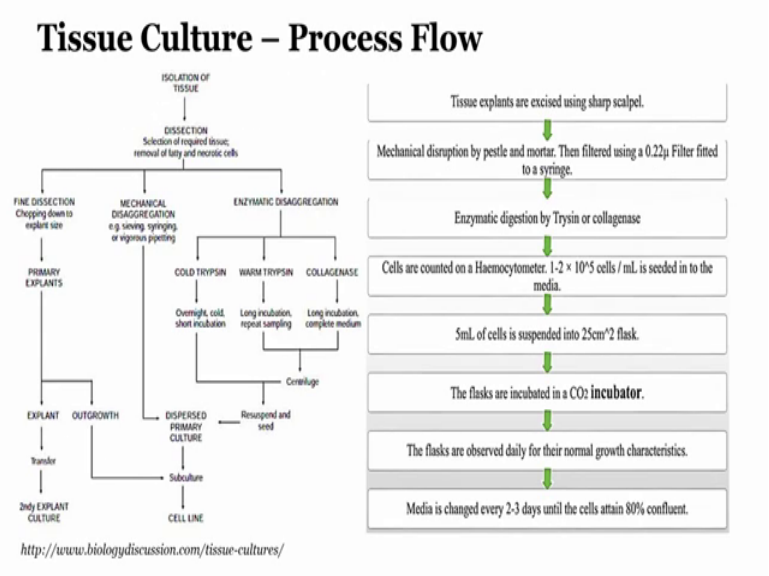

And then what are the how are the tissue culture process flow works.

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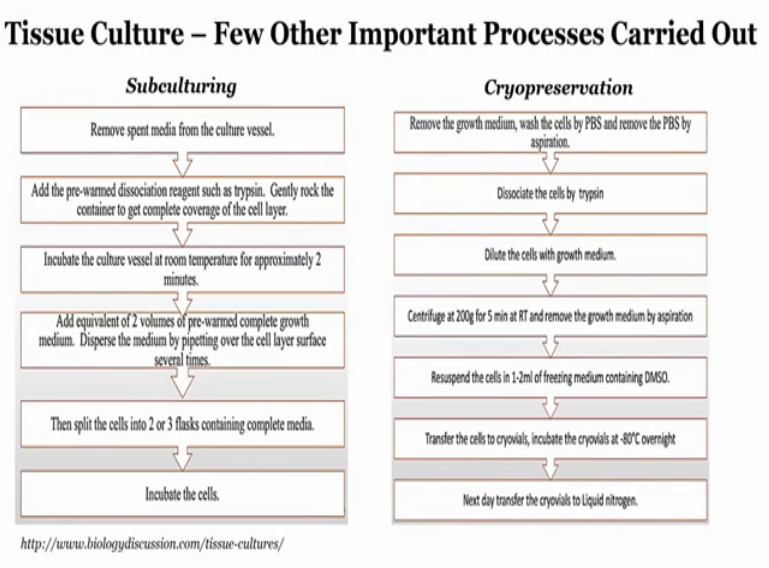

So, we have seen 2 different approach, we also seen how the sub culturing works end cryopreservation works. So, if you want to preserve these cells for a long time, what is the process flow correct?

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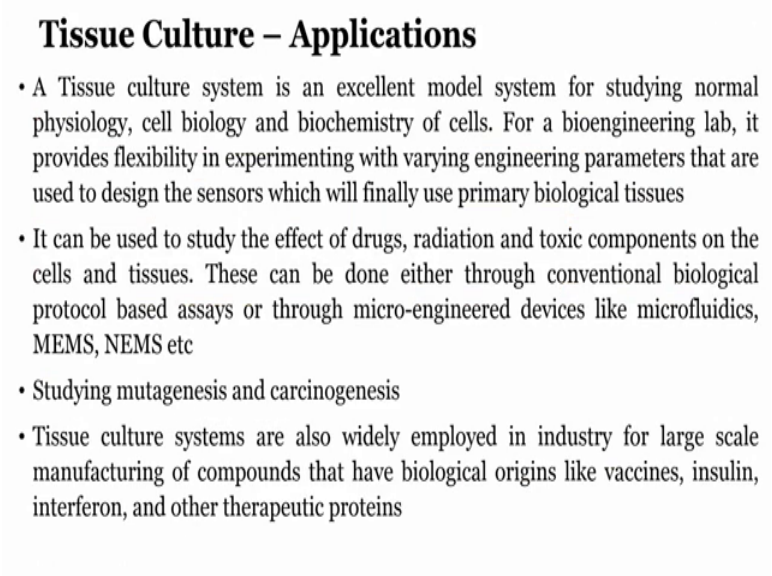

So, today what we are focusing on is what are the application of this tissue culture, why we should understand this tissue culture and how it is added to cancer diagnosis right.

So, let us see today a tissue culture system is an excellent model system for studying normal physiology, cell biology and biochemistry of cells right. So, for a bioengineering lab right; if I want to work in a bioengineering lab a bio is biology, engineering is engineering laboratory. Now if you see recently when you talk about bioengineering it is a mixer, it is an interdisciplinary branch, where the engineers work with scientists right. Now when I say that; that means, that a electronics engineer working with a biologist or a mechanical engineer working with the biologist or you know robotics engineer working with the biologist to mimic a biomedical robots.

So, when you talk about interdisciplinary research is not just about 2 different fields; it is how we can merge the expertise of different labs and to work on a particular problem. Let me give you few examples. Suppose I want to understand of from given set of drugs which drug would be effective or which drug has a higher efficacy for a for the patient right. If that is my idea, I need to understand how the how we can take out the cells from the patient and how can we load the cells, how can we make the in-vivo situation that can be happen only when we have expertise from the cell biologists group. But when you want to understand how the fluid will flow in the body and how can we mimic this in

your situation on the in-vitro platform, we require micro fluidics and that is why the engineering comes into picture right.

It includes the chemical engineering, it includes a materiel science, it includes the electronics engineering. Because you it includes packaging of the device finally, what we want to see is we have to control the amount of liquid flowing or drug flowing through the channel such that it infuses into the cells and based on the efficacy based on the effectiveness of the drug the cells will die or it will sustain. So, if the cell dies we say the drug is more effective, how you know cells are dying right? Can we create a platform? That is what my point about bioengineering would be when we study different projects with different, different problems and there is a indeed interdisciplinary research.

So, it provides a facility in experimenting with the varying engineering parameters I just discussed that are used to design the sensors which will finally, used for primary biological tissues. Now as I said it can be used to understand the effect of drugs, radiation, toxic components onto the cells and tissue.

So, we can use either conventional biological protocol or we can use micro engineering devices, microfluidics, MEMS, NEMS, etcetera right. So, studying mutagenesis and carcinogenesis is another understanding of tissue culture helps us to understand both the problems or to study both the problems well how the carcinogenesis works and then we can also understand that this tissue culture systems are widely employed for large scale manufacturing compounds that has biological reasons like vaccines, insulin, interferon and other therapeutic proteins. So, if I want to give you a few examples how we can use this tissue culture, let me show it to you few examples here I will open a new slide and let me show you a few examples ok.

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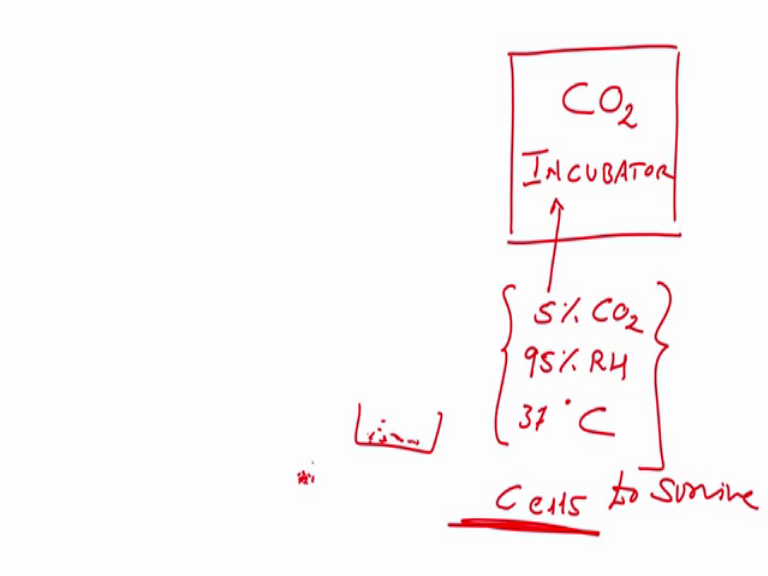

So, suppose, there is a equipment call CO 2 incubator, CO 2 incubator the parameter within it is 5 percentage CO 2, 95 percent RH, 37 degree centigrade. So, if you see this these are the parameters or the environment within the CO 2 incubator what is, why it is 5 percentage CO 2 95 percent relative humidity and 37 degree centigrade? Because this will help the cells to survive right and if the cells are there if you lower the cells in a you bottom plate right and you put the media with growth components, then the cells will clump together to form a spheroid alright. So, again what is the point, this is a cell culture technique that we have a CO 2 incubator, we have 5 which has the following parameters.

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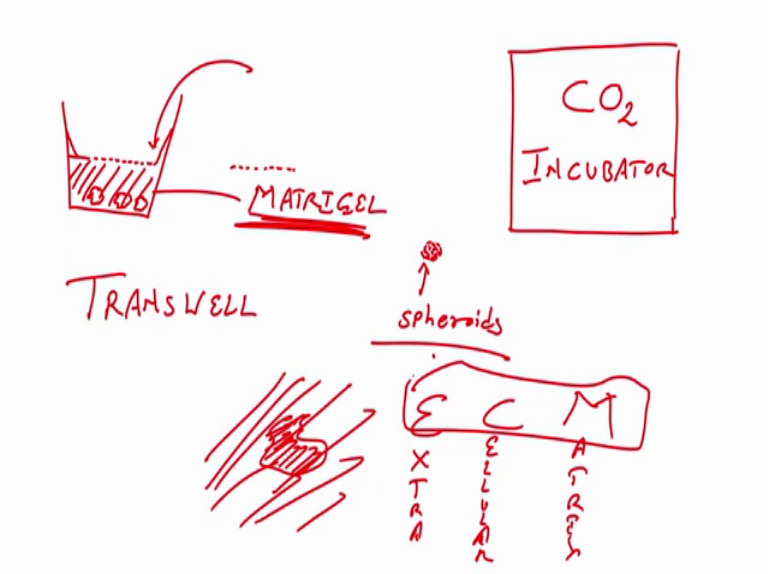

Now, these parameters are because these are the same parameters that are used or that is existing within a human body right. If you see our temperature is 37 degree centigrade, 5 percentage CO 2 95 percentage relative humidity that is how the cells are surviving the cells are growing right. So, if I take these cells and if I want to understand how can I implement a micro engineering or engineering idea to study the cells or to study the drug that is our question ok. So, now what will I do? I have something called a transwell, T R A N S W E L L transwell consists of a well. So, this is a well right here and there is a mess, this is a mess right like this.

So, if I load my spheroids, what is spheroid group of cells together forming within the incubator right spheroid. If I load the spheroids with matrigel, what is called matrigel, M A T R I G E L matrigel, what is matrigel? So, when you see a cancer within a body is always surrounded by Extra Cellular Matrix, Extra Cellular Matrix Extra Cellular Matrix ok. This Extra Cellular Matrix provides a nutrition and the remaining growth hormones to the cancerous tissue. So, this matrigel has similar constituents like this ECM ok.

So, what we are doing is, we are growing the cells within the co 2 incubator with the help of cell culture techniques and then this growing of cells and clumping it together forms of spheroid, this spheroid I will take it and I will load in a transwell right. And this transwell is having along with the spheroid; it has a matrigel alright. This was easy.

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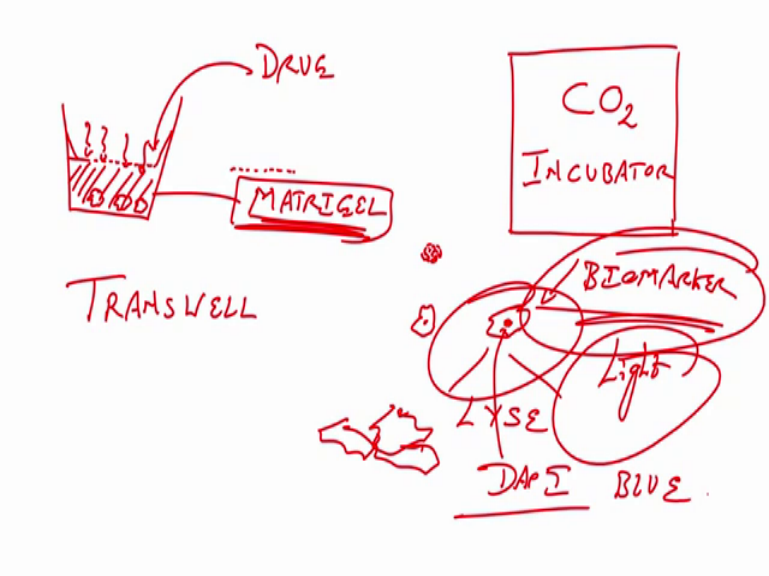

Now, what I will do? Now I will load drug here drug here and if the drug is effective this drug with diffuse through matrigel drug diffuse through matrigel, your drug diffuses through matrigel. What will happen if the drug is effective this cell? Let us say we take up one single cell and there is a nucleus right and if the cell if the drug is effective the cell will LYSE or it is fragmented defragment and it broke breaks into pieces as you mean a very easy language assuming in a very easy language what I said their cell will get lysed.

Now if the cell is Lysed the constituent within the cells will come out. So, we can use 2 different ways one is called biomarker if I use a biomarker, then I can understand when the cell is breaking the fluorescence will change. There will be creation of light why, because we have use a biomarker such that the biomarker will highlight these cells and it is cytoplasm or it is nucleus. So, if I want and if I want to know that this is a cell then I will use something called dapi DAPI; dapi will stain the nucleus with blue color with blue color ok. Let us not go into this detail right now we do not worry about this, I will teach you when the time comes right.

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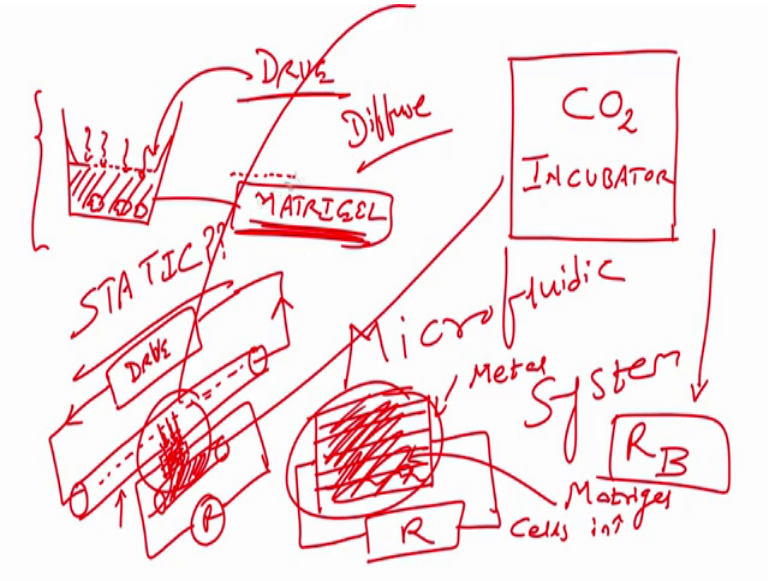

Now, let us understand that this is my current way of understanding the efficacy of drug, if the drug is effective the cells would die ok. So, the question is every time here the cell and the drug are in a static contact; it is a static platform right static there is nothing moving static.

But if I can if I consider human body, is human body static? No right. The blood continuously flows in our body it is a dynamic system.It is a dynamic model, then how can we trust a static model when our body itself is a dynamic model. So, in our body when there is a drug is it in continuous contact with the matrigel or with the cells continuous no it is flowing right in entirely through the body. So, can we create a platform that can mimic the in-vivo situation situations within the body on to the in vitro. So, this is potomac in- vitros because it is in the laboratory. So, such that it can have or it can have the properties of the dynamic model that is our body. So, what can we do for that? We can develop the micro fluidic system; micro fluidic alright micro fluidic system all right. So, let us see how we can clear this micro fluidic system ok.

So, I have a channel I will teach you how to create it ok, right now you assume that with the help of micro engineering, you have formed microfluidic channel. This is a channel in which you have a drug, in a reservoir right and you are created inlet, it flows through here, it comes out here and it flows continuously alright. Now this is your channel which one this is your channel. Here I have inter-digitated electrodes. What is inter digitated

electrodes? When you take a metal and you pattern the metal pattern the metal I will teach you how to do this one, this becomes our inter digitated electrodes and you can measure resistance. So, these are all metal lines metal lines metal lines. Now this inter digitated electrodes right. Now if I want to measure resistant of this since the lines are not sorted, they are open; the resistance would be infinite right. If I place cells on this along with matrigel I will have some value of resistance; let us say, resistance R 1 or base resistance R B right when I am placing when I am placing the cells in matrigel alright. So, when I place cells in matrigel onto this chip, then I will have resistance R B right.

So, let us assume that there are cells in this that the group of cells right which are on this inter digitated electrodes all right this was easy. So, I have resistance R B correct base resistance. Now if I flow the drug so, this is when the drug is not there is when I am not playing the drug I have based the resistance R B. Now if I start flowing the drug what will happen, the drug will diffuse through matrigel it will diffuse diffusion diffuse right. How you can I give an example of diffusion is example is when you lead a scented stick right, in Hindi we call agarbathi right. Then what happens that even in the corner of a room when you liteks scented stick the other corner of the room you will feel the fragrance of that right.

The phenomenon by which this the fragrance comes to the another side of the room is a diffusion phenomena right. These are the diffusion occurs. This is an example of a diffusion there are many examples of diffusion and this giving very easy example alright. So, now with the phenomena of diffusion the drug molecules will start diffusing into the matrigel. And what is there in the matrigel? There are cells in the matrigel. So, this drug if you see the slide this drug when it goes through diffuses through the matrigel, what will happen if I magnify this one right?

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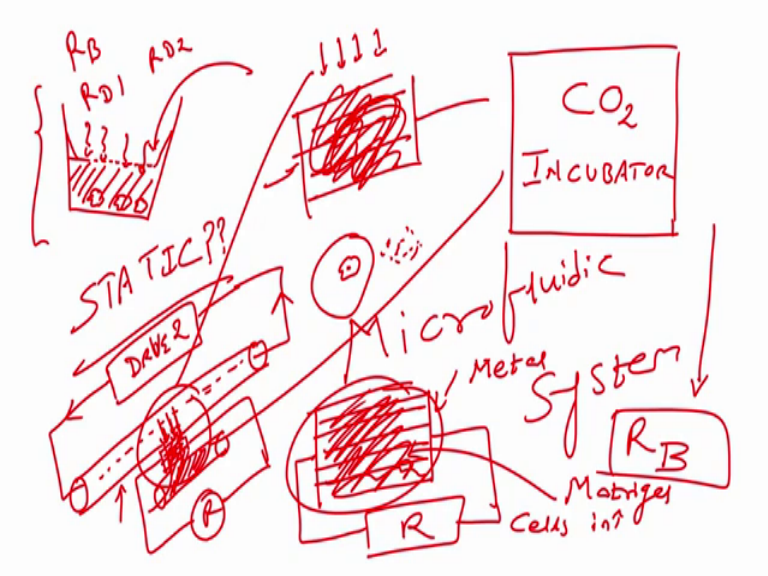

If I magnify this one this is what happens that, there is a electrodes, there is this matrigel with cancer cells and the drug is diffusing onto the chip. When drug diffuses the cells would if the drug is effective your drug is effective the cells would lies right; it will be defragmented. So, when the cells are defragmented the constituents within the cell will come out and that causes change in the conductivity. Now that change in conductivity will change the resistance right. So, we have a new resistance value; initial resistance value was R B. You have a new resistance in presence of drug 1 let us say so, R D 1 correct.

So, if I use the similar platform and flow drug 2 this is drug 1 right. Now instead of drug 1; if I flow drug 2, then depending on the efficacy depending on the effectiveness of the drug again the drug will diffuse and depending on the effectiveness of the drug again the cells would lies and I will see a change in resistance R D 2. This is from second platform, you understand for each drug. We will use a separate platform. So, if I just delete everything. So, I can explain you quickly in a easier way what we discussed is that if we have multiple drugs ok.

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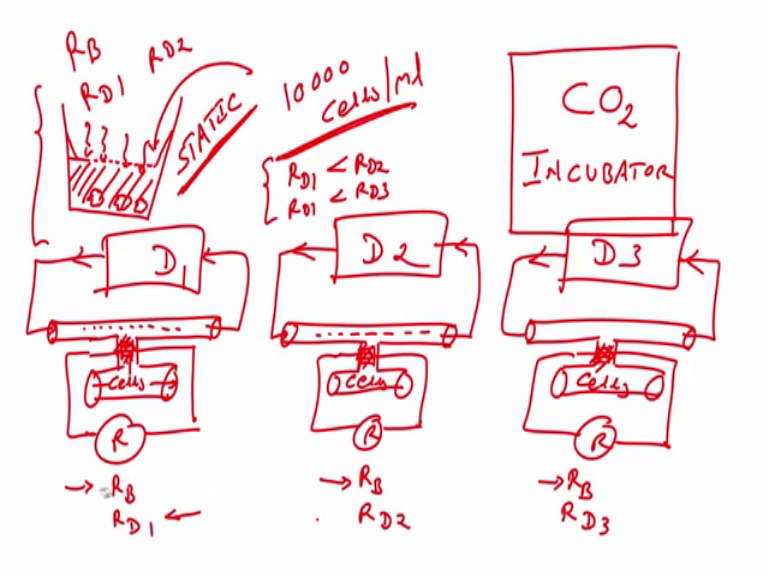

We have let us say drug 1, drug 2 and drug 3 correct. Now we should use a multiple channels multiple microfluidic platform like this and here what we have is, inter digitated electrodes like this correct we have inter digitated electrodes. Now this drug 1 is loaded into deserver 1, deserver 2, deserver 3 and we are flowing the drug into microfluidic platform and drug will come back to the deserver or deserver ok.

So, this is my microfluidic platform, what I am looking at initially a resistance values which is R B base resistance, R B, R B when I load cells with matrigel. This is my resistance value. See without loading any cells, I should have the resistance infinite right. When I load cells with matrigel, I have a resistance R B right. How many cells I am loading? Fepending on the microfluidic platform I can load few thousands of cells per ml about let us say 10000 cells per milliliter that is our concentration of the cells. Where the cells we loaded? We loaded the cells here in this particular platform alright.

So, now, you assume that when we flow drug 1, the drug 1 diffuses to the matrigel and I will have resistance R D 1.This R D 1 would be different than R B if depending on how effective our drug is, you know drug is effective the conductivity would increase. Because the cell would lies when the cell lies is the constituent within the cell comes out when the constituent within the cell comes out the conductivity increases and that caused the resistance to decrease alright. Now similarly when I flow the drug 2 it diffuses to the matrigel I will have R D 2, I will float drug 3; I will have resistance R D 3 right, base the

distance resistance when drug is flowing in each case. As I said if the drug is effective the cells would lies and conductivity would be higher resistance would be lower. So, using this platform what we can do is, we can understand which drug is effective right so, easy because depending on the change in resistance we can see that what is the how many cells would have been lies, where to do our multiple experiments to understand the quantification how many cells are lies.

But we can do or we can use this platform as a screening platform. What mean by screening? Drug screening platform right if you see back what I mean is. So, now, if the R D 1 that resistance R D 1 that is right that I down here R D 1 is less than R D 2 is less than R D 3 right then R D 1 is less than R D 2 and also I find that R D 1 is less than R D 3, then I can see that the resistance of the chip right this introduced electrodes right is less when I use drug 1 compared to drug 2 or drug 3. If that is the case then my drug 1 is more effective compared to drug 2 and drug 3 and for the cells that we have extracted from the patients we should use this particular micro fluidic platform and the drug 1 or based on the micro fluidic platform we can select drug 1 over drug 2 and drug three; that means, we have screen which drug would be effective for a given patient.

Now the point here is that why we should understand cells in tissue culture because when you take out the cells from the patient right we have to keep the cells alive and how can you keep the cells alive using the tissue culture or cell culture technology. If I want to study the group of cells like spheroid I to grow the cells within the CO 2 incubator again cell culture you got it. The micro fluidic platform is based on micro engineering and that is why it is MEMS based device Micro Electro Mechanical Systems based device. Understood this is just one example out of many yeah on ongoing research in lot of laboratory around the world and what we see here is that we can use the platform for drug screening right.

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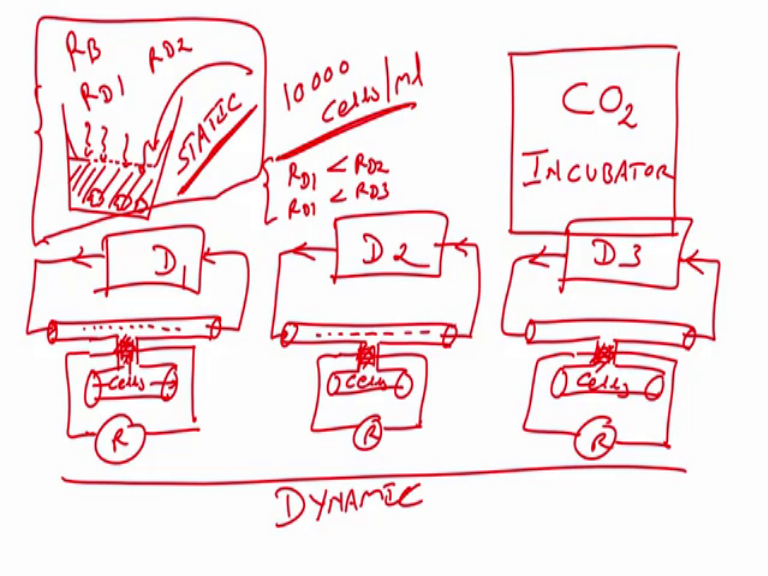

Now I give an example here that this is a static platform if you see the slide static platform right while all 3 here macfluidic 1, 2 and 3 is a or our dynamic platforms these are dynamic models right dynamic. The transwell this one is a static platform you understand the difference. The dynamic platform would be better compared to a static platform for studying the drug scanning since we can mimic the in your situation better. In this particular case, as we are flowing the media instead of blood we can also use blood and load the drug and then you flow the drug into the microphone a chip.

So, there can be multiple applications as well ok. So, let us go to the next slide and what we see this is the applications.

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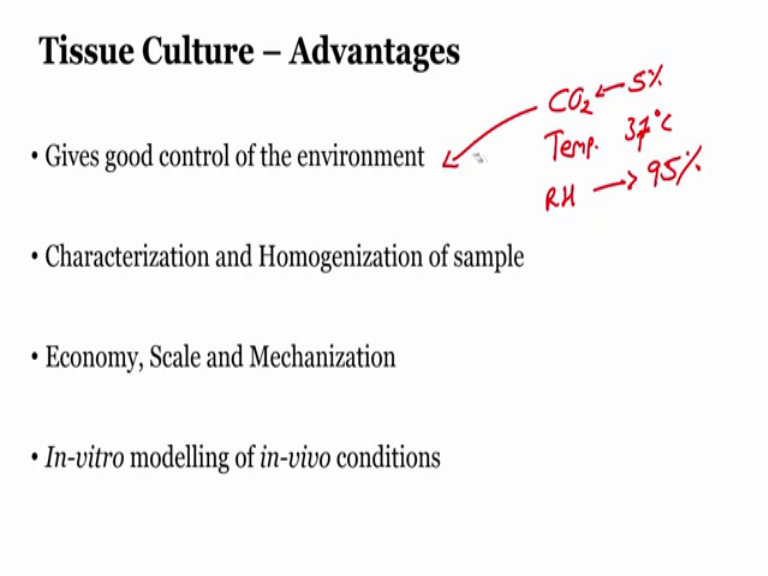

So, now let us see the advantages of tissue culture technique. The first advantage is the, it gives a good control of the environment then why good control of environment because like I said we can grow the cells with a similar environment like our body right like our body ok. Now this is not this is the incubator that helps to control the growth and the cell culture right.

But when you understand the tissue culture when you get the tissue, then the environment when you grow the tissue within the laboratory would be similar to what is there in the body that. So, the good control of the environment means it will retain most of the property of how these cells would be there within the body that that is how the one of the advantages there. Second is that characterization and homogenization of this tissue sample and the third one is the economic scale and mechanization; we can always induce that. And finally, in vitro modeling and in vivo conditions we can use in vitro modeling and in vivo conditions right.

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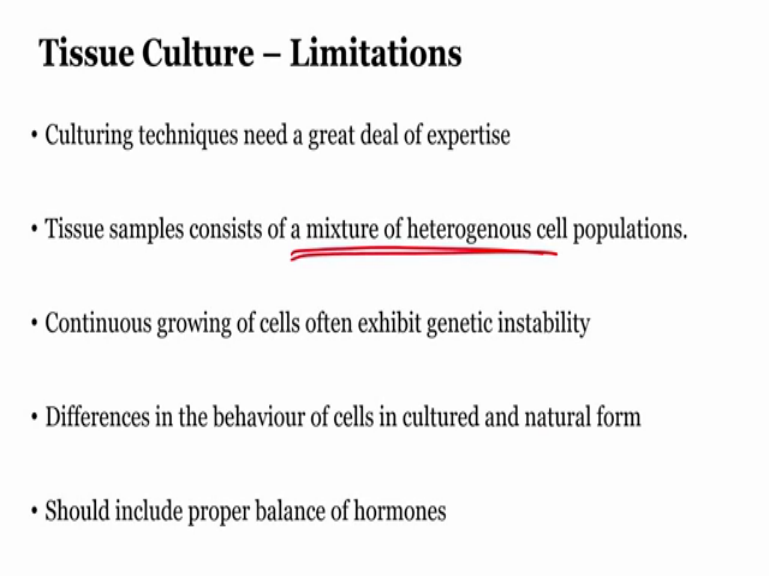

So, if you see the next slide, what are the limitations right? So, the limitations are the cell culture techniques need a great deal of expertise. If there is a contamination, if there is a matrigel contamination cells will die. So, we require a semi skilled or not semi scale actually a skilled person or skilled personnel to operate the tissue culture laboratory ok. So, culturing technique requires a great deal of expertise tissue sample consists of mixture of heterogeneous cell populations because you can have different cells and you want to grow a tissue out of it you can use that continuous growing of cells often exhibit genetic instability then. So, the why this is limitation mixture of heterogeneous cell population because if I want to understand a study a particular cells out of which the tissue is from I and if I have the multiple tissue samples may consist of multiple cells like heterogeneous a heterogeneity, then the homogeneity is less and then I cannot study a particular cells or group of cells; I had to rely on the heterogeneity of the cell.

So, it is kind of difficult because I do not know what to study that right. So, that is the limitation continuous growing of cells often exhibit genetic instability, then we have differences in the behavior, cells in cultured and natural form and finally, it should include proper balance of hormones.

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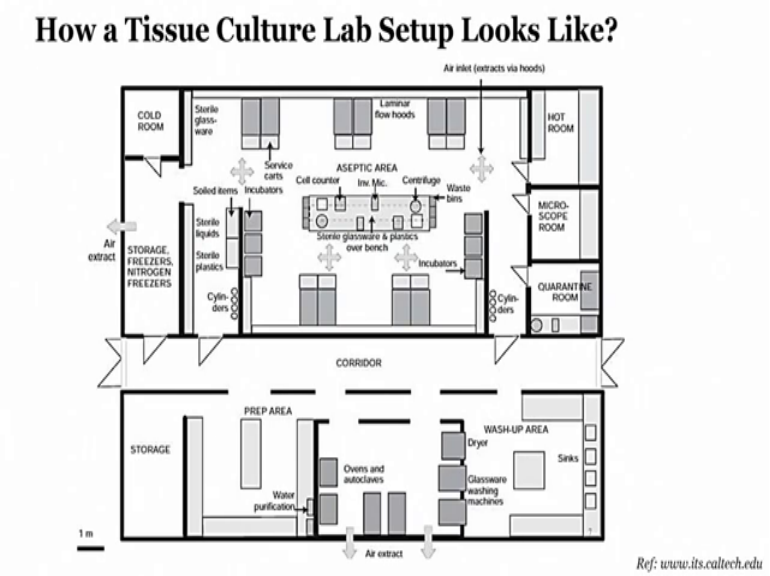

So, if you want to see how a tissue culture lab setup looks like. This is an example from caltech; however, we have the similar kind of facility in our institute and a small facility in my department in my laboratory which I will show it to you as a part of your lab class ok. So, if you see the tissue culture lab setup there is a cell counter centrifuge right. There are incubators which are shown here, there are waste bins right, then there is a hot room, hotter ways to grow the bacteria, there are microscope room, quarantine room then we have a glass trial where we have laminar flow hoods. We can have a storage freezer for nitrogen, nitrogen freezers. These freezers are for the crab preservation of the cells, then we have a preparation area ovens autoclaves right, wash up area and there are things.

So, the most of the facilities right they are kind of in a clean room environment not really required. Now when you are that depends on what kind of you know applications you are working on, otherwise it is always good to maintain a clean room for this kind of study since you are taking care of removing the bacterial contamination as far as possible the quality of air is better compared to a normal laboratory environment right. Now there is a hot room if you are seeing a hot room, why hot room is required because we have to keep these cells alive at 37 degree centigrade. If you want grow the cells grow the bacteria right, we require a room which can maintain that is degree centigrade right.

Why we require autoclave, autoclave is required to clean wash right kill the remaining bacteria if any ourselves if any from the class well before we discard into the bio waste bag. We have oven to heat the matrigel of course, you know it we have several cylinders we have a nitrogen cylinder, CO 2 cylinder, we sometimes also use a compressed air.

So, we have multiple cylinders over there and there the systems depends on a stability on non cellular components some of the applications requires non sterile, some of the applications required sterile components right. All the operations in operation theater they are all sterile that is why where you will see or when you are using a robotic arm to perform the surgery. I do not exactly you are using what I mean is when the surgeon is using the robotic arm for performing surgery, you see it is covered by a lot of plastic and lot of other things right, only the tip of that is access to the environment. This is to maintain the sterility the most like the infection the matrigel infection generally occurs because of it transmitted to our hand and that is why it is very important to keep our self sterile as well as the equipments today and like I said bacteria is enemy to cell. So, if you are growing a cancer cell; if you put bacterial cells for dying right so, we need to be careful when we are operating or using this kind of facility.

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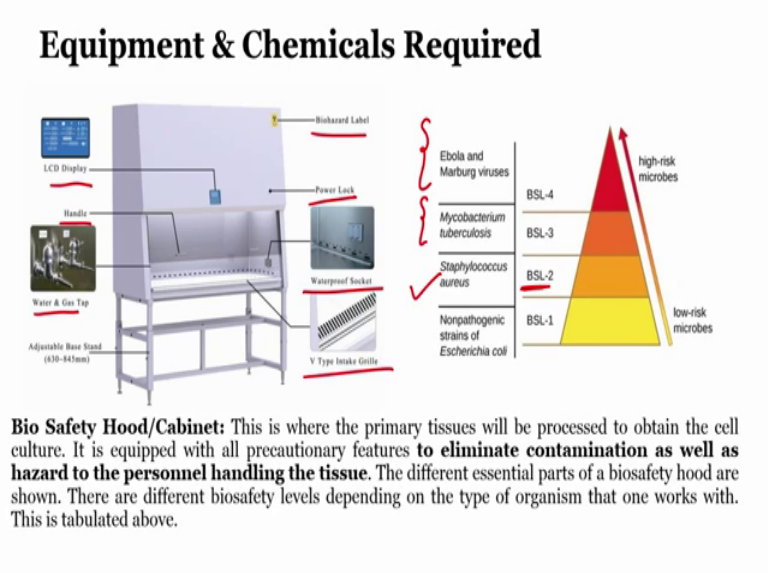

Now, if you go to the next one these are from the equipment and chemicals required for working in a bio safety class and the class of this bio safety hood is divided into 4. First is BSL 1 where we can use a non pathogenic strains of equal high. If there is a BSL 2

which is bio safety level 2, we can use staff or us where if it is bio safety level 3. We can use micro bacterium tuberculosis and if it is bio safety level 4 then and then only we can use Ebola or Marburg viruses and Zika this kind of stuff. So, this way I will not see in our course this is also we will not see will see bio safety level 2, I will show it to you how the system operates in a real situation in my laboratory ok.

Just to understand it has a LCD display to maintain the flow rate. It has a power lock water proof socket there is water and care step as adjustable stand for the height of the for the personal. And you have to put a bio safe biohazard level when you are using bio you know it is always a good idea to put a biohazard label whenever we are working with a biology sample. Now this is what is called bio safety level 2.

So, what is this bio safety hood or cabinet? This is where the primary tissues are processed to obtain the culture. It is equipped with all precautionary features to eliminate contamination right as well as hazard to the personal and the link that issue. The different essential parts of bio safety hood are shown in the figure like I said water gas tap, LCD display, handle, power log, bio safety labeled, water proof socket and V type in take grille right let us see.

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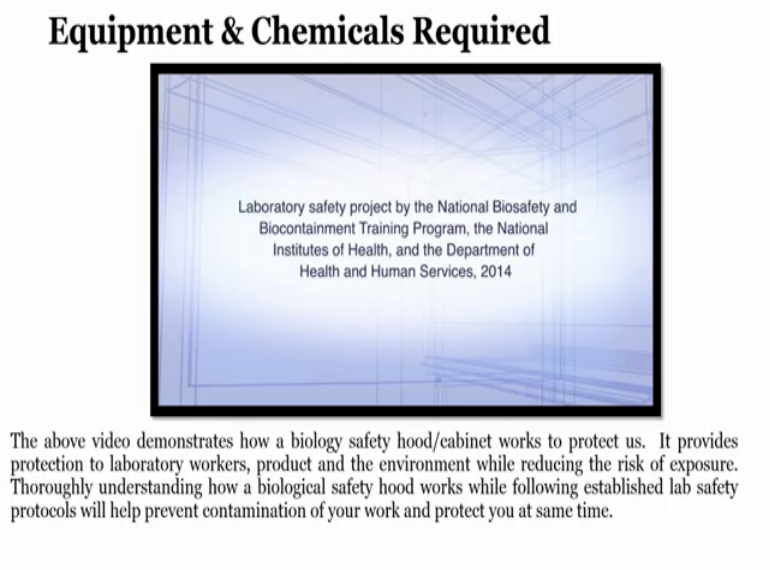

So, this video that I will play now demonstrates how a biological safety hood cabinet works to protect us and how it is helping the laboratory workers by reducing the risk of exposure right. We need to understand the biological safety hood as when we work in

this particular hood there is lab safety protocols which will help prevent contamination of our work and protect us at the same time.

So, it is very important you see this video in detail right and then we will continue the next slide. So, let me play the video.

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This video demonstrates how biological safety cabinets work to protect you. Providing protection to laboratory workers product and the environment while reducing the risk of exposure thoroughly understanding how a biological safety cabinet works while following established lab safety protocols will help prevent contamination of your work and protect you at the same time.

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When used correctly a properly installed and certified biological safety cabinet provides personnel, environmental and product protection for work with biological materials including infectious agents and recombinant DNA.

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This video depicts free standing class 2 type A 2 biological safety cabinet or BSC.

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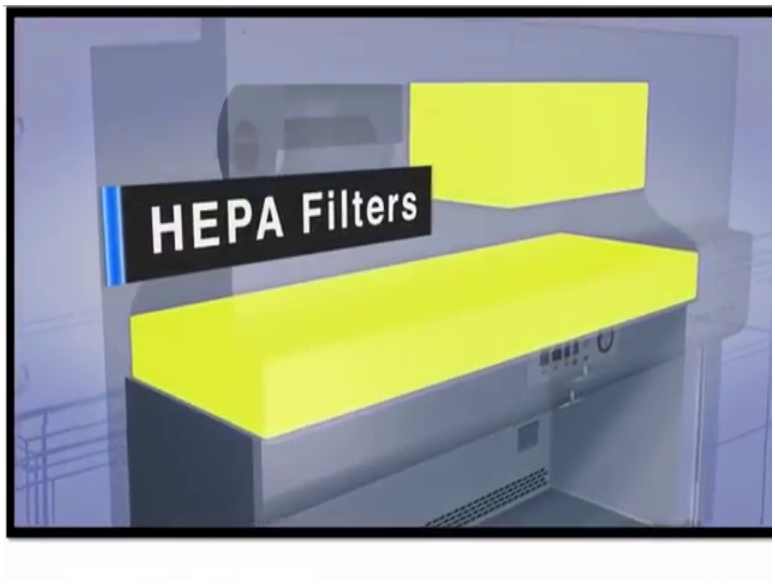

It includes HEPA filters for exhaust and supply air.

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The work surface.

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The opening to the work surface.

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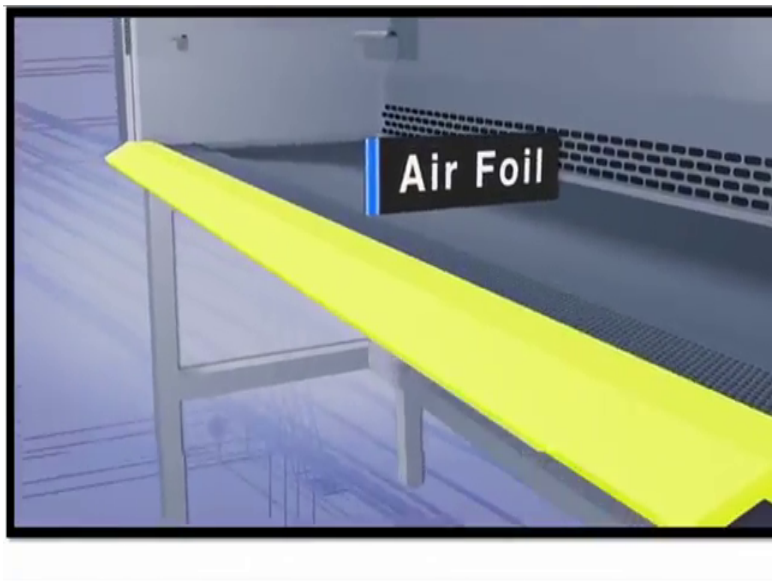

The Air Foil.

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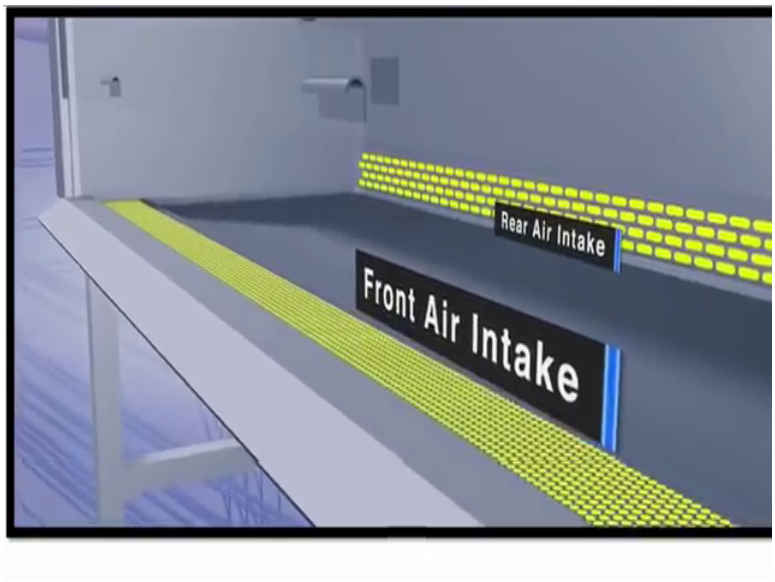

Front and rear air intake grilles.

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The plenum.

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The BSCs air filtration system works to keep potentially contaminated air from seeping back onto the worker, air flows through the window opening into the front grille through the plenum.

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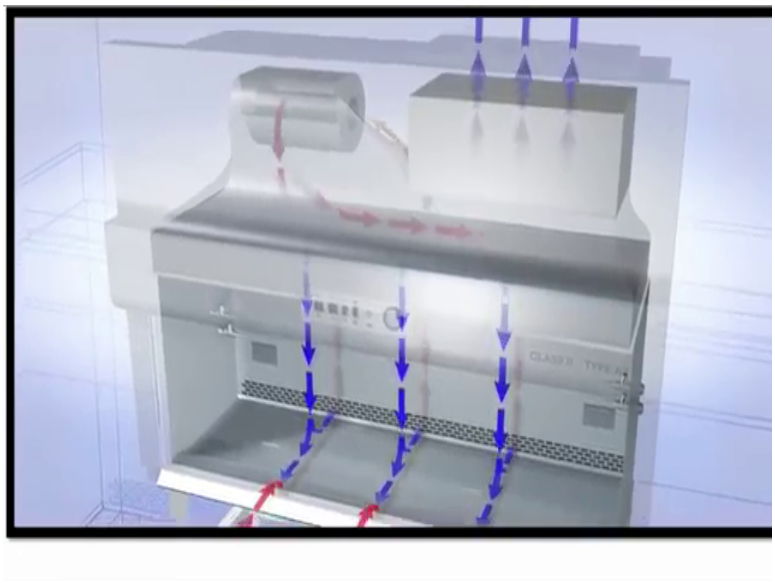

Then through the HEPA filters 30 percentage of the filtered air is exhausted, the remaining 70 percentage which is now HEPA filtered is recycled back into the workspace to ensure maximum protection.

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In using a BSC here are some essential reminders. 1, if the cabinet has been turned off you must turn it on and wait at least 15 minutes before beginning your work.

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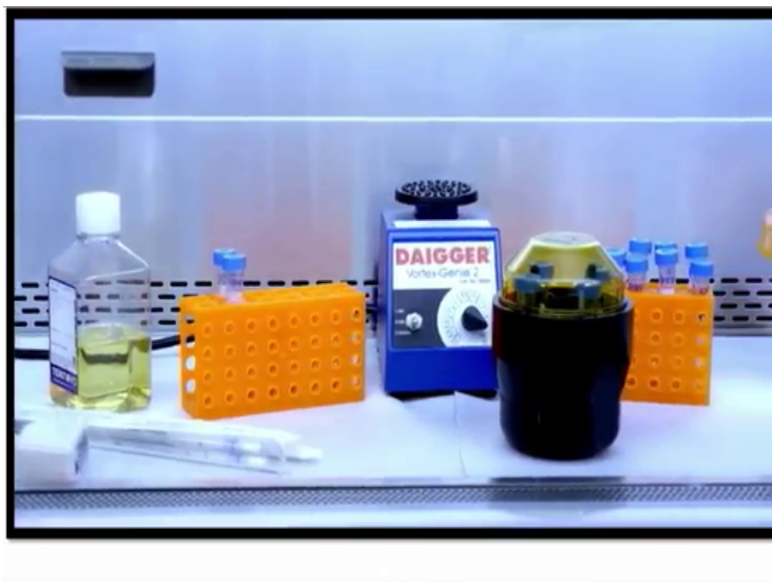

2, set up the interior workspace to work from clean to dirty.

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And work consistently from one direction toward the other to prevent cross contamination.

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3, place your chair at a comfortable height and in the middle of your workspace to ensure you can reach everything you need inside the cabinet without discomfort.

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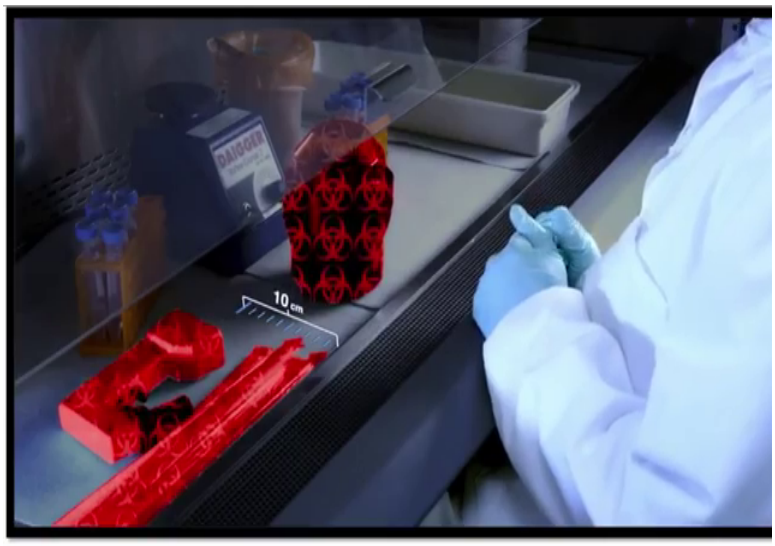

Please keep in mind that you must work at least 10 centimeters inside the BSC.

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To guarantee uninterrupted airflow cabinets should never be overcrowded, over crowding the BSC can block air grilles.

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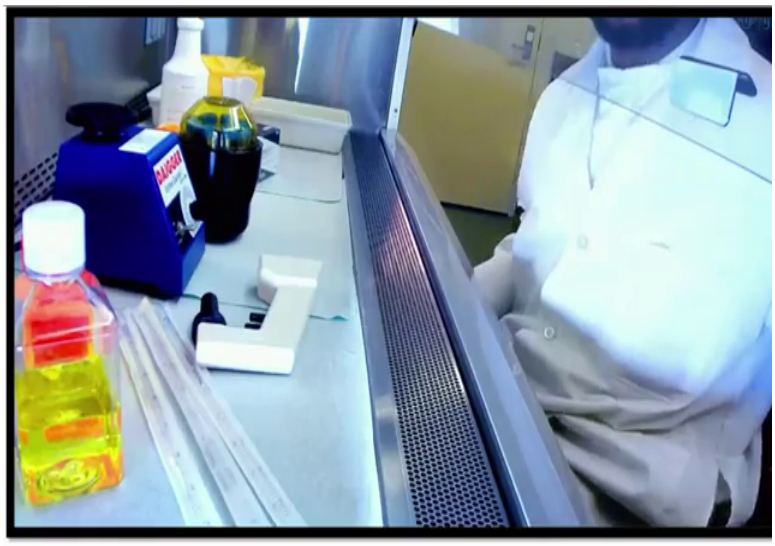

Airflow can also be disrupted by sudden or sweeping movements.

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Slow direct movements work best too much foot traffic can cause problems as well and should be kept at a minimum.

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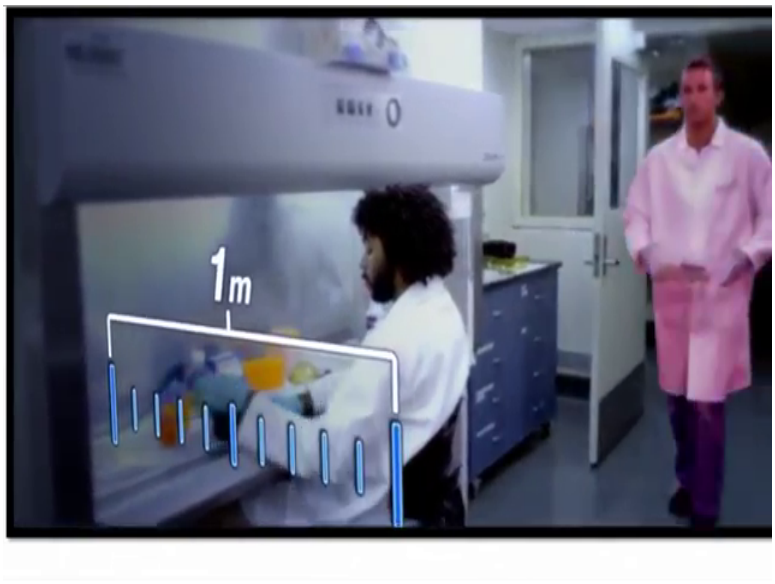

If pedestrians are unavoidable keep people at least 1 meter from your BSC and remember to check nearby doors or supply vents to determine if they disrupt the cabinets airflow. When you have completed your work any reusable items should be wiped down with disinfectant before removing them from the BSC. Next the interior surfaces of the BSC, you should be decontaminated using the appropriate disinfectant for a contact time recommended for the agent used to be sure a second decontamination is advisable.

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In summary there are a small number of best practices to follow in using a biological safety cabinet.

Let us go over them one last time.

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Work at least 10 centimeters inside of the BSC.

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Do not block front and rear grilles.

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Too many objects in the BSC can disrupt the airflow.

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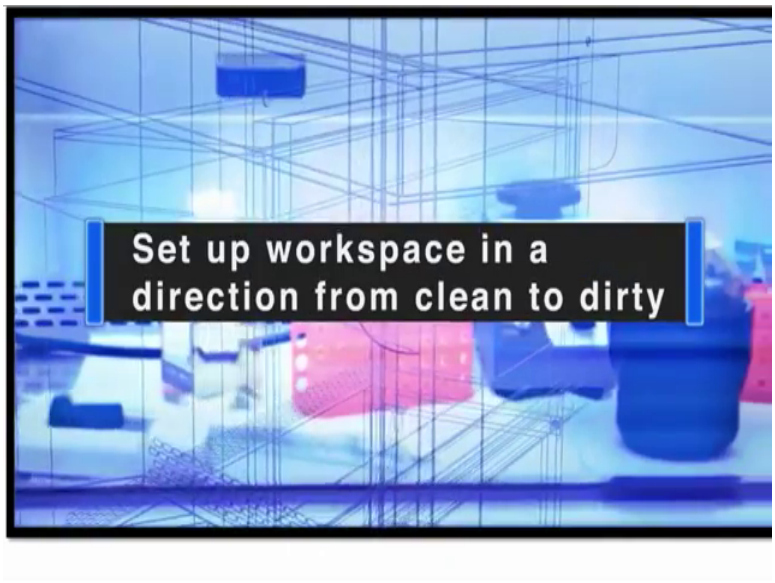

Set up workspace in a direction from clean to dirty.

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Use slow direct movements.

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Minimize foot traffic within one meter of the BSC.

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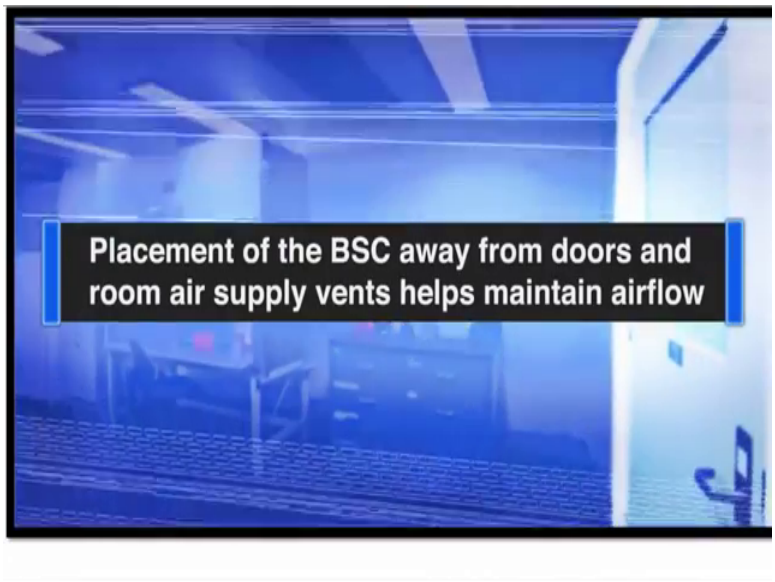

Placement of the BSC away from doors and room air supply vents helps maintain airflow.

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So, now having seen the video now you have all the idea that how a bio safety hood works right now I like I said the focus of the today's lecture is to understand the bios the cell and tissue culture; however, it is also to understand what are the equipment and chemicals required and such one such equipment like we discussed in the previous example is a CO 2 incubator. As you can see the CO 2 incubator from thermo scientific

is shown right over here and you can see here the T 75 flask T 75 or T 25 flasks are there along with petri dish, along with transwell, you can see here right the petri dish with cells loaded here these are T 75 flasks with cells loaded and there is a media that is why because of the media you can see a red color within the glass webs..

Now the use of CO 2 incubator is that we can grow the cells in a controlled environment and like I discussed the parameters usually are 37 degree centigrade temperature, 95 percentage humidity, 5 percentage CO 2 and this is the core equipment of any tissue culture laboratory.. It gives a control over contamination which is a major issue in tissue culture labs and now the size can vary from tabletop to those that can fill an entire small room, some of the incubators can be programmed to cycle different temperature and humidity level.

If you see here we can program different temperature and different humidity level depending on the application of the work ok. So, the to this let us finish our this particular module and we will continue understanding tissue and cell culture in one more module and then we will finish the this particular topic. Then we will actually go to understand what exactly is cancer and how can we develop several systems related to cancer right. It is more of understanding of how tissue and cell culture laboratory looks like, what are the equipment, that are used to maintain these cells and or to grow the tissues within a controlled environment right. And then you take care, I will see you in the next class.