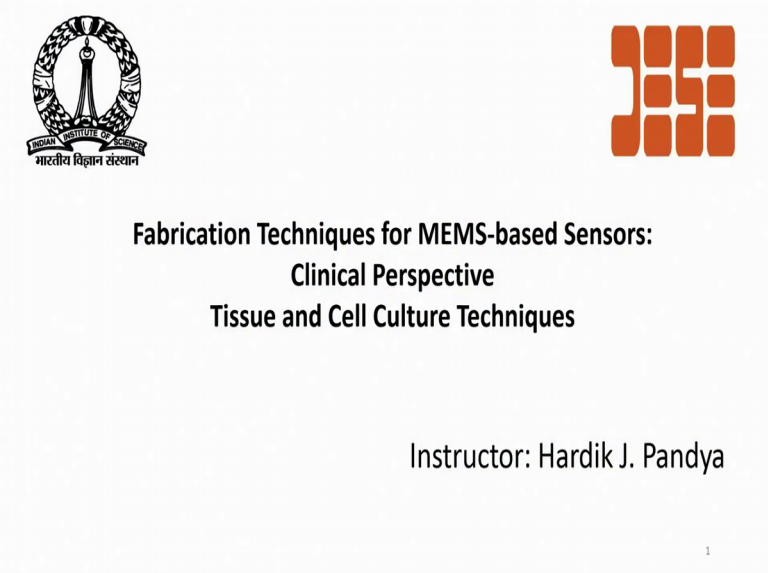
Fabrication Techniques for Mems-based Sensors: Clinical Perspectives

Prof. Hardik J Pandya
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore

Lecture - 33
Tissue and Cell Culture Techniques

(Refer Slide Time: 00:25)

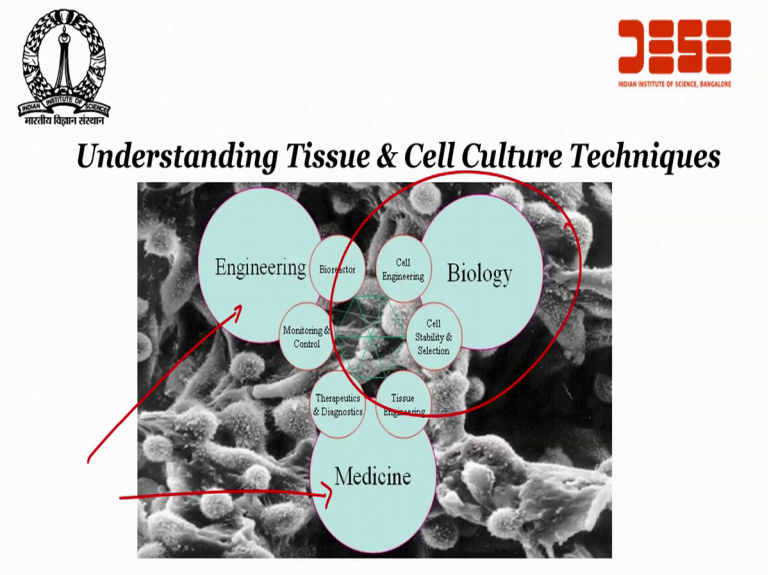


Hi, welcome to this class. This class is extremely important, since we will be talking about tissue and cell culture. Now, tissue and cell culture when we talk about tissue and cell culture it is important, because when we the our topic itself, our course itself says that we are developing a fabrication techniques for MEMS base sensors from clinical prospective. So, clinical problems are all the problems related to our heath and when we talk about our health about human body, human body finally when you go down it is all about cells right.

So, if you have device and you want load the cells. If have the device and if you want to load organized or fluorides. If you have a device to test the tissue properties, then how to culture those tissues and cells right and what kind of laboratory could handle this kind of tissue and cell culture. What kind of equipments should be there, and what kind of media that you will be using to grow the cells and then what kind of technique is used to scrape the cells, what how many cells have grown in particular Petridis or t75 flowers a lot of the things similar to that we will be learning in this particular lecture right, little bit of

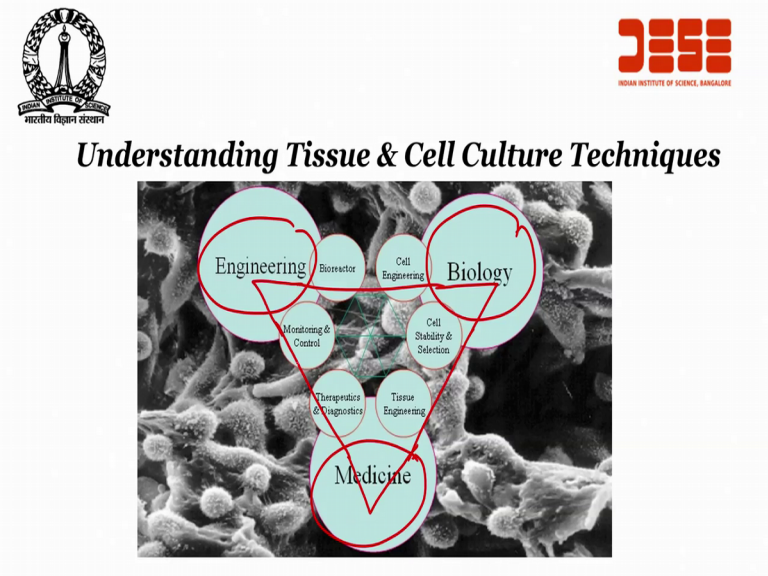
biology, but interesting right engineer talking biology's. So, it will be interesting of course, biologist when the talk the biology, it will be even more interesting. So, let us go through this journey and see as a tissue and cell culture techniques.

(Refer Slide Time: 01:55)



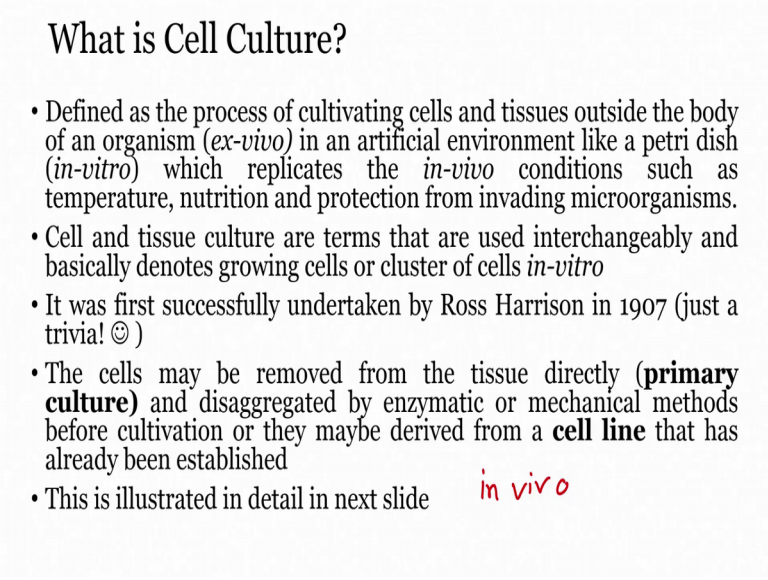
So, when we talk about understanding tissue and cell culture techniques, you are not only talking biology, but your also talking engineering, you are talking about medicine right, and then you talking about biology. And when you talk about all these things and they are all interrelated, it is a like no one can leave without the another one right. See bioreactor, monitoring control, therapies, diagnostics, tissue engineering, cell stabilities elections, cell engineering all these things are interrelated ok.

(Refer Slide Time: 02:49)



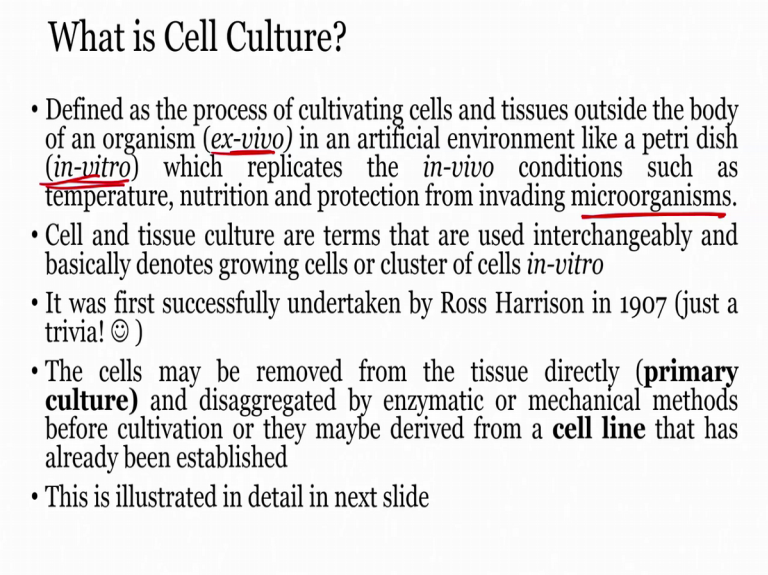
So, we will be we will be looking at this from engineering point of view, and medicine point of view, keeping biology in the centre point of the talk. So, because our device is that we are designing or engineering devices the problems that we are addressing or medical problems and the techniques that we had to use or biology techniques. Thus all these three are interlinked right; a triangle that interlinks is not finished, let us finish the triangle, cool.

(Refer Slide Time: 03:09)



So, what is cell culture, so what is cell culture? First thing defined as a process of cultivate cultivating cells and tissues outside the body of an organism right. Let me tell you few terms, first term is in-vivo; in vivo, in vivo, it is within body.

(Refer Slide Time: 03:41)

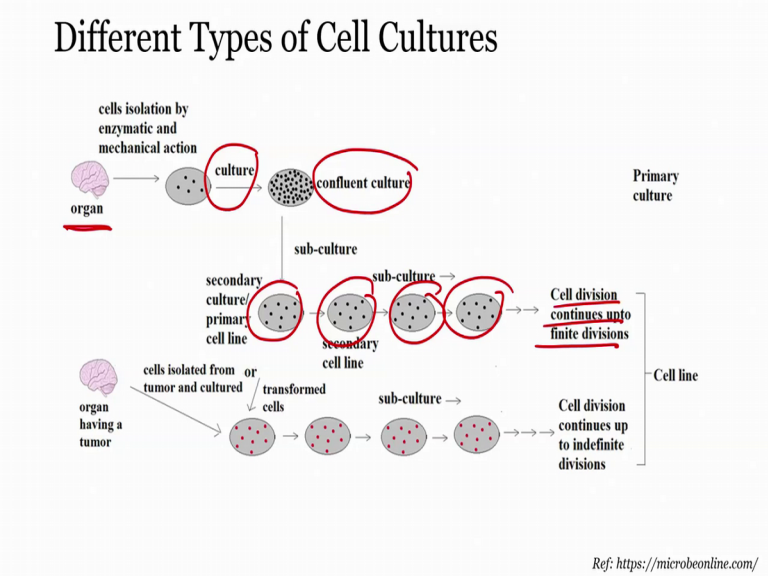


Next term ex-vivo, outside the body; next term in-vitro right or in laboratory environment, artificial environment in-vitro. So, let us see defined what is cell culture? Defined as a process of cultivating cells and tissues outside of the body of an organism that is ex-vivo, in an artificial environment like a Petri dish which is in-vitro right. Which replicates the in-vivo conditions such as temperature, nutrition and protection from invading microorganisms.

Cell and tissue culture are terms that are used interchangeably and basically denotes growing cells or cults cluster of cells in vitro right. You take as a load in Petri dish, load a particular media and will start growing right. So, it can be it as a interchangeable terms that we use cell and tissue culture.

It was first time successful undertaken by Ross Harrison in 1907 just a trivia [FL]. So, the cells may be removed from the tissue directly like primary culture and this aggregated by enzymatic or mechanical methods before cultivating it right. And then may be derived from a cell line that has already been established, there are two ways of taking of the cells. So, let us see both ways in the next slide.

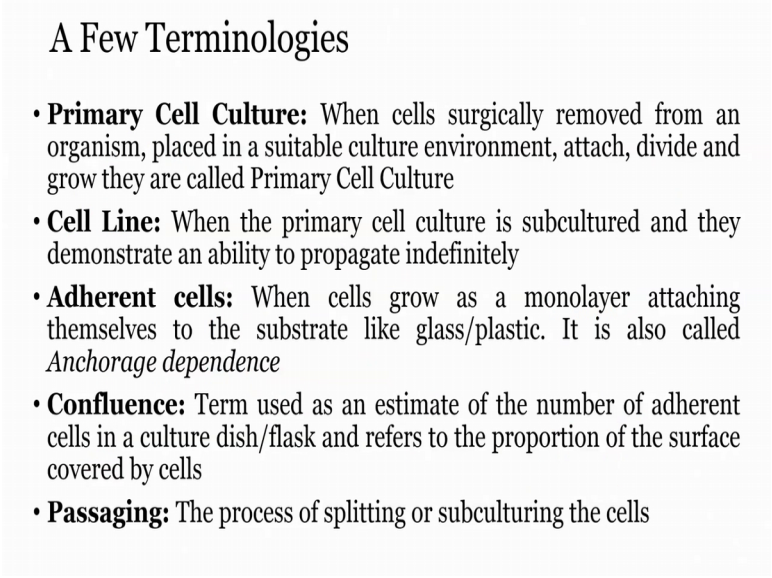
(Refer Slide Time: 05:29)



You see here, first one organ; so, cells isolation by enzymatic or by mechanical action, it is cultured and confluent culture is ready. Once a confluence culture is ready, the secondary culture or a primary line is found right. Secondary line subculture right, we go and bring it cell division continues up to finite divisions right, the primary culture it is a primary culture you got it.

So, what we do is we take the tissue; we take out the cells from the tissue either by enzymatic or mechanical actions. Once the tissues are once the cells is out, we culture those cells and once is which is confluence state we do secondary culture and perform a secondary lie cell line to finally, we keep on culturing it until cell division until some finite divisions. Second way of doing it, organ having a tumor, cell isolated from tumor and cultured right, transformed cells, sub-culture, cell division continues up to indefinite divisions right, these about primary culture.

(Refer Slide Time: 06:53)



So, what is primary culture? When cell or surgically removed from an organism, placed in a suitable culture environment, attach, divide and grow they are called primary cell culture. These terminologies we need to understand, so that when we talk about tissue culture is easily understandable, when these kind of terminologies are used; like cell culture, primary cell culture, cell line, adherent cells, confluence, passaging right. So, we need to understand.

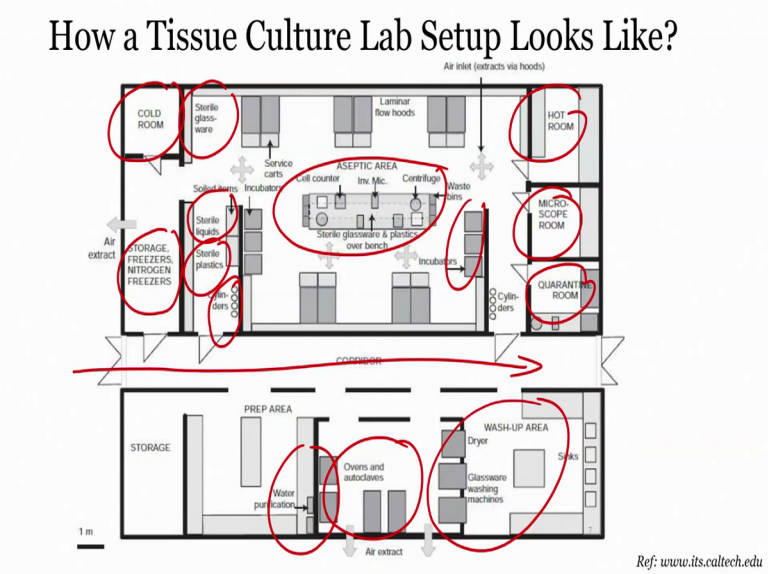
So, when we talk about primary cell culture as we have seen cell surgically removed from an organisms and displaced in the cultural environment and that will attach divide and grow. Cell line, what is cell line? Cell line when the primary cell culture is subculture and they are demonstrated an ability to propagate in definitely is called the cell line.

Then there are adherent cells, adherent cells are when cells grow as a monolayer attaching themselves to the substrate like glass or plastic, it is called anchorage dependence. That is like anchor right anchoring so, because this cells will stick to the glass or plastic in a monolayer.

Next terms comes to as confluence, what is confluence? Terms used as an estimate of the number of adherent cells in a cultural dish or flask and refers to the proportion of the surface covered by cells is called confluence. Passaging the process of splitting or sub culturing the cells is called passaging right. Now, once we know this some terms right,

passaging, cell line, confluence, cell culture what kind of laboratory or a tissue culture lab setup looks like.

(Refer Slide Time: 09:21)



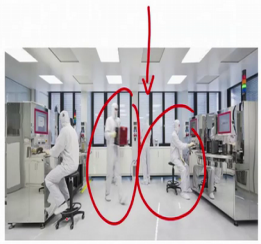
So, basic tissue culture lab should look like the one that I am showing here; however, there are several kind of different setup also. So, just to see you see here there is a cold room, then there are air storage freezers, stereo glassware are kept here, liquid sterile liquid, sterile plastic, cylinder are kept right. Then, this is the area where people work; there are incubators or all this sides right, there are microscope room, there is a hot room, there is a quartertone room, then this is the corridor right.

Again water purification, oven and autoclaves, dryer, glassware, washing machine, sinks right is typically how was setups looks like, we have taken example from Caltech, but similar kind of short facility or a smaller facility, we are also developing right in my department which is electronic system engineering in IISC right. And it will not have the cell culture lab, but it will also have a micro engineering lab, where you can fabric the device and within the same environment you can test a device in this kind of facility.

(Refer Slide Time: 10:43)

Clean Room Specifications

- The tissue culture room needs to be preferably housed in a clean room environment. A typical clean room looks as shown in figure aside
- Clean rooms are classified based on the amount of particulate matter contained within the target space. There is ***ISO*** classification as well as ***FED*** classification for the same. This is enlisted below



Class	maximum particles/m³						FED STD 209E equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1 µm	≥5 µm	
ISO 1	10	2.37	1.02	0.35	0.083	0.0029	
ISO 2	100	23.7	10.2	3.5	0.83	0.029	
ISO 3	1,000	237	102	35	8.3	0.29	Class 1
ISO 4	10,000	2,370	1,020	352	83	2.9	Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1.0×10^6	237,000	102,000	35,200	8,320	293	Class 1,000
ISO 7	1.0×10^7	2.37×10^6	1,020,000	352,000	83,200	2,930	Class 10,000
ISO 8	1.0×10^8	2.37×10^7	1.02×10^7	3,520,000	832,000	29,300	Class 100,000
ISO 9	1.0×10^9	2.37×10^8	1.02×10^8	35,200,000	8,320,000	293,000	Room air

Trivia

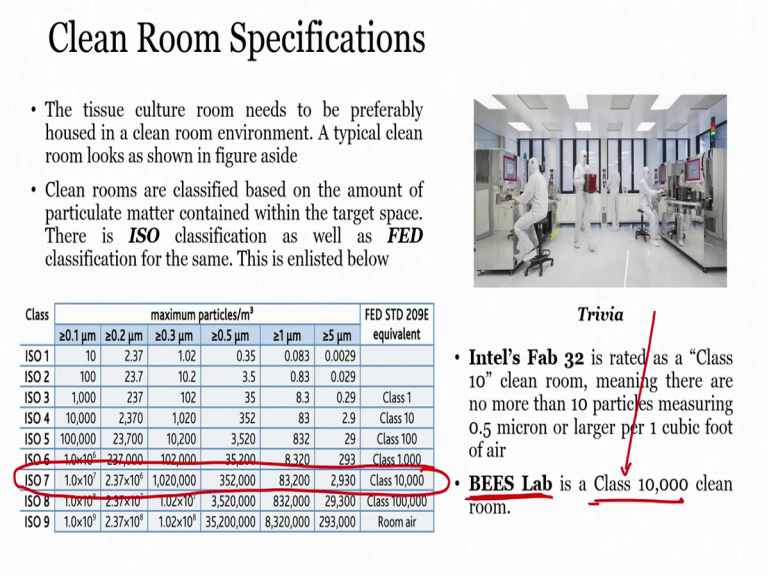
- **Intel's Fab 32** is rated as a "Class 10" clean room, meaning there are no more than 10 particles measuring 0.5 micron or larger per 1 cubic foot of air
- **BEES Lab** is a Class 10,000 clean room.

Now, we always talked about clean room right so, what is clean room and why it is important? So, generally a tissue culture room next to be preferably house in a clean environment and around 10000 class 10000 would be ideal environment for such kind of activity. A typical clean room looks a shown in figure here, you can see here this is a clean room right; and you can see that you have ware gown right to avoid any contamination right is a typical clean room for fabricating device ok. But we can work around if we have class 10000 clean room environment.

Now clean rooms are classified based on the amount of particular matter obtain within the target space. So, with in a space how much particular mater is obtain, also there are ISO classification as well as FED classification, you can see here in the table we talk about ISO 1 then it is really clean about 10 greater than 0.1 micro meter particles, 2.37 for 0.2, 1.02 for 0.3 and this maximum particles or per meter cube; a cubic meter or per meter cube.

When we talk about class 1 right, so that will be ISO 3 and it is a clean room which has about 1000 particles which has grater then 0.1 micron, but 237 greater than 0.2 and so on till 0.5; it till 5 microns. But when we talk about fab lab that is a inter class room, Intel fab 32 that is rated as a class 10 clean room and if you see 10 class clean room, class 10 clean room is here right.

(Refer Slide Time: 12:51)



So, it would have this particular specification. Then comes class 100, then comes class 1000, then comes class 10000, then comes class 100000 and then our laboratory which is cleaner that is out room air right. So, for biology, for pharmacy generally class 10000 is used for experiments right. We have and we will show you, we will give a tool to the to my laboratory here in my department, which is the class 10000 clean room facility, and like I said we are in process of developing another laboratory with a cell culture, as well as the micro fabrication facility which will be class 1000 clean room.

But for now for this particular the lecture and course we will be understanding class 10000 and will be looking at class 10000 in reality, and what kind of equipment are there with in the class 10000 clean room environment right. So, like well if you see the screen, what is see here is intel fab 32 is rated as class 10 clean room, meaning there are no more than 10 particles measuring 0.5 micron or larger per 1 cubic foot of air right, see class 10; no more than 10 particles.

(Refer Slide Time: 14:41)

Now, generally when you see a fab lab, generally see a fab lab, you will find that in a conventional clean room facility which is 100, 1000, 10000 right, you will find several equipment such as LP-CVD, we have just talked about CVD right, Chemical vapor Deposition, low chemical vapor deposition. There is PECVD, which you can see here right it is from oxford, I think yes, it is from oxford. Then we have Tystar CVD for your wet oxidation, dry oxidation. Then you have your CMP for polishing the wafer, then you have dicing saw for dicing the chips, then you have bonder for bonding silicon with silicon, so you need that kind application when we are making a macro fluidic chip.

Then you have thermal evaporator, then you have a parylene deposition, there is plastic deposition, polymer deposition; you have a sputtering system, you may have a E-beam evaporator, you may have a Denton E-beam or thermal evaporator as a thermal evaporation, you have a photoresist spin station, you have a polymer spin station like SU8, you have a EVG 620 mask aligner, you can also have MJB-4 mask aligner; and like you see here it is MJB, MJB-3 you can have MJB- 3,4 whatever mask aligner.

In general mask aligner, you will see then you will see plasma system for bonding glass with PDMS, then you may have wet etching, you have a RI, you may have fluorine and chlorine, and you may have DRI. So, this are the like few of the equipment that you generally find in a clean room, conventional clean room which is 100, 1000, 10000 even class 10.

(Refer Slide Time: 16:35)

But, what we also see, a clean room where the biology experiments have to be performed. In such a kind of clean room, you may have where you can also design, you can design the device, you can fabricate the device and you can perform experiments right. Such kind of facilities are available in few of the top class universities across the world and we are trying to replicate a small facility over here, as well so that kind of clean room where a person can work interface of biology, medicine and engineering.

You will fee[l] you will see a thermal evaporator, you can see a pathology work session, there will be 3-D cell culture, bio safety hood right, it will be a imaging microscope, there can be spin cotter, there can be peristaltic pump, there can be upright microscope uses it peristaltic pump, spin cotter, upright microscope, mycological microscope, bio safety hood you can see here right, you can see fume hood here, you can see micromanipulator here, you can see a incubator or a oven right, DI plant, oxygen plasma system.

(Refer Slide Time: 18:03)

So, is not limited to only this many equipment, there can be if you more like there can wet aching right. It is acid wet aching and can be solve one right. Then you have a mask aligner, you have a inverted microscope, florescent microscope, you have HEPA of filters right, you have a autoclave, you have the you have the system for tissue right tissue microwave and it also called micro tomb by the way.

And of course, you have the vacuum oven that you can see here. So, lot of things you will see in reality in the laboratory so, let me tell you what you will be looking at; when you actually go to the fab lab, we will show it you part of this course at will show you the microscope.

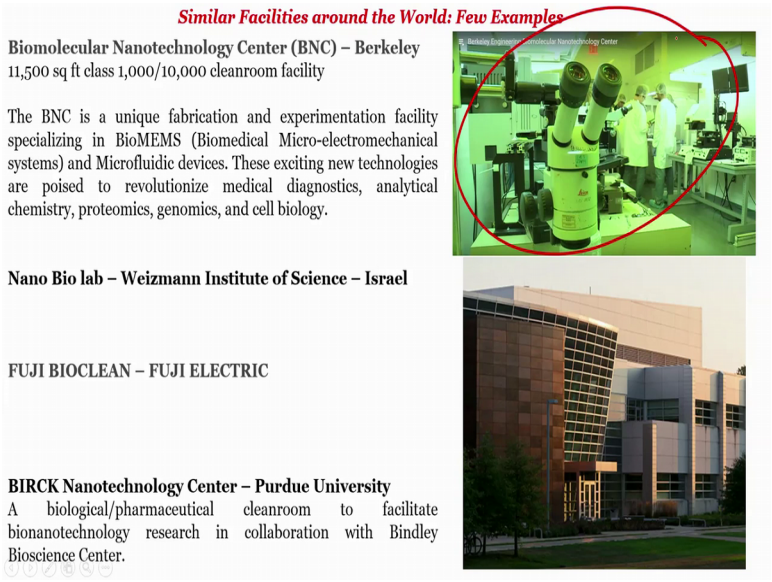
(Refer Slide Time: 18:49)

All three microscope will show you; metallurgical microscope, inverted microscope and stereo microscope, will show you this bio safety hood right. We will show you micro manipulator, we will show you oven, we will show you incubator, we will show you peristaltic pump right. Then, we will also show you HEPA filters we will show you oven and we will show you autoclave right.

So, it will be like half an hour and 1 hour on each of the system so you understand, how it can be operated; how it can be used, not just to show you what facilities should contain; what equipment are there? No, to show you how to operate that equipment right if you have to entered in a clean room, what kind of preclusion you should take, what kind of garment you should wear right? You wear a full sleeve, you should have a toe cover shoe right; it should cover your toe, you should wear coat lab coat, gloves right, a beard cover, head cap right, goggles to protect your eye.

So, how can you how to enter laboratory? Once you enter the laboratory, what kind of equipment are there? Once you see the equipment, how to use this equipment, so that is idea that will be part of this particular course as well. So, when we if you see the slide, when we talk about this equipment; let us see some similar facility around the world.

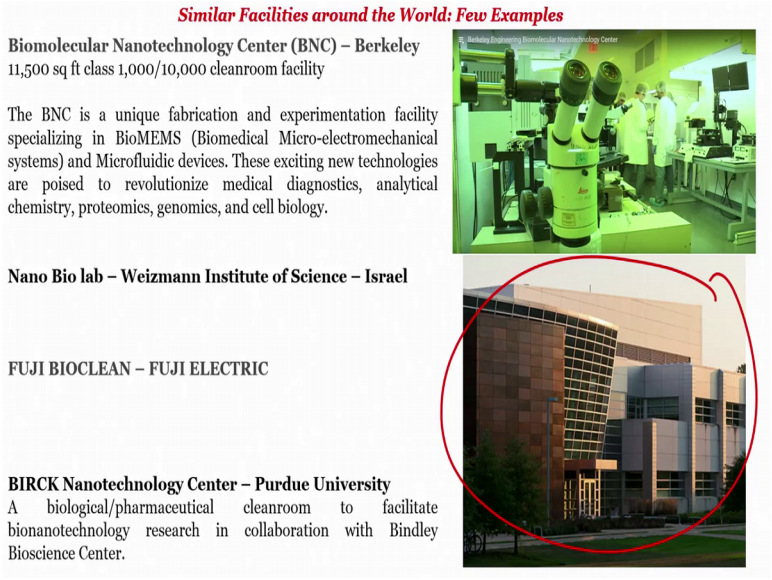
(Refer Slide Time: 20:35)



So, if you see this screen, what you see is bio molecular nanotechnology center, which it at Berkeley you can see here right. This is unique fabrication and experimental facilities specializing in Bio MEMS, bio medical micro-electromechanical system microfluidic devices. These exciting technologies are poised to revo[lutionize] revolutionize medical diagnostics, analytical chemistry, proteomics, genomics, and cell biology. You can see, biology experiments that should be done in a clean room, class 10000 right.

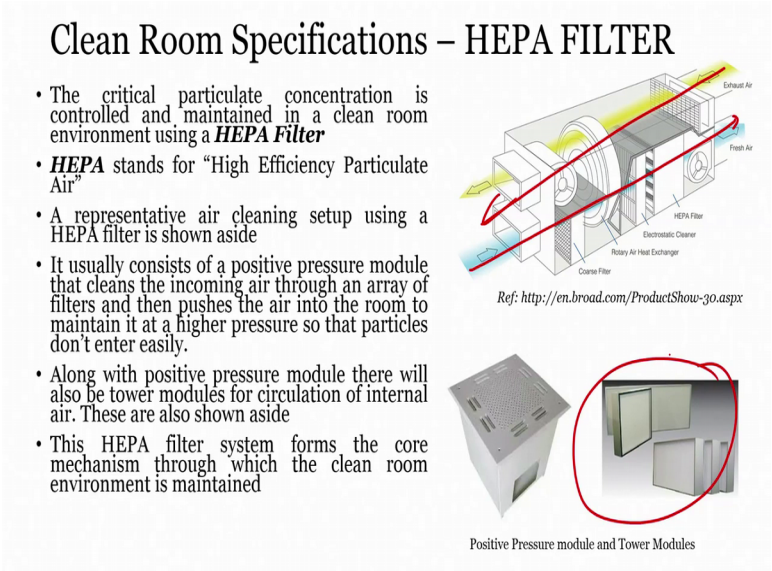
A same way if you see Nano bio lab at Weizmann institute of science, Israel. They have the similar kind of facility, you we have similar facility at Fuji electric.

(Refer Slide Time: 21:27)



And we also have separate Brick Nanotechnology center at Purdue University for especially, this whole center dedicated for biology pharmaceutical clean room to facility bio nanotechnology center collaboration with mainly bio center, bio science center. So, point is do you require such a big thing, I am not saying we should have this right. What I am saying is, if class 10000 environment a clean room environment is provided, then we can do research in multiple areas of biology as well as medicine and pharmacy and work on bio medical devices and so and so forth.

(Refer Slide Time: 22:01)



So, now when we talk about clean room? The most important equipment or a tool is HEPA filter right. So, what exactly HEPA filter is, so the critical particular concentration is controlled and maintained in a clean room environment using a HEPA filter. And HEPA stands for High Efficiency Particulate Air.

So, generally when you see it works like this. So, there is a exhaust air that goes through here, and there is course filter, there is a rotary head air heat exchanger, there is electro static cleaner, there is a HEPA filter and fresh air goes like this, the exhaust air goes back right and this is it works.

There is positive pressure module and there are tower modules that can apply positive pressure. Representative air cleaning setup is shown here right,. it is visually consist of positive pressure module that cleans the incoming air, either the role of positive pressure of module is to clean the incoming air. And area filters that than pushes the air into the room and to maintain it at a higher pressure, so that particles do not easily enter right, so that is the advantage of high pressure; so that particles from outside would not entre the room.

Along with positive pressure module there will be at tower module first circulating of internal air, these are also shown right, you can see here on the in the figure. This HEPA filter system forms core mechanism through which the clean room environment is maintain. HEPA filters are used to keep the clean room environment stable right.

So, for this particular module let us stop here. And we have to see another part of this particular lecture in the next module where you will be looking at equipments, some chemical relate to it then how they are use right, how the tissue culture is done, how the cell culture is done, we will take in the next module, as a part of this same class. Till then you just go through it, this like more informative lecture, so that you understand what kind of facilities are there and how you can culture the cells as well as tissue right.

Till then you take care, I will see you in the next class. Bye.