Electronic Systems for Cancer Diagnosis

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Lecture – 24
Cytology - A detail study on Spin Coater and Cytospin

Hi, welcome to this particular module. And if you remember in the last module what we have discussed, we have discussed how we can understand the cell and tissue morphology, in particular we were interested in cell morphology to delineate between normal and cancer you know cells. So, what is a role of that, the role of understanding the cell morphology is to help the help in rapid screening of the patients.

Now, when we are talking about patients, what kind of disease we are talking about we are talking about oral cancer. So, in oral cancer if we can developed a system that can rapidly screen the patient then it will be useful. So, the current way of performing this procedure is that the cells are taken from the oral cavity, they are kept in PBS, and then it is shift to the oncopathologist where he or she will smear the cells of the glass slide, look at the glass slide.

Of course, this smearing before smearing and do HNE which is a biomarker, look at the glass slide, understand the cell morphology. And if the cells morphology is different, then the result comes as atypical. When the result comes as atypical, then the patient is advised to go for histology where the biopsy is done and tissue is taken out from the suspected region, and further the biomarkers are studied that is the procedure right.

What we are talking, we are talking that can we develop a system that you can place in a primary health care center, where once the cells are taken, you can do the smearing. And the cyst you can place a slide on the system, and there will be auto scanning of the cells. And based on image analysis and you know machine learning algorithm that we will discuss in later part, we can delineate the cells based on the cell morphology, so that when you have a slide, the system will scan the slide, if the cells are having a different morphology, it will send those cells to the remote oncopathologist.

Now, why we had to do this kind of procedure, why there is a need of building such kind of system. The need is because we have very few clinicians in our country as we know

right. And we have even fewer oncopathologist to look at this slides and that is why the load on the oncopathologist is really high. At the same time to ship the sample from the remote area of our country to the place where we have the facility it is a time consuming task right. And when a oncopathologist is looking at this slide, there can be human errors, there can be false positive, there can be false negative, sometimes the atypical cells are missed, pre malignant region in particular. If you see there is malignancy is cancer, pre malignancy pre cancer. Precancerous cells they look very similar to benign.

But when you use the machine to look at the cells, you can identify whether the cells is changing a shape even a slight of it. So, if we understand the cell morphology, then we can help the oncopathologist to come up with a better diagnosis right, or to improve the area which is pre malignant and screen it in a proper way that is the idea right. So, like I said once you take out the cells, what is the procedure, you have to smear the cells on the glass slide and that is the problem is that because of the clumping of cells. If the cells are clumped, it is difficult to understand the morphology right. We require cells to be separated uniformly, so that one can study individual cells.

So, what is a system that can be used to study these cells, the system is called cytospin. And you have seen the video in the last module, let us discuss little bit more about cytospin before we go to the cell morphology that how the cell changes, and how we can understand that point. Along with that in today's module I also want to cover something on photolithography, very important point because in photolithography with the help of photolithography system, we can fabricate lot of device.

And I have already taught you photolithography in earlier modules, but the today's idea is to understand one part of it which is spin coater unit. You see the cytospin also work on the spinning and the centrifugal force right. Spin coater also works on the same principle. So, can we develop a system that can act as a cytospin and can act as a spin coater right?

So, let us see first the importance of understanding or of building the cytospin, and then we will move on to the polymer coating applications. Polymers are nothing but your photoresist, positive photoresist, negative photoresist, SU-8 these are all polymers p dot pss that we have seen is a polymer, how to coat it by using spin coater. So, we can develop a system that can work as a spin coater, and can develop a can work as a

cytospin. So, if you see the slide our today's idea is to understand a system that can work as cyto-centrifugation and for polymer coating.

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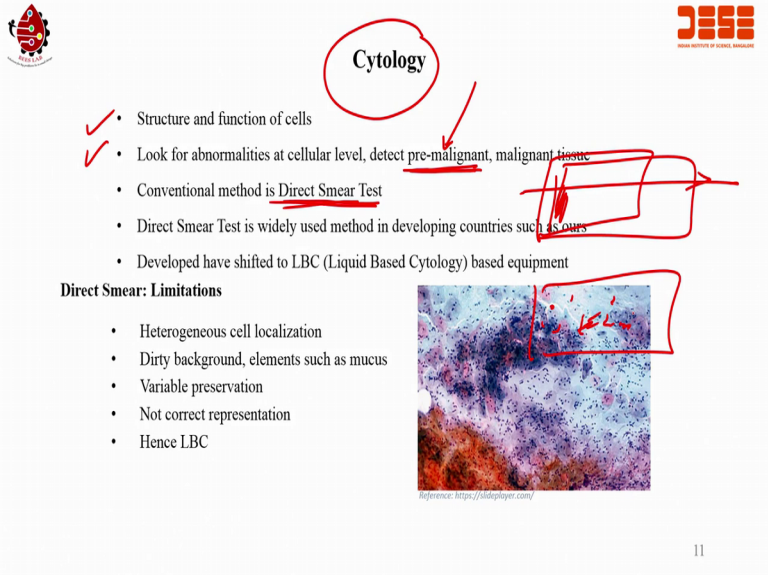

Cytology, when we talk about cytology, we have to understand that we are talking about structure of the structure and functions of cell, look for abnormalities a cellular level, detect pre-malignant, this is extremely important and malignant tissues. Pre-malignancy is very important, because with the current method the pre-malignant detection is extremely difficult. Conventional method is a direct smear test, direct smearing. You take the slide and you put a drop of the sample. And you take another slide right and just smear it across the bottom slide. What will happen that you will see cells all the way like this and some cells are clamped right.

So, the right the correct pronunciation is clumped, c l u m p e d. So, you can see here one such slide right. It is so difficult because the cells are clumped because you are smearing the slide with the help of direct smear test. So, we need a system that can that can help us to smear the cells in a much more uniform fashion without clumping right. So, direct smear test conventional is direct smear test is widely used method in developing countries such as ours. And developed have shifted to LBC, which is liquid based cytology based equipment right. So, now, the developed countries have been now focusing on using liquid based cytology or the equipment that can be used to spin coat or the smear the smear the cells onto the glass slide using the liquid based cytology right.

So, why what are the limitations of the direct smear that limitations like I said at heterogeneous cell localization, dirty background elements such as mucus, variable preservation not correct representation, and hence we had to go for liquid bio based cytology or LBC, cool all right.

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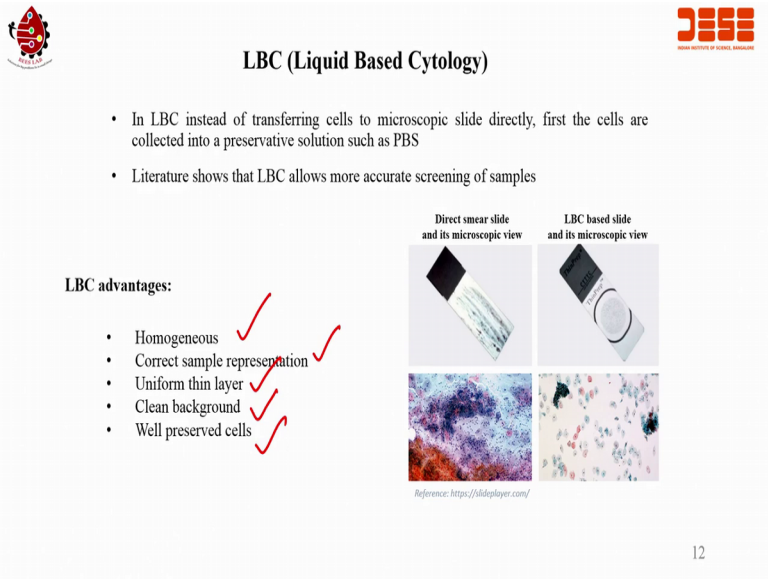

Let us go to next slide. Next slide is LBC in liquid base cytology first is liquid base cytology instead of transferring the cells to microscopic slide directly. First the cells are collected into preservative solution such as phosphor buffer saline which is PBS. Now, the study shows that LBC allows more accurate screening of sample. You can see here a clear difference between the direct smear slide and the LBC slide in the microscopic view. You can see the how uniform the cells are distributed here, and you see this case it is very different correct. So, now, this is easier to understand each cell and study the cell morphology.

If you just see in detail you will understand that each cell has a this black dot in center, it is a nucleus of the cell. So, what are the advantages of LBC? LBC advantages are it is homogeneous, correct sample representation, uniformly thin layer so no clumping, clean background and well preserved cells. These are the advantages of the LBC equipment over direct smear testing; these are the advantages ok, so having this much of information let us go for the next one.

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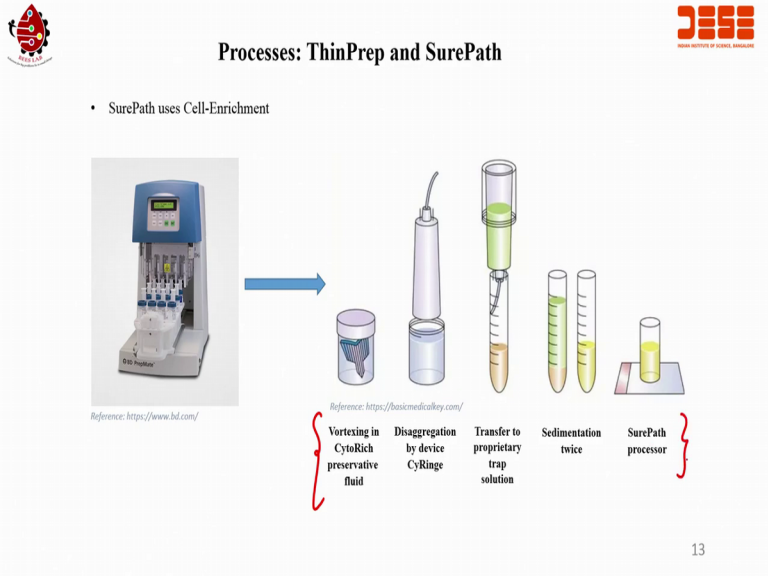

So, what are the equipment? One such equipment is sure paths using cell enrichment technology. So, the process is called thin preparation and sure path. So, in this first we are to vertex in a cytorich preservation fluid that the brush, the brush is kept in the brush which contains the cell is vertex in the cytorich preservative fluid, then it is this in aggregated by device called syringe, it is transferred to a propriety trap solution it is sedimented twice. And finally, Sure Path processor puts it on the last slide, this is the procedure.

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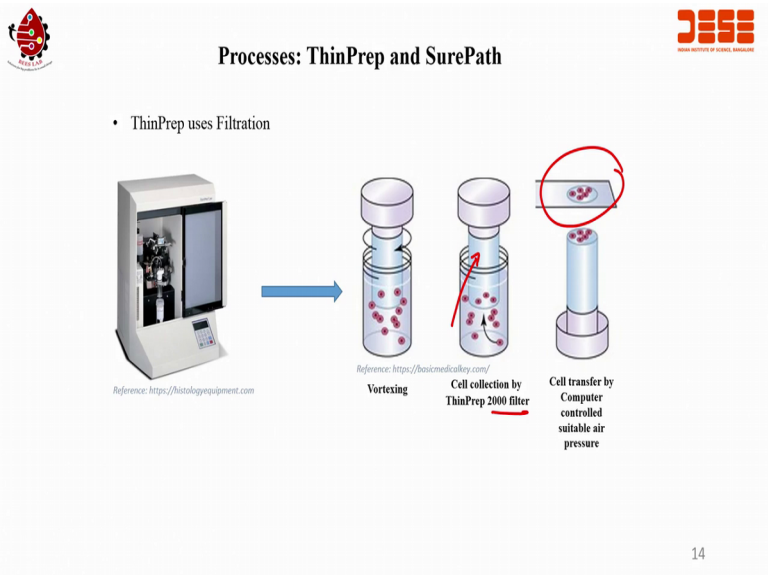

If you talk about another system which is using the thin film thin prep for preparing thin prep, it uses filtration technique. And here the first the cells are vertex like you have see here then cell collection by thin prep 2000 filter, the cells are collected by this filter. And then cells are transferred by computer controlled suitable air pressure onto the glass slide. This is another way of using the equipment for LBC.

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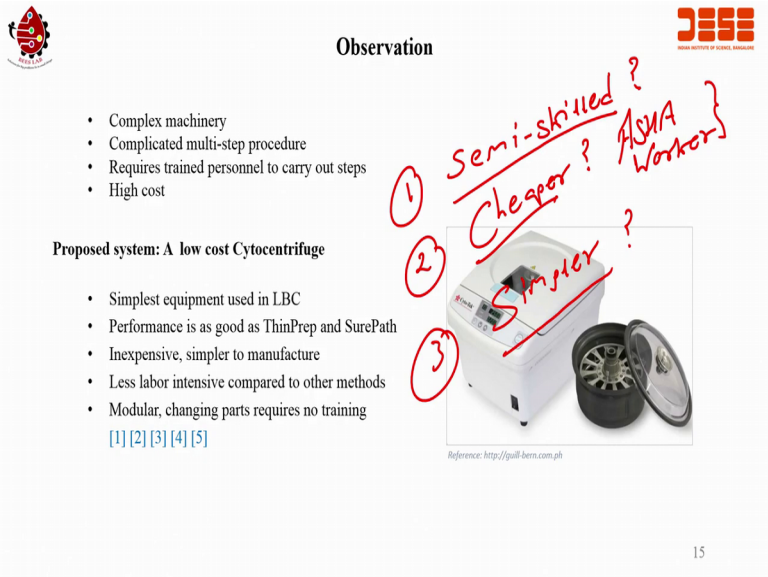

So, what are the observations with the current technology? The observations are that the machinery is complex; it is multi-step procedure which is complicated. It requires a skilled person to carry out steps, and it is a high cost ok. So, why it is important for us to develop such a system because of this particular factor, also we want like I said we have very few oncopathologist just can a semi-skilled person, semi-skilled person can operate the system that is our first question. Second question is can we make a cheaper system?

And third question is can we make it simpler? If I can make a system that can be operated by a semi skilled person like ASHA worker aggregated social health you know workers. And these are semi-skilled persons right compared to oncopathologist. And can we use social health services people who are associated with that to take out the cells and load on the glass slide, and or take out the cells and smear the cells using this cytospin. So, can we develop a system, there can be cheaper, can be simpler, can be operated by semi-skilled person. This is the, these are the questions in front of us.

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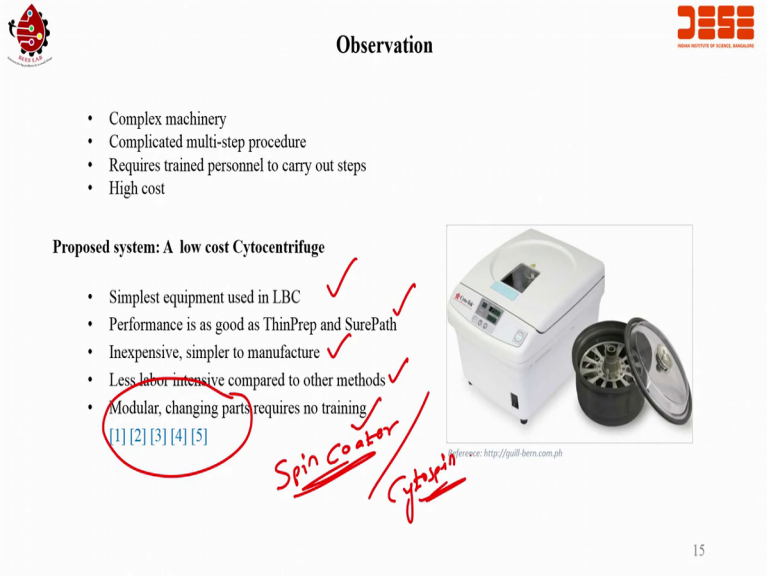

So, let us see the proposed system or the one that we are now building and that is important is simplest equipment that can be used in LBC. Performances should be as good as thin prep and sure path, inexpensive simpler to manufacture, less labor intensive and molecular changing modular changing parts requires no training. Why we are require modular changing parts, because we want to never develop a system that can work as a lithography system or spin coater as a spin coater or as a cytospin or end, it can work as a cytospin.

So, you have to change the parts if you want to use a spin coater, you have to change the parts; if you do a cytospin, you have to change you have to area enough put another parts. So, can we develop such a system that is another question. Some of the videos of how cytospin works and how the spin coater works, I will show you at the end of this particular module.

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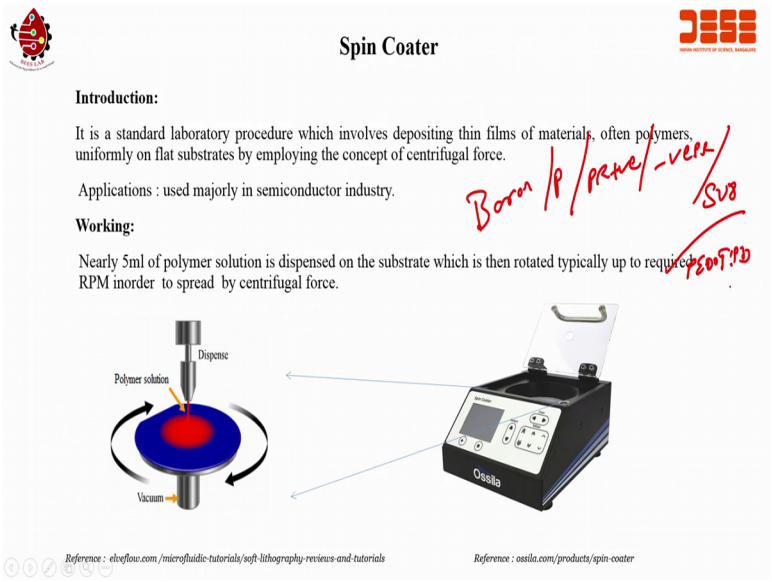

So, if you go to the next slide, we are talking about spin coater. Spin coater is a it is a standard laboratory procedure which involves depositing thin films of material, often polymers, uniformly on flat substrates by employing the concept of centrifugal force; the applications is used majorly in the semiconductor industry. Now, when you talk about polymers you see we can also deposit semiconductors or we can also deposit some dopants.

So, for example, I can spin coat boron liquid boron, I can spin coat liquid phosphor, I can spin coat positive photo resist, I can spin coat negative photo resist, I can spin coat SU8, I can spin coat P dot PSS right. So, there are many things that we can use spin coater for and that is why it is extremely important equipment in a laboratory, which works on the micro fabrication. The working is nearly 5 ml of polymer solution is dispensed on the substrate which you can see right over here right.

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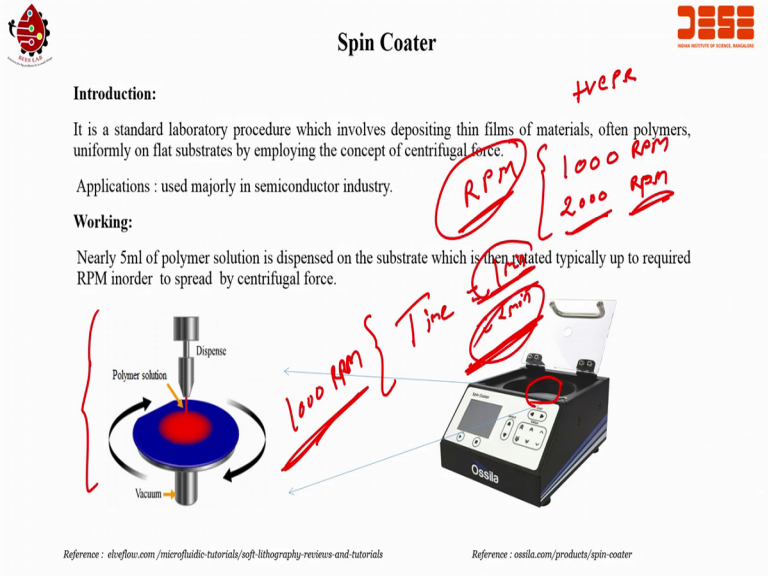

And which is then rotated typically up to required RPM in order to spread by centrifugal force and this particular system is within the system here ok. So, the spin coater consist of there are different steps that we can select, initially you can use a lower RPM. RPM stands for Rotations Per Minute, RPM stands for Rotations Per Minute. Now, if I use the particular photo resist let us say positive photo resist, and I spin coat it for 1000 RPM. And I spin coat for 2000 RPM, what will happen for 1000 RPM I will get a thick film compared to 2000 RPM.

So, if your rotations per minute is higher than your film is thinner, if your rotations per minute it is less your film would be thicker. If the time now let us say time, time is 1 minute in one case, another case time is 2 minutes. And the rotations is 1000 RPM for 1000 RPM rotation if I spin coat for 1 minute and if I spin coat for 2 minutes that the film that is coated for two minutes will be thinner that film that is coated for one minute would be thicker right, it is obvious.

So, either you if you increase the rotation film be thinner or if you decrease the if you increase the time if you increase the time or increase the rotations per minute, your film would be thinner; if you decrease the rotations per minute and your time is less, your film would be thicker that we are comparing these two parameters all right. So, I like I said, I will show it to you actual procedure of how the spin coater is used in the in the laboratory all right.

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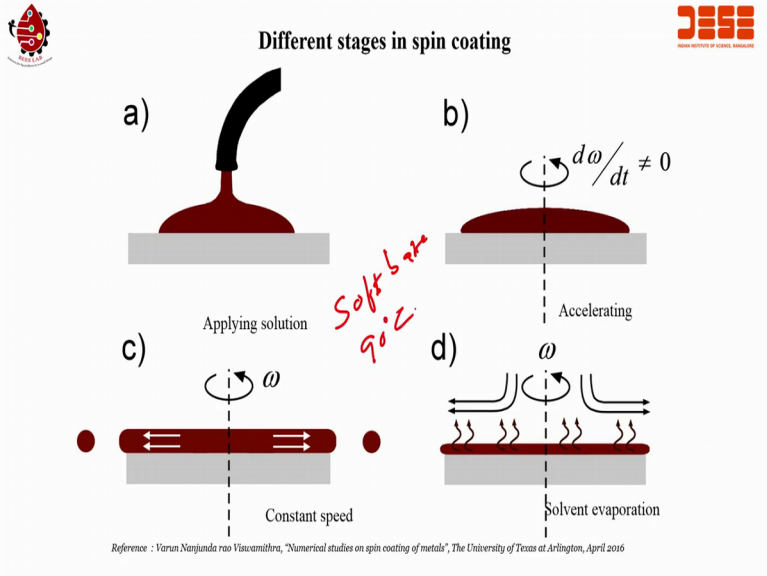

So, I will go to the next slide now. Spin coat different stages in spin coating has a first is applying solution, then you have to accelerate, then you have to maintain a constant speed and finally there is a solvent evaporation that is what we were talking always, there is solvent evaporation this is one way. And then after this, what happens after the solvent is evaporated, we are doing a soft bake, you remember soft bake. The soft bake is after this, soft bake at 90 degree centigrade if it is a positive photo resist right for 1 minute on a hot plate. So, soft baking is done after this d cool all right. Let us go to the next slide.

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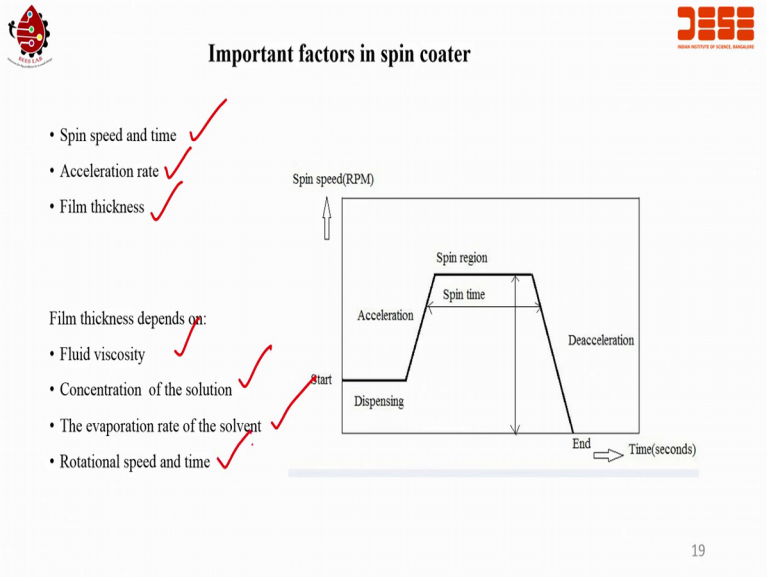

So, what are important factors when we talked about spin coater? The important factors are spin speed and time, acceleration rate and film thickness right. So, if you see speed versus time, then you start the system, you dispense you know polymer and then you accelerate it to a certain you know speed, maintain the speed and you have to deaccelerate. So, to this is your end of the spin coating time right. So, this is your spin coating time where you are maintaining the speed all right.

So, film thickness depends on fluid viscosity right. There is a thin photo resist that are thick photo resist, so that is a viscosity of the fluid concentration of the solution, the evaporation rate of the solution and rotational speed and time ok. Let us go to the next slide.

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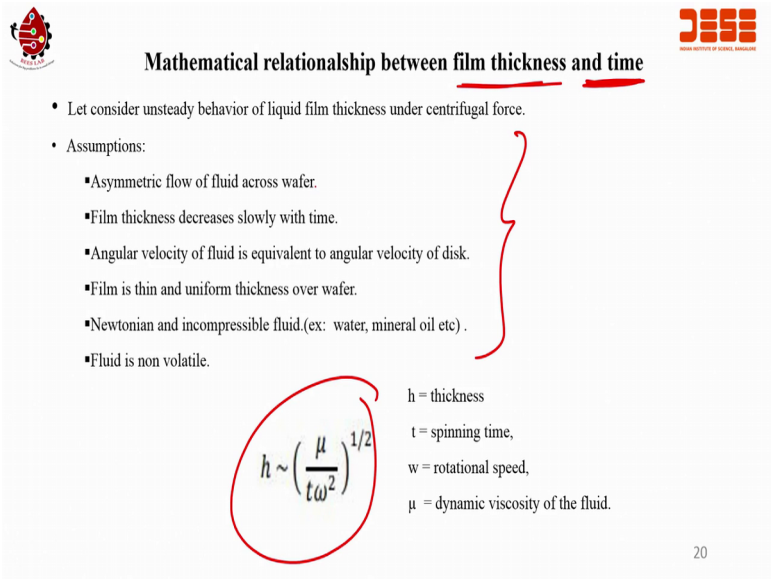

So, mathematical relationship between film thickness and time; if I want to have relationship between film thickness and time, then these are the things that we have to remember. First is the assumptions are, because you see if you see the unsteady behaviour of liquid film thickness under centrifugal force, then what are the assumptions; the asymmetrical flow of fluid across the wafer will happen, film thickness decreases slowly with time, angular velocity of fluid is equivalent angular velocity of disk, film is thin and uniform thickness over wafer right.

And Newtonian and incompressible fluid, example water, material, mineral oil, etcetera and fluid is non-volatile these are the assumptions, when we are and when we are

understanding the mathematical relation between film thickness and time. And here you can see, how the film thickness is related to the dynamic viscosity of the fluid, it is rotational speed of the system and the time of the spinning right. So, this is the mathematical relation if you guys are interested in.

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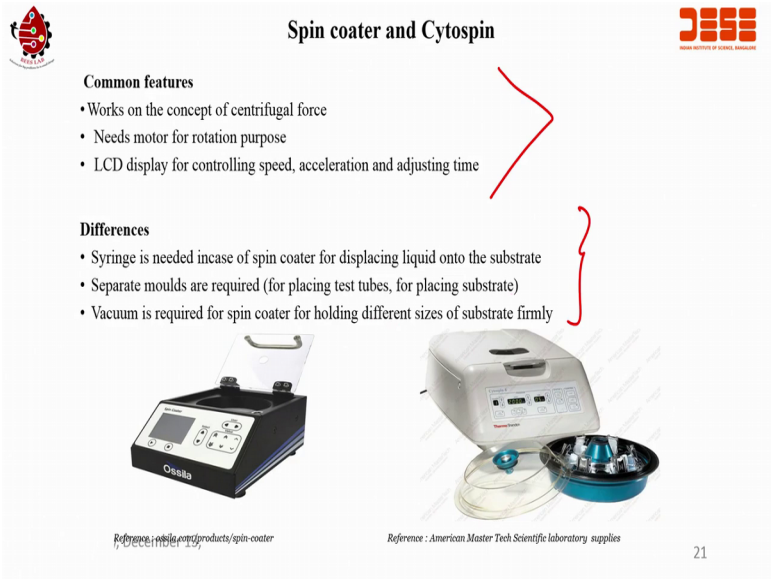

So, what are the common features when I talk about spin coater and when I talk about cytospin? So, the common features are works on concept of centrifugal force, both needs motor for rotation purpose, LCD display for controlling speed, accelerating and adjusting time that are common features. What are the differences? The differences are that syringe is needed in case of spin coater for displacing liquid on to the substrate right. You see this one, this one dispenser right we require dispenser that is what is say, dispenser is required.

Separate moulds are required for placing test tubes or placing substrate. And vacuum is required for spin coater for holding different size of the substrate firmly, well in case of cytospin we do not require such a system, we do not require vacuum.

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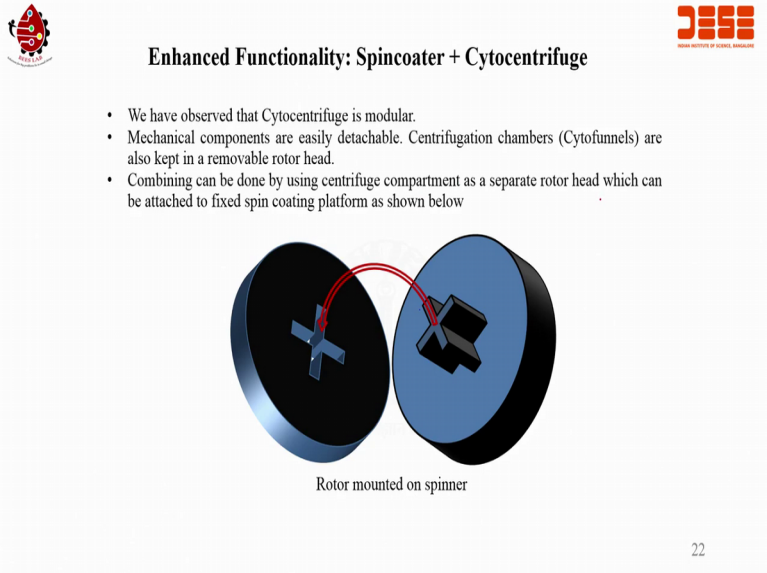

So, cytocentrifuge is modular mechanical components are easily detachable, this is a one system that you can developed. If you want to use the system as a cytospin and as a spin coater, the centrifugation chambers is cytofunnels are also kept in a removable rotor head. So, the idea is that can we just place this guy on this, and work as a cytospin, if he remove this, then it becomes a spincoater or vice versa. So, we can developed our own substrate holder, combining can be done by using a centrifuge compartment as separate rotor head, which can be attached to a fixed spin coating system as shown below.

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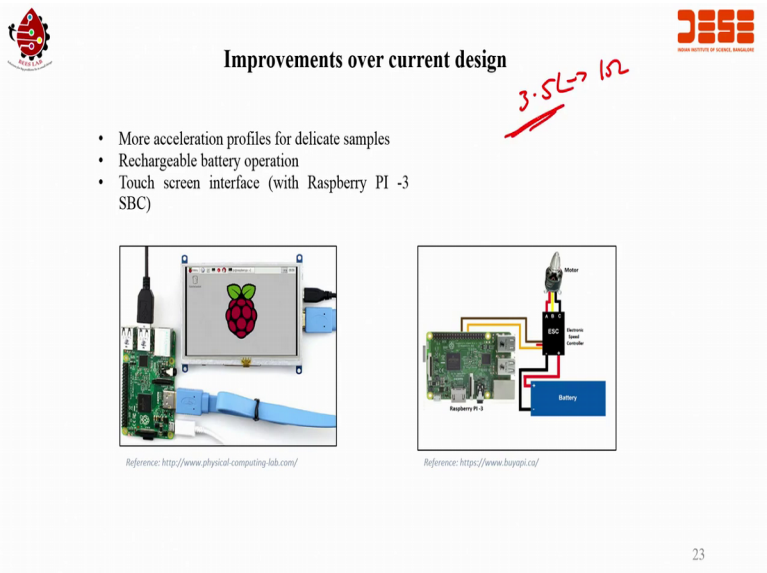

Now, if I want to if you want to mix as a system, what things you require you require. First is you need to understand, what can be the improvement over the current design, because there are already cytospin available in market. The cytospin cost about 3.5 lakh all the way to 15 lakhs.

Now, still this is very high cost for lot of primary health centers, and that is why our target is to make a low cost cytospin system. Now, for that what are the improvements more acceleration profiles, and for delicate samples, there is a rechargeable battery operation, touch screen interface. Rechargeable battery operation is very important because of the power situation within our country; there is lot of power fluctuation. And if there is no power, then systems is not stop in between right, so that is another thing that we need to take care.

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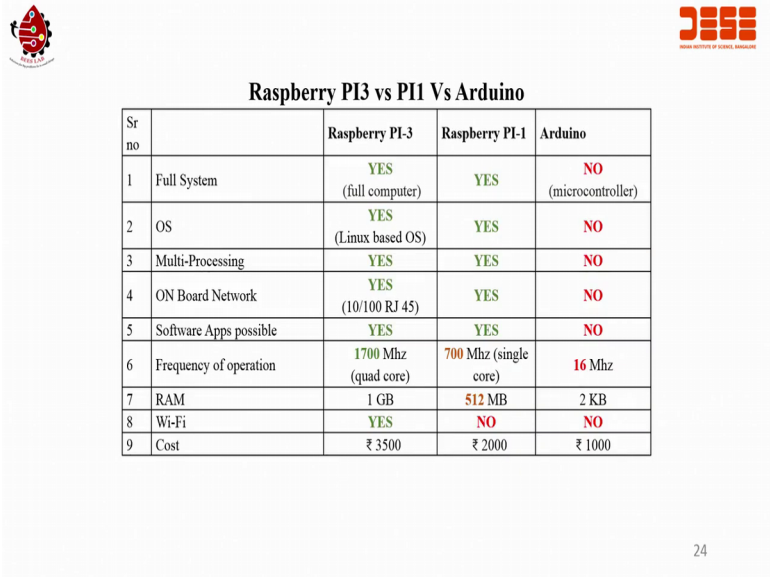

So, if I want to select a a in a system for programming, what will I select over Raspberry PI and Arduino? I will go for Raspberry PI which is three 3rd version, if we talk about Arduino, you know the OS is Linux based OS. Here also Raspberry PI is the same thing, right multi-processing is possible in Raspberry PI 3, where arduino is not possible right, software apps possible. In both the you know a Raspberry PI, where here it is not possible in Arduino. When you run over frequency operator is a quad core is a single core with the raspberry PI 3 is better than Raspberry PI 1.

When you talk about RAM, it is a 1 GB ram over 512 megabytes RAM, here is just two kilobytes ram. So, this is better over the Raspberry PI 1. Finally, if we talk about Wi-Fi, then also there is a Wi-Fi option in Raspberry PI 3, where it is not there in both the Raspberry PI 1 and Arduino. The cost when you talk about, the cost is about 3500 for using the Raspberry PI 3 controller.

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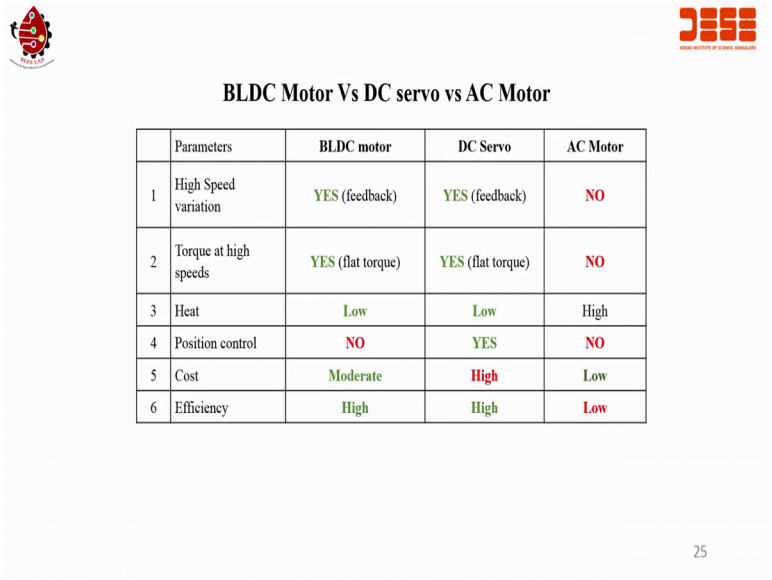

Now, we also had to understand that which one we want to select for the as a rotor for the system right, we are we are understanding system building. So, when you talk about system building, you need to understand that should I use BLDC motor or this is a a servo or AC motor. So, when I compare the speed, torque, heat, position control, cost, and efficiency, I see that the BLDC motor can help us to solve the problem. Same thing we can we can think of using the DC servo also. So, so you have the option of comparing and then selecting, which particular system you can use it.

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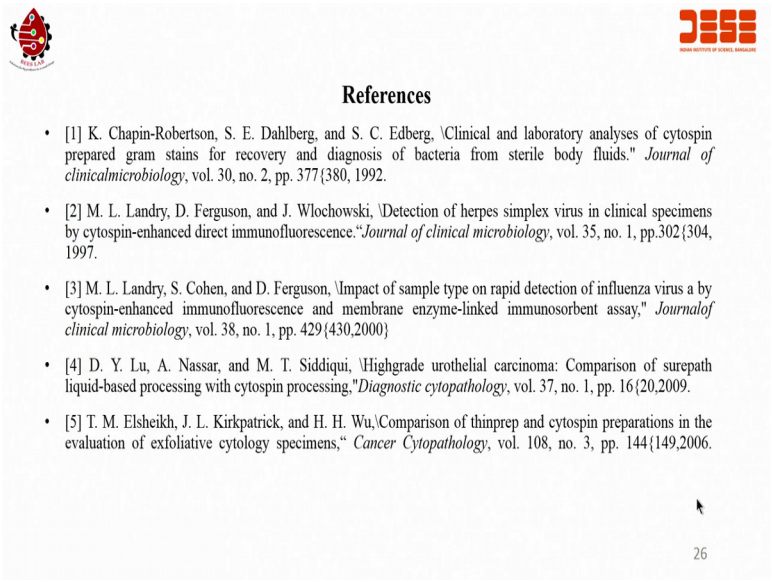

So, please use this slide as a reference, and you can read the papers. So, you know more about what is the importance of the cytospin in case of the cytology based screening all right. Now, this is the end of this particular module, and that is why I want to show it to you two videos, which you will see at the as a part of understanding the cytospin and spincoater in detail. So, I will just show you two videos, where you will see how the spin coating is done, and how we can use cytospin in reality. So, we can put a sample, then we see how the cells are smear on the glass slides all right.

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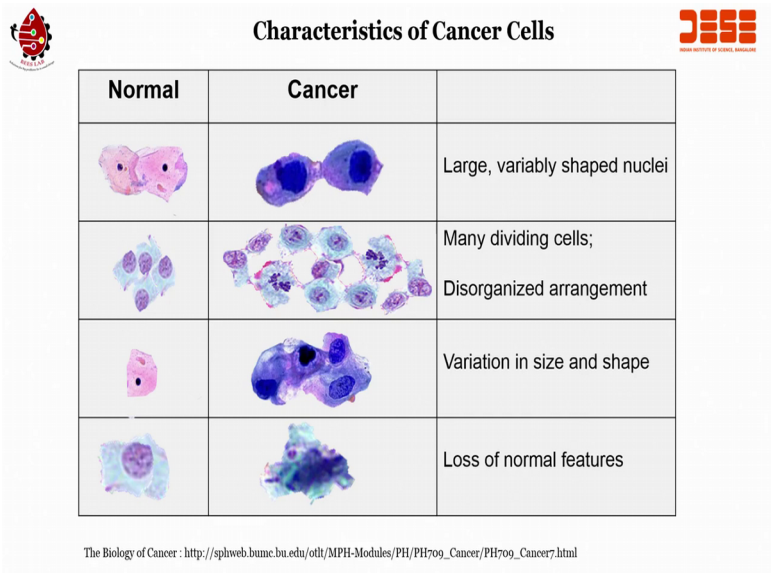

So, I will I will complete my module now. And let us see the videos, and then we will continue next module next time right. In the next module, I want to show it to you how the normal cells and the cancer cells can be delineated based on the different factors like dividing of cells, variation in shape and size, normal features, it is a variably shaped nuclei and so on and so forth ok. We will we will see these thing in a next module, let us run the videos.

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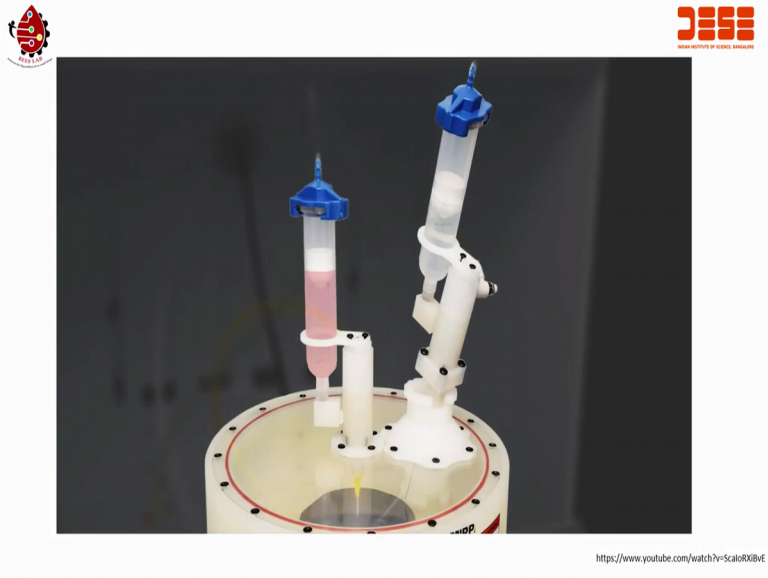

Our automatic universal dispenser is available as a fixed (Refer Time: 25:07) unit or in the off center adjustable UD-3b mount. The UD-3b is used as an additional coating dispense unit of automated edge bead removal. An edge bead is a layer of thicker material that builds up the edge of a substrate, during coating process. It can interfere with further processing steps, and is best removed as part of the coating program.

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It is most important to begin with a correctly centered substrate. Here we used a laurel technologies alignment tool to guarantee consistent accurate centering. Consistent centering allows you to set up the UD-3b to accurately remove, just the edge bead without having to reset for each substrate or remove more of your coating than is absolutely necessary.

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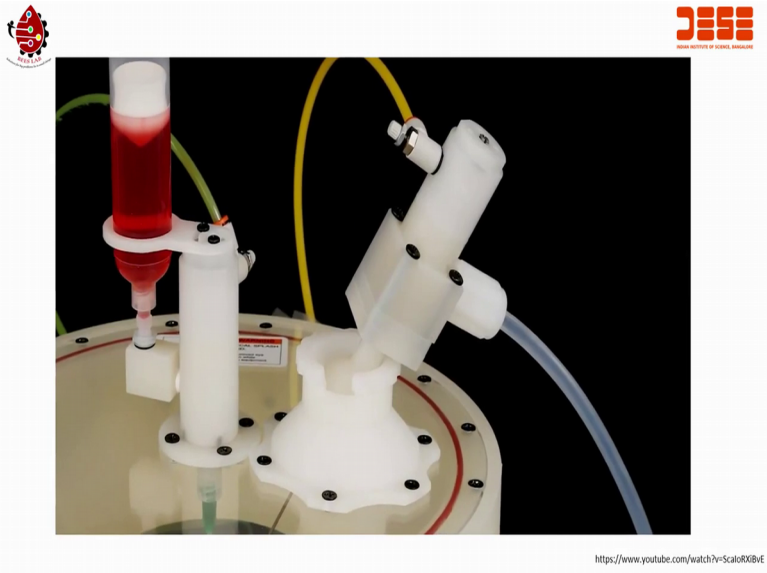

The UD-3b we are using has the solvent fed by an air operated syringe. There are options available that allow solvent to be fed from a pressure vessel or from a bottle through a bottle pump.

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A fine needle is used and in combination with a low syringe dispense pressure. This creates a consistent stream of solvent that can be accurately directed at the edge of the substrate. The tip of the needle should be one to two millimeters from the surface of the substrate and should be angled, so that the stream of solvent flows in the direction of rotation. Laurel systems normal direction of rotation is counterclockwise.

Here we show an example of a coating program with automatic edge bead removal. The center universal dispenser delivers the coating material. And the substrate is accelerated to the final speed needed to achieve the required film thickness, and dry the substrate. The substrate is slowed to around 1000 Rpm, and the solvent is dispensed with three seconds removing the edge bead. The substrate is then accelerated to 3000 Rpm to dry the edge.

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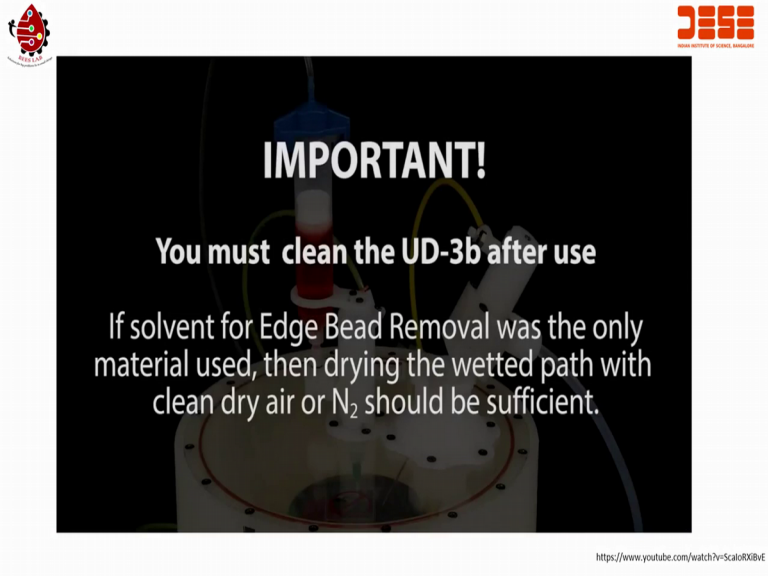

We recommend you clean the UD-3b at the end of every day. If all that has been used is solvent for edge bead removal, then drying the wetted path with clean dry air or nitrogen should be all that is necessary.

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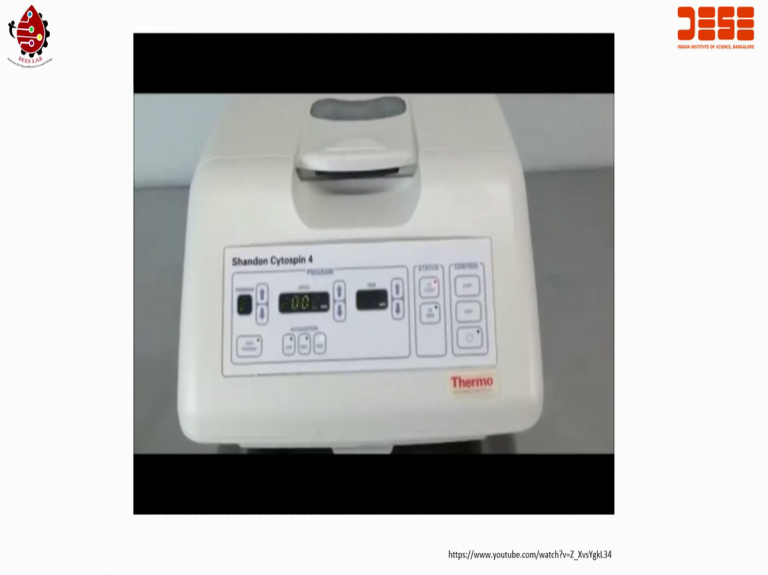

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Yeah. So, now you have seen the video right. So, you can you can see how the cytospin, and how the spin coater works as a individual unit, and how you can design a system that can integrate both the stuff right. So, this is the end of this module. I will see you in the next module. Till then you take care. Bye.