Electronic Systems for Cancer Diagnosis
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Lecture – 55
Demonstration of Cleanroom Equipments
Hot plate & Microcentrifuge

Hello everyone, welcome to the course on Electronic Systems for Cancer Diagnosis. This is a continuation to the previous module, where we have gone through the different essential or the must need equipments required in a biomedical research facility, where you study, the study and understand the change in tissue biomechanics, the underlying structural, features, the stiffness elasticity, property. And, how the electrical thermal and mechanical properties of these tissues can be leveraged using the biosensors, what we intend to fabricate.

So, coming to the next set of equipments what would, what would be essential during the process of fabrication or even during the analysis, where the cell and tissue cultures, which are cultured and then extracted. And, then analysed and during the entire process, there are several equipments which we would be utilising. So, let us get our hands on these equipments and understand how these can be used and what is the purpose of this. So, coming today, I am sure you all studied, understood about lithography process, the soft lithography process, which is a very important step during micro fabrication.

I am sure Professor Hardik has covered the detailed procedure of the fabrication of various sensors what was, what have been fabricated by him, what is the steps, what is the process flow of the entire fabrication. So, when we are talking about an, one important step is lithography, that is how you get these features on to the resist or on to your, the device. So, how do you get a develop these features and that is where soft lithography comes into place. So, when we are talking about soft lithography, I am sure there you heard about the pre-bake and post bake during your lecture classes.

So, what is this pre bake and post bake and why do you have to carry the in a specific time interval or there is a procedure, which has to be followed. So, there is a most important or a widely known photo resist the SU-8 photoresist, which is a negative photoresist. When I say negative say what happens? There is a difference between the

photoresist that is positive and negative, there are two variants and what happen when you say negative photoresist; that is the exposed the you create a mass in such a way that the photo resist, which is exposed to UV light the cross link enhances and then it becomes more solid. The chemical bond, the chemical bonding between the structures, which are exposed to UV become strong and then there the remaining part, which gets; the path which remains un exposed can be dissolved easily, while you are putting them in a developer solution and the other way around is your positive photo resist.

So, we when we are talking about SU-8, which is an a proxy based negative photo resist, I am sure this is the most widely used photo resist to develop features or to create moulds, while you want to fabricate your devices. So, when we have these, when you have the SU-8 photoresist, you have to, how do you develop the features? This is, there is a process flow. Let me show you this figure.

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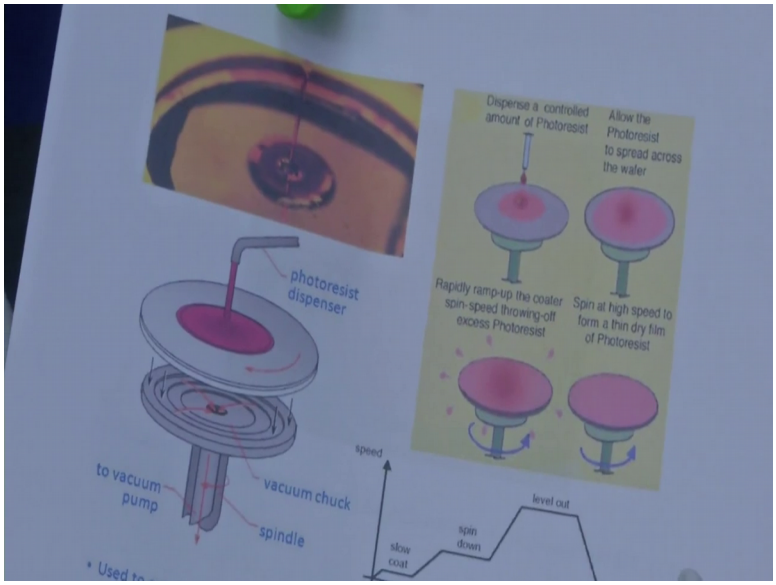

So, let us say, this is your disk for, let us consider this is your wafer. It could be the glass wafer, silicon wafer and what happens is you dispense the photo resist. The amount of resist disposed, a dispensed onto your wafer. The distance between the two has to be maintained minimal, because you do not want any air to come and contaminate your samples. So, there should not be any kind of air bubble formation. So, this here is the chuck which is holding your wafer. When we say there is a vacuum chuck here and the

spindle, which rotates it is controlled by motor and this rotates and this holds your wafer tightly here and then the dispenser flows your photoresist.

So, what is a photo resist we are talking about here is the SU-8 photoresist. So, as you and then once it has been dispense for the required amount, the spinning happens and then there is a uniform spread and then there is a formation of a thin film of photo resist across your wafer. And now, after this step once there is a uniform deposition of this SU-8 onto the wafer, you have to bake it. So, this process is called post baking. Why do you have to do post baking? In order to remove any amount of dissolved solvents in the SU-8 photoresist, you bake it for the desired temperature. It could be like 80 degrees or depending on the type of photoresist what you use.

You set it to over a certain temperature and it could take a minute or two just on the hot plate and then you remove the dissolved solvents and this is an essential step. I show you why this becomes an essential step. You have to remove the dissolved solvents in this step and then the resist which is more liquid in nature. When you heat it, it solidifies not too hard, but it is now semi solid. So, that you get the required features developed on this photoresist.

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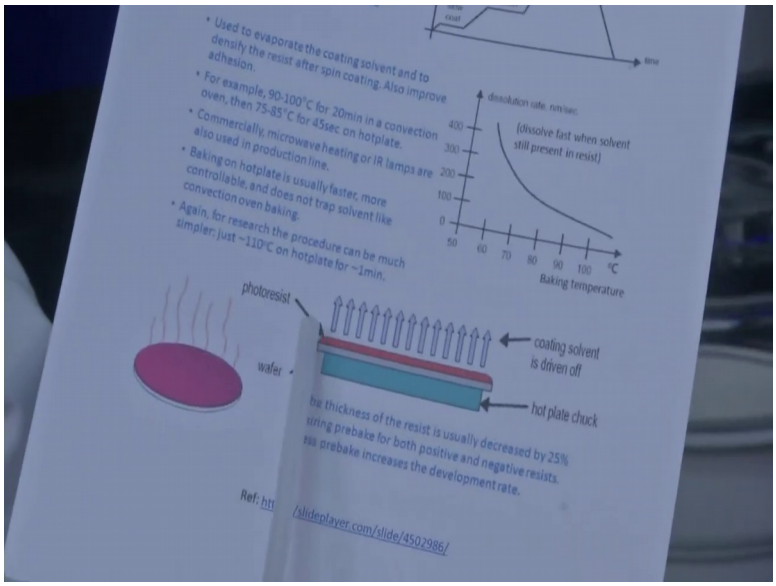

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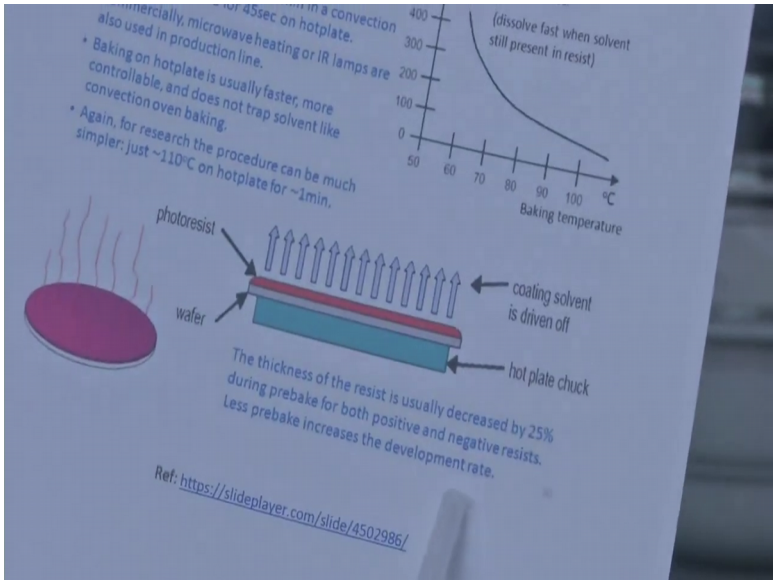

Now, that we have seen this, if you could see this. Here, say this is your wafer and then the photo resist and the solvent is driven off this becomes an essential step. You do not want any solvents while even before you develop features ensure that this process is followed. There is a point here now, this entire thing those reference is here kindly in case of in case you require further information. You could always look into these and this is a reference from where I have taken the material and the important point here is the microwave heating are also used in production line.

However, baking on hot plate is usually faster, more controllable and does not trap solvents like convection oven on baking. So, this point needs to be taken care. Now, in the clean room facility we have seen, when we are talking about baking, you could even think of using an oven.

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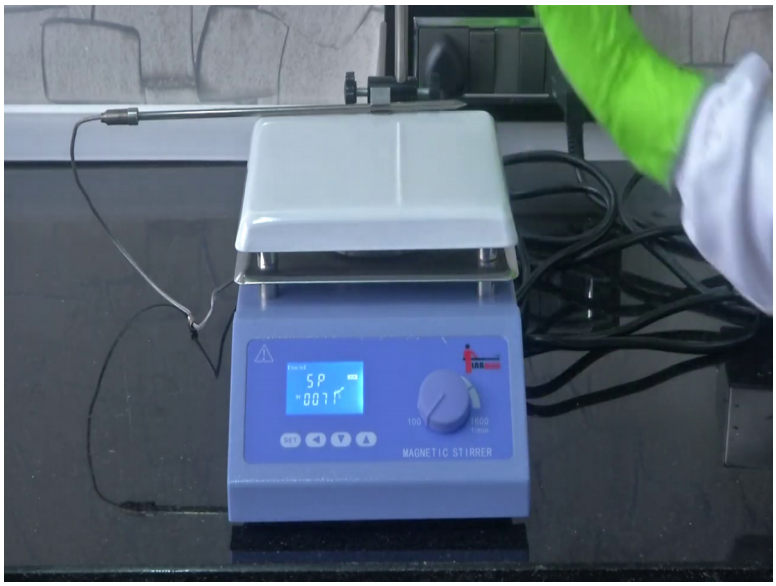

Right behind me here is the hot plate. The composition of the plate here could be ceramic, the one which you can see here or it could even be aluminium. This is a magnetic stirrer along with a hot plate; you could set the desired temperature and time for which heating is required. So, these are the options here. So, let me turn it on. So, as you can see that display here, you could set the desired temperature, for the required time using the hot plate.

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Now, along with the hot plate is the oven, which we had used it in one of the modulus for PDMS moulding and you had used to bake the PDMS. Once, the process once the PDMS and the cross linking agent were mixed n is to 1 ratio, you and the features in and then you once it was poured on to the mould, you put it on to this at 70 degree Celsius for two hours. So, that is when you have used an oven; if you remember previously in one of the modules, where we had used this.

So, there are two things when we are talking, that is one kind of baking. So, PDMS baking so, so there two things; we have the hot plate and we have the oven. So, what do you want to choose when you want to bake. Like, if you would see I mean, I have used this I have used this for the PDMS stuff that is fine, but when you are talking about SU-8 at e the photo resist and when like I mentioned post baking requires usage of hot plate and not the oven also there are several advantages.

I am the, the oven has several compartments, different laws, it can accommodate multiple wafers, it can accommodate many things, but when you see the small hot plate, it can accommodate just a single wafer; however, it is always important that you choose and prefer using the hot plate, when you are doing the post baking. I will also explain what is the pre baking and the post baking.

So, always choose the hot plate over the oven, why? When you are using an oven there, the among the heat, what is generated inside the oven, goes from the surrounding environment into your it flows, you know through your resist and flows through the centre it flows from the top to down.

So, that is not what you want this does not ensure removal of solvents, the dissolve solvents which are there in the photo resist which has been uniformly quoted on the wafer. However, when you prefer using the hot plate, what happens is it is more like the convection heating, the conventional the straw based cooker cookers or any other utensils you have the heat generator at the bottom and then there is they are in contact. In the same way the hot plate is in contact with your device.

So, what happens when the heat is generated from below any solvents are taken away. So, the heat generated from the bottom is taken up. So, it forces the solvents to move away from the surface. So, this ensures complete removal of solvent from your photoresist. I hope you have understood the importance of using hot plate over a

microwave oven. So, what happens if you do not use say, say that is desired time, you set the desired time you overheat him, but there are chances of features they could be cracks with could be formed on the photoresist what has been developed like, let me show you an example.

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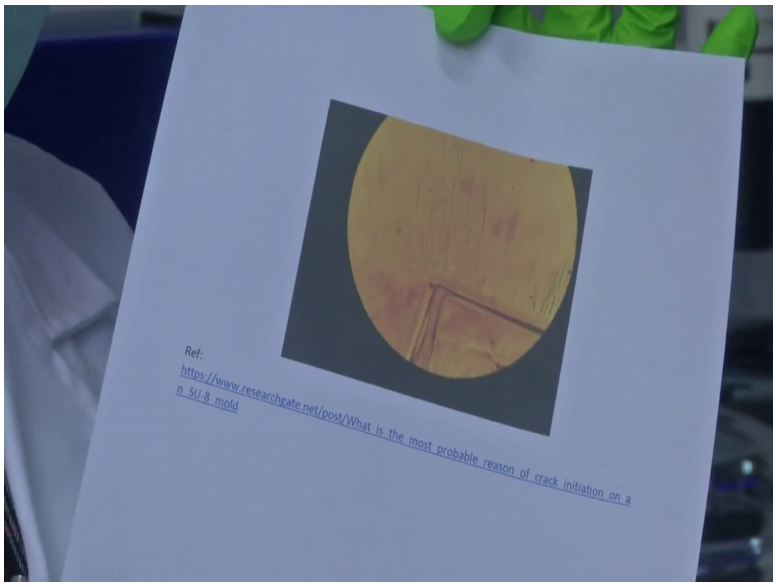

This here; so here, if you could see these tiny cracks, which are formed and they even air bubbles improper. So, this is your feature and then there are cracks across the SU-8 photo resist. This is not what is what is what we want. These features could hamper your device functionality. Assume your device is here and then there are cracks which could be formed. So, the device remain the device you could not use your device anymore and it hampers the entire functionality and this is an undesired feature, which has to be avoided and taken care while you do the, while you do the backing process.

So, this is again taken from a reference here. In case, if you want to study more about the process flow and what has caused these. So, now, you know the importance of uniform heating. Uniform heating is guaranteed when you use a hot plate and always prefer using a hot plate when you want to do the baking process up instead of using the oven, which could be there in your, which could be used, but otherwise more efficient is your hot plate.

Now, coming to the pre baking and post baking process: Why do you need to pre bake? Pre bake in order to remove the dissolved solvents and ensure good cross linking of the desired features and then when does the post baking come?

Once you, once the SU-8 has the photo resist has been dispatched on to your wafer and it is spin coated uniformly onto your wafer and then you put it on your hot plate for a desired temperature for a minute or two. And, this pre-baking is an essential step and once this is done, only this ensures removal of solvents and thoroughly cross linkage and then the ensures cross linking in future. So, once this is done then you expos it to UV while your mask is in between it could be contact or non contact based depending on the process you follow and then expose UV and once the features are formed, you have to an immediately after the UV exposure again put it on the hot plate and this is the post exposure process.

Why is this step carried out and why is it important is, even before you put them into the developer solution once you after exposure, you put the wafer onto your hot plate and this ensures that the path the depth of the features, which has to be developed, when you heat it the chemical composition changes such that there is a there is a highly efficient cross link which is formed between the features, which are to be formed and the ones which are not exposed would be easily soluble in the developer solution. In order to ensure this process happens, you have to bake it after the UV exposure and then set the desired temperature time.

And, once you bake it, take it out, put it on to your developer solution and then after developing you would see the right features on to your wafer, which is the desired features. So, this becomes an important step. Now, hope you understood the importance of using the microwave oven, when it comes to moulding, PDMS moulding and the importance of using a hot plate, when you want to pre bake and post bake. So, this was what was intended to be thought in this module. Now, let us see the other device, which is another essential component while we are working with some biology cell and tissue modules.

The next equipment what we would be talking about is the micro centrifuge. Like the term said micro, it is a small, compatible, benched of module, which we would get a angstrom very quickly centrifuge when I say it is like a device, which rotates and then if

the working principle is more like the centripetal force like say, I spin a ball around my hand, when it is, when there is a string attached to it, what happens is as it revolves, it is always directed towards the centre, the way where I am holding the force. So, that is your centripetal force. So, on a same working principle is the centrifuge. What happens is; as it rotates the samples, what we keep would rotate and bend. The different constituents in a sample would have different mass and density

So, the amount of force that is acting on each of this is different and what happens while it revolve is; the tendency of the tensor objects to settle down across you know the radial force would be higher when compared to this lighter once. So, this phenomenon is what is used in order to separate different particles of different density, when you have a solvent or a solution, a solution mixture which has to be separated out. So, let us see, why the micro centrifugation is required for us and let us see the different features, a micro centrifuge device can offer.

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I am sorry. So, this is the micro fudge, what I was talking about and like I said centrifugation is a applying centrifugal force, in order to separate different particles from a solution based on their size, shape, density, viscosity and other features.

Now, what happens here is; if we want to study the entire, let us let us see the different features the centrifuge offers as you can see this is a small compact, based of configuration of the centrifuge. Now, there are some factors for, which you have to

consider while you choose a centrifuge. Even before we see what are the factors you have to consider, let us see what are the features the centrifuge offers. Here, is the speed setting.

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Like, I mentioned it would rotate, spinning action of the different sample use, which would be placed inside happens. So, in order to adjust the speed; the knob here and then the time, the time for which the desire time for which the spinning has to happen.

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So, let us open the centrifuge and now, there is a warning that is written here, moving parts may cause injury. Do not open the lid until rotor stop. Always ensure you follow this protocol, because what you spin would be hazardous material, it would be any kind of biohazard, which you do not want to inherited, it might explore to the surface around and contaminate the entire devices. So, ensure that you stop, wait for the device to stop and then open the lid.

Now, you can see so, this is the rotatable, this supported on the rotor and on this are these timing 2 ml, here it has 1.5 ml.

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So, there are different sample tubes what this can support. So, it supports up to 2 ml and 1.5 ml. So, it is very small amount of samples, which are taken in the sample tool. So, here is the 2.1 0.5 ml tube, you could also use a 2 ml. So, anything less than 2 ml is water device, can support these sample tubes will hold the sample. It could be any kind of cell, tissue culture, where in which centrifugation is required, when centrifugation is done to separate out different particles. Now, how many does, how many slots does it supports? So, there is 1 2 3 4 5 7 8, 8 slots are supported.

So, you could use 8 into 1.5 ml tubes and there is a lid for each of this tube and why is this precaution taken? Because you could see, that the spinning rate is very high. So, let us let us switch it on and see the operating speeds. So, it is the (Refer Time: 23:19) the multiplying by 100 RPM. So, when they are rotating at a very high speed, you do not

want your samples to be statured out. Hence, there is a lid that is provided on each of this as a safety measure as a safety measure and now, that this supports, there are variations in the micro centrifuge.

There could be multiple options here, like I said it supports a few sample holder and there would be multiple options, it could even support much more numbers. It all depends on how much of testing is required, the amount of time that is required and the study purpose you, how long would it lasts. So, if the process takes the day, what is the amount of sample, which has to be tested. Based on all these parameters, you could choose the centrifuge tube on and the amount of loss it could support.

Now, that we have seen the different casing, this is one sort of a revolving structure with these things, but otherwise, there are other centrifuge which could even support different structure, different rotating structure. It could be a plate like structure where you want to have your culture or any kind of centrifugation, which is required that could also be attached to this for centrifugation. In this case the, you can see the kind of structures, what we are using.

Now, let us turn it on, you could see how robust the casing is like I have already mentioned, it is very essential when you are dealing with hazardous cultures like bacterial culture, virus, HIV or any other cultures, which could cause contamination. In case there is a break or a leakage or a damage that could be cause either to the solvent tube, the small sample tubes what you have seen. The damage to the either to the cost that or the damage could be cost to the lead and we would be exposed to the bio hazard. In order to ensure all of this we have a thorough robust casing like this and their different casing like you can see here, this is a plastic casing. There are others centrifuge, which have a metal casing; however, they have their own advantage and disadvantage. When it comes to these, there are more transferred and you could directly visualise it.

In case there is a leakage and accordingly, take precautions even before you stop or open the lid and when it comes to metal, it is more robust; however, there they are they are opaque and hence you cannot see the samples, in case there is a leakage what happens inside. Like the metal casings, they are very strong and more robust; however, in order to prevent any type of corrosion, you would prefer the plastic casing. And, another option is when you when you need a more robust structure you could go with the metal casing and

in case it corrode, you always have the other things like the autoclave or you could even use the ultrasonic path. I like I mentioned earlier, how it can be used to remove the corrosion from metals.

So, you could clean them and then re-use in case you require metal casing for centrifugation. So, now, let us see, let us assume I have a culture medium, inside each of the samples tubes and then I want to do the centrifugation for the desired time. Now, let me turn it on. So, now, we have understood that the, we follow the instructions what are provided you close the lid and then now, we set the RPM. Now, that the lead is showing RPM, let us set the RPM to 10, 10 implies 10 in to 100. So, we are setting at as 1000 RPM. Now, let us set the time for a minute.

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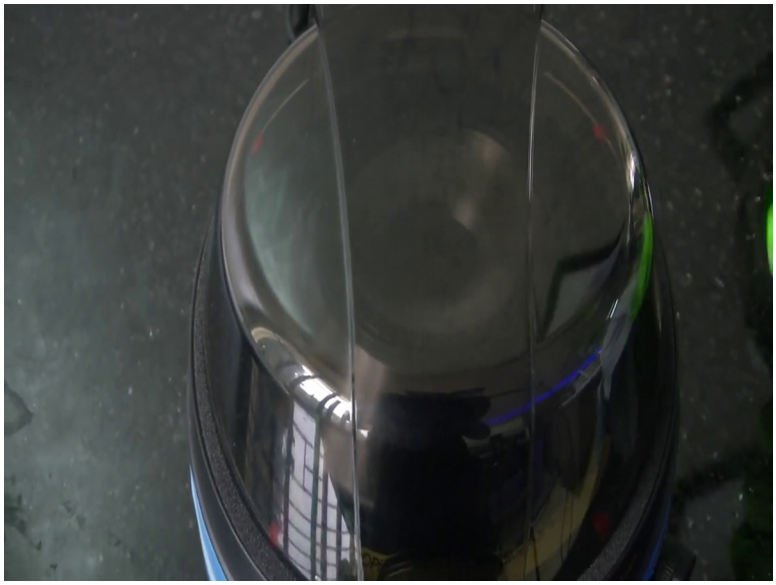

Now, you can see how the rotor, which is holding the three sample tubes, what I have placed is rotating at 1000 RPM and this would continue for a minute. So, it always depends on the amount of through put what you need. Now, as I mentioned it has this 1.52 ml tubes and you it could spend some amount of sample. So, based on your requirement and based on the amount of through put, you could choose the different centrifuge which has, which could accommodate more number of slots. So, this is one kind of adapter. So, to there are manufactures which can provide you with different adaptors. So, you can switch between the two.

So, that you accommodate different size tube. This is a small size tube, you could in case required, you could use the different size tube or different type like I said, the plate model can be used with the help of an adaptor, which can be fixed. So, this was the centrifuge. Always ensure you follow the safety by you spin, because there could be hazardous materials like in case of medical laboratories, they may need to process blood during the micro centrifugation and these samples are unsettling, they in order to in order to safely work with them, because occasionally like I already mentioned, the sample tubes can break now, that we have stopped it, we can open the lid.

These sample tubes what I mentioned could break at the bottom and could be could splash or spread on the surface, which could cause contamination and sometimes, it is also required to have some radioactive labels across them. There could be biohazard bacteria, HIV, any other kind of virus. Hence, you should always choose a rotor lid that fits well enough to ensure proper protection is certified, when you are using biohazard materials.

Now, that you have seen how the micro centrifuge can be used for centrifugation process, what are the different features you have to choose and what among the different things. Try the different adaptors in case you want to do the centrifugation with sample tubes of bearing size. You could choose a base of the amount of through put you need all of that can be considered the type of casing like I mentioned the advantages of using plastic or using a metal casing, considering all the this you come to an optimal solution of what is a kind of centrifuge is can be used for your purpose.

So, in this case we have the table top centrifuge. I have shown you the how the working principle and why the centrifugation is required. In case you want to separate different culture from separate different part different particles from a medium you use the micro centrifuge. I hope this has thrown enough light on the purpose, the choice and how to use a micro centrifuge.

Thank you.