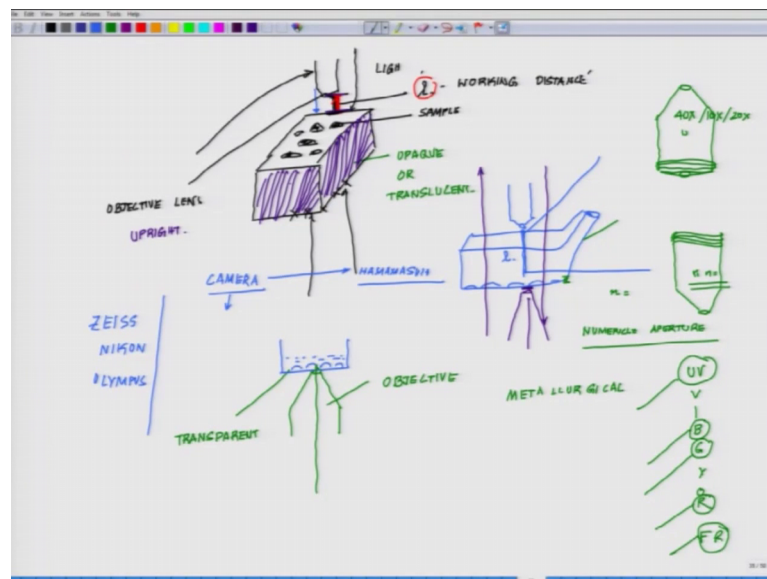


Cell Culture Technologies
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Lecture - 12
State of the art facility in cell culture Lab – II

So, welcome back to the lecture series on Cell Culture. So, we are into the week 3 and we have finished our first class. So, we are going to the second class. So, in the last class if you recollect we were talking about the microscopy. So, we have talked about the challenges of or the need for an upright as well as the inverted system, and there is something in the end I told you about the numerical aperture this is something which will be written on the lens.

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So, if you see on objective if you something like this or objective like this or you can and you will see this is where you fit the objective. So, you have all the groves which are cut there or if you see it from the top something like this, or you were seeing it like this.

Something likes this. So, out here there is some small things which most of us and you only look for it is written 40 x or 10 a x or you know 20 a x like you know likewise so and so forth, but (Refer Time: 01:43) that you will see something very small written like n, which is essentially small n which is essentially call the numerical aperture is called numerical aperture. This numerical aperture is the one which will tell you whether this

objective is best suited for a viewing through glass or through plastic or some other material not getting to the physics of it at this point, because this is not will in the v of it, but those of you are interested you can open up and check how that numerical aperture is being because if this is property of the lens itself inside the objective and this is something it is my experience that every time the big joint microscope sellers kind of do not tell you about it.

They just wanted to push their stuff. So, be very careful on this because these objectives most of that top companies whether you talk about Zeiss Nikon aianthus all of them charges a fortune for individual objective. Everything is in somewhere in the range of the say one lakh or something, as individual objective I am not have been talking about the microscope. Microscope budget goes anywhere between a standard good quality microscope if we talk about somewhere between, you know say 15 to 30 lakhs this is the kind of budget we talk about good quality microscope, you can get some of the cheaper version unless I mean they may not be the best, but you can work out with them within a range of say 5 lakhs.

But whenever you have to plan for microscope, we have to really plan in advance, whether in 5 lakhs this is not something which is like you ask for it and you get it. So, we have to very careful about planning of how really you want this stuff to be. So, again you no no mass up with it; if you have two kind of samples say for example, you have plastic sample you know that will be taking yourself on talk about plastic and you have a glass vessels, the you may need to have two different kind of objectives with different numerical aperture.

(Refer Time: 04:34) see microscope that is not an initial, but you have to have different objectives. Now suppose there is a third thing about the microscopy, if you want to if you are working especially there are several labs sitting in it there are you know there are handful of labs who work on metallurgical sample, for cell related study or some people work on electrodes integration of cell on top of electrodes for them or they may work on you know micro electrode arrays and field if a transistor chips, those are completely opaque systems, you may off for another version of upright which is a metallurgical microscope you may think over that.

But again I am just means there metallurgical microscope. So, again you have to realize couple of a stuff, it is the neck requirement and most importantly as I told you say about the budget what are we talking about. So, budgetary constants we will always be there. So, we have to ensure that within your available budget you are playing your gain. So, that is why I told you in the beginning that lets have a roadmap like you know I will achieve this by this time, this time likewise unless we have a proper road map you will a stumbled upon consequences, which you do not want to get into and this is I am talking from my soul after at least developing 5 to 6 independent state of the art facility across the world, that I have gone through that roller coaster ride that you know lab has this much fund. So, now, (Refer Time: 06:50) but in a very early phases I mean I had no one to tell me that you know I mean it is a job.

So, that is get this, but did not kind of take into account that you know what here you are going to face a roadblock, what will going do to. So, please plan things plan things neatly and you should have a margin like you know after one year I will be getting this, after two years I am going to getting this after three years I am going to. So, if you have a lay out plan for 10 years you are good to go. Now with a microscope there is another aspect which answer, do you want. So, there is (Refer Time: 07:37) do you want provision to attach flowers and facility or do you want the flowers and facility. Because most of the modern days will biology depends heavily on different kind of antibody conjugated markers or people use nano particles flowers and nanoparticles, different kind of conjugations, different kind of astrology, where you have these markers different kind of calcium binders, different kind of movement or the drift of different kind of ions.

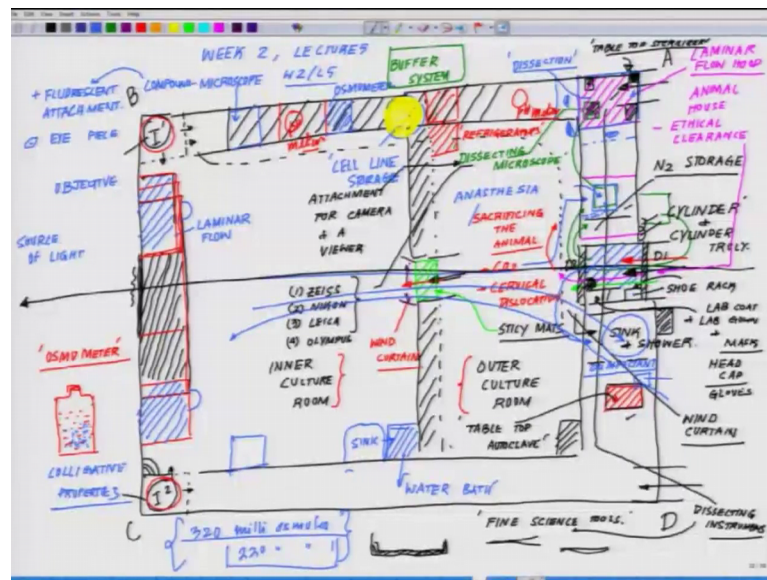
So, I on a slide biasness we will recommend go for fluorescence if your budget permits. So, in other word then get a really good microscope, where you have a integration of the fluorescence. And again with the fluorescence we will come across the interesting thing, which all wavelength you will be getting most of them you will come for you know you will be able to visualize anything which is fluorescing red or green, but you may not be able to visualize something which fluoresce blue, because those lasers are costly. So, again these are some of the points what you have, to take into account those laser diodes which are therefore, the blue are costly as compared to red or green or you may even go for a far raid.

So, there are four zones we can play away u v blue you have if you put it like this, it will make easy, you have blue a u v before that, you have green you have red and you have far red, this is the kind of zone where you are playing this game. So, be careful what you are asking whatever you will ask you will get that. So, this fluorescent assembly is a costly affair, but as I told you with my biasness I will recommend you have a provision for adding fluorescence you will be benefited by it do not leave it because this is needed. Any library setup its a chemical biology lab or a bioengineering facility or a tissue engineering facility or a development biology facility in or by material cell interface facility fluorescent will be essential.

So, do not become fugal on it instead just get it. So, keep in mind what kind of laser diodes your getting and if you have any confusion grab proper manual on microns copy read through them, and all those very nut shell what I have told you will be very clear to you what I am trying to tell you. And much of the things what I am telling you are the practical hurdles you are going to face which the books we will tell, but you will over look most likelihood and the seller will never tell you. Because seller wants to sell his stuff they are now I am going to tell that here now this is the problem you want going to face. So, this problem you want going to face with this they will just you know you ensure that you buy the stuff and then they will ask you have to add up this oh you have to add up this.

So, do not fall in the truck. So, these are some of I may have missed out something and if I have recollect during the (Refer Time: 11:56) of this course. I will definitely put it down there, but these are certain things what in practical day to day life I have faced and I will not wish you young people face that problem. Now from here we will talked about few other instruments which you will be needing pretty (Refer Time: 12:15) in your facility.

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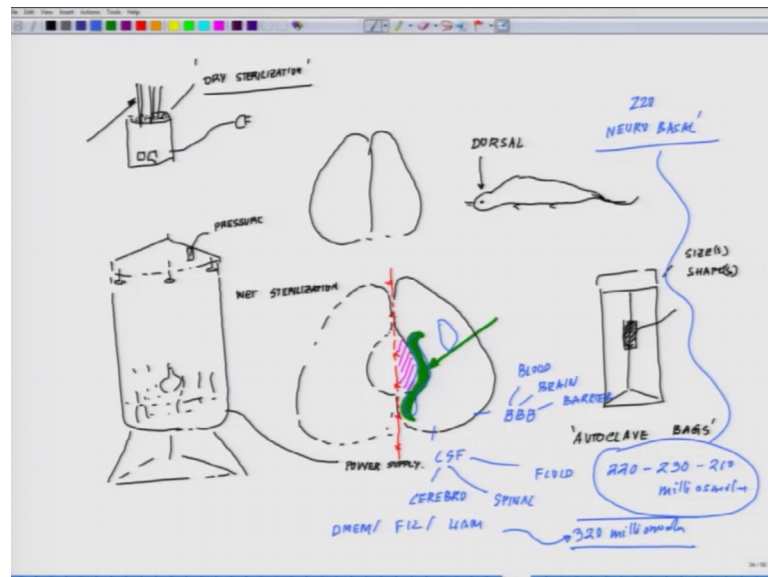
One of the things which you will be needing in both sides here also will be PH meter because when I whenever you are dealing with any kind of medium any kind of buffer system, you have to have a PH meter absolute essential by a grid local quality beach meter and keep it in a safe corner or safe place very does not fall down put in a stand and ensure that its standards because PH meters comes with standards like you know 11 page on page 7 page 10 likewise you will have a standard (Refer Time: 13:10) in a they are kept properly closed and you have a kind of spot where you can measured.

Next in that line apart from PH meter, will be another instrument which most of the labs I visit time to time do not carry with something which is very very important that is called Osmometer. So, Osmolarity in a layman language; that means, suppose here I have a bottle and this is fluid, and I has certain other kind of particles in it the green ones are showing the particles, and these particles generates a pressure which is part of something guys must have studied called Colligative properties of material. Osmometer is being used using something called freezing point depression process, and I am not going to get into the freezing point depression process because it falls under the studying the Colligative property.

If you give you how much proportion in a simplest language how much proportion of solute is present there, and if you talk about our body fluid it how was around 320 milliosmoles, that is essentially your extra cellular fluid as compare to if you look at your

cerebrospinal fluid which is at least 100 magnitude lesser than that it is around 220 milliosmoles. Why this values an important and why am I taking the pain to tell you this once again. So, that means suppose you are working on developing a medium for neural culture neurons right. So, neurons are if you see this picture.

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So, for example, you want culture they have hippocampal neuron or cortical neuron or any part of the brain.

So, these are protected by blood brain barrier BBB, and they are bathe in cerebrospinal fluid CSF Cerebro Spinal Fluid blood brain barrier. So, if you are developing a medium for the neuronal cells, then you should know that what is the milliosmolarity. So, most of the neurons grows better between 2 hun 220 to 230 or even up to 260 milliosmoles, as against the other cell types of the body which are indirect contact with the blood which are around 320 milliosmole. So, many a times your cells dies specially fragile sense like neurons or neuron derivatize cells, because your cell has a very high osmolarity or you do not see the proper growth. So, for example, once we will go to the medium you will realize medium like d m a m all these have F 12, HAM these are different medium names they all have pretty high osmolarity values.

As compared to some of the mediums in recent times what have been developed like neuro base cell exclusively developed by professor brier and southern (Refer Time: 18:09) medical school. These are hovering around in a 220 250 milliosmoles. So, this is

something which time to time you may need to check that what is the Osmolarity value of the medium what you are using. So, you have to just take like in a Eppendorf tube or you take a small sample of the medium mister royal manner and you test it I mean this device it is a very interesting colligative property and its fun. So, this is kind of to keep track. So, you have to have a spot where you will be putting the osmometer ok.

So, see now slowly we are covering the space what we have dedicated for the countertop. So, now, a PH meter you have a osmometer. Now we needed something what I have an mentioned you will be needing somewhere, because you have to thaw the cells if you are using cell lines you have to thaw the cell lines, if you are using some frozen medium you have to thaw it. So, the best idea is to have a small water bath somewhere in the again in the corner has to be careful wherever you keep a water bath, because there are lot of water spill happens at the zone. So, either and second thing forgot this before I forget this you have to have a sink close by. So, you needed to curve out another sink somewhere out here in the inner cell culture facility, a small sink and adjacent to the sink you have to have a water bath because you have to fill this water bath.

So, you do not want to carry water in something and go and (Refer Time: 20:10) in on it or you have other option is that you keep a water bath somewhere out here outside you may need one. So, think over it what kind of water bath you are going and where you want to place the water bath because you will be needing water bath. So, in that case you needed to have a sink along with a water bath adjacent to it. That way you will be ensuring that you know you are not spelling out things and it is always a good idea to have another sink inside may not be a bad idea because you are working on a wash your hands and you know go to the next room, or you are coming from the next one two inside wash your hands or if you want to really minimize you can have one sink the again. These are all function of the amount of a space your having right without that I mean it is tricky.

So, think over it what all you needed. So, definitely will be needing on water bath that is for sure there is no question on that. So, now, the thing is that either you can get away with one water bath out here, somewhere out here you can place a water bath or you can have a have a water bath here, but in that case we will have to travel back and fourth like this. And one more thing which I forgot is while you are working out or kind of you know one second while you are wrestling through using or buying a camera.

So, the companies which you may like to you know look forward for good quality cameras will be Zeiss Nikon, you may even think of Olympus, but again it is all about your budget where you are hitting upon and how far you want to hit on this, and one more thing. Do you want a camera on top of your microscope? Again out of my farsightedness I will recommend go for it because that is really really helpful because then you have a recording like say for example, you want to take wonderful florescent picture you will be doing later some confocal microscopy or something, but you want to take that picture or your cells adhering to some florescent nanomaterial or some other material. So, it is always a good idea to have the camera and with the camera also you have to be careful which company's camera you are going to go through.

However going to fit the camera want will be the camera resolution what. So, there are companies like Hamamatsu they really sell good quality camera, but then the very movement you are having a camera into the system you have to have a monitor adjacent to you because in order take pictures now right. So, your microscope will have a setting adjustment to it computer setup and then of course, the seller of the compute of the microscope we will provide you the software, and you have to image processor image processing software along with it. So, you are saying how the price is continuously jacking up and these are I am telling these are bare essential when you are competing or when you are trying to pull out really good quality work.

So, that is why it becomes very much more imperfective that they 0, when you start to build up the facility you think twice or some of the colleagues who knows it discuss among the user group who will be using it. It is very very very essential think through it thoroughly; I will close in here in the next class we will add up. So, new paradigm into this what will make it much better and more beautiful facility.

So, let us recollect what all we have talked today we have talked about the some of the fundamental information about the objectives, we have talked about the need for an Osmometer, we have talked about the need for multiple PH meters, we have talked about water baths which has to be kept there and in the end we talked about to have a camera setup along with the basic set up of the microscope. And the need for having a monitor along with image processing software's, which partly will be provided by the microscope makers and rest you may have to have a photoshop or something installed in it where you transfer the images or you found to make a video what all facilities you have ok.

Thank you.