Electronic Systems for Cancer Diagnosis
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Lecture – 25
Techniques in oral cytology studies

Hi, welcome to this module. This module is in continuation with the previous module where we are understanding the cell and tissue morphology. Now we have seen two different techniques of how we can design a spin coater and how we can change the design of a spin coater to a cytospin. We have also seen two different videos in fact, three different videos about how the cytospin works, about how a spin coater works right.

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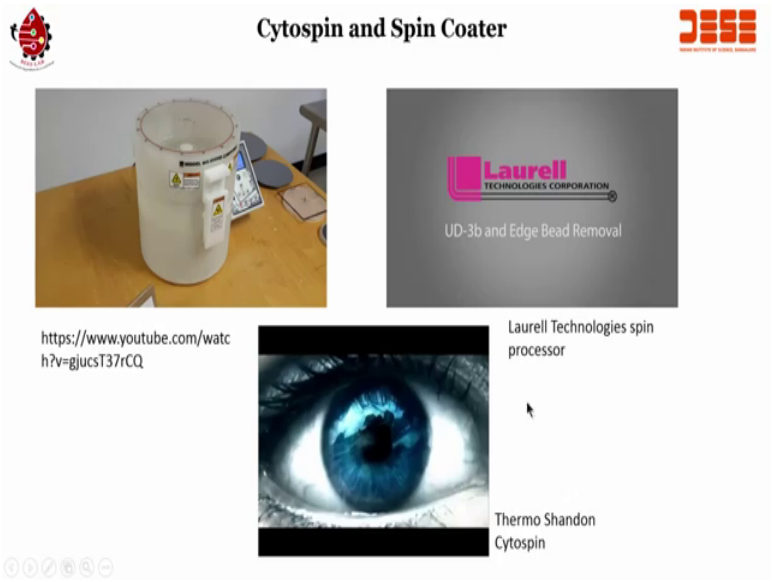

And into three different videos like I said, a spin coater here a cytospin here and a detail about the cytospin here as well correct.

Now, let us see further that how the difference in the characteristics of a cancer cells would be there compared to normal cell, or in another language how you will delineate normal cells with respect to cancer cells.

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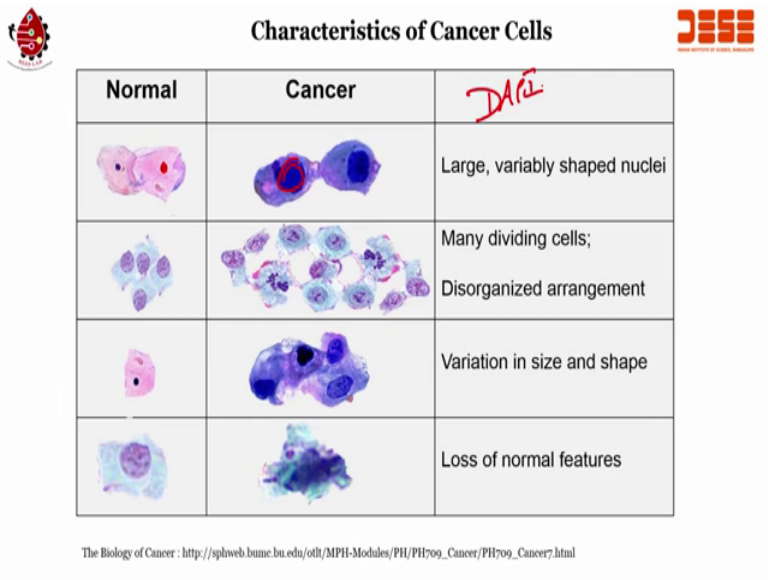

So, there are several factors that we need to understand, as you can see here this thing this images are taken from the biology of cancer, the link is provided over here where you want to understand or read in detail, but gist of is of this particular slide is that if you get a normal cell and if you have a cancer cell, then you can delineate normal and cancer cells based on the morphology. So, what are the morphologies? First thing is that the cancer cells would have a large variably shaped nuclei compared to normal cells.

You can see here the nuclei of a normal cell right you can see here the nuclear of a cancer cell. So, the blue color is because of the biomarker called DAPI; D, A, P, I DAPI. That stains the nucleus with blue color. So, you can see here this thing and then another thing is how we can understand is that there are many dividing cells disorganized arrangements. You can see here where you can see this particular case you can see they are organized in a particular form right.

Then next one is that there is a variation in size and shape. You can see all the normal cells would be having uniform size where if you take the cancer cells, then there will be variation in the shape and the size of the cells.. The next one would be loss of normal features. You see cytoplasm, nucleus, here there can be double nucleation, the cell morphology would look different, the shape would be different and that is the normal features which you can find in a normal cell would not be there.

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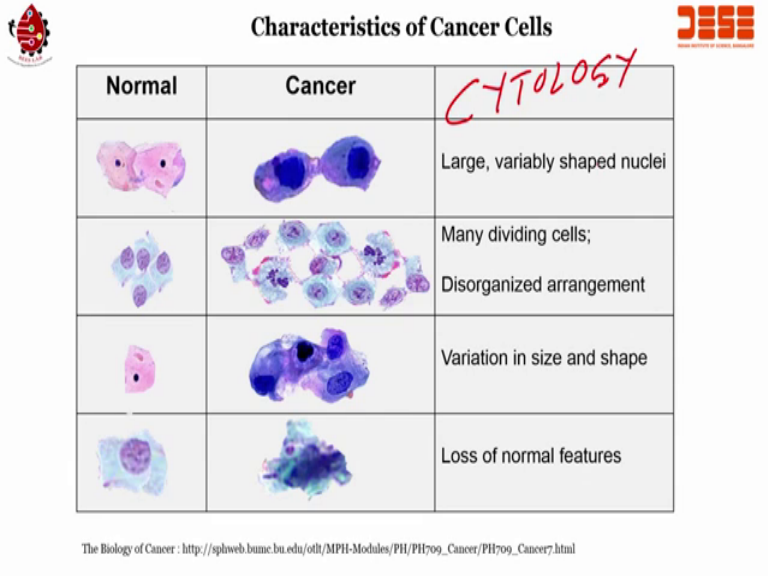

So, thus if you can understand this property or this properties of a cell then, you can design a platform that can help us to delineate between cancer cells and normal cells again this is all cytology based techniques. What? Cytology. These are all cytology based techniques ok alright. Let us go to the next slide.

So, techniques for cancer diagnosis: here what we see is that there are several techniques, as you can see in the slide which are called diagnostic techniques.

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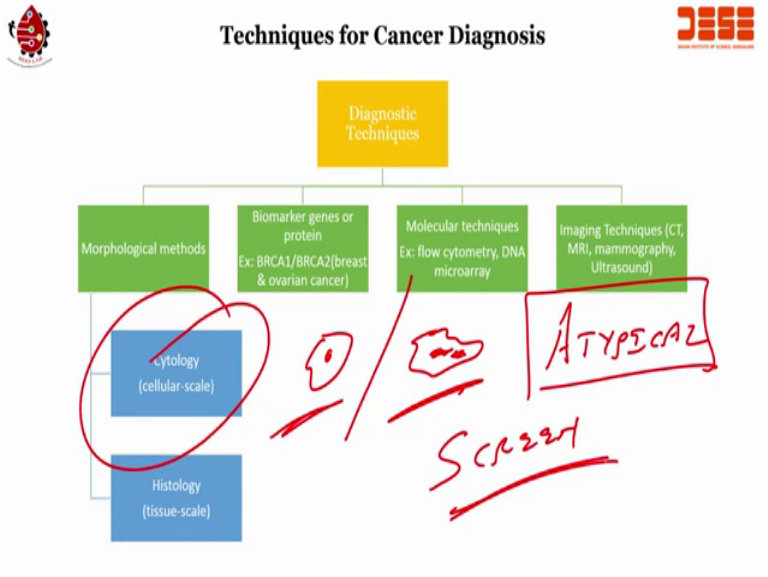

The first one is called morphological methods, change in the morphology of the cells and tissues, the second one is on biomarkers genes or protein, what are the changes in the protein, the third one is on the molecular techniques like flow cytometer, DNA microarray, the fourth one is an imaging technique like CT, MRI mammography, ultrasound right. So, these are the four different diagnostic techniques, generally like if you see have seen the earlier lectures that MRI and mammography the women are advice to go and do the testing once in a year or twice in a year based on the age group. And the mammography is where it can show the suspected region and further a person has to go for a biopsy.

Now, the another way of understanding if it is a oral cancer or ovarian cancer in fact, is if the cells are changing their morphology and that will help us to screen the patient. So, further what we will be focusing on is the cytology which is cellular scale and histology which is a tissue scale. So, once the cells are out and if you can see a normal cell versus a cancerous cells right a double nucleation or something or cell to cytoplasm ratio then you can understand that a person is having atypical cells. The the diagnosis will be ATYPICAL; A T Y atypical ATYPICAL ok.

Atypical is a diagnosis. It is not saying the person has a malignant or pre malignant it is just saying it is atypical; it is not normal. Then once the atypical diagnosis is done, then the patient is asked to go for the histology. So, our idea is to understand the cytology properties of a cell and to develop a device that can help to screen the patient faster, screen the cases faster. This will reduce the burden on the hospitals as well as help the patients to make sure that they also sees, why we are doing this? Because if you see the videos, I will show you two different videos how the cells are taken from the mouth, we do not have that many experts like I said oncopathologist onsite also it will take long time for sending the cells from the ground level to the oncopathology lab.

So, there is a waste of time, in and sometimes a patient has to go to that laboratory. So, we want to develop a system or we are trying to develop a system using engineering techniques, so, that we can do the onsite screening of the patient. When it is a case of oral cancer or a ovarian cancer.

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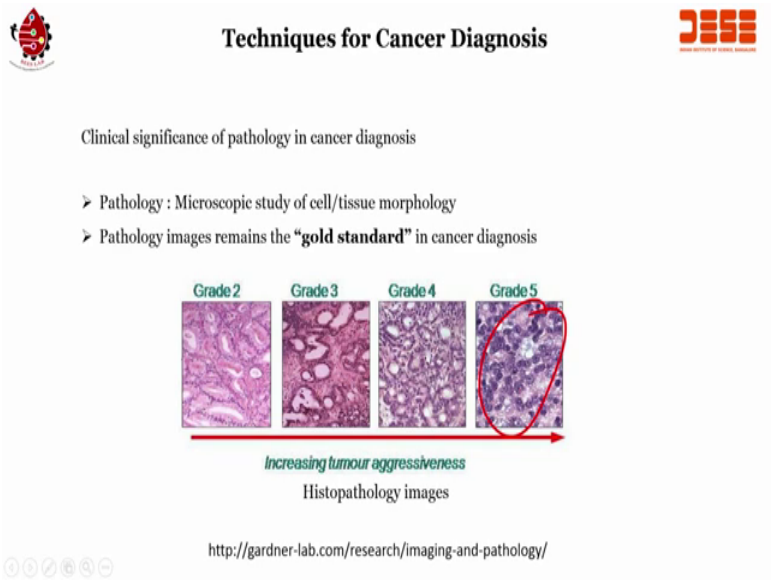

Now, if you go for the histology. So, if the patient is diagnosed with atypical, then the clinical significance of pathology in cancer diagnosis is the tissue based morphology and the pathology is microscopy study of cells or tissue and pathology images remains the gold standard, right which is right over here.

And these are the histopathology images. You can see that from grade 2 to grade 5, the how the tumor is aggressive and this you can further find from the images from this particular link and read more about that that how the gold standard is decided and how and how the staining is done. Here you can see there are many cells with you know on different morphology, abnormal growth right. And you can you can based on this gold standard, the diagnosis will be done that which grade the tumor is within.

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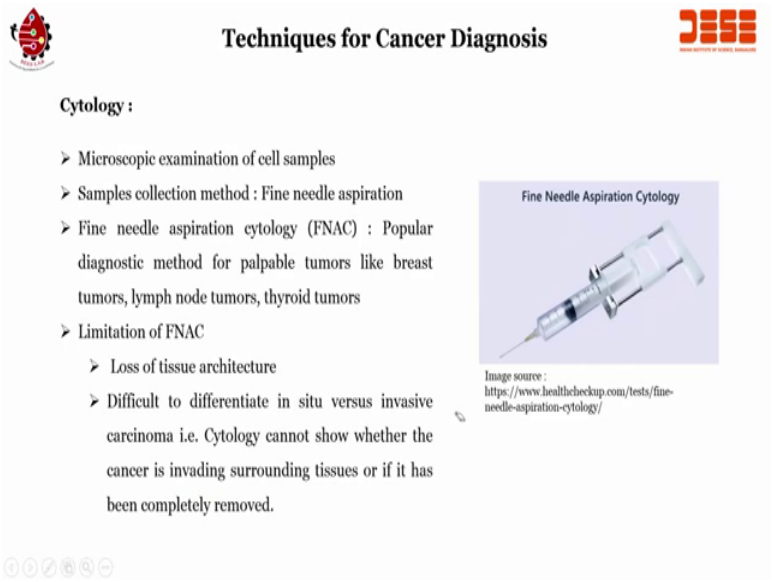

Now, cytology we will focus on cytology here, in this particular course. And or in this particular lecture I should say, where the cytology is a study using microscope. So, it is a microscopic examination of cell samples, a study of cells using microscope I should say and sample collection method is fine needle aspiration which is right over here. And then we can see which is also called fine needle aspiration cytology or FNAC, popular diagnostic method for palpable tumors like breast tumors, lymph node tumors, thyroid tumors ok. This is where we want to take the cells from the suspected region from the breast cancer case or a thyroid tumors or a lymph node tumors.

But we talk about oral cancer then we can just use a brush biopsy. I will show you what exactly brush biopsy means. Now what are the limitation of the fine needle aspiration cytology? The limitations are that the loss of tissue architecture and difficult to differentiate in situ versus invasive carcinoma. That is cytology cannot show whether the cancer is invading surround the tissues or if it has been completely removed. So, that is why we had to rely on the histology techniques ok. These are limitations.

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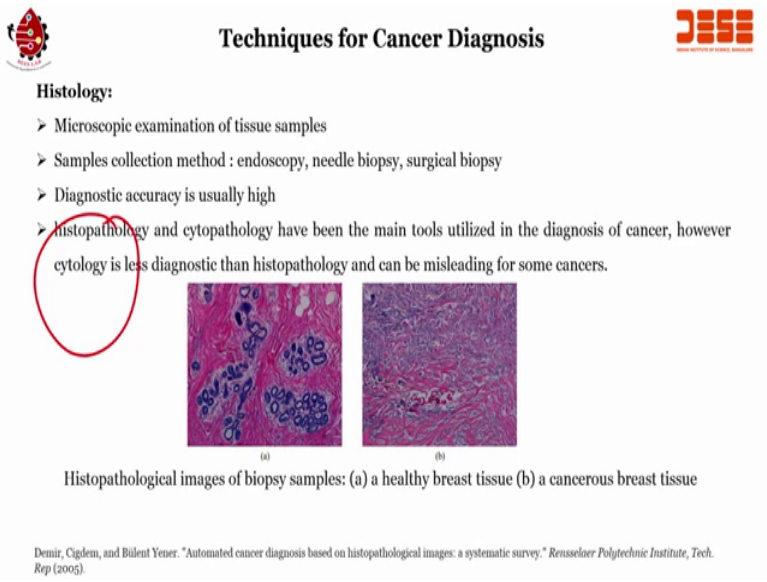

Now, if we go for histology; if we go for histology what we find is that histo pathological images of the biopsy tissues you can see here one is the healthy breast tissue, right. This is this one, while another one is a cancerous breast tissue. You can clearly see the change in the morphology of the tissue right. Also microscopic examination of tissue is what the histology means, sample collection method is either endoscopy or needle biopsy or a surgical biopsy.

Then diagnostic accuracy is usually high in this case. Histopathology and cytopathology have been main tools utilized in diagnosis of cancer. Diagnosis of cancer has been done using two main tools; one is the histopathology and second one is cytopathology. We cytopathology is where we are studying the cell morphology. Histopathology is where we are understanding the tissue morphology as well as the biomarkers.

However cytology is less diagnostic, then histopathology can be misleading for some cancers and that is why cytology cannot be considered as a gold standard. However, cytology can be used to screen the patient quickly alright that is the reason of understanding the cytology. That is the study of cells.

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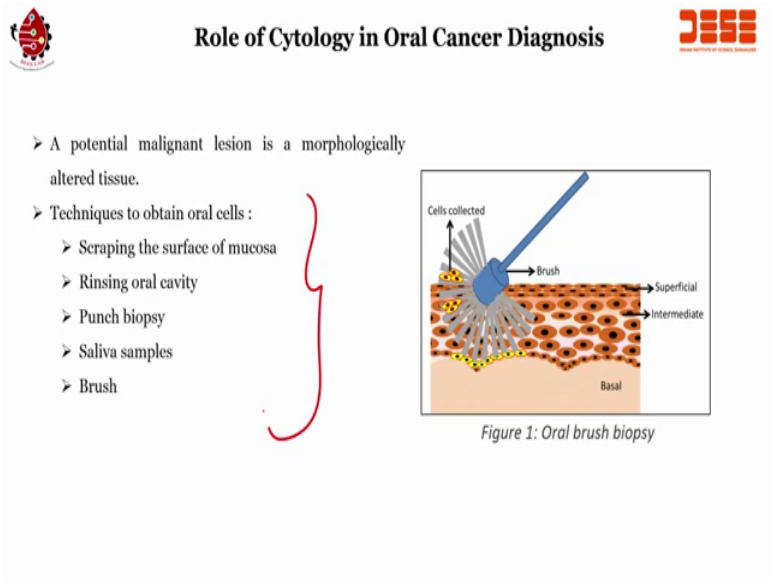

Now, let us see role of cytology in oral cancer diagnostics. So, if I want to consider just oral cancer diagnostics then this is how it is happen. If you take the oral brush biopsy that is our mouth then you can see there is a basal membrane, there is a intermediate and there is a superficial layer.There is a brush which is you know turned and the cells are collected in the brush, and we will see how the process is done in the next few slides. But the point is the a potential malignant lesion is a morphological altered tissue techniques to obtain these oral cells are scraping the surface of mucosa rinsing oral cavity or punch biopsy or saliva sample or using the brush biopsy the images for the oral brush biopsy. So, these are the techniques to obtain the oral cells.

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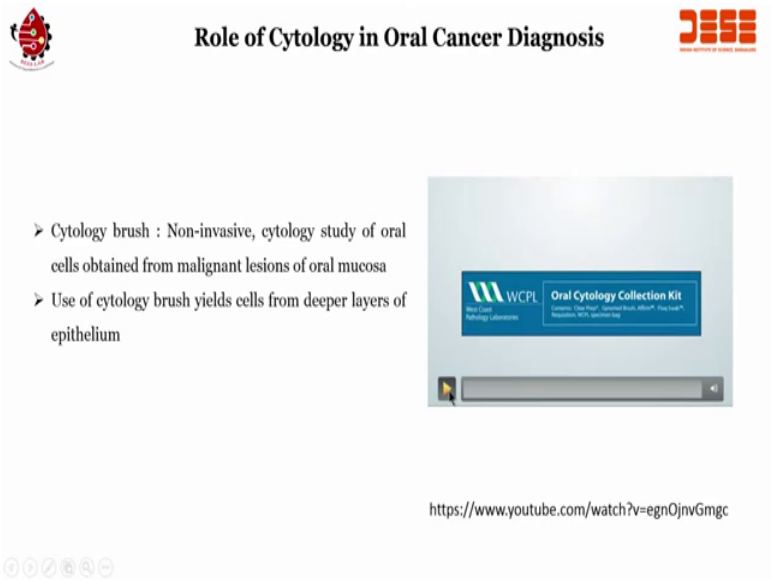

Now, if I go further you can see the cytology brush or a non invasive cytology study of oral cells obtained from malignant lesions of oral mucosa that is the cytology brush that it is a non invasive cytology study of oral cells and use of cytology brush yields cells from deeper layers of the epithelium. That is advantage of cytology, brush cytology. And let us see the video this is the oral cytology collection kit how it can be used. So I will play the video and this is from West Coast Pathology laboratories the Youtube link is given over here.

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So, you can go to the Youtube and look at it later on also. So, let me play the video and then we will continue the module. Open the oral collection kit lid and remove the collection vial and brush. Label the vial with the patients name and date of collection. Remove the lid from the vial and set aside. Take the brush provided and retract the cannula off of the brush exposing the bristles. If sampling a visible oral cavity lesion, rub the bristles onto the surface of the lesion, rotating the bristles back and forth. If sampling for hpv or infectious disease rub and rotate the bristles randomly, over the entire oral cavity buccal mucosa, buccal gutter, retromolar trigon and nasal pharynx if possible.

Next place the brush into the collection vial fluid. Quickly open and close the cannula over the brush several times while submerged using a plunging motion to remove all cellular material, from the bristles. Discard the brush replace the lid and tightened place the vial into the specimen bag was completed requisition form and forward to escos laboratory for analysis. Ok.

So, you have seen that video right. Now what are the advantages of oral biopsy or oral brush biopsy in the oral cancer diagnosis? So, let us focus on this particular topic now, where we are considering what are the advantages of the oral of the brush biopsies.

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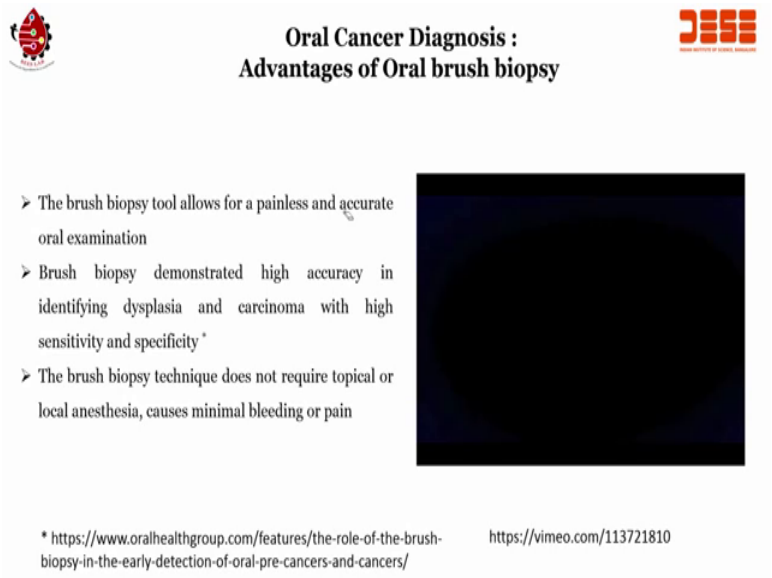

So, if you see the slide the brush biopsy tool allows for painless and accurate oral examination. That is the first advantage that it is painless and it is accurate. Second is brush biopsy demonstrated high accuracy in identifying dysplasia and carcinoma with

high sensitivity and specificity right. You can see here the details about what we are discussing here is in the link right you can go to the link find out the details and so, that you can understand what we are discussing about the dysplasia and the carcinoma.

Finally the brush biopsy techniques does not require topical or local anesthesia causes minimal bleeding or pain. Now in other cases you if you use the biopsy technique, you have to go for anaesthetize we are anaesthetize the patient, and it causes sometimes lot of bleeding, but in the case of brush biopsy we do not have to really anesthetize the patient and that is why local anaesthetize not required and also there is a minimal bleeding or deep pain. So, that is the advantage of the brush biopsy. So, let us see the video how exactly brush biopsy is done in the actual case.

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How to perform the oral CDx brush biopsy? Each test kit includes all of the components needed to perform one brush biopsy sample. It is applied in a prepaid mailer for ups return to the lab; complete instructions and a patient information card are included with each kit.

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When sampling the spot. Rotate the brush while applying pressure until pinpoint bleeding is observed.

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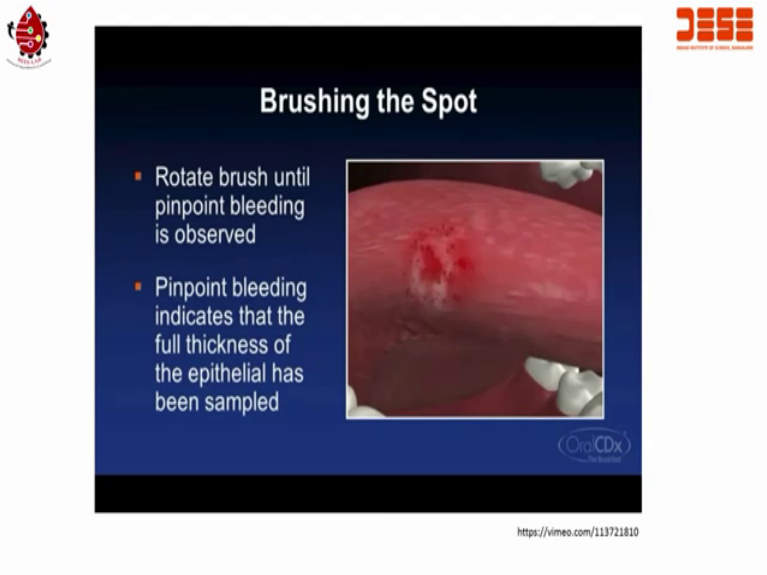

This is important as it indicates that the entire epithelium has been sampled. While it is generally easier to apply pressure using the flat side of the brush, the circular end of the brush can be used for hard to reach areas.

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Once the sample is obtained, it is important to immediately transfer the cellular material from the brush with the slide. Rotate the brush using a painting motion over the entire length of the slide, while remaining within the bracketed area. Once the transfer to the slide is complete, immediately fold the fixative pouch in half and squeeze the contents over the slide flooding the specimen. Any delay may result in air drying of the specimen, which compromises the quality of the cells. Insert the brush into the vial, for cell block preparation at the lab, brush the lesion and obtain another complete tissue sample. This sample is not transferred to the glass slide.

But instead the brush is inserted directly into the supplied vial. Seal the vial and gently shake it. Then place it into the enclosed bag. Packaging, while filling out the test form be sure to complete the shaded areas. Missing information may delay the processing of the specimen. Insert the slide holder with the slide and the vial into the kit bag. Finally, seal the prepaid mailer for shipment to CDx diagnostics v a u p s ok.

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Now, this is a oral CDx square the brush biopsy technique. Let us see this video as well because we need to see multiple videos to understand how exactly brush biopsy is done. I will play this video as well.

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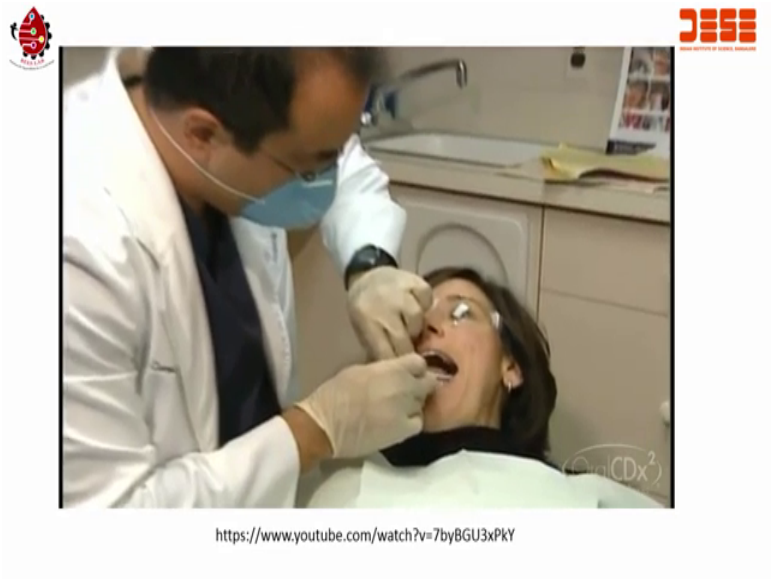

All CDx allows for the easy testing of any oral spot or tissue change in the oral cavity. Oral CDx obtains a complete epithelial sample in a virtually painless manner, allowing for oral spots to be easily tested. So, that dysplasia can be detected while it is still harmless. Once the sample is collected it is sent to CDx diagnostics, where a

sophisticated neural network of computers assists in the analysis of the specimen. This highly advanced imaging system is designed to detect even a handful of oral precancerous or cancerous cells scattered among tens of thousands of normal. So now, you have seen both the videos right. So, what we had to see now is, let us compare if you are given a cytology that is brush biopsy what is cytopathology, and how comparative studies in front of us looks like?

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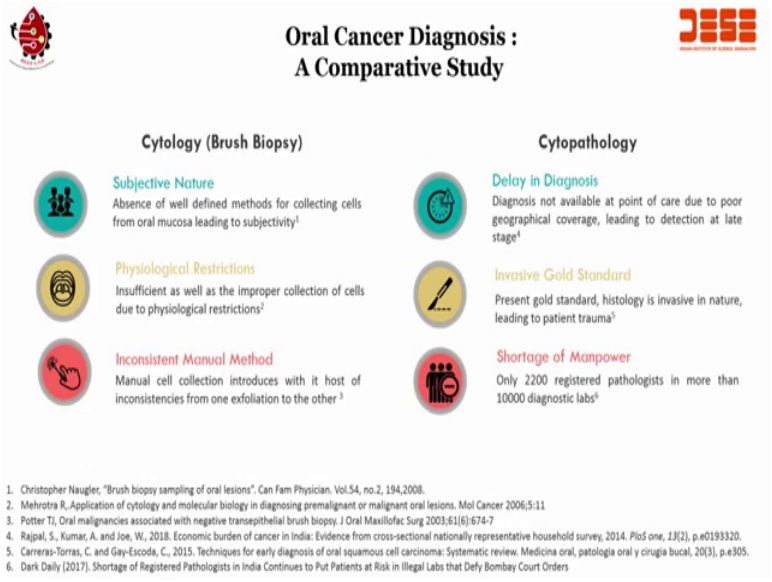

So, if you see the slide if you compare the cytology versus cytopathology, these are few of the papers, see guys it is always good to read as many research papers as you can, because that is will help you to understand in detail what we are talking in this lecture is 30 hours of lecture covering lot of topics or 20 hours of lecture covering a lot of topics will not help you to go in depth. It will cover lot of things which will help you to understand when you want to go in depth, you have to understand and read more number of research papers. That is why few research papers for you can you can read this papers and you can understand the details about what we are talking about on what we are talking about ah.

So, if I compare the brush biopsy in terms of cytology versus cytopathology, then you can see first this subjective nature absence of well defined methods for collecting for from oral mucosa leading to the subjectivity issues. While here if you compare cytopathology delay is diagnosis, diagnosis not available at a point of care due to poor

geographical coverage. Particularly we are talking from our country point of view, that the point of care techniques when we, when we when we are talking about cytopathology is difficult. One is geographical coverage and that leads to detection at a late stage ah.

Second is, if you come here which is cytology then physiologicalrestrictions are insufficient as well as improper collection of cells due to say physiological restrictions, how you can access the oral cavity right and if you are a trained or on or on semi trained person depending on that you can collect a proper number of cells. In case of the cytopathology invasive gold standards present gold standard histology is invasive in nature and leading to patient trauma. When you are doing any invasive surgery it causes some kind of trauma to the patient. When we are talking about the third point which is the inconsistent manual method, because the manual circulation introduces with it is host of inconsistencies from one exfoliation to the other. So, if one person you know take out the cell from a mouth, and the second person who is again a trained physician will take out the cell from the mouth, there is a inconsistent in case of the cell collected using manual method. So, what we want is, every time when you are collecting the cells on the mouth it should be uniform. If there is a less number of cell or more number of cells as a variation in the cells that is not good so, manual method has some kind of restriction.

So, we will look into this particular module how to develop a automated brush biopsy like a atom motorized stage for the brush biopsy. So, that these the difficulty of inaccuracy in collecting the cells using manual method can be reduced. Alright. So, we look into this in few slides after this, but right now let us see the third one which is shortage of manpower, like i said only 2200 registered pathologist in more than 1000 diagnostics lab. You see that this is the this is shortage of manpower.

Now, when we talk about a population of our country any similar countries where there is huge population and we do not have that many oncopathologist. Same way for the clinicians, we have a less number of patients. So, there is a need of developing a electronic system or a or a medical system that can be placed in a primary health care center and can help the oncopathologist or can do the screening at a faster rate. That is why I am stressing more that we require this kind of platforms and that is why the whole idea of proposing this lecture or this course is to make you understand that what kind of alternative systems you can develop in your laboratory if you have a right team and a right collaboration with a clinician Alright.

Because the clinician and oncopathologist are the one who will give you the problem and will help you to implement your device to test your device with their existing gold standard right. So, having said that let us go to the next slide.

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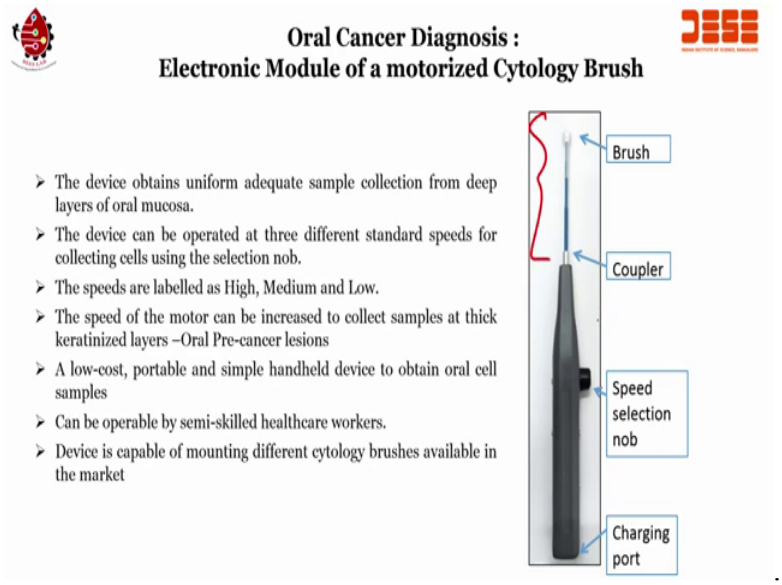

And this is what I was talking about i will show you actual working of this particular brush this is called a motorized stage or electronic model of a motorized cytology brush. And it has a you can you can you can insert the brush at the tip, there is a coupler there is a speed selection knob and there is a charging port.

So, we have developed this system right in this laboratory, in i a s e in my department electronic systems engineering. Alright this is the logo of my department D E S E. This is the logo of a laboratory BEES lab. If you want to know more about the laboratory and that what we are working, you can write down bees lab IASE and you will get into details. So, this particular motorized stage. What is the advantage? The device contains, or using this particular motorized stage what we had to do? The person or even a semi skilled person can use this one. Because you have to replace the brush inside them out and you can change the speed we will give you the speed at what speed what cells you can connect whether you can go deep into the layer or you can just collect from the surface everything can be because we are selected the torque of the motor accordingly.

Now, the device obtains or the system this particular electronic system or module with a motorized brush can obtain uniform adequate sample collection from deep layer of oral

mucosa one. Second the device can be operated at three different standard speeds for collecting cells using the selection knob, you can change the speed right. Then what are the speed high medium and low. The speed of the motor can be increased to collect the samples at a thick keratinized layers or oral pre cancer lesions a low cost this system we had develop right in the laboratory. It is just a three d printer and some electronics and a battery, I will show it in the next slide what are the electronics things within this particular system.

.Or components within this particular tool a low cost portable and simple handle device to obtain oral cell samples, can be operated by semi skilled health workers devices capable of mounting different cytology brushes available in the market. The this is the advantage that whatever brush you have you can mount on this device and use the device very simple to use.

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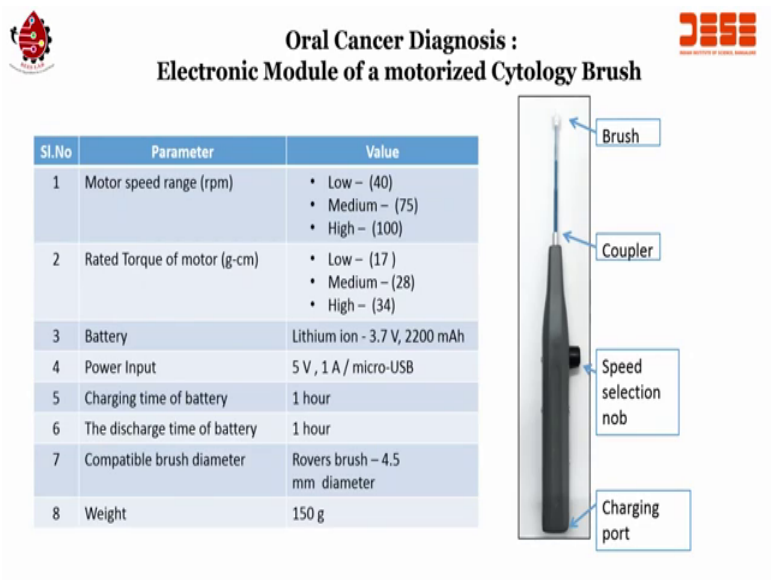

So, what are the values? Let me give this specifications as well the first is motor speed range: low, medium, high at various rpm we have 40 to 100 rpm. The rated torque of the motor is 17 to 34 g the cm battery is 3.7 volts to one 22000 milliamp mm power input is about 5 volts 1 Ampere. And you can use micro USB charging time of batteries about 1 hour, discharging time the discharge time battery about 1 hour, compatible brush diameter robust 4.5 millimeter brush, with the weight of the device is just about 150 grams these are the specification whenever you do develop any tool you need to get these

specifications. Because specifications are compared with the existing tool there is no such tool, if you want to have the, if you want to sell your tool. You need to understand what are the different specifications and the parameters that your device can work on.

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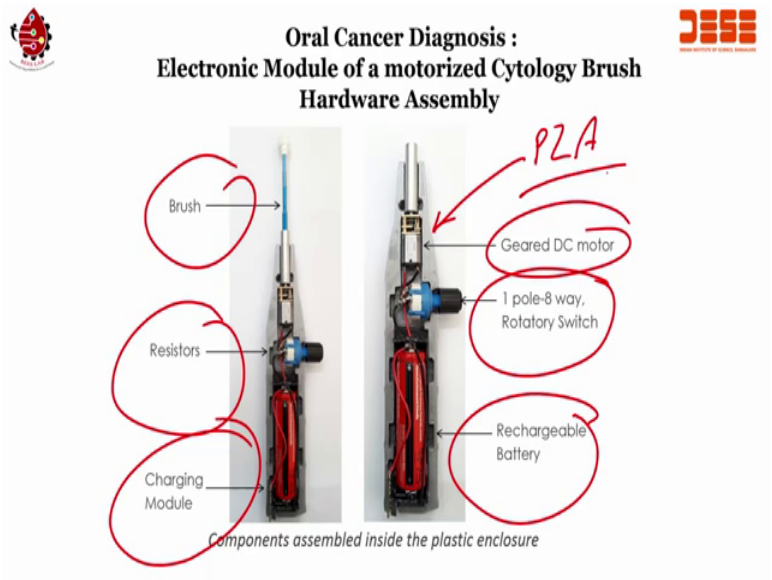

This is the details about the brush as you can see we have a three d printed casing. This is a 3D printed casing. There is a gear for dc motor. There is a 1 pole-8 way, rotary switch. There is a rechargeable battery, there is there is registers this is a brush and these are charging module. Right now everything is assembled inside a plastic enclosure, you can use these three d printed using P L A.You can use a metalcasing as well, right by using the molding technique and the idea of developing this particular system is to make it available and at a low cost and can be used by a semi skilled person. That is the main idea of developing this particular device or tool right. Because of the limitations that we have seen with the manual method that the cell obtained will not be uniform.

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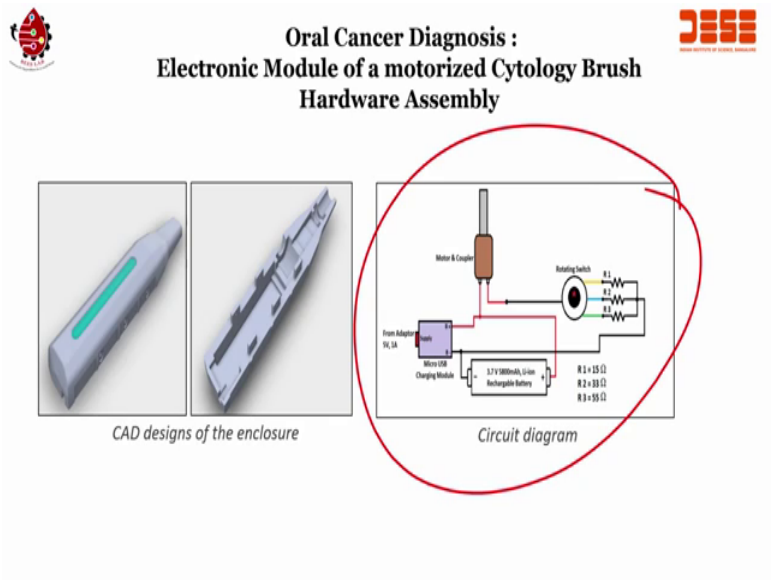

In this case what we found is that I will show you in the next slide. What exactly the data we got from the brush versus a manual method. But if i talking about the further design, then these are the cad design all of you learn at some point of your engineering studies the cad designs right. So, if you use the cad design, you can design it and then you can use the three d printing to print it out. When it comes to circuit diagram we have a micro USB charging module, we have the rechargeable battery, lithium ion battery, we have rotating switch with three different resistors, resistor values are given over here, we have a motor and a coupler which is which is right over here.

So, this is a circuit diagram and you can see it is a very simple circuit to work on to make this tool in a operating state right. So, it is very simple circuit you have to just to make sure that these kind of things are not available in the market. So, that will be the novelty.

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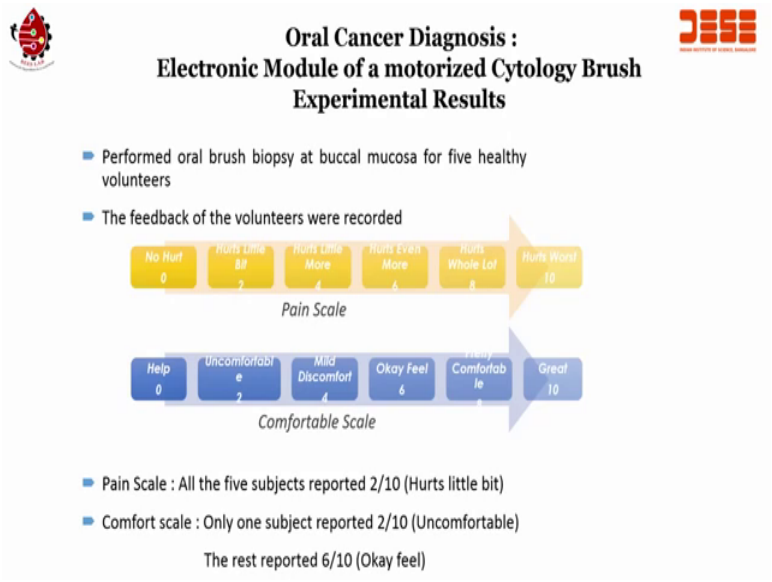

So, then what we have done is that performed oral brush biopsy and buccal mucosa for five healthy volunteers. Feedback of volunteers were recorded right. Pain scale from is no hurt, is 0. Where it hurts the worst is 10 and comfortable scale again we had to help is at 0 and it is great it is 10 alright. So, this is what we have you know marked for from the pain scale and comfortable scale point of view ah. So, you have no hurt, hurts little bit, hurts little bit more, hurts even more, hurts whole lot and hurts worst. Same way in the comfortable scale we need a help it is not comfortable at all, immediately the volunteer will raise the hand is uncomfortable, it is a mild discomfort feel it is pretty comfortable and it is great.

So from that, what we obtained is that out of 10 pain scale all the five subjects reported it hurts a little bit hm. So, hurts a little bit, and when you comfort scale the only one subject reported to where it is uncomfortable while the rest so, out of seven actually that the remaining said it is 6 on 10. So, the 6 is about feel. This is what we obtain from the volunteers, again the this the volunteers are free to give their report it is a blind study where we do not know what volunteer will write and will record it.

So, it was a very interesting experiment to understand how the tool feels like, whether it is really comfortable and how it works in the pain scale. So, like I said out of 10 we got 1 person, five subjects reported about 2 which is hurts a little bit. Because when you want to use it at a high speed it has to take out the cell from the keratinized layer, which is

deep and that is why it will hurt a little bit. But on the comfortable scale most of them were saying it is alright.

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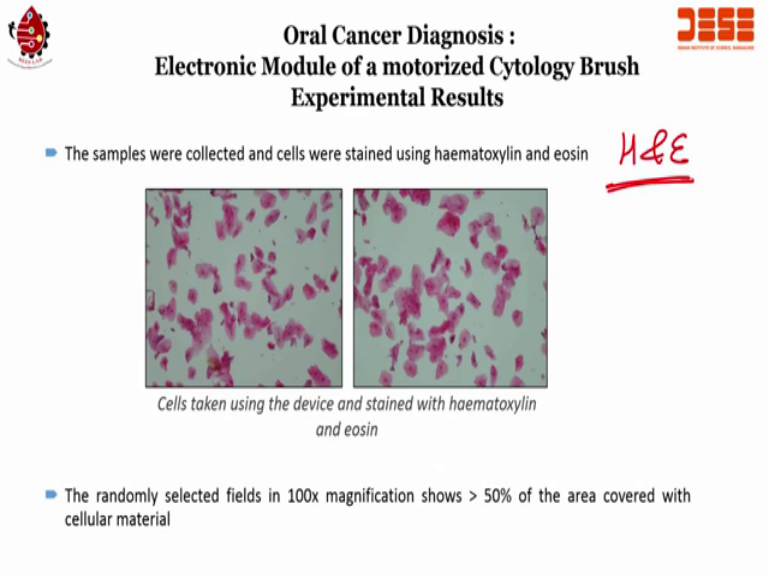

So, the cells were collected and cells were stained using haematoxylin and eosin it we also call in short H and E staining. H and E staining. So, cells are taken using the device and stained with h and e and you can see the h and e stain cells right over here, right the randomly selected fields in 100 x magnification shows that 50 percent greater than 50 percent of area covered with the cellular material right.

So, these are all cells and that is a good sign that using thus using the brush we are able to extract the cells, you can see that you know each cell has the nucleus here right. This that means, that they are cells only. That means, that whatever we are taking out a more than 50 percent of the area is covered by cells ok.

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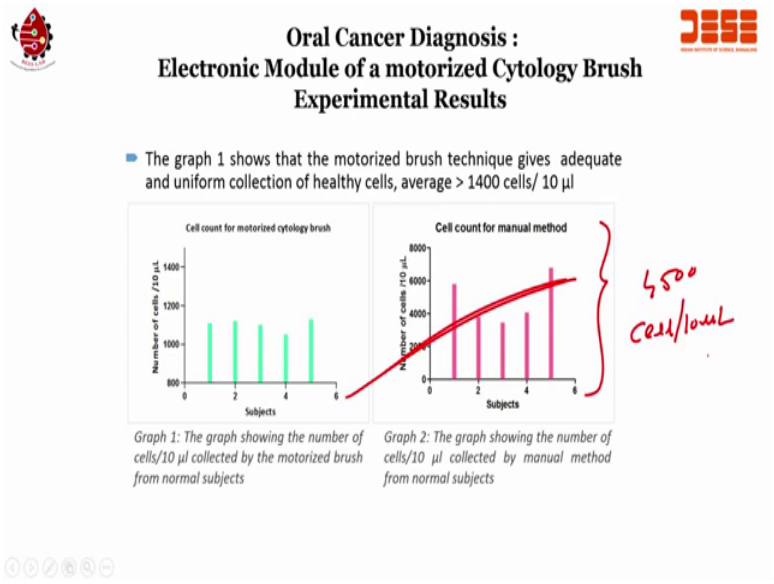

Now, further this is very interesting. That if you count cell using manual method, the average comes out to be 4500 cells; 4500 cells right in 10 microliter of sample right.

B ut we do not require those many cells to understand the cytology we require about 1000 or less, to understand whether the there is a atypical cells or not in a given slide ok. So, if you once we so, this manual method was carried out by a professional, a physician and what we found is from the five subjects the average was about 4500. While the brush was used in present of a finish, present to a physician by a non skilled person. Or you can say semi skilled person. And what we found if you see the slide is that,we obtained on an we obtained about 100 close to 1000 cells per 10 microliter. You know when it was used when the motorized brush was used from the normal subjects again this case we have only perform experiments on normal subjects ok. So, this is very interesting because, the cells obtained by a semi skilled person it is almost similar. You can see almost similar. While you see the range here, right this is what a manual method versus our method is there, you can see here down this down sorry this is not a correct way of representing you were here, then here, then here again down again up sorry you had to go from here then down, then up, then up, like this right. When you see here it is almost similarly little bit down, little bit, down and then up again hm.

But, but the count is close to 1100, here at count is close to 4500 which is which is really high. So, the uniformity the manual method only expert can do, brush a semi skill person

can operate alright. The system is very simple you all know now what is the electronic circuit within it right and there is a uniform collection, right low cost right and easy to use. These are the advantages of the motorized cytology brush over the existing things alright.

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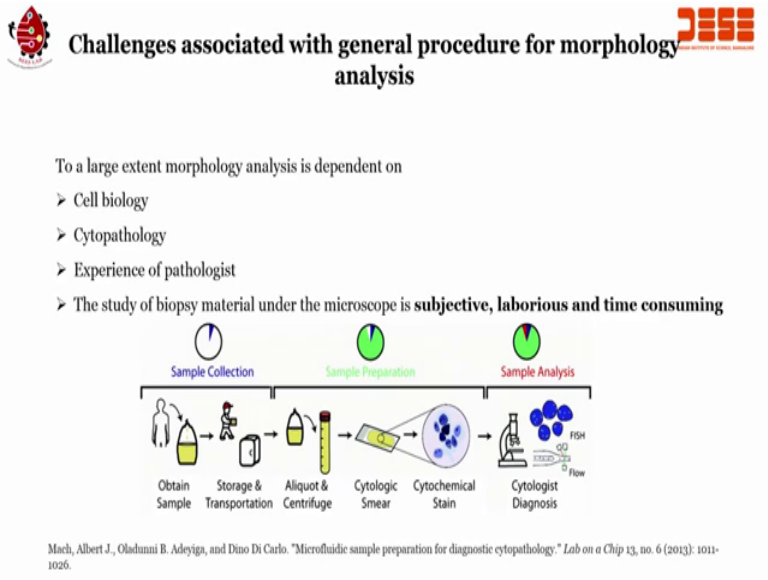

So, if I go further right. I would tell you what are the challenges associated with general procedure for morphology analysis. But before that, let us understand an end this particular module at this stage so, that we can go for the next module and understand that what are the challenges associated with the procedure for morphology analysis alright. So, till then you go through this module understand the videos look at the videos understand what exactly cytology means, how these cells and tissues changes will help us to deal in it, between normal and cancer cells.

Also understand how we can collect the cells using a brush biopsy and in the next slide I will show you another electronic module or system where once the cell is taken out, you can place the slide and you can do automated image analysis by scanning the slide and getting the atypical cells with the slide. So, it is very interesting I will see you in the next module till then, you take care bye.